Advances in Experimental Medicine and Biology 1154

Rafael Toledo
Bernard Fried *Editors*

Digenetic Trematodes

Second Edition

Springer

Advances in Experimental Medicine and Biology

Volume 1154

More information about this series at http://www.springer.com/series/5584

Rafael Toledo • Bernard Fried
Editors

Digenetic Trematodes

Second Edition

Springer

Editors
Rafael Toledo
Área de Parasitología
Departamento de Farmacia y
Tecnología Farmacéutica y Parasitología
Facultad de Farmacia
Universidad de Valencia
Valencia, Spain

Bernard Fried
Department of Biology
Lafayette College
Easton, PA, USA

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-030-18615-9 ISBN 978-3-030-18616-6 (eBook)
https://doi.org/10.1007/978-3-030-18616-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Digenetic trematodes are a large group of parasites of vertebrates that have significant medical, veterinary, and economic importance. Over 100 species of digenetic trematodes have been recorded to be infecting humans, and the list is very extensive considering the trematode species that parasitize animals. The morbidity and mortality caused by several species in humans can be considered mild or even benignant, though other species have important implications for human health. For example, schistosomiasis affects more than 200 million people, and it has been estimated that schistosomes cause approximately 280,000–500,000 deaths every year and the DALY ("disability-adjusted life years") index of schistosomiasis is estimated as 3.3 million per year. Moreover, it is well established that several species of trematodes act as promoters of malignancy. Despite these facts, human trematode infections have been neglected for years, probably in relation to their limited distribution to low- and middle-income countries. However, this picture has been changing in recent years. Factors such as the migration flows, increased international tourism, changes in alimentary habits, and the globalization of food markets are expanding their geographical limits and the population at risk worldwide. Apart from their importance in human and animal health, digenetic trematodes have a great interest in experimental biology due to their other characteristics such as the following: (1) the complex systematics of this group of parasites in relation to the morphological similarity between members of different taxa and the inadequate or poor specific diagnosis (or both) of several newly established taxa and (2) the complexity of the life cycles that have led to the development of an important number of adaptive strategies to enhance parasite survival and transmission.

In 2014, the first edition of the present book was published to provide coverage to all these aspects. The main goal of the second edition is to present the scientific updates in the field during the latest years and to complete some aspects that could be overlooked in the first edition. To this purpose, it has been divided into four parts. The first part is devoted to analyze the general concepts on the biology and systematics of trematode. The second part focuses on the groups of trematodes that have important implications for human health. Each of the six major groups of human trematodes and the corresponding diseases (schistosomiasis, fascioliasis, paragonimiasis, opisthorchiasis, and clonorchiasis and the intestinal trematodes) are dealt with under separate chapters.

In these chapters, emphasis is placed on recent advances and gaps in our understanding that must be filled to complete the knowledge of these trematodes. In the third part of the book, the main groups of trematodes of veterinary and wildlife interest are analyzed. As mentioned above, the list of potential trematodes that might be discussed in this section is vast. For convenience, we have chosen to focus the chapters on those groups of trematodes that are also recognized to have implications for human health. In the fourth part, clinical aspects such as epidemiology and diagnosis of trematode infections are specifically addressed. Moreover, a new chapter on the genomics and proteomics of the trematode infections has been included in this fourth part to complete a modern view of these important parasitic diseases.

In summary, the main goal of the book is to provide an update of the current status of knowledge on these important parasites in the context of modern parasitology.

Valencia, Spain — Rafael Toledo
Easton, PA, USA — Bernard Fried

Contents

Contributors

Maria Alvárez-Izquierdo Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Ross H. Andrews CASCAP, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Faculty of Medicine, St Mary's Campus, Imperial College London, London, UK

Lawrence R. Ash Infectious and tropical Diseases, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA, USA

Mª Dolores Bargues Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

David Blair College of Science and Engineering, James Cook University, Townsville, QLD, Australia

Matthew G. Bolek, MS, PhD Department of Integrative Biology, Oklahoma State University, Stillwater, OK, USA

J. Bulantová Department of Parasitology, Faculty of Science, Charles University, Prague 2, Czechia

Alba Cortés Carbonell Departament de Farmàcia, Tecnologia Farmacèutica i Parasitologia, Facultat de Farmàcia, Universitat de València, Valencia, Spain

Jong-Yil Chai Institute of Parasitic Diseases, Korea Association of Health Promotion, Seoul, Republic of Korea

Seoul National University College of Medicine, Seoul, Republic of Korea

Jillian T. Detwiler, MS, PhD Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

J. Guillermo Esteban Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

M. Carmen Ferreras Departamento de Sanidad Animal, Facultad de Veterinaria, Instituto de Ganadería de Montaña (CSIC-ULE), Universidad de León, León, Spain

Bernard Fried Department of Biology, Lafayette College, Easton, PA, USA

Sudeep Ghatani Department of Zoology, Sikkim University, Gangtok, Sikkim, India

Petr Horák Department of Parasitology, Faculty of Science, Charles University, Prague 2, Czechia

Bong-Kwang Jung Institute of Parasitic Diseases, Korea Association of Health Promotion, Seoul, Republic of Korea

Nadda Kiatsopit Department of Parasitology, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Aneta Kostadinova Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

Alex Loukas Centre for Molecular Therapeutics, Australian Institute for Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia

Philip T. LoVerde Department of Biochemistry and Structural Biology, University of Texas Health, San Antonio, TX, USA

Department of Pathology and Laboratory Medicine, University of Texas Health, San Antonio, TX, USA

M. Yolanda Manga-González Departamento de Sanidad Animal, Instituto de Ganadería de Montaña, Consejo Superior de Investigaciones Científicas (CSIC-ULE), León, Spain

Santiago Mas-Coma Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Libor Mikeš Department of Parasitology, Faculty of Science, Charles University, Prague 2, Czechia

Carla Muñoz-Antoli Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Mark S. Pearson Centre for Molecular Therapeutics, Australian Institute for Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia

Ana Pérez-del-Olmo Unitat de Zoologia Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Parc Científic, Universitat de València, Valencia, Spain

Trevor N. Petney CASCAP, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Department of Paleontology and Evolution, State Museum of Natural History, Karlsruhe, Germany

Bishnupada Roy Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya, India

Weerachai Saijuntha Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham, Thailand

Jollin Andrea Shylla St. Anthony's College, Shillong, Meghalaya, India

Paiboon Sithithaworn Department of Parasitology, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Javier Sotillo Centre for Molecular Therapeutics, Australian Institute for Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia

Heather A. Stigge Department of Biology, College of Saint Mary, Omaha, NE, USA

Veena Tandon Biotech Park, Lucknow Uttar Pradesh, India

Rafael Toledo Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Mª Adela Valero Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain

Part I

General Aspects of the Trematodes

Form and Function in the Digenea

1

Alba Cortés Carbonell and Bernard Fried

1.1 Introduction

For our coverage on form and function in the Digenea, we have relied heavily on the former chapter on this topic in the first edition of this book (Peoples and Fried 2014). Herein, we have considered the major organ systems covered in that previous work, and followed the same strategy for literature search. Briefly, the words Digenea and Trematoda were each paired with the following search terms: tegument, tegumentary system, sense organs, sensory structures, parenchyma, lymph system, lymphatic system, nervous system, neuromuscular system, holdfast organs, alimentary tract, excretory system, osmoregulation, male reproductive system, and female reproductive system.

Emphasis in this chapter is based on the novel literature since the coverage by Peoples and Fried (2014) and places accent on salient findings between 2012 and the first half of 2018. The layout of this chapter will proceed as follows: for every body system, an overview is given followed by a discussion of the recent literature as it applies to (1) its morphology and (2) its functionality in adult digeneans and, if applicable, in some larval stages. For background information on the subject matter covered in this chapter, two earlier works by Fried and Haseeb (1991) and Fried (1997) should be consulted.

1.2 Tegumentary System

The tegument of digeneans is a living tissue consisting of a distal syncytial cytoplasm and the nucleated cell bodies (or cytons) that lie under a layer of peripheral muscles. A network of cytoplasmic bridges connects the cytons with the distal cytoplasm (Roberts and Janovy 2009). Tegument-associated cells are the primary differentiation progeny of somatic stem cells (or neoblasts) in the schistosomatid *Schistosoma mansoni* (Collins et al. 2016). In an elegant study, Collins and co-authors showed that depletion of stem cells resulted in a delayed downregulation of a set of genes encoding tegument-associated proteins, thus indicating that neoblasts are required to sustain the expression of these genes. Moreover, pulse-chase experiments labeling proliferating cells showed that neoblasts differentiate mainly into a population of short-lived tegument-associated cells, which is rapidly and continuously renewed. Whether this cell population represents

A. Cortés Carbonell (✉)
Departament de Farmàcia, Tecnologia Farmacèutica i Parasitologia, Facultat de Farmàcia, Universitat de València, Valencia, Spain
e-mail: alba.cortes@uv.es

B. Fried (✉)
Department of Biology, Lafayette College, Easton, PA, USA
e-mail: friedb@lafayette.edu

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_1

terminally differentiated tegumental cell bodies or short-lived progenitors to terminally differentiated cells is difficult to demonstrate; irrespective of this, the authors suggested that neoblasts contribute to parasite longevity by way of sustaining tegument turnover and regeneration of host-inflicted damage (Collins et al. 2016).

Key changes in tegument structure along the development of larval stages are described in the first edition of this book (Peoples and Fried 2014). Furthermore, topographic and ultrastructure descriptions of the tegument of digenetic trematodes can be found elsewhere. For instance, recent studies have provided insights on the tegumental organization of the adult stages of the microphallid *Maritrema feliui* (Świderski et al. 2013), the cryptogonimid *Aphallus tubarium* (Antonelli et al. 2014) or the paramphistomes *Carmyerius spatiosus* (Anuracpreeda et al. 2015), and *Orthocoelium parvipapillatum* (Anuracpreeda et al. 2016), among others. The tegumental surface is often corrugated with ridges and furrows, and penetrated by deep pits (Świderski et al. 2013; Anuracpreeda et al. 2015, 2016); some species bear short microvilli (Anuracpreeda et al. 2015, 2016). Ornamentation such as papillae and spines is commonly present in some areas of the body (Świderski et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016) although some species have been reported to be spineless (Anuracpreeda et al. 2016). The syncytial tegument normally contains organelles, such as mitochondria and lysosomes, as well as a number of morphologically diverse vesicular inclusions (commonly referred as tegumental granules or secretory bodies) that arise from the underlying cytons (e.g., Świderski et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016). The function of these vesicles still remain unclear although it has been suggested that they may contribute to the maintenance, restoration, and modification of tegumental membranes (Skelly and Wilson 2006).

Certain proteins with key roles in membrane biology are essential for tegument development and the maintenance of its structural integrity. Suppression of members of the tetraspanin family resulted in malformation of the tegument of *S. mansoni* (Tran et al. 2010) and the opisthorchiid *Opisthorchis viverrini* (Piratae et al. 2012), and impaired parasite survival (Tran et al. 2010). Similarly, suppression of vesicle-associated membrane protein 2 (VAMP2), which is presumably involved in the normal cargo trafficking across the tegument, affected tegument structure and transtegumental glucose uptake in the schistosomatid *S. japonicum*, thus reducing parasite viability (Han et al. 2017). Myoferlin, in contrast, has been regarded to participate in vesicle trafficking and fusion during the repair of the external membrane of *S. japonicum* (Xiong et al. 2013).

From a functional perspective, the tegument of digenetic trematodes is involved in multiple vital processes, including osmoregulation, nutrient acquisition, synthesis and secretion of substances, and protection from external damage (Roberts and Janovy 2009). Furthermore, the apical membrane also contains a number of proteins, lipids, and glycoconjugates that are important for host–parasite interactions across developmental stages (e.g., Skelly and Wilson 2006). Recently, a role for the tegument in the secretion of extracellular vesicles has been documented. Multivesicular bodies have been observed in the syncytial tegument of the echinostomatid *Echinostoma caproni*, the fasciolid *Fasciola hepatica*, and the dicroceliid *Dicrocoelium dendriticum* (Marcilla et al. 2012; Bernal et al. 2014). These structures, which have an endosomal origin and are likely to be constitutively produced by flukes (Cwiklinski et al. 2015a, b), fuse with the apical membrane to discharge exosome-like intraluminal vesicles into the environment (Marcilla et al. 2012; Bernal et al. 2014). Parasite-derived extracellular vesicles can transfer their cargo (mainly proteins and small RNAs) into mammalian host's cells (Marcilla et al. 2012; Chaiyadet et al. 2015) and alter their protein profiling (Chaiyadet et al. 2015); therefore, these tegument-derived elements are crucial to interspecific communication between parasites and their hosts.

Being the most external organ, the tegument plays also a pivotal role in immune evasion. Several tegumental proteins of *S. japonicum* are able to bind host immunoglobulins in a non-specific manner (i.e., via the Fc domain), which

may serve to mask specific antigen-recognition sites and avoid immune attachment (Wu et al. 2015). In contrast, host antibodies bind specifically to the surface of *E. caproni* and become trapped by excretory/secretory products; that facilitates antibody degradation by parasite-derived proteases and may hamper the antibody-mediated attack (Cortés et al. 2017). On the other hand, tegumental ATP diphosphohydrolase expressed by intravascular stages of *S. mansoni* works as an apyrase, degrading external pro-inflammatory and pro-thrombotic signals (ATP and ADP, respectively) and minimizing host immune responses (Da'dara et al. 2014).

1.3 Sensory System

Ultrastructure studies have revealed a vast array of presumed sensory receptors in the tegument of both larval and adult stages of digeneans. These organs harbor fine nervous terminations branched from the peripheral nervous system that end beneath the surface layer of the tegument. Although experimental evidence is lacking, a variety of functions have been ascribed to these receptors based on accurate structural descriptions. In particular, sensory endings have been regarded to be sensitive to touch (tangoreceptors), light (photoreceptors), fluid currents (rheoreceptors), and specific molecules and ions (chemoreceptors). Form, function, and distribution of these receptors throughout the body of the parasite largely depend on the species and life stage and may reflect the sources of stimuli to which the parasite have to react to Halton (2004).

Two types of sensory structures displaying different morphology and localization have been described on the tegument of the deropristid *Deropristis inflata* (Filippi et al. 2013) and the cryptogonimid *A. tubarium* (Antonelli et al. 2014). In both species, type 1 receptors are button-like uniciliated structures consisting of a nerve bulb from which a cilium extends from a centriole. The nerve bulb contains numerous mitochondria and nerve fibers, and is connected to the cytoplasm by a hemidesmosome located at the top of the bulb. A long striated rootlet lies underneath the centriole in type 1 receptors of *D. inflata* (Filippi et al. 2013), but is lacking in those of *A. tubarium* (Antonelli et al. 2014). Type 2 receptors are dome-like non-ciliated structures; however, their internal ultrastructure varies between the two species. While type 2 receptors of *D. inflata* enclose an ovoid electron-dense structure (Filippi et al. 2013), in the case of *A. tubarium*, nerve bulbs are filled with electron-lucent vesicles (Antonelli et al. 2014). Hemispherical electron-dense collars are observed on the top of the nerve bulbs in type 2 receptors of *A. tubarium*, and most of them display a gland-duct opening to the outside (Antonelli et al. 2014). Similar uniciliated (type 2) and non-ciliated (type 1) structures have been described on the surface of paramphistomes *C. spatiosus* (Anuracpreeda et al. 2015) and *O. parvipapillatum* (Anuracpreeda et al. 2016).

Sensory receptors show a specific distribution throughout the surface of the parasite and may appear both singly or in groups. For instance, either type 1 or type 2 receptors of *C. spatiosus* and *O. parvipapillatum* are grouped on the top of dome-shaped papillae displaying a nipple-like tip, which, in turn, can also appear clustered or isolated (Anuracpreeda et al. 2015, 2016). Type 1 receptors of *A. tubarium* are grouped (Antonelli et al. 2014), while in *D. inflata* this type of receptors can be observed either individually, or in groups of two to three in a single papilla (Filippi et al. 2013). Regarding their distribution throughout body of the parasite, the density of sensory receptors is generally higher in the ventral side of the flukes and, in particular, in the surroundings of the suckers, suggesting that they may have roles in feeding and attachment (Filippi et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016). Intrategumental receptors (i.e., non-ciliated) are suggested to act as tangoreceptors sensing pressure, while ciliated receptors could possibly be chemoreceptors, as well as rheo- or mechanoreceptors (Filippi et al. 2013; Antonelli et al. 2014; Anuracpreeda et al. 2015, 2016).

Sensory organs are particularly important for the free-living stages of digeneans (mainly miracidia and cercariae), which heavily rely on external stimuli, such as light and gravity, to rapidly

find an appropriate host and keep the life cycle going (Roberts and Janovy 2009). Three sensory structures have been described in the miracidia of the strigeid *Cardiocephaloides longicollis*: two types of peripheral sensory endings, namely uniciliated and multiciliated deep-pit-like sensory papillae, and a pair of eyespots (each one of a different size). These structures are located in the anterior part of the miracidium and are presumed to be adaptations for chemoreception, tangoreception, and photoreception in the active location of the snail host (Born-Torrijos et al. 2017).

1.4 Neuromuscular System

According to classical descriptions, the somatic musculature of digenetic trematodes generally consists of the body-wall musculature, which comprises a layer of circular muscles laying just under the basal lamina of the tegument plus longitudinal and diagonal muscular bundles beneath the circular muscles, and the internal or parenchymal musculature, mostly formed by dorsoventral muscle fibers (Roberts and Janovy 2009). However, a study conducted by Krupenko and Dobrovolskij (2015) on the organization of the muscular system in digeneans, suggests that further groups of muscle fibers do exist in both the body-wall and the inner musculature, which appear consistently across distant taxa (Krupenko and Dobrovolskij 2015). In particular, anteroradial, posteroradial, and anterolateral muscle fibers and U-shaped muscle sets are abundant in the body-wall musculature at the ventral surface, and are associated with the acetabulum. Furthermore, eight main types of additional internal musculature have been defined, with presumable functions in the movement of the suckers and pharynx (Krupenko and Dobrovolskij 2015). Indeed, both body-wall and parenchymal musculature are generally more prominent in the preacetabular part of the flukes, from cercaria to adult. Preacetabular musculature is regarded to be involved in the movements of the anterior part of the body, including ventral and oral suckers and the anterior organ in the cercarial stage (Krupenko and Dobrovolskij 2015).

Species lacking of a ventral sucker, such as notocotylids, display adaptations of the musculature that may aid in parasite attachment via the ventral concavity (Krupenko and Gonchar 2017a, b). Analogs for acetabulum-associated groups of muscles are found in the ventral concavity of these flukes, including muscle groups that are characteristic for the Notocotylidae family. For instance, a set of ventrolateral longitudinal musculature with a U-shaped pattern works like the circular musculature of a sphincter, while the oblique internal musculature may perform the function of the radial muscle fibers of a sucker. Moreover, additional body-wall and internal musculature at the anterior region are also specific for this family in Digenea and may provide the oral sucker with diverse movements (Krupenko and Gonchar 2017a, b).

The structure of the muscular system usually shows a general pattern through the hermaphroditic generations of digeneans (Krupenko and Dobrovolskij 2015; Krupenko and Gonchar 2017b). However, the musculature of diplostomids, and in particular that of *Diplostomum pseudospathaceum*, undergoes a substantial rearrangement during metacercarial development, which is particularly extensive in the body-wall and the anterior region of the parasite; this transformation may reflect the different roles the musculature plays in cercarial and adult worms (Petrov and Podvyaznaya 2016). Developmental changes in the body-wall musculature of *D. pseudospathaceum* occur in three consecutive phases, including (1) the expansion of the longitudinal musculature; (2) the replacement of cercarial musculature and the increase in the number of longitudinal and circular body-wall muscles; and (3) the separation of the diagonal muscles into two distinct sets on the dorsal and ventral sides of the body, each of them working in an antagonistic manner. Circular and longitudinal muscles seem also likely to form two separate sets although this configuration has not been confirmed. Furthermore, dorsoventral muscles become particularly abundant in the infective metacercariae (Petrov and Podvyaznaya 2016).

The digenean nervous system typically has an orthogonal, or ladder-like, conformation, with

pairs of longitudinal nerve cords extending anteriorly and posteriorly from the cerebral ganglia and cross-linked at intervals by transverse ring commissures. Nervous terminations branch from the longitudinal trunks and provide motor and sensory innervation to the muscles and tegument (Roberts and Janovy 2009). The nervous system of trematodes have been traditionally reported to consist of a single pair of cerebral ganglia; however, presence of two of these structures has been reported in adult *S. mansoni* by targeting specific neuronal markers, including the biogenic amine octopamine (El-Sakkary et al. 2018). Each pair of ganglia is associated to one of the two suckers (oral or ventral) in the adult worm, suggesting a particular adaptation for an independent control of each sucker (El-Sakkary et al. 2018).

Neuronal cell types in Digenea are mainly multi- and bipolar, and highly secretory in nature; the composition of these secretions have been reported to include cholinergic, aminergic, and peptidergic messenger molecules (Halton and Maule 2004). Although early experimental evidence supported an inhibitory role for acetylcholine (ACh) in trematodes (Barker et al. 1966; Holmes and Fairweather 1984), functional ACh-activated receptors were not identified until many decades later. A study conducted in *S. mansoni* characterized for the first time a family of ACh-gated chloride channels (SmACCs), which play an inhibitory role in the neuromuscular activity of this parasite (MacDonald et al. 2014). These channels are formed by chloride-selective nicotinic ACh receptor subunits and are located mainly in minor nerve fibers that innervate the body-wall musculature, rather than directly on the muscles. This pattern of distribution suggests that SmACCs mediate their inhibitory effects in an indirect manner, presumably modulating the release of other neurotransmitters (MacDonald et al. 2014). Genomic evidence exists for the presence of closely related putative nicotinic ACh receptor chloride channels in the opisthorchiid *C. sinensis* (Huang et al. 2013), which lends credit to the hypothesis of MacDonald and co-workers (2014) about the existence of a unique clade of platyhelminth-specific nicotinic chloride channels.

A second ACh receptor has been described in *S. mansoni* (SmGAR), a constitutively active G protein-coupled receptor, which suppression led to the unexpected inhibition of motility in the schistosomula (MacDonald et al. 2015). The way in which this receptor modulates the movement of the parasite remains unknown. Nonetheless, it seems plausible that, as in other organisms (Dittman and Kaplan 2008), SmGAR works to repress the activity of ACh via a mechanism of negative feedback; therefore, suppression of the negative feedback provided by the receptor would result in increased ACh signaling with the consequent reduction in motility (MacDonald et al. 2015).

Serotonin (5-hydroxytriptamine: 5-HT) is an important myoexcitatory neurotransmitter in trematodes, which is present both in the central nervous system, as well as in peripheral nerve fibers and the plexuses that supply the body-wall musculature. Furthermore, 5-HT stimulates glucose uptake and carbohydrate metabolism, thus increasing energy availability for muscle activity (reviewed by Ribeiro et al. 2005). Two studies have started to shed light on the mechanisms of signaling of 5-HT and its mode of action in *S. mansoni* (Patocka and Ribeiro 2013; Patocka et al. 2014). A functional receptor specific for 5-HT (Sm5THR) has been identified in this schistosomatid, which is involved in the control of motor activity (Patocka et al. 2014). Sm5THR is a rhodopsin-like G protein-coupled receptor, signaling through which involves coupling of a stimulatory G protein and activation of adenylate cyclase, with the subsequent increase in cytosolic cyclic AMP. This receptor is broadly distributed through the body of the worm although it is particularly abundant throughout the nervous system and, particularly, closely to 5-HT-containing neurons. Suppression of the gene encoding for Sm5HTR resulted in a reduced motility of both larval and adult schistosomes, which is consistent with the excitatory role of 5-HT. Overall, localization and functional features of Sm5HTR suggest that it controls parasite's movement by modulating the release of other myoexcitatory neurotransmitters, which in turn initiate muscle contraction. Moreover, its distribution in the

central nervous system indicates that additional 5-HT-regulated mechanisms may be operating at this level and contribute to motor control (Patocka et al. 2014). A second putative 5-HT receptor has been identified in the genome of *S. mansoni*, which has yet to be confirmed by functional analysis (Patocka et al. 2014).

Another component of the serotonergic system has been characterized in *S. mansoni*: a specific plasma membrane transporter (SmSERT), with a potential role in the inactivation of 5-HT. This transported is distributed parallel to 5-HT throughout the nervous system, in particular at neuronal varicosities, where neurotransmitters are released to exert their function and then sequestered back into the neurons. Furthermore, disruption of SmSERT activity caused schistosome larvae to become hyperactive, which is consistent with it mediating the re-uptake of 5-HT to terminate signaling. These features suggest that SmSERT is a key player in the serotonergic control of parasite motility, which main function should be removing 5-HT from the extracellular space to control the intensity and duration of signaling (Patocka and Ribeiro 2013).

Besides ACh and 5-HT, further neuroactive molecules such as dopamine, histamine, octopamine, and neuropeptides are also involved in nervous and neuromuscular activities in digeneans (e.g., Eriksson et al. 1996; El-Shehabi and Ribeiro 2010; Tolstenkov et al. 2010; El-Shehabi et al. 2012; El-Sakkary et al. 2018). In regard to the later, two groups of native neuropeptides have been identified in trematodes, including pancreatic polypeptide-like neuropeptide F and FMRFamide-related peptides, whose localization and potential functions were covered in the first edition of this book (Peoples and Fried 2014). Nevertheless, it is worth mentioning that genes with potential functions in neuropeptide signaling are highly transcribed in the gonads of adult *S. mansoni*, particularly in the testes, suggesting that neural processes may play roles in growth and differentiation of the gonads, and intervene the reproductive biology via endocrine and paracrine mechanisms (Lu et al. 2016).

1.5 Alimentary System

Trematode feeding varies depending on the habitat within the host, generally consisting in blood, mucus, or tissues. The alimentary system consists of two differentiated parts, the foregut and the gut, or ceca. The former typically includes the mouth (surrounded by the oral sucker), the pharynx, and the esophagus, which connects with the ceca. Some species display a short pre-pharynx, while others, however, have no pharynx. The lining of the foregut displays a tegument-like structure, but without spines, and is responsible for food suction and early break down. Right after the esophagus, the alimentary tract usually bifurcates in two ceca, where digestion and nutrient uptake occur. Digestion is predominantly extracellular, occurring at the lumen of the ceca, although intracellular events may occur in some species. The ceca are lined by a simple epithelium, called gastrodermis, which can be cellular or syncytial, and whose cytoplasm is highly folded with the purpose to increase the absorptive surface (Bogitsh et al. 2013; Roberts and Janovy 2009). Some genera in the Digenea, however, may not have a digestive system. In particular, while early descriptions of the dicroceliid genus *Metadelphis* reported these flukes to have a poorly visible, although still existing, digestive system (Travassos 1944, 1955), recent reexaminations have suggested that this statement was mistaken, and members of the genera *Metadelphis* and *Parametadelphis* actually lack of a digestive system (Tkach et al. 2018).

To our knowledge, the latest fine ultrastructural descriptions of the whole alimentary tract in Digenea were carried out on the cercarial stages of the diplostomid *D. pseudospathaceum* (Podvyaznaya 2006) and the bucephalid *Prosorhynchoides borealis* (Podvyaznaya 2011), and both were well covered in the first edition of this book (Peoples and Fried 2014).

In the past few years, studies on the alimentary system of digeneans have focused on the esophagus of human schistosomatids *S. mansoni* and *S. japonicum*. The esophagus of these flukes appears as a glandular tissue, organized in two

well-demarcated compartments. The lining of the anterior esophagus is typical of the syncytial tegument, with cytoplasmic folds projecting towards the lumen (Li et al. 2013). In *S. japonicum*, the surface area of the anterior esophagus is greatly increased by abundant corrugations displaying thread-like extensions of cytoplasm on their tips. These filiform projections have been proposed to work to entangle blood cells during the feeding process, thus increasing their retention time for interacting with esophageal secretions (Li et al. 2014). The posterior esophageal syncytium is characterized by the presence of copious plate-like extensions containing unique crystalloid vesicles that are emptied between the plates, where they form extracellular aggregates. These cytoplasmic extensions are closely disposed in such a way that only plasma proteins are allowed to enter the interplate spaces. This singular organization, typical of transporting epithelia, led the authors to suggest that it may serve for the generation massive ion-fluxes. Alternatively, it is also plausible that this structure functions to sequester the aforementioned crystalloid secretions, thus enabling a more efficient interaction between them and the ingested blood (Li et al. 2013).

Food ingestion in these schistosomes occurs in two steps (Li et al. 2013). First, blood accumulates in the lumen of the anterior esophagus, before being pushed as a bolus to the posterior compartment; a network of muscular fibers propels this transition. Red blood cells appear to be lysed rapidly after they enter the posterior esophagus. However, a plug of leucocytes at different degrees of degradation is tethered in the lumen of this compartment, while blood streams around. Presumably, this cellular mass works to minimize the entrance of leucocytes into the gut and avoid the detrimental consequences that this event might have for the parasite (Li et al. 2013).

Ultrastructural studies suggest that the esophagus of these blood flukes possesses a high secretory activity since different types of vesicular inclusions are abundant in the cytoplasmic projections of both the anterior and posterior compartments (Li et al. 2013, 2014). The importance of the secretory pathway in this organ is supported by transcriptomic data, showing the enrichment of transcripts from genes involved in the intracellular vesicle transport (Wilson et al. 2015). Other transcripts overrepresented in this region come from microexon genes and genes encoding proteases and lysosomal hydrolases, which are secreted into the esophagus lumen (Li et al. 2013, 2018; Wilson et al. 2015). Predicted structure for the protein products of microexon genes suggests that they may perform several functions, including leucocyte trapping in the esophageal lumen, protection against immune attack, hemolysis, and antimicrobial roles (Wilson et al. 2015; Li et al. 2018). Some families of microexon genes are also represented in the bird schistosomatid *Trichobilharzia regenti*, suggesting that these genes have an ancient origin related to blood feeding (Li et al. 2018). Lysosomal hydrolases and proteases are regarded to work in blood processing, and in preventing blood clotting (Wilson et al. 2015).

1.6 Respiratory System

The life cycle of digeneans consist in a sequence of several developmental stages inhabiting a range of aerobic/anaerobic environments; therefore, these organisms need to undergo constant transitions in energy metabolism to adapt themselves to the changing environment (Takamiya et al. 2010). The expression profile of key metabolic enzymes revealed that fasciolid *F. hepatica* shifts from an aerobic to an anaerobic metabolism during the development of newly excysted juveniles into adult flukes, which has been related to a reduction in the diffusion of oxygen into the parasite tissues as it grows (Cwiklinski et al. 2015a, b). Transcriptomic analyses of the opisthorchids *Clonorchis sinensis* (Huang et al. 2013) and *O. viverrini* (Young et al. 2014) showed that the adult stages of these liver flukes express genes associated with both aerobic and anaerobic respiration.

1.7 Excretory System

The excretory system in Digenea is of protonephridial type, i.e., a tubular system composed of flame cells that opens only at the distal end by way of a pore. Flame cells (or bulbs) are flask-shaped units, each containing a tuft of fused flagella and opening in a terminal tubule. Beating of the flagella creates a pressure gradient that draws the excretory fluid through a filtering weir out of the cell into the collecting tubule. Terminal tubules of several flame cells converge to form larger collecting tubules, eventually emptying into a common excretory bladder. In adult digeneans, the excretory bladder opens to the exterior through the excretory pore, usually located near the posterior end of the body. This system is presumed to serve both excretory and osmoregulatory functions. Notwithstanding, in some trematodes the walls of the collecting ducts display structural adaptations such as microvilli, thus suggesting that additional absorptive/secretory functions may also occur (Roberts and Janovy 2009).

Excretion comprises a series of processes aiming at (1) removing waste products of metabolism and other unnecessary substances; (2) regulating the internal osmotic pressure and ionic composition; and (3) inactivating and/or eliminating injurious compounds (Beklemishev 1969). The latter purpose raises a particular interest in the context of parasitic helminths due to its importance in the development of drug resistance (e.g., Sato et al. 2004; Kumkate et al. 2008). A study conducted on the opisthorchiid *O. felineus* identified four putative genes encoding for P-glycoproteins, which are active in the excretory system of the adult stage and are presumably involved in the elimination of drugs via this system (Mordvinov et al. 2017). P-glycoproteins belong to the ABC transporter superfamily of proteins and are responsible for the efflux of a wide range of endo- and xenobiotics across cellular membranes (reviewed by Greenberg 2013). Members of this family had been previously identified in other digeneans, including the fasciolids *F. gigantica* (Kumkate et al. 2008) and *F. hepatica* (Wilkinson et al. 2012), and the schistosomatid *S. mansoni* (Messerli et al. 2009). In these species, P-glycoproteins have been implicated in drug excretion and the generation of anthelmintic resistance (Kumkate et al. 2008; Messerli et al. 2009; Wilkinson et al. 2012; Kasinathan et al. 2014; Pinto-Almeida et al. 2015).

The metabolic system cytochrome P450 is also associated with the protonephridia in *O. felineus*, where it participates in the biotransformation of xenobiotics. Moreover, suppression of the gene encoding for this system caused a significant enlargement of the excretory channels and bladder, and increased worm mortality. These results suggest that cytochrome P450 is potentially involved in worm metabolism and detoxification, and evidence the importance of this system for the parasite lifestyle (Pakharukova et al. 2015).

Although the term excretory system is usually employed as a synonym for protonephridia, excretion in digeneans, as in other flatworms, involves additional body systems, such as the alimentary tract and the tegument (Hertel 1993). For instance, unlike in *O. felineus* (Mordvinov et al. 2017), the aforementioned P-glycoproteins have been localized in the ceca and tegumental cells of *F. gigantica* (Kumkate et al. 2008), and the gut of *S. mansoni* (Messerli et al. 2009).

The tegument play a particularly important role in osmoregulation throughout the various developmental stages of trematodes, and tegumental aquaporins are crucial for this function, facilitating the selective passage of water and small solutes through the external membrane (e.g., Faghiri and Skelly 2009; Thanasuwan et al. 2014). Furthermore, aside of maintaining the osmotic pressure, a role for some tegumental aquaporins in the excretion of nitrogenous waste products, such as urea and ammonia, have been reported in the opisthorchiid *C. sinensis* (Geadkaew et al. 2015) and the schistosomatid *S. japonicum* (Huang et al. 2016). In addition to transporter-assisted excretion, wastes can be also excreted via simple diffusion across the tegument and the gut epithelium, or by the means of exocytic vesicles that flush residues produced within the organism outwards (Hertel 1993).

1.8 Reproductive Systems

Digenetic trematodes display obligate alternation between sexual and clonal reproduction over their life cycles. With the exception of members of the family Schistosomatidae, which have evolved separate sexes, trematodes are hermaphroditic. However, although self-fertilization may occur in some species, cross-fertilization is the most extended form of reproduction during the adult stage. Within the definitive host, flukes find each other by means of chemoattractants, presumably cholesterol, or a closely related steroid, except in the case of schistosomes (Roberts and Janovy 2009). Exceptional cases of asexual reproduction by parthenogenesis have also been reported in adult trematodes (Whitfield and Evans 1983; Nollen 1997; Van Herwerden et al. 1999).

1.8.1 Male Reproductive System

The male reproductive system typically consists of two testes, although some species have only one testis, while others are multitesticular. The position, relative orientation, and shape of the testes vary among species and are generally useful for the identification of trematode species. Each testis has a vas efferens that connects with others to form a unique vas deferens, eventually ending in a genital pore, that is usually located within the common genital atrium on the midventral surface of the fluke. Before entering the genital pore, the deferent duct normally goes into a muscular cirrus sac, where it expands to form an internal seminal vesicle. Distally, this vas constricts again to form an ejaculatory duct, normally bounded by prostate gland cells; it extends until the end of the cirrus sac, forming the male copulatory organ, or cirrus, which can evaginate from the surrounding pouch to discharge the sperm into the female reproductive system. Specialized modifications of the distal part of the system, such as absence of cirrus sac and/or prostate glands, or external location of the seminal vesicle (i.e., out of the cirrus sac) are observed in some trematodes (Roberts and Janovy 2009).

The spermatological characteristics in Digenea have been extensively reviewed by Bakhoum and co-authors (Bakhoum et al. 2017), who proposed five types of spermatological models according to the main features of the mature spermatozoa. Commonly, male gametocytes are elongated cells without discernible external organization, containing two axonemes, four attachment zones, at least one mitochondrion, a posterior nucleus, and two longitudinal bundles of parallel cortical microtubules; membrane ornamentations and spine-like bodies can be observed in some species (Bakhoum et al. 2017). The axonemes typically exhibit a 9 + '1' trepaxonematan pattern, consisting in 9 peripheral doublets of microtubules disposed around a central core. Nonetheless, particular variations of this structure are found in schistosomes and some didyzomids (Bakhoum et al. 2017). Additional ultrastructural features such as presence or absence of a lateral expansion, the arrangement and localization of cortical microtubules, or the existence of external ornamentations on the plasma membrane, as well as their location and association with cortical microtubules, are also variable across the spermatozoa of digeneans and may be useful for phylogenetic purposes (Bakhoum et al. 2017). For instance, the ultrastructure of the posterior spermatozoon extremity supports molecular data suggesting that the family Acanthocolpidae is not closely related to the clade Opecoelidae + Opistholebetidae (Littlewood et al. 2015). Notably, while the posterior ending of spermatozoon lacks of cortical microtubules and only contains the nucleus in the studied acanthocolpids *Stephanostomum murielae* and *Stephanostomoides tenuis* (Bakhoum et al. 2015), the presence of cortical microtubules in the posterior extremity has been reported in opecoelids and opistholebetids (Miquel et al. 2000; Levron et al. 2004; Quilichini et al. 2010). In a like manner, the first ultrastructural characterization of the mature spermatozoa of a member of the genus *Sclerodistomum* (*S. italucum*) showed specific features that are observed only in other representatives of the superfamily Hemiuroidea. These features include the simultaneous presence of two types of extramembranous

ornamentations, the presence of short cortical microtubules in the anterior part of the spermatozoon, and a single bundle of longer cortical microtubules in the medium part of the sperm cell (Ndiaye et al. 2017).

Spermiogenesis is generally homogeneous across digenean species. It starts with the formation of the differentiation zone, which is bordered by cortical microtubules and contains numerous mitochondria, as well as the nucleus and a pair of centrioles that give rise to two initially free flagella. Then, a cytoplasmic process is formed between the two flagella, along which the nucleus, mitochondria, and cortical microtubules migrate. Finally, the flagella arrange parallel to and fuse with the median cytoplasmic process, and the spermiogenesis terminates with the constriction at the differentiation zone, and the release of the young spermatozoon (Bakhoum et al. 2017). High-throughput transcriptomics carried out in the opistorchiid *C. sinensis* (Huang et al. 2013) and the schistosomatid *S. mansoni* (Lu et al. 2016) has revealed that the vast majority of transcripts are concentrated in the gonads in these digeneans. According to Lu and co-authors, this observation could be explained by the storage of mRNA in spermatozoa and its transport into the oocyte via sperm (Lu et al. 2016).

1.8.2 Female Reproductive System

The female reproductive system consists of two parts: a structural complex called oogenotop (or egg-forming apparatus) and the uterus, a long, often convoluted tube, through which the eggs are transported to the exterior. Most trematodes have a single ovary, connected to a short oviduct via a proximal sphincter, called ovicapt, that regulates the passage of oocytes down into the oviduct. A seminal receptacle emerges as an out pocket of the oviduct wall and provides with sperm for fertilization. Vitelline glands communicate also with the oviduct and contribute with mature vitelline cells, which are essential for eggshell formation and nourishment of the forming embryos. Following sperm penetration and association with several vitelline cells, oocytes go into the ootype, an expansion of the oviduct surrounded by numerous unicellular Mehlis' glands that discharge their secretions into it via miniscule ducts. The function of the material provided by Mehlis' glands is not fully understood; presumed functions include contribution to eggshell formation and hardening, activation of sperm, and activation of vitelline cells to release shell material. Beyond the ootype, the female duct expands to form the uterus, which extends to the female genital pore. In some species, the distal end of the uterus becomes muscular and serves as an ovijector and a vagina; this structure is termed metraterm. During the copula, the cirrus is inserted into the distal end of uterus and the sperm swims towards the seminal receptacle, where it is stored. A narrow tube, called Laurer's canal, often arises at the base of the seminal receptacle; it ends either blindly in the parenchyma or opens outward through the tegument, and is thought to be a non-functional vestigial vagina (Roberts and Janovy 2009).

Oogenesis and vitellogenesis follow general patterns in all digeneans although some variations have been reported. For instance, Greani and co-authors (2016), studied for the first time the evolution of these two processes in a member of the family Allocreadiidae, namely *Crepidostomum metoecus.* Compared to other digeneans, fully mature vitellocytes of *C. metoecus* contain large amounts of nutritive reserves and material for shell development; this, together with the low protein composition of mature oocytes, led the authors to suggest that in this species oocytes do not participate in the nourishing of the developing embryo (Greani et al. 2016).

A comparative study of oogenesis between diploid and triploid isolates of the fasciolid *F. hepatica* evidenced that synaptonemal complexes are formed early in primary oocytes of all individuals, irrespective of their chromosomal complement (Hanna et al. 2016). The formation of these complexes in triploid specimens indicates that meiosis is also initiated in this type of flukes, which, a priori, are unable to undergo conventional meiosis (Fletcher et al. 2004). Furthermore, in all the isolates studied by these authors, the meiosis of oocytes completes within eggs in the

uterus, in which distal end, oocytes typically enclose two equal-size pronuclei; this represents the most advanced stage of development of eggs within the fluke, before being shed into the environment. In diploid individuals, each pronucleus originates from male and female gametocytes, respectively. In contrast, parthenogenesis is believed to occur in triploid organisms, which are aspermic (Hanna et al. 2016). In these specimens, besides the segregation of synapsed homologous chromosomes, meiosis 1 is thought to involve the allocation of the unsynapsed chromosomes (i.e., the third copy) in the two daughter nuclei, resulting into an unequal distribution of the genetic material between them. Hanna and co-authors (2016) postulated that in these flukes the zygote might be restored to the triploid condition prior to proceed into meiosis 2, by way of re-fusing the two pronuclei resultant from the first mitotic division. This mechanism might restore the unbalancing effects of meiosis 1 and ensure success in the cleavage division at meiosis 2, thus enabling the survival and clonal expansion of polyploids (Hanna et al. 2016). According to these authors, the same mechanism of parthenogenesis may operate in a facultative manner in diploid *F. hepatica* if sperm is not available (Hanna et al. 2016).

In regard to the functioning of the female reproductive system, particular attention has been paid in the last 5 years to specific molecules and pathways that play key roles in regulating the reproductive biology of dioecious trematodes of the genus *Schistosoma*, in which egg production has special relevance due to the role of eggs in chronic pathology. Several signal transduction processes are now known to play essential roles in controlling mitosis, cell differentiation, and egg production via the regulation of key transcriptional targets. In particular, cooperation between Src-kinase- and TGFβ receptor I-containing pathways has been reported to influence mitotic activity and egg production in female *S. mansoni* since the inhibition of these pathways suppressed both processes in paired females (Buro et al. 2013). Furthermore, the mitogen-activated protein kinase (MAPK) cascade also plays an important role in regulating the reproduction of this species of schistosome. Suppression of the gene coding for the extracellular signal-regulated kinase (SmERK) caused a significant reduction in egg production, in addition to morphological alterations in the female reproductive system, such as smaller ovaries that accumulated immature oocytes and accumulation of mature oocytes in the uterus (Andrade et al. 2014). Additional protein kinases, such as SmTK3, SmTK4, SjTK4, and SmTK6, have also been reported to play roles in gonad-associated processes in female schistosomes (Beckmann et al. 2010a, 2011; Buro et al. 2017; Hahnel et al. 2017; Ding et al. 2017).

Venus kinase receptors (VKRs) are unusual receptor tyrosine kinases of invertebrates, displaying a particular protein structure (Vicogne et al. 2003). Two VKRs are expressed in *S. mansoni* (SmVKR1 and SmVKR2), which play different roles in oogenesis and egg formation (Vanderstraete et al. 2014). Each of these receptors is activated by a different ligand; specifically, L-arginine activates SmVKR1, while Ca^{+2} activates SmVKR2 (Vanderstraete et al. 2014). However, a novel way of activation has been discovered for SmVKR1, which is independent of ligand binding and works by interaction of the receptor with a signaling complex, including a beta integrin receptor Smβ-Int1 (Gelmedin et al. 2017), for which a role in oogenesis had been previously reported (Beckmann et al. 2012). Ligand-independent activation of SmVKR1 results in downstream activation of several signaling pathways, and the blockade of this type of activation affects ovary structure, oocyte integrity, and egg formation (Gelmedin et al. 2017). In particular, formation of this signaling complex is crucial to control the differentiation status of oocytes by regulating apoptosis (Gelmedin et al. 2017).

Schistosome micro(mi)RNAs also play important regulatory roles in male–female pairing, gametogenesis, and egg production (Zhu et al. 2016). A number of miRNAs are differentially expressed between male and female *S. japonicum*, and across different stages of maturation; among them, miRNAs with regulatory roles on mRNA targets involved in signal transduction for sexual maturation and egg production have been identified. In particular, functional evidence has

been proved for miR-31 and bantam, whose suppression, as well as the suppression of their mRNA targets, resulted in severe defects in ovary development (Zhu et al. 2016). A role in regulating the sexual development of *S. mansoni* females have also been suggested for members of the miR-277 family of miRNA since their target genes display different expression profiles between paired (mature) and unpaired (immature) females (Protasio et al. 2017).

Beyond sexual maturation of worms, a proper formation of the eggshell is crucial for egg viability and successful transmission of the infection. In *S. mansoni*, the production of the major eggshell protein (Smp14) is controlled by epigenetic modifications at the promoter region of the corresponding gene. In particular, two histone acetyltransferases (SmGCN5 and SmCBP1) are recruited at the *Smp14* promoter by nuclear receptors and acetylate the H3 to activate the transcription of this gene, an essential step for egg development. Indeed, inhibition of these transferases resulted in disrupted eggshell integrity and defective egg structure (Carneiro et al. 2014). Moreover, this inhibition impacted negatively on the development of the ovary and vitellaria, and the maturation of oocytes (Carneiro et al. 2014). Other processes involving chromatin remodeling have been proved relevant for the function in the sexual development of *S. mansoni* females. Concretely, suppression of the members of the family of high mobility group box proteins (HMGBs: SmHMGB2 and SmHMGB3) impaired ovary development and egg laying (de Abreu da Silva et al. 2016).

1.9 Trematode Body Systems as Targets for Drug Discovery

One of the biggest issues faced by current human and veterinary parasitology is the limited range of drugs available for combating trematode infections in the face of emerging resistance against conventional chemotherapeutics (Kelley et al. 2016; Vale et al. 2017). This concern evidences the necessity of developing next-generation antitrematodal drugs, a challenging task that comprises not only the discovery and/or characterization of anthelmintic compounds, but also the identification of parasite molecules that can be exploited as drug targets (Keiser and Utzinger 2007; Ferreira et al. 2015). Each of the body systems covered in this chapter performs vital functions for the parasite biology, thus having the potential for novel treatment opportunities. Nonetheless, besides a critical functional relevance, drug-target candidates are required to meet additional criteria, such as known or predicted druggability and parasite specificity, i.e., lack of host orthologues or, at least, low structure similarity (McVeigh et al. 2011). Reviewing the recent advances in this field is out of the scope of this chapter. Notwithstanding, brief comments on some few highlights concerning the targeting of trematode body systems for the discovery of novel therapeutic and control opportunities are provided.

Aiming at identifying candidate molecules for their validation as new drug targets, particular emphasis is being placed on neural, excretory, and gonad-associated processes in the schistosomatid *S. mansoni*. Neurotransmitter signaling is subject of an extensive research (e.g., Patocka and Ribeiro 2013; Patocka et al. 2014; MacDonald et al. 2014, 2015; Chan et al. 2016a, b; Hahnel et al. 2018; McVeigh et al. 2018a). Neuropeptides and other small neuronal transmitters are key for the control of the neuromuscular activity, beside performing additional functions in metabolism and nutrient transport (reviewed by Ribeiro et al. 2005 and Marks and Maule 2010); therefore, by disrupting neural signaling, disabling effects on vital functions such as locomotion, attachment, feeding, or sensory perception can be achieved. Neurotransmitter transporters, transmitter-activated ion channels receptors, and G protein-coupled receptors (GPCRs) are known targets for a number of neuroactive compounds and have a well-contrasted druggability potential, thus becoming promising drug targets for anthelmintic development [see McVeigh et al. (2011, 2018b), Ribeiro and Patocka (2013) and Greenberg (2014) for reviews].

Targeting the reproductive function represents also an interesting treatment option for control purposes since it impacts directly on life cycle progression and parasite transmission. Moreover, this approach is particularly interesting to combat the egg-associated pathology of schistosomiasis (Beckmann et al. 2010b), which is reflected in the great number of publications on this subject that have arisen in the past few years (see Sect. 1.8.2). Several proteins have been proposed as potential therapeutic targets based on transcriptional profiling and/or functional evidence (e.g., Beckmann et al. 2010b; Buro et al. 2013; Andrade et al. 2014; Lu et al. 2016; Hahnel et al. 2018; McVeigh et al. 2018a). Among these options, GPCRs displaying essential functions in trematode reproductive and developmental biology (Hahnel et al. 2018; McVeigh et al. 2018a) are excellent candidates for applied studies due to their great structural diversity and pharmacological properties that enable the design of highly selective ligands (reviewed by McVeigh et al. 2018b).

On the other hand, although the inactivation and elimination of injurious compounds are key functions of the excretory system of all animals, xenobiotic-metabolizing enzymes and, specially, drug transporters are known to contribute to low drug efficacy and the development of anthelminthic resistance (Kotze et al. 2014; Matoušková et al. 2016). Biotransformation enzymes, such as cytochrome P450 or glutathione S-transferase, are potential drug targets due to their essential roles in parasite metabolism (reviewed by Matoušková et al. 2016). In turn, targeting drug transporters may represent an opportunity to increase the efficacy of other anthelmintics (reviewed by Kotze et al. 2014). Aside performing fundamental functions for parasite biology, these groups of proteins display additional desirable properties for being considered as potential therapeutic targets (Kotze et al. 2014; Matoušková et al. 2016).

In addition to the abovementioned biological functions, the maintenance of the structural integrity of the tegument is vital for trematodes; therefore, this organ also represents a prominent target for the rational development of flukicide drugs and, more important, of anti-helminth vaccines (see Leow et al. 2015 and Sotillo et al. 2017 for reviews).

References

Andrade LF, Mourao Mde M, Geraldo JA, Coelho FS, Silva LL, Neves RH, Volpini A, Machado-Silva JR, Araujo N, Nacif-Pimenta R, Caffrey CR, Oliveira G (2014) Regulation of *Schistosoma mansoni* development and reproduction by the mitogen-activated protein kinase signaling pathway. PLoS Negl Trop Dis 8:e2949

Antonelli L, Quilichini Y, Foata J, Marchand B (2014) Topography and ultrastructure of the tegument of *Aphallus tubarium* (Rodolphi, 1819) Poche, 1926 (Digenea: Cryptogonimidae), intestinal parasite of the common *Dentex dentex* (Linnaeus 1758) from Valinco Gulf. Acta Parasitol 59:615–624

Anuracpreeda P, Phutong S, Ngamniyom A, Panyarachun B, Sobhon P (2015) Surface topography and ultrastructural architecture of the tegument of adult *Carmyerius spatiosus* Brandes, 1898. Acta Trop 143:18–28

Anuracpreeda P, Chawengkirttikul R, Sobhon P (2016) Surface histology, topography, and ultrastructure of the tegument of adult *Orthocoelium parvipapillatum* (Stiles & Goldberger, 1910). Parasitol Res 115:2757–2769

Bakhoum AJ, Quilichini Y, Justine JL, Bray RA, Ba CT, Marchand B (2015) Ultrastructural study of sperm cells in Acanthocolpidae: the case of *Stephanostomum murielae* and *Stephanostomoides tenuis* (Digenea). Peer J 3:e744

Bakhoum AJS, Miquel J, Ndiaye PI, Justine JL, Falchi A, Ba CT, Marchand B, Quilichini Y (2017) Advances in spermatological characters in the Digenea: review and proposal of spermatozoa models and their phylogenetic importance. Adv Parasitol 98:111–165

Barker LR, Bueding E, Timms AR (1966) The possible role of acetylcholine in *Schistosoma mansoni*. Br J Pharmacol Chemother 26:656–665

Beckmann S, Buro C, Dissous C, Hirzmann J, Grevelding CG (2010a) The Syk kinase SmTK4 of *Schistosoma mansoni* is involved in the regulation of spermatogenesis and oogenesis. PLoS Pathog 6:e1000769

Beckmann S, Quack T, Burmeister C, Buro C, Long T, Dissous C, Grevelding CG (2010b) *Schistosoma mansoni*: signal transduction processes during the development of the reproductive organs. Parasitology 137:497–520

Beckmann S, Hahnel S, Cailliau K, Vanderstraete M, Browaeys E, Dissous C, Grevelding CG (2011) Characterization of the Src/Abl hybrid kinase SmTK6 of *Schistosoma mansoni*. J Biol Chem 286:42325–42336

Beckmann S, Quack T, Dissous C, Cailliau K, Lang G, Grevelding CG (2012) Discovery of platyhelminth-

specific alpha/beta-integrin families and evidence for their role in reproduction in *Schistosoma mansoni*. PLoS One 7:e52519

Beklemishev WN (1969) Origin and development of the excretory apparatus. In: Kabata Z (ed) Principles of comparative anatomy of invertebrates, vol 2. Oliver & Boyd, Edinburgh, pp 327–352

Bernal D, Trelis M, Montaner S, Cantalapiedra F, Galiano A, Hackenberg M, Marcilla A (2014) Surface analysis of *Dicrocoelium dendriticum*. The molecular characterization of exosomes reveals the presence of miRNAs. J Proteomics 105:232–241

Bogitsh BJ, Carter CE, Oeltmann TN (2013) General characteristics of the Trematoda. In: Bogitsh BJ, Carter CE, Oeltmann TN (eds) Human parasitology, 4th edn. Academic Press, Waltham, pp 153–178

Born-Torrijos A, Holzer AS, Raga JA, van Beest GS, Yoneva A (2017) Description of embryonic development and ultrastructure in miracidia of *Cardiocephaloides longicollis* (Digenea, Strigeidae) in relation to active host finding strategy in a marine environment. J Morphol 278:1137–1148

Buro C, Oliveira KC, Lu Z, Leutner S, Beckmann S, Dissous C, Cailliau K, Verjovski-Almeida S, Grevelding CG (2013) Transcriptome analyses of inhibitor-treated schistosome females provide evidence for cooperating Src-kinase and TGFbeta receptor pathways controlling mitosis and eggshell formation. PLoS Pathog 9:e1003448

Buro C, Burmeister C, Quack T, Grevelding CG (2017) Identification and first characterization of SmEps8, a potential interaction partner of SmTK3 and SER transcribed in the gonads of *Schistosoma mansoni*. Exp Parasitol 180:55–63

Carneiro VC, de Abreu da Silva IC, Torres EJ, Caby S, Lancelot J, Vanderstraete M, Furdas SD, Jung M, Pierce RJ, Fantappie MR (2014) Epigenetic changes modulate schistosome egg formation and are a novel target for reducing transmission of schistosomiasis. PLoS Pathog 10:e1004116

Chaiyadet S, Sotillo J, Smout M, Cantacessi C, Jones MK, Johnson MS, Turnbull L, Whitchurch CB, Potriquet J, Laohaviroj M, Mulvenna J, Brindley PJ, Bethony JM, Laha T, Sripa B, Loukas A (2015) Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. J Infect Dis 212:1636–1645

Chan JD, Acharya S, Day TA, Marchant JS (2016a) Pharmacological profiling an abundantly expressed schistosome serotonergic GPCR identifies nuciferine as a potent antagonist. Int J Parasitol Drugs Drug Resist 6:364–370

Chan JD, McCorvy JD, Acharya S, Johns ME, Day TA, Roth BL, Marchant JS (2016b) A miniaturized screen of a *Schistosoma mansoni* serotonergic G protein-coupled receptor identifies novel classes of parasite-selective inhibitors. PLoS Pathog 12:e1005651

Collins JJ III, Wendt GR, Iyer H, Newmark PA (2016) Stem cell progeny contribute to the schistosome host-parasite interface. Elife 5:e12473

Cortés A, Sotillo J, Muñoz-Antolí C, Molina-Durán J, Esteban JG, Toledo R (2017) Antibody trapping: a novel mechanism of parasite immune evasion by the trematode *Echinostoma caproni*. PLoS Negl Trop Dis 11:e0005773

Cwiklinski K, Dalton JP, Dufresne PJ, La Course J, Williams DJ, Hodgkinson J, Paterson S (2015a) The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. Genome Biol 16:71

Cwiklinski K, de la Torre-Escudero E, Trelis M, Bernal D, Dufresne PJ, Brennan GP, O'Neill S, Tort J, Paterson S, Marcilla A, Dalton JP, Robinson MW (2015b) The extracellular vesicles of the helminth pathogen, *Fasciola hepatica*: biogenesis pathways and cargo molecules involved in parasite pathogenesis. Mol Cell Proteomics 14:3258–3273

Da'dara AA, Bhardwaj R, Ali YB, Skelly PJ (2014) Schistosome tegumental ecto-apyrase (SmATPDase1) degrades exogenous pro-inflammatory and pro-thrombotic nucleotides. Peer J 2:e316

de Abreu da Silva IC, Carneiro VC, Vicentino AR, Aguilera EA, Mohana-Borges R, Thiengo S, Fernandez MA, Fantappie MR (2016) The distinct C-terminal acidic domains of HMGB proteins are functionally relevant in *Schistosoma mansoni*. Int J Parasitol 46:253–262

Ding H, Liu F, Zhu L, Wu F, Liu Q, He S, Shao W, Du Y, Ren C, Shen J, Liu M (2017) Tyrosine kinase 4 is involved in the reproduction of the platyhelminth parasite *Schistosoma japonicum*. Parasit Vectors 10:498

Dittman JS, Kaplan JM (2008) Behavioral impact of neurotransmitter-activated G-protein-coupled receptors: muscarinic and GABAB receptors regulate *Caenorhabditis elegans* locomotion. J Neurosci 28:7104–7112

El-Sakkary N, Chen S, Arkin MR, Caffrey CR, Ribeiro P (2018) Octopamine signaling in the metazoan pathogen *Schistosoma mansoni*: localization, small-molecule screening and opportunities for drug development. Dis Model Mech 11:dmm033563. https://doi.org/10.1242/dmm.033563

El-Shehabi F, Ribeiro P (2010) Histamine signalling in *Schistosoma mansoni*: immunolocalisation and characterisation of a new histamine-responsive receptor (SmGPR-2). Int J Parasitol 40:1395–1406

El-Shehabi F, Taman A, Moali LS, El-Sakkary N, Ribeiro P (2012) A novel G protein-coupled receptor of *Schistosoma mansoni* (SmGPR-3) is activated by dopamine and is widely expressed in the nervous system. PLoS Negl Trop Dis 6:e1523

Eriksson KS, Johnston RN, Shaw C, Halton DW, Panula PA (1996) Widespread distribution of histamine in the nervous system of a trematode flatworm. J Comp Neurol 373:220–227

Faghiri Z, Skelly PJ (2009) The role of tegumental aquaporin from the human parasitic worm, *Schistosoma mansoni*, in osmoregulation and drug uptake. FASEB J 23:2780–2789

Ferreira LG, Oliva G, Andricopulo AD (2015) Target-based molecular modeling strategies for schistosomiasis drug discovery. Future Med Chem 7:753–764

Filippi JJ, Quilichini Y, Marchand B (2013) Topography and ultrastructure of the tegument of *Deropristis inflata* Molin, 1859 (Digenea: Deropristidae), a parasite of the European eel *Anguilla anguilla* (Osteichthyes: Anguillidae). Parasitol Res 112:517–528

Fletcher HL, Hoey EM, Orr N, Trudgett A, Fairweather I, Robinson MW (2004) The occurrence and significance of triploidy in the liver fluke, *Fasciola hepatica*. Parasitology 128:69–72

Fried B (1997) An overview of the biology of trematodes. In: Fried B, Graczyk TK (eds) Advances in trematode biology. CRC, Boca Raton, pp 1–30

Fried B, Haseeb MA (1991) Platyhelminthes, Aspidogastrea, Monogenea, and Digenea. In: Harrison FW, Bogitsh BJ (eds) Microscopic anatomy of invertebrates, vol 3. Wiley and Liss, New York, pp 141–209

Geadkaew A, von Bulow J, Beitz E, Tesana S, Vichasri Grams S, Grams R (2015) Bi-functionality of *Opisthorchis viverrini* aquaporins. Biochimie 108:149–159

Gelmedin V, Morel M, Hahnel S, Cailliau K, Dissou SC, Grevelding CG (2017) Evidence for integrin - venus kinase receptor 1 alliance in the ovary of *Schistosoma mansoni* females controlling cell survival. PLoS Pathog 13:e1006147

Greani S, Quilichini Y, Marchand B (2016) Ultrastructural study of vitellogenesis and oogenesis of *Crepidostomum metoecus* (Digenea, Allocreadiidae), intestinal parasite of *Salmo trutta* (Pisces, Teleostei). Parasite 23:47

Greenberg RM (2013) ABC multidrug transporters in schistosomes and other parasitic flatworms. Parasitol Int 62:647–653

Greenberg RM (2014) Ion channels and drug transporters as targets for anthelmintics. Curr Clin Microbiol Rep 1:51–60

Hahnel S, Parker-Manuel R, Dissous C, Cailliau K, Grevelding CG (2017) First characterization of SmOPG1, a novel protein involved in gonad-associated processes in *Schistosoma mansoni*. Mol Biochem Parasitol 213:22–25

Hahnel S, Wheeler N, Lu Z, Wangwiwatsin A, McVeigh P, Maule A, Berriman M, Day T, Ribeiro P, Grevelding CG (2018) Tissue-specific transcriptome analyses provide new insights into GPCR signalling in adult *Schistosoma mansoni*. PLoS Pathog 14:e1006718

Halton DW (2004) Microscopy and the helminth parasite. Micron 35:361–390

Halton DW, Maule AG (2004) Flatworm nerve-muscle: structural and functional analysis. Can J Zool 82:316–333

Han Q, Jia B, Hong Y, Cao X, Zhai Q, Lu K, Li H, Zhu C, Fu Z, Shi Y, Lin J (2017) Suppression of VAMP2 alters morphology of the tegument and affects glucose uptake, development and reproduction of *Schistosoma japonicum*. Sci Rep 7:5212

Hanna RE, Moffett D, Forster FI, Trudgett AG, Brennan GP, Fairweather I (2016) *Fasciola hepatica*: a light and electron microscope study of the ovary and of the development of oocytes within eggs in the uterus provides an insight into reproductive strategy. Vet Parasitol 221:93–103

Hertel LA (1993) Excretion and osmoregulation in the flatworms. Trans Am Microsc Soc 112:10–17

Holmes SD, Fairweather I (1984) *Fasciola hepatica*: the effects of neuropharmacological agents upon *in vitro* motility. Exp Parasitol 58:194–208

Huang Y, Chen W, Wang X, Liu H, Chen Y, Guo L, Luo F, Sun J, Mao Q, Liang P, Xie Z, Zhou C, Tian Y, Lv X, Huang L, Zhou J, Hu Y, Li R, Zhang F, Lei H, Li W, Hu X, Liang C, Xu J, Li X, Yu X (2013) The carcinogenic liver fluke, *Clonorchis sinensis*: new assembly, reannotation and analysis of the genome and characterization of tissue transcriptomes. PLoS One 8:e54732

Huang Y, Li W, Lu W, Xiong C, Yang Y, Yan H, Liu KC, Cao P (2016) Cloning and in vitro characterization of a *Schistosoma japonicum* aquaglyceroporin that functions in osmoregulation. Sci Rep 6:35030

Kasinathan RS, Sharma LK, Cunningham C, Webb TR, Greenberg RM (2014) Inhibition or knockdown of ABC transporters enhances susceptibility of adult and juvenile schistosomes to praziquantel. PLoS Negl Trop Dis 8:e3265

Keiser J, Utzinger J (2007) Advances in the discovery and development of trematocidal drugs. Expert Opin Drug Discov 2(sup1):S9–S23

Kotze AC, Hunt PW, Skuce P, von Samson-Himmelstjerna G, Martin RJ, Sager H, Krücken J, Hodgkinson J, Lespine A, Jex AR, Gilleard JS, Beech RN, Wolstenholme AJ, Demeler J, Robertson AP, Charvet CL, Neveu C, Kaminsky R, Rufener L, Alberich M, Menez C, Prichard RK (2014) Int J Parasitol Drugs Drug Resist 4:164–184

Kelley JM, Elliott TP, Beddoe T, Anderson G, Skuce P, Spithill TW (2016) Current threat of triclabendazole resistance in *Fasciola hepatica*. Trends Parasitol 32:458–469

Krupenko DY, Dobrovolskij AA (2015) Somatic musculature in trematode hermaphroditic generation. BMC Evol Biol 15:189

Krupenko D, Gonchar A (2017a) Musculature arrangement and locomotion in notocotylid cercariae (Digenea: Notocotylidae) from mud snail *Ecrobia ventrosa* (Montagu, 1803). Parasitol Int 66:262–271

Krupenko D, Gonchar A (2017b) Ventral concavity and musculature arrangement in notocotylid maritae (Digenea: Notocotylidae). Parasitol Int 66:660–665

Kumkate S, Chunchob S, Janvilisri T (2008) Expression of ATP-binding cassette multidrug transporters in the giant liver fluke *Fasciola gigantica* and their possible involvement in the transport of bile salts and anthelmintics. Mol Cell Biochem 317:77–84

Leow CY, Willis C, Hofmann A, Jones MK (2015) Structure-function analysis of apical membrane-associated molecules of the tegument of schistosome parasites of humans: prospects for identification of

novel targets for parasite control. Br J Pharmacol 172:1653–1663

Levron C, Ternengo S, Marchand B (2004) Spermiogenesis and sperm ultrastructure of *Poracanthium furcatum* (Digenea, Opecoelidae), a parasite of *Mullus surmuletus* (Pisces, Teleostei). Acta Parasitol 49:190–200

Li XH, de Castro-Borges W, Parker-Manuel S, Vance GM, Demarco R, Neves LX, Evans GJ, Wilson RA (2013) The schistosome oesophageal gland: initiator of blood processing. PLoS Negl Trop Dis 7:e2337

Li XH, Stark M, Vance GM, Cao JP, Wilson RA (2014) The anterior esophageal region of *Schistosoma japonicum* is a secretory organ. Parasit Vectors 7:565

Li XH, DeMarco R, Neves LX, James SR, Newling K, Ashton PD, Cao JP, Wilson RA, Castro-Borges W (2018) Microexon gene transcriptional profiles and evolution provide insights into blood processing by the *Schistosoma japonicum* esophagus. PLoS Negl Trop Dis 12:e0006235

Littlewood DTJ, Bray RA, Waeschenbach A (2015) Phylogenetic patterns of diversity in cestodes and trematodes. In: Morand S, Krasnov B, Littlewood DTJ (eds) Parasite diversity and diversification: evolutionary ecology meets phylogenetics. Cambridge University Press, Cambridge, pp 304–319

Lu Z, Sessler F, Holroyd N, Hahnel S, Quack T, Berriman M, Grevelding CG (2016) Schistosome sex matters: a deep view into gonad-specific and pairing-dependent transcriptomes reveals a complex gender interplay. Sci Rep 6:31150

MacDonald K, Buxton S, Kimber MJ, Day TA, Robertson AP, Ribeiro P (2014) Functional characterization of a novel family of acetylcholine-gated chloride channels in *Schistosoma mansoni*. PLoS Pathog 10:e1004181

MacDonald K, Kimber MJ, Day TA, Ribeiro P (2015) A constitutively active G protein-coupled acetylcholine receptor regulates motility of larval *Schistosoma mansoni*. Mol Biochem Parasitol 202:29–37

Marcilla A, Trelis M, Cortés A, Sotillo J, Cantalapiedra F, Mínguez MT, Valero ML, Sánchez del Pino MM, Muñoz-Antolí C, Toledo R, Bernal D (2012) Extracellular vesicles from parasitic helminths contain specific excretory/secretory proteins and are internalized in intestinal host cells. PLoS One 7:e45974

Marks NJ, Maule AG (2010) Neuropeptides in helminths: occurrence and distribution. In: Geary TG, Maule AG (eds) Neuropeptide systems as targets for parasite and pest control. Springer Nature, Gewerbestrasse, pp 49–77

Matoušková P, Vokřál I, Lamka J, Skálová L (2016) The role of xenobiotic-metabolizing enzymes in anthelmintic deactivation and resistance in helminths. Trends Parasitol 32:481–491

McVeigh P, Atkinson L, Marks NJ, Mousley A, Dalzell JJ, Sluder A, Hammerland L, Maule AG (2011) Parasite neuropeptide biology: seeding rational drug target selection? Int J Parasitol Drugs Drug Resist 2:76–91

McVeigh P, McCammick E, McCusker P, Wells D, Hodgkinson J, Paterson S, Mousley A, Marks NJ, Maule AG (2018a) Profiling G protein-coupled receptors of *Fasciola hepatica* identifies orphan rhodopsins unique to phylum Platyhelminthes. Int J Parasitol Drugs Drug Resist 8:87–103

McVeigh P, McCusker P, Robb E, Wells D, Gardiner E, Mousley A, Marks NJ, Maule AG (2018b) Reasons to be nervous about flukicide discovery. Trends Parasitol 34:184–196

Messerli SM, Kasinathan RS, Morgan W, Spranger S, Greenberg RM (2009) *Schistosoma mansoni* P-glycoprotein levels increase in response to praziquantel exposure and correlate with reduced praziquantel susceptibility. Mol Biochem Parasitol 167:54–59

Miquel J, Nourrisson C, Marchand B (2000) Ultrastructure of spermiogenesis and the spermatozoon of *Opecoeloides furcatus* (Trematoda, Digenea, Opecoelidae), a parasite of *Mullus barbatus* (Pisces, Teleostei). Parasitol Res 86:301–310

Mordvinov VA, Ershov NI, Pirozhkova DS, Pakharukov YV, Pakharukova MY (2017) ABC transporters in the liver fluke *Opisthorchis felineus*. Mol Biochem Parasitol 216:60–68

Ndiaye PI, Quilichini Y, Marigo AM, Ba CT, Tkach VV, Marchand B (2017) Ultrastructural characteristics of the mature spermatozoon of the digenean *Sclerodistomum italicum* (Stossich, 1893) (Hemiuroidea, Sclerodistomidae) intestinal parasite of *Hypocanthus amia* (Teleostei, Carangidae). Tissue Cell 49:15–21

Nollen PM (1997) Reproductive physiology and behavior of digenetic trematodes. In: Fried B, Graczyk TK (eds) Advances in trematode biology. CRC, Boca Raton, pp 117–147

Pakharukova MY, Vavilin VA, Sripa B, Laha T, Brindley PJ, Mordvinov VA (2015) Functional analysis of the unique cytochrome P450 of the liver fluke *Opisthorchis felineus*. PLoS Negl Trop Dis 9:e0004258

Patocka N, Ribeiro P (2013) The functional role of a serotonin transporter in *Schistosoma mansoni* elucidated through immunolocalization and RNA interference (RNAi). Mol Biochem Parasitol 187:32–42

Patocka N, Sharma N, Rashid M, Ribeiro P (2014) Serotonin signaling in *Schistosoma mansoni*: a serotonin-activated G protein-coupled receptor controls parasite movement. PLoS Pathog 10:e1003878

Peoples RC, Fried B (2014) Form and function in the Digenea. In: Toledo R, Fried B (eds) Digenetic trematodes, 1st edn. Springer, New York, pp 3–20

Petrov A, Podvyaznaya I (2016) Muscle architecture during the course of development of *Diplostomum pseudospathaceum* Niewiadomska, 1984 (Trematoda, Diplostomidae) from cercariae to metacercariae. J Helminthol 90:321–336

Pinto-Almeida A, Mendes T, Armada A, Belo S, Carrilho E, Viveiros M, Afonso A (2015) The role of efflux pumps in *Schistosoma mansoni* praziquantel resistant phenotype. PLoS One 10:e0140147

Piratae S, Tesana S, Jones MK, Brindley PJ, Loukas A, Lovas E, Eursitthichai V, Sripa B, Thanasuwan S, Laha T (2012) Molecular characterization of a tetraspanin

from the human liver fluke, *Opisthorchis viverrini*. PLoS Negl Trop Dis 6:e1939

Podvyaznaya IM (2006) An ultrastructural study of alimentary tract development in the cercariae of *Diplostomum pseudospathaceum* (Digenea: Diplostomidae). Parasitol Res 99:362–367

Podvyaznaya I (2011) An ultrastructural study of alimentary tract development in the cercariae of *Prosorhynchoides borealis* (Digenea, Bucephalidae). *Acta Zool* 92:170–178

Protasio AV, van Dongen S, Collins J, Quintais L, Ribeiro DM, Sessler F, Hunt M, Rinaldi G, Collins JJ, Enright AJ, Berriman M (2017) MiR-277/4989 regulate transcriptional landscape during juvenile to adult transition in the parasitic helminth *Schistosoma mansoni*. PLoS Negl Trop Dis 11:e0005559

Quilichini Y, Foata J, Justine JL, Bray RA, Marchand B (2010) Ultrastructural study of the spermatozoon of *Heterolebes maculosus* (Digenea, Opistholebetidae), a parasite of the porcupinefish *Diodon hystrix* (Pisces, Teleostei). Parasitol Int 59:427–434

Ribeiro P, El-Shehabi F, Patocka N (2005) Classical transmitters and their receptors in flatworms. Parasitology 131:S19–S40

Ribeiro P, Patocka N (2013) Neurotransmitter transporters in schistosomes: structure, function and prospects for drug discovery. Parasitol Int 62:629–638

Roberts LS, Janovy J Jr (2009) Trematoda: form, function, and classification of Digeneans. In: Roberts LS, Janovy J Jr (eds) Foundations of parasitology, 8th edn. McGraw-Hill, Dubuque, pp 219–245

Sato H, Kusel JR, Thornhill J (2004) Excretion of fluorescent substrates of mammalian multidrug resistance-associated protein (MRP) in the *Schistosoma mansoni* excretory system. Parasitology 128:43–52

Skelly PJ, Wilson AR (2006) Making sense of the schistosome surface. Adv Parasitol 63:185–284

Sotillo J, Doolan D, Loukas A (2017) Recent advances in proteomic applications for schistosomiasis research: potential clinical impact. Exp Rev Proteomics 14: 171–183

Świderski Z, Montoliu I, Feliu C, Gibson DI, Miquel J (2013) A transmission electron microscopical study of the tegument of *Maritrema feliui* (Digenea: Microphallidae). Acta Parasitol 58:478–485

Takamiya S, Fukuda K, Nakamura T, Aoki T, Sugiyama H (2010) *Paragonimus westermani* possesses aerobic and anaerobic mitochondria in different tissues, adapting to fluctuating oxygen tension in microaerobic habitats. Int J Parasitol 40:1651–1658

Thanasuwan S, Piratae S, Brindley PJ, Loukas A, Kaewkes S, Laha T (2014) Suppression of aquaporin, a mediator of water channel control in the carcinogenic liver fluke, *Opisthorchis viverrini*. Parasit Vectors 7:224

Tkach VV, Achatz TJ, Hildebrand J, Greiman SE (2018) Convoluted history and confusing morphology: molecular phylogenetic analysis of dicrocoeliids reveals true systematic position of the Anenterotrematidae Yamaguti, 1958 (Platyhelminthes, Digenea). Parasitol Int 67:501–508

Tolstenkov OO, Terenina NB, Serbina EA, Kreshchenko ND, Maklakova L, Jashina A (2010) The spatial relationship between the musculature and the 5-HT and FMRFamide immunoreactivities in cercaria, metacercaria and adult *Opisthorchis felineus* (Digenea). Acta Parasitol 55:123–132

Tran MH, Freitas TC, Cooper L, Gaze S, Gatton ML, Jones MK, Lovas E, Pearce EJ, Loukas A (2010) Suppression of mRNAs encoding tegument tetraspanins from *Schistosoma mansoni* results in impaired tegument turnover. PLoS Pathog 6:e1000840

Travassos L (1944) [Revisão da familia Dicrocoeliidae Odhner, 1910] *Imprensa Nacional* (in Portuguese)

Travassos L (1955) Sôbre dois novos Dicrocoeliidae de Chiroptera. An Acad Bras Cienc 27:561–565. (in Portuguese)

Vale N, Gouveia MJ, Rinaldi G, Brindley PJ, Gärtner F, Correia da Costa JM (2017) Praziquantel for schistosomiasis: single-drug metabolism revisited, mode of action, and resistance. Antimicrob Agents Chemother 61:e02582–16

van Herwerden L, Blair D, Agatsuma T (1999) Genetic diversity in parthenogenetic triploid *Paragonimus westermani*. Int J Parasitol 29:1477–1482

Vanderstraete M, Gouignard N, Cailliau K, Morel M, Hahnel S, Leutner S, Beckmann S, Grevelding CG, Dissous C (2014) Venus kinase receptors control reproduction in the platyhelminth parasite *Schistosoma mansoni*. PLoS Pathog 10:e1004138

Vicogne J, Pin JP, Lardans V, Capron M, Noel C, Dissous C (2003) An unusual receptor tyrosine kinase of *Schistosoma mansoni* contains a Venus Flytrap module. Mol Biochem Parasitol 126:51–62

Whitfield PJ, Evans NA (1983) Parthenogenesis and asexual multiplication among parasitic platyhelminths. Parasitology 86:121–160

Wilkinson R, Law CJ, Hoey EM, Fairweather I, Brennan GP, Trudgett A (2012) An amino acid substitution in *Fasciola hepatica* P-glycoprotein from triclabendazole-resistant and triclabendazole-susceptible populations. Mol Biochem Parasitol 186:69–72

Wilson RA, Li XH, MacDonald S, Neves LX, Vitoriano-Souza J, Leite LC, Farias LP, James S, Ashton PD, DeMarco R, Castro-Borges W (2015) The schistosome esophagus is a 'hotspot' for microexon and lysosomal hydrolase gene expression: implications for blood processing. PLoS Negl Trop Dis 9:e0004272

Wu C, Hou N, Piao X, Liu S, Cai P, Xiao Y, Chen Q (2015) Non-immune immunoglobulins shield *Schistosoma japonicum* from host immunorecognition. Sci Rep 5:13434

Xiong Y, Zhang M, Hong Y, Wei M, Ai D, Meng P, Han Y, Fu Z, Shi Y, Yang J, Lin J (2013) Characterization analysis of *Schistosoma japonicum* plasma membrane repair relative gene myoferlin. PLoS One 8:e66396

Young ND, Nagarajan N, Lin SJ, Korhonen PK, Jex AR, Hall RS, Safavi-Hemami H, Kaewkong W, Bertrand D, Gao S, Seet Q, Wongkham S, The BT, Wongkham C,

Intapan PM, Maleewong W, Yang X, Hu M, Wang Z, Hofmann A, Sternberg PW, Tan P, Wang J, Gasser RB (2014) The *Opisthorchis viverrini* genome provides insights into life in the bile duct. Nat Commun 5:4378

Zhu L, Zhao J, Wang J, Hu C, Peng J, Luo R, Zhou C, Liu J, Lin J, Jin Y, Davis RE, Cheng G (2016) MicroRNAs are involved in the regulation of ovary development in the pathogenic blood fluke *Schistosoma japonicum*. PLoS Pathog 12:e1005423

The Systematics of the Trematoda

2

Aneta Kostadinova and Ana Pérez-del-Olmo

2.1 Introduction

The Trematoda Rudolphi, 1808, is a class of the phylum Platyhelminthes that comprises two subclasses, the Aspidogastrea Faust & Tang, 1936, and the Digenea Carus, 1863. The subclass Aspidogastrea is a small group (4 families, 12 genera considered valid, *c.*80 species) parasitic in molluscs, fishes and chelonians (see Rohde 2001, 2002). Aspidogastreans like the digeneans use molluscs as first obligate hosts but are characterised by being external rather than internal parasites of these hosts and by having single-generation life-cycles lacking asexual reproduction and a stage comparable to the cercaria (Gibson 1987; Cribb et al. 2003; Rohde 2002). Key information on the aspects of morphology, life-cycles, taxonomy, systematics and phylogeny of the aspidogastreans can be found in Rohde (1972, 1994, 2001, 2002), Gibson (1987), Gibson and Chinabut (1984) and Zamparo and Brooks (2003).

A. Kostadinova (✉)
Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria
e-mail: aneta.kostadinova@uv.es

A. Pérez-del-Olmo
Unitat de Zoologia Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Parc Científic, Universitat de València, Valencia, Spain
e-mail: ana.perez-olmo@uv.es

The subclass Digenea comprises a large and diverse group (*c.*2500 nominal genera, *c.*18,000 nominal species; see Bray 2008) of cosmopolitan platyhelminths that are obligatory parasitic in invertebrate intermediate and vertebrate definitive hosts. Digeneans are found in all vertebrate classes but are less diverse in agnathans and chondrichthyans (Cribb et al. 2001; Bray and Cribb 2003). The subclass is characterised by a number of autapomorphies, associated with the unique complex digenean life-cycle: (i) acquisition of a vertebrate host as a terminal addition to the life history; (ii) alternation of sexual and asexual reproductive generations; (iii) a series of asexual generations within the first intermediate host (typically mollusc); (iv) free-swimming cercaria with a tail; (v) tiers of ectodermal cells on the miracidium; (vi) lack of digestive system in the miracidium and mother sporocyst (Gibson 1987; Cribb et al. 2003; Littlewood 2006). For details and apomorphies at lower taxonomic levels, see the review by Cribb et al. (2003). Although the complexity of digenean life-cycles may have influenced the expansion of the Digenea rendering it the most speciose group among Platyhelminthes (see Littlewood 2006), the mainstay of digenean systematics has been the information obtained from examination of the sexual generation, i.e. the adults from vertebrates (see Nolan and Cribb 2005).

The classification of the Digenea has long been a challenge especially because of the

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_2

difficulties in establishing relationships and finding diagnostic characters for identification keys of the higher taxa (Gibson 1987; Gibson and Bray 1994; Gibson 2002a). Thus, whereas most groupings established at lower taxonomic levels using adult morphology have been widely accepted, the search of apparent non-homoplasious morphological characters at the higher taxonomic levels has been the subject of debate and (sometimes heated) discussions [for details, see Gibson (1987); Pearson (1992) and Gibson and Bray (1994)].

The early attempts for the classification of the digeneans relied upon sucker arrangements initially at the generic level (i.e. *Monostoma* Zeder, 1800; *Distoma* Retzius, 1786; *Amphistoma* Rudolphi, 1801; and *Gasterostomum* von Siebold, 1848) were unsatisfactory (Gibson 2002a), whereas later treatments have incorporated more morphological characters including features of the daughter sporocyst/redia and/or cercaria and life history patterns (Poche 1926; La Rue 1957; Odening 1961; Pearson 1972; Cable 1974; Odening 1974; Brooks et al. 1985; see Gibson 1987 for a detailed discussion on the aspects of the evolution of the Trematoda).

2.2 Keys to the Trematoda

Perhaps one of the most important endeavours of this century in the field of digenean taxonomy is the publication of the *Keys to the Trematoda*, a series on the systematics and identification of the platyhelminth class Trematoda (see Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008). The three volumes provide detailed historical background and novel concepts for the systematics and taxonomy at the generic and suprageneric levels and a reappraisal of the generic diagnoses via re-examination of type- and/or other representative species. Considering just these two aspects makes the series an essential unique source of information on the Trematoda well into the 21st century. Furthermore, although the superfamily was treated as the basic unit of classification, the editors have made a substantial effort towards a classification reflecting a natural system of the Digenea considering morphological evidence in conjunction with phylogenies inferred from molecular data. This provides a sound basis for future molecular studies addressing phylogenetic relationships at the suprageneric level.

There are 148 families with 1577 genera considered valid in the *Keys to the Trematoda*. An examination of the distribution of generic diversity (estimated as the number of valid genera) across digenean superfamilies related to their complexity (estimated as the number of families) illustrates a general trend of association between the two estimates (Fig. 2.1). The lower extreme of the complexity gradient is represented by six monotypic superfamilies [1–6, characterised by poor generic richness (one to nine genera with only superfamily Bucephaloidea Poche, 1907, containing 25 genera)]. The upper extreme comprises the most complex superfamilies, i.e. Hemiuroidea Looss, 1899; Microphalloidea Ward, 1901; and Plagiorchioidea Lühe, 1901 (comprised of 13–23 families), with generic richness varying between 107 and 196 genera. The variability in the middle range is due to two patterns indicating the need of further systematic work. Thus, two superfamilies, the Opisthorchioidea Looss, 1899, and Allocreadioidea Looss, 1902, are characterised by a large number of genera (129 and 113, respectively) whose familial affiliations require further scrutiny. Notably, these are among the superfamilies recovered to contain paraphyletic taxa, i.e. Heterophyidae Leiper, 1909 + Opisthorchiidae Looss, 1899 (see Olson et al. 2003; Thaenkham et al. 2012) and Opecoelidae Ozaki, 1925 + Opistholebetidae Fukui, 1929 (see Olson et al. 2003), respectively, and this supports our suggestion (also see below).

The second pattern observed in Fig. 2.1 is associated with a relatively low generic richness that is unequally distributed among the families: Gymnophalloidea Odhner, 1905 (42 genera among 5 families); Pronocephaloidea Looss, 1899 (48 genera among 6 families); and Brachylaimoidea Joyeux & Foley, 1930 (24 genera among 8 families). The large number (relative to content) of the families within these higher taxa indicates higher rates of "splitting", and the molecular phylogeny of Olson et al. (2003) has

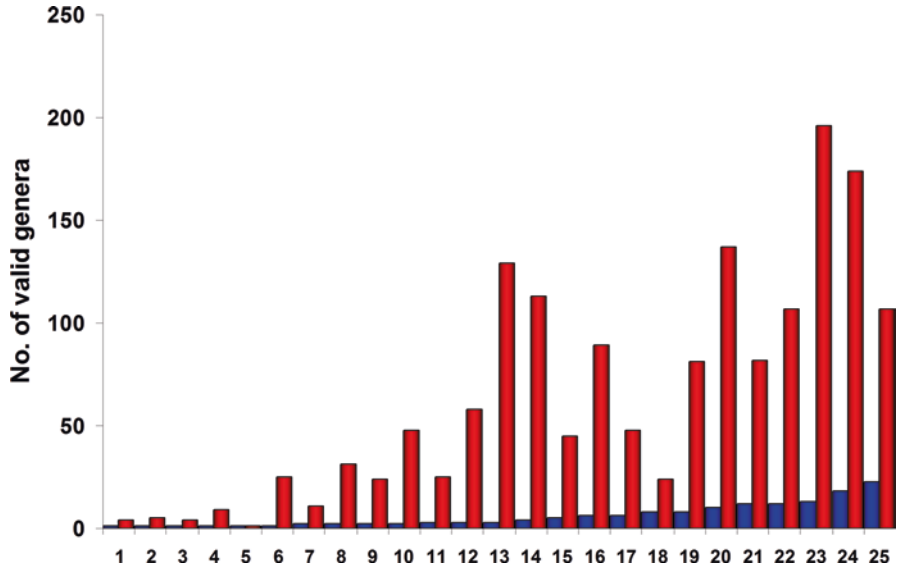

Fig. 2.1 The distribution of digenean generic diversity (assessed as the number of the genera considered valid, *red bars*) along a gradient of increasing superfamily complexity (assessed as the number of constituent families, *blue bars*). Data extracted from the *Keys to the Trematoda* (Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008). *Order of superfamilies*: 1, Azygioidea; 2, Bivesiculoidea; 3, Transversotrematoidea; 4, Haplosplanchnoidea; 5, Heronimoidea; 6, Bucephaloidea; 7, Clinostomoidea; 8, Haploporoidea; 9, Microscaphidioidea; 10, Monorchioi-dea; 11, Cyclocoeloidea; 12, Schistosomatoidea; 13, Opis-thorchioidea; 14, Allocreadioidea; 15, Gymnophalloidea; 16, Diplostomoidea; 17, Pronocephaloidea; 18, Brachylaimoidea; 19, Echinostomatoidea; 20, Lepocread-ioidea; 21, Gorgoderoidea; 22, Paramphistomoidea; 23, Hemiuroidea; 24, Microphalloidea; 25, Plagiorchioidea

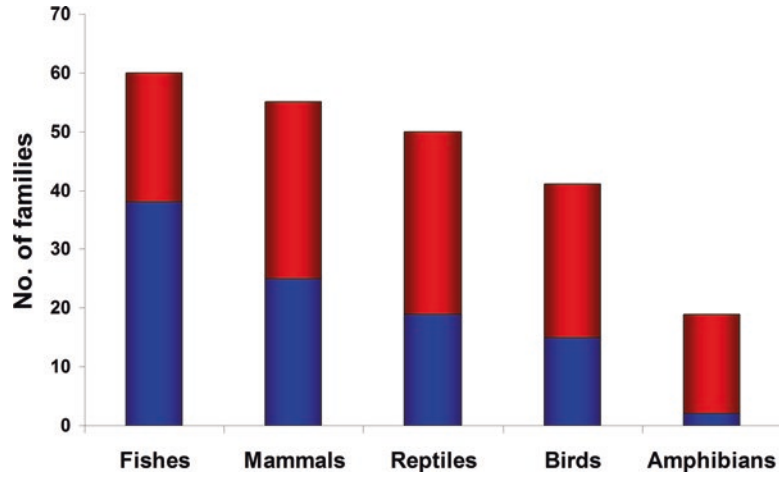

Fig. 2.2 The distribution of digenean diversity (assessed as the number of families) among the major definitive host groups [data from Bray (2008)]. Highlighted in *blue* are the digenean families found exclusively in a given host group, and the remaining (highlighted in *red*) are found in more than one group

demonstrated that this is the case at least in one occasion, i.e. the Brachylaimidae Joyeux & Foley, 1930 was recovered as paraphyletic, with the Leucochloridiidae Poche, 1907 nested. However, a molecular-based hypothesis based on denser taxon sampling within these superfamilies is required to test this suggestion.

Regarding the distribution of digenean taxonomic diversity among the major definitive host groups, 99 (67%) of the digenean families are parasitic in a single vertebrate group (highlighted in blue in Fig. 2.2), whereas the remaining are found in more than one host group (highlighted in red in Fig. 2.2). Data from Bray (2008) plotted

in Fig. 2.2 illustrate that the highest number of the digenean families that occur in fishes represent exclusively fish parasites, whereas the number of families found only in amphibians is the lowest; the remaining vertebrate groups occupy intermediate position with respect to their exclusive associations with the digenean families.

2.3 Molecular Approaches to Digenean Phylogeny: Higher Taxa

Molecular data from nucleic acid sequences represent a useful source of independent data for phylogenetic inference. As predicted by Gibson and Bray (1994), our understanding of the diversity, systematics and evolutionary relationships of the Digenea has substantially advanced as a result of the expansion of molecular-based studies in the past 15 years. Ribosomal RNA (rRNA) genes (and their spacer regions) and mitochondrial (mt) genes have been the most popular markers used in the systematic studies of digeneans at several nested taxonomic scales; this is associated with the varying rates of evolution of the gene regions. Whereas rRNA genes have been used for inferring relationships at higher taxonomic levels, the internal transcribed spacer (ITS1 and ITS2) separated by the 5.8S rRNA gene has been utilised for exploring species boundaries in a range of studies related to 155 species of 19 digenean families [see Nolan and Cribb (2005) for an exhaustive review]. Another relatively recent review on the advances and trends in the molecular systematics of the parasitic platyhelminths covers comprehensively studies on the Digenea at several taxonomic scales (Olson and Tkach 2005). Here we highlight selected examples with significant contribution towards the development of the molecular phylogeny of the Digenea at the higher taxonomic levels rather than provide an account of the investigations at the lower levels.

The first studies of the relationships within the Digenea at the suprageneric scale used the most conserved of the nuclear ribosomal RNA genes, the genes encoding the 18S subunit (Blair and Barker 1993; Blair et al. 1998; Fernandez et al. 1998; Hall et al. 1999). Soon focus has been placed on the 5′ variable domains (D1–D3) of the 28S rRNA gene as suitable data source for inferring relationships at several levels, i.e. among species, genera and closely related families (Barker et al. 1993), and the first studies at the suprageneric level proved to be influential.

Tkach et al. (2000a) developed a molecular phylogeny of one of the most diverse digenean groups, the formerly recognised suborder Plagiorchiata, based on partial 28S rDNA sequences for 28 species of 13 families. They demonstrated the derived position of Plagiorchiata in relation to all major digenean lineages considered in their analysis. Tkach et al. (2000a) also found that Plagiorchiata of the hypotheses based on morphological and life-cycle characters (Odening 1964; Brooks et al. 1985, 1989; Brooks and McLennan 1993) is paraphyletic and suggested as a solution the exclusion of the superfamilies Opecoeloidea, Dicrocoelioidea and Gorgoderoidea. These authors considered Plagiorchiata *sensu stricto* to comprise the superfamilies Plagiorchioidea (including the Plagiorchiidae Lühe, 1901, Haematoloechidae Freitas & Lent, 1939, Telorchiidae Looss, 1899, Brachycoeliidae Looss, 1899, and Leptophallidae Dayal, 1938) and Microphalloidea (including the Microphallidae Ward, 1901, Prosthogonimidae Lühe, 1909, Lecithodendriidae Lühe, 1901 and Pleurogenidae Looss, 1899). Their analyses also indicated close relationships between the genera *Macrodera* Looss, 1899 and *Paralepoderma* Dollfus, 1950, *Leptophallus* Lühe, 1909 and *Metaleptophallus* Yamaguti, 1958, and *Opisthioglyphe* Looss, 1899 and *Telorchis* Lühe, 1899. The first four genera were later (in the *Keys to the Trematoda*) placed in the family Leptophallidae (see Tkach 2008c) and the latter two were accommodated within the family Telorchiidae (see Font and Lotz 2008).

In an updated analysis of partial 28S rDNA sequences using a larger number of diverse taxa (51 species belonging to 27 families), Tkach et al. (2001a) assessed the relationships of Plagiorchiata with 14 digenean families. The results of their study confirmed the main group-

ings (and their content), i.e. the Plagiorchioidea and Microphalloidea, found in Tkach et al. (2000a) and revealed a basal position of the families Schistosomatidae Stiles & Hassall, 1898, Diplostomidae Poirier, 1886, Strigeidae Railliet, 1919, Brachylaimidae and Leucochloridiidae, and a sister-group relationship between the Renicolidae Dollfus, 1939 and Eucotylidae Cohn, 1904, both associated with the superfamily Microphalloidea.

Tkach et al. (2003) used partial 28S rDNA sequences to explore the phylogenetic interrelationships of 32 species belonging to 18 genera and 4 families of the superfamily Microphalloidea with members of the Plagiorchioidea (eight species of six genera) as outgroups. They demonstrated that the representatives of the Microphalloidea form three main lineages corresponding to the families Lecithodendriidae, Microphallidae and Pleurogenidae + Prosthogonimidae and suggested synonymies at the generic level (*Floridatrema* Kinsella & Deblock, 1994 with *Maritrema* Nicoll, 1907; *Candidotrema* Dollfus, 1951 with *Pleurogenes* Looss, 1896; and *Schistogonimus* Lühe, 1909 with *Prosthogonimus* Lühe, 1899). Whereas the first synonymy was not accepted by Deblock (2008), the latter two were considered in the *Keys to the Trematoda* (Lotz and Font 2008; Jones 2008).

All of the above-mentioned studies concern solving pieces of the puzzle of digenean relationships at higher taxonomic levels. The first step to a more inclusive analysis of digenean phylogeny is that of Cribb et al. (2001) who attempted a combined evidence approach using morphological characters for all stages of the digenean lifecycle and complete 18S rDNA sequences for 75 digenean species of 55 families. Analyses of this first morphological dataset with a published character matrix identified the Bivesiculidae Yamaguti, 1934 + Transversotrematidae Witenberg, 1944 as the sister group to the remainder of the Digenea and the Diplostomoidea Poirier, 1886 + Schistosomatoidea Stiles & Hassall, 1898 as the next most basal taxon. The combined evidence solution of Cribb et al. (2001) was found to exhibit greater resolution than morphology alone with the predominant effect of the molecular data on tree topology. Analyses of the combined data found no support for a basal position of the Heronimidae Ward, 1917 and revealed that the earliest divergent digeneans include the Diplostomoidea (Diplostomidae and Strigeidae) and Schistosomatoidea (Sanguini-colidae von Graff, 1907 and Schistosomatidae) with the Transversotrematidae and Bivesiculidae progressively less basal.

Although Cribb et al. (2001) found poor resolution of higher digenean taxa, the relationships at the superfamily level were well resolved. These include the superfamilies:

- The Hemiuroidea, with the Azygiidae Lühe, 1909 as basal, the Sclerodistomidae Odhner, 1927, Accacoeliidae Odhner, 1911, Syncoeliidae Looss, 1899, Derogenidae Nicoll, 1910 and Didymozoidae Monticelli, 1888 in one clade, and the Hemiuridae Looss, 1899 (recovered as paraphyletic) and Lecithasteridae Odhner, 1905 in the other.
- The Paramphistomoidea Fischoeder, 1901 [including the Paramphistomidae Fischoeder, 1901, Diplodiscidae Cohn, 1904, Microscaphidiidae Looss, 1900 (as Angiodictyidae Looss, 1902) and Mesometridae Poche, 1926].
- The Opisthorchioidea (including the Cryptogonimidae Ward, 1917, Heterophyidae and Opisthorchiidae).
- The Echinostomatoidea Looss, 1899 (including the Echinostomatidae Looss, 1899, Fasciolidae Railliet, 1895, Philophthalmidae Looss, 1899 and Cyclocoelidae Stossich, 1902).
- The Acanthocolpoidea Nahhas & Cable, 1964 (including the Acanthocolpidae Lühe, 1906, Campulidae Odhner, 1926 and Nasitrematidae Ozaki, 1935).
- The Lepocreadioidea Odhner, 1905 [with the Lepocreadiidae Odhner, 1905 (recovered as paraphyletic), Enenteridae Yamaguti, 1958 and Gyliauchenidae Fukui, 1929 but not the Apocreadiidae Skrjabin, 1942 which grouped with the Haploporoidea Nicoll, 1914 and Monorchioidea]; there was no support for a close relationship between the superfamily Haploporoidea and the Haplosplanchnidae Poche, 1926.

- The Haploporoidea (the Haploporidae Nicoll, 1914 and Atractotrematidae Yamaguti, 1939).

Cribb et al. (2001) found weak support for the Fellodistomoidea (containing the Tandanicolidae Johnston, 1927 and Fellodistomidae Nicoll, 1909) and the Plagiorchioidea (containing a subgroup formed by the Plagiorchiidae, Brachycoeliidae and Cephalogonimidae Looss, 1899; and Microphallidae, Pachypsolidae Yamaguti, 1958, Zoogonidae Odhner, 1902 and Faustulidae Poche, 1926). On the other hand, the Opecoelidae and Opistholebethidae Fukui, 1929, were strongly related as well as there was a strong sister relationship between the Monorchiidae Odhner, 1911 and the enigmatic genus *Cableia* Sogandares-Bernal, 1959, which has variously been placed in the Lepocreadiidae, Opecoelidae, Enenteridae and the Acanthocolpidae.

The most comprehensive phylogeny of the Digenea to date is that of Olson et al. (2003); it is also the first re-evaluation of relationships at higher taxonomic levels that has affected digenean classification. These authors estimated digenean relationships after adding a substantial number of novel sequences for complete 18S and partial (variable domains D1–D3) 28S rRNA genes (80 and 124, respectively). Their combined dataset which was found to yield the most strongly supported results thus comprised a rich and diverse array of taxa representing all major digenean groups (163 species of 77 families) (see Table 2.1). One important outcome of this study is the first molecular-based classification proposed based on the results from Bayesian analysis of the combined dataset; the authors went further by considering in association of putative synapomorphies that add morphological or ontological support to the molecular data.

Table 2.1 Classification of the Digenea of Olson et al. (2003) and of the *Keys to the Trematoda* (Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008)

Olson et al. (2003)		*Keys to the Trematoda*
Superfamily	Family	Different superfamilial placements and/or additional families
Order Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Suborder Diplostomata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Brachylaimoidea Joyeux & Foley, 1930	Brachylaimidae Joyeux & Foley, 1930 + Leucochloridiidae Poche, 1907[a]	Hasstielsiidae Hall, 1916; Leucochloridiomorphidae Yamaguti, 1958; Moreauiidae Johnston, 1915; Ovariopteridae Leonov, Spasskii & Kulikov, 1963; Panopistidae Yamaguti, 1958; Thapariellidae Srivastava, 1953
Diplostomoidea Poirier, 1886	Diplostomidae Poirier, 1886 + Strigeidae Railliet, 1919	Bolbocephaloididae Strand, 1935; Brauninidae Wolf, 1903; ∗Cyathocotylidae Mühling, 1898; ∗Proterodiplostomidae Dubois, 1936
Schistosomatoidea Stiles & Hassall, 1898	Schistosomatidae Stiles & Hassall, 1898	
	Clinostomidae Lühe, 1901	**Clinostomoidea Lühe, 1901** (also including ∗Liolopidae Odhner, 1912)
	Sanguinicolidae von Graff, 1907	
	Spirorchiidae Stunkard, 1921	
Order Plagiorchiida La Rue, 1957		
Suborder Apocreadiata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Apocreadioidea Skrjabin, 1942	Apocreadiidae Skrjabin, 1942	**Lepocreadioidea Odhner, 1905**
Suborder Bivesiculata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Bivesiculoidea Yamaguti, 1934	Bivesiculidae Yamaguti, 1934	

(continued)

Table 2.1 (continued)

Olson et al. (2003)		*Keys to the Trematoda*
Superfamily	Family	Different superfamilial placements and/or additional families
Suborder Bucephalata La Rue, 1926		
Bucephaloidea Poche, 1907	Bucephalidae Poche, 1907	
Gymnophalloidea Odhner, 1905	Fellodistomidae Nicoll, 1909	Gymnophallidae Odhner, 1905; Botulisaccidae Yamaguti, 1971
	Tandanicolidae Johnston, 1927	
Suborder Echinostomata La Rue, 1926		
Echinostomatoidea Looss, 1899	Echinostomatidae Looss, 1899 + Fasciolidae Railliet, 1895	Calycodidae Dollfus, 1929; ∗Cathaemasiidae Fuhrmann, 1928; ∗Rhopaliidae Looss, 1899; Rhytidodidae Odhner, 1926
	Philophthalmidae Looss, 1899	
	Psilostomidae Looss, 1900	
	Cyclocoelidae Stossich, 1902	**Cyclocoeloidea Stossich, 1902** (also including Typhlocoelidae Harrah, 1922)
Suborder Haplosplanchnata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Haplosplanchnoidea Poche, 1926	Haplosplanchnidae Poche, 1926	
Suborder Hemiurata Skrjabin & Guschanskaja, 1954		
Azygioidea Lühe, 1909	Azygiidae Lühe, 1909	
Hemiuroidea Looss, 1899	Hemiuridae Looss, 1899 + Lecithasteridae Odhner, 1905	Bathycotylidae Dollfus, 1932; Dictysarcidae Skrjabin & Guschanskaja, 1955; Hirudinellidae Dollfus, 1932; Isoparorchiidae Travassos, 1922; Ptychogonimidae Dollfus, 1937; Sclerodistomoididae Gibson & Bray, 1979
	Accacoeliidae Odhner, 1911	
	Derogenidae Nicoll, 1910 (*Hemiperina* Manter, 1934; *Derogenes* Lühe, 1900)[b]	
	Didymozoidae Monticelli, 1888	
	Sclerodistomidae Odhner, 1927	
	Syncoeliidae Looss, 1899	
Suborder Heronimata Skrjabin & Schulz, 1937		
Heronimoidea Ward, 1917	Heronimidae Ward, 1917	
Suborder Lepocreadiata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Lepocreadioidea Odhner, 1905	Lepocreadiidae Odhner, 1905	Deropristidae Cable & Hunninen, 1942; Liliatrematidae Gubanov, 1953; Megaperidae Manter, 1934
	Enenteridae Yamaguti, 1958	
	Gorgocephalidae Manter, 1966	
	Gyliauchenidae Fukui, 1929	
Suborder Monorchiata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Monorchioidea Odhner, 1911	Monorchiidae Odhner, 1911	
	Lissorchiidae Magath, 1917	
	Cableia Sogandares-Bernal, 1959	
Suborder Opisthorchiata La Rue, 1957		
Opisthorchioidea Looss, 1899	Heterophyidae Leiper, 1909 + Opisthorchiidae Looss, 1899	
	Cryptogonimidae Ward, 1917	

(continued)

Table 2.1 (continued)

Olson et al. (2003)		*Keys to the Trematoda*
Superfamily	Family	Different superfamilial placements and/or additional families
Suborder Pronocephalata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Pronocephaloidea Looss, 1899	Pronocephalidae Looss, 1899	Nudacotylidae Barker, 1916
	Labicolidae Blair, 1979	
	Notocotylidae Lühe, 1909	
	Opisthotrematidae Poche, 1926	
	Rhabdiopoeidae Poche, 1926	
Paramphistomoidea Fischoeder, 1901	Cladorchiidae Fischoeder, 1901	Paramphistomidae Fischoeder, 1901; Balanorchiidae Stunkard, 1925; Brumptiidae Stunkard, 1925; Choerocotyloididae Yamaguti, 1971; Gastrodiscidae Monticelli, 1892; Gastrothylacidae Stiles & Goldberger, 1910; Olveriidae Yamaguti, 1958; Stephanopharyngidae Stiles & Goldberger, 1910; Zonocotylidae Yamaguti, 1963; Zygocotylidae Ward, 1917
	Microscaphidiidae Looss, 1900 + Mesometridae Poche, 1926	**Microscaphidioidea Looss, 1900**
	Diplodiscidae Cohn, 1904	
Suborder Transversotremata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Transversotrematoidea Witenberg, 1944	Transversotrematidae Witenberg, 1944	
Suborder Xiphidiata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Allocreadioidea Looss, 1902	Opecoelidae Ozaki, 1925 + Opistholebetidae Fukui, 1929	Allocreadiidae Looss, 1902; Batrachotrematidae Dollfus & Williams, 1966
	Acanthocolpidae Lühe, 1906[b]	**Lepocreadioidea Odhner, 1905**
	Brachycladiidae Odhner, 1905	**Lepocreadioidea Odhner, 1905**
Gorgoderoidea Looss, 1899	Gorgoderidae Looss, 1899	Anchitrematidae Mehra, 1935; Braunotrematidae Yamaguti, 1958; Collyriclidae Ward, 1917; Cortrematidae Yamaguti, 1958; Mesocoeliidae Dollfus, 1929; Prouterinidae Foreyt, Schell & Beyer, 1996
	Callodistomidae Odhner, 1910	**Gymnophalloidea Odhner, 1905**
	Dicrocoeliidae Looss, 1899	
	Encyclometridae Mehra, 1931	**Plagiorchioidea Lühe, 1901**
	Haploporidae Nicoll, 1914 + Atractotrematidae Yamaguti, 1939	**Haploporoidea Nicoll, 1914**
	Orchipedidae Skrjabin, 1913	
	Paragonimidae Dollfus, 1939	
	Troglotrematidae Odhner, 1914	

(continued)

Table 2.1 (continued)

Olson et al. (2003)		*Keys to the Trematoda*
Superfamily	Family	Different superfamilial placements and/or additional families
Microphalloidea Ward, 1901	Microphallidae Ward, 1901 (*Maritrema* Nicoll, 1907, *Microphallus* Ward, 1901)	Anenterotrematidae Yamaguti, 1958; Diplangidae Yamaguti, 1971; Eumegacetidae Travassos, 1922; Exotidendriidae Mehra, 1935; Gyrabascidae Macy, 1935; Leyogonimidae Dollfus, 1951; Phaneropsolidae Mehra, 1935; Renschetrematidae Yamaguti, 1971; Stomylotrematidae Poche, 1926; Taiwantrematidae Fischthal & Kuntz, 1981
	Eucotylidae Cohn, 1904	**Cyclocoeloidea Stossich, 1902**
	Lecithodendriidae Lühe, 1901	
	Pachypsolidae Yamaguti, 1958	
	Pleurogenidae Looss, 1899	
	Prosthogonimidae Lühe, 1909	
	Renicolidae Dollfus, 1939	
	Zoogonidae Odhner, 1902 + Faustulidae Poche, 1926	
Plagiorchioidea Lühe, 1901	Plagiorchiidae Lühe, 1901 (*Glypthelmins* Stafford, 1905, *Skrjabinoeces* Sudarikov, 1950, *Haematoloechus* Looss, 1899)	Dolichoperoididae Johnston & Angel, 1940; Echinoporidae Krasnolobova & Timofeeva, 1965; Gekkonotrematidae Yamaguti, 1971; Glypthelminthidae Cheng, 1959; Haematoloechidae Freitas & Lent, 1939; Leptophallidae Dayal, 1938; Meristocotylidae Fischthal & Kuntz, 1964; Mesotretidae Poche, 1926; Ocadiatrematidae Fischthal & Kuntz, 1981; Opisthogonimidae Travassos, 1928; Orientocreadiidae Yamaguti, 1958; Reniferidae Pratt, 1902; Styphlotrematidae Baer, 1924; Thrinascotrematidae Jue Sue & Platt, 1999; Urotrematidae Poche, 1926
	Auridistomidae Stunkard, 1924	
	Brachycoeliidae Looss, 1899	**Gorgoderoidea Looss, 1899**
	Cephalogonimidae Looss, 1899	
	Choanocotylidae Jue Sue & Platt, 1998	
	Macroderoididae McMullen, 1937	
	Omphalometridae Looss, 1899	
	Telorchiidae Looss, 1899	

Different superfamilial placements are indicated in bold; underlined are families for which molecular data are required; asterisks indicate novel data gathered for some of these families after the first edition of the chapter (see text for details)
[a]Paraphyletic relationships in the analysis of Olson et al. (2003) are indicated with a +
[b]Polyphyletic in Olson et al. (2003)

Generally, the molecular phylogenetic analyses of Olson et al. (2003) supported the most recent classification of the Digenea provided in the *Keys to the Trematoda* at the familial and superfamilial levels (but see the differences in superfamilial placements highlighted in Table 2.1) but provided strong evidence for a different subdivision (and membership in some cases) at the higher taxonomic levels. This has led to the recognition of a number of new taxa at the ordinal and subordinal levels (one order and nine suborders; see Table 2.1).

Key in the new classification is the reflection that the molecular phylogeny of the Digenea does not support its traditional division into three groups at the ordinal level, i.e. the Strigeida La Rue 1957, the Echinostomida La Rue 1957, and the Plagiorchiida La Rue 1957 (see La Rue 1957; Gibson and Bray 1994; Gibson 1996). Olson et al. (2003) split the subclass Digenea into two major groups, the order Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003 and the order Plagiorchiida La Rue 1957 [these were referred to as superorders by Cribb et al. (2003)] thus confirming the prediction of Gibson and Bray (1994) and the results of Cribb et al. (2001). The Diplostomida is comprised of three superfamilies whereas the Plagiorchiida has a more complex structure with 13 suborders [referred to as orders by Cribb et al. (2003) and Littlewood (2006)] containing a total of 19 superfamilies (see Table 2.1 for details). The four more inclusive suborders in the phylogeny of Olson et al. (2003) are:

- The Hemiurata Skrjabin & Guschanskaja, 1954 represented by two superfamilies, the Azygioidea Lühe, 1909 (monotypic) and the Hemiuroidea [seven families as in Cribb et al. (2001), see above].
- The Bucephalata La Rue, 1926 represented by two superfamilies, the Bucephaloidea (monotypic) and the Gymnophalloidea (including two families).
- The Pronocephalata Olson, Cribb, Tkach, Bray & Littlewood, 2003 represented by two superfamilies, the Paramphistomoidea (including four families) and the Pronocephaloidea (including five families).
- The Xiphidiata Olson, Cribb, Tkach, Bray & Littlewood, 2003 represented by four superfamilies, the Gorgoderoidea (monotypic), the Microphalloidea (including nine families), the Allocreadioidea (including four families) and the Plagiorchioidea (including eight families, Table 2.1).

An important outcome of the development of a molecular phylogeny of the Digenea is that inferences can be made on the origin and evolution of the digenean life-cycle. Cribb et al. (2003) used the hypothesis and the classification of Olson et al. (2003) and life-cycle traits derived from a large database (*c.*1350 species) of information on the life-cycles for the Digenea to explore the evolution of the digenean life-cycle. Cribb et al. (2003) inferred that gastropods were the basal host group for the Digenea, parasitism of bivalves being a result of host-switching that has occurred multiple times, and found no convincing evidence for a deep level coevolution between the major digenean clades and their molluscan hosts. Regarding the second intermediate hosts, these authors illustrated a great diversity with discontinuous distributions on the phylogeny of different host types and concluded that three-host life-cycles have been derived from two-host life-cycles and adopted repeatedly. With respect to definitive hosts, these authors suggested an origin for the Digenea in association with teleosts followed by host-switching into chondrichthyans and provided alternative explanations for parasitism in tetrapods based on the topologies of relationships within the Xiphidiata and Diplostomida.

2.4 Towards Greater and Focused Representation of Digenean Diversity in Phylogenies

The development of the molecular phylogeny of the Digenea coincided with that of the *Keys to the Trematoda* so that a full consensus with the classification based on the molecular results of Cribb et al. (2001), Olson et al. (2003) and Tkach et al. (2000a, 2001a, 2003) has been reached in the

treatment of the five superfamilies in the third volume (Bray 2008). Overall, a comparison between the two classifications of the Digenea summarised in Table 2.1 shows a considerable congruence. The superfamilial placement of 12 families (8%, highlighted in bold in Table 2.1) in the *Keys to the Trematoda* departed from that inferred from the molecular phylogeny of Olson et al. (2003).

Although the analysis of Olson et al. (2003) represents the broadest sampling of the Digenea to date (52% of a total of 148 currently recognised digenean families), a number of omissions (families highlighted in Table 2.1) were depicted (Olson et al. 2003; Olson and Tkach 2005). We here comment upon the taxa involved in the molecular phylogeny of the Digenea in association with the content of the *Keys of the Trematoda* focusing on additional important omissions rather than on those previously identified in an attempt to outline the suprageneric taxa that require further exploration in a molecular phylogeny. We also highlight important recent advancements published after the first edition of this chapter.

Olson et al. (2003) did not include in their analysis representatives of the type-families of the Allocreadioidea (the Allocreadiidae Looss, 1902), Gymnophalloidea (the Gymnophallidae Odhner, 1905) and Paramphistomoidea (the Paramphistomidae). Therefore, the basis of each of these superfamilies was not actually established and should not be considered definitive; also see Cribb (2005). At the lower taxonomic level, the same problem exists, i.e. lack of data from the type-genera of the families Cryptogonimidae, Opisthorchiidae, Strigeidae and Plagiorchiidae. Notably, the first three taxa were recovered in clades in which paraphyly was detected (Olson et al. 2003). The Plagiorchioidea represents a special case. Formally, the type-family has been sampled at the time of the study of Olson et al. (2003). However, the three genera whose representatives have been sequenced (*Glypthelmins* Stafford, 1905, *Skrjabinoeces* Sudarikov, 1950 and *Haematoloechus* Looss, 1899) were transferred to different families, recognised in 2008, i.e. the Glypthelminthidae Cheng, 1959 and the Haematoloechidae (see Tkach 2008a, b). Therefore, the Plagiorchioidea also needs re-establishment preferably based on molecular data from representatives of the type-genus *Plagiorchis* Lühe, 1899, of the type-family Plagiorchiidae.

A number of superfamilies characterised by high taxonomic diversity at the generic and suprageneric levels have been underrepresented in the broad phylogeny of the Digenea by Olson et al. (2003). These are (in order of increasing generic richness, data from the *Keys to the Trematoda*; see also Fig. 2.1): Echinostomatoidea, Diplostomoidea, Paramphistomoidea, Plagiorchioidea, Allocreadioidea, Opisthorchioidea, Lepocreadioidea and Microphalloidea.

Using only a small fraction of the actual generic/familial diversity in the phylogeny of the Digenea by Olson et al. (2003) has typically led to problems in resolving relationships. Thus the family Echinostomatidae [correct name given in the taxonomic listing of the taxa studied and the trees but referred to as "Echinostomidae" [*sic*] elsewhere in the text; see Olson et al. 2003] was represented by just two genera, *Echinostoma* Rudolphi, 1809 and *Euparyphium* Dietz, 1909 [sequence for *Euparyphium melis*, a synonym of *Isthmiophora melis* (Schrank, 1788); see Kostadinova and Gibson (2002) and Kostadinova (2005a)] and found to be paraphyletic. The family represents a diverse and complex group comprised of 43 genera belonging to 10 subfamilies (Kostadinova 2005a), and we predicted in the first edition of this chapter that denser sampling would likely lead to a better resolution of the relationships within the superfamily Echinostomatoidea [molecular data for 7 out of 81 genera available in the phylogeny by Olson et al. (2003)]; we suggested that effort should also be focused on representation of the four families not sampled to date (i.e. the Calycodidae Dollfus, 1929, Cathaemasiidae Fuhrmann, 1928, Rhopaliidae Looss, 1899, and Rhytidodidae Odhner, 1926, Table 2.1). This gap in our knowledge is now substantially narrowed as a result of the first exhaustive molecular analysis of the Echinostomatoidea by Tkach et al. (2016) (see Section 2.5 for details) which also included representatives

of two of the families indicated above, i.e. the Cathaemasiidae and Rhopaliidae.

The superfamily Diplostomoidea was represented in the phylogeny of Olson et al. (2003) by five out of 89 genera, two diplostomid (*Alaria* Schrank, 1788 and *Diplostomum* Nordmann, 1832) and three strigeid genera (*Apharyngostrigea* Ciurea, 1927, *Cardiocephaloides* Sudarikov, 1959 and *Ichthyocotylurus* Odening, 1969) and the members of these genera were found intermingled in the clade of Diplostomoidea; the type-genus of the Strigeidae was not sampled (see Olson et al. 2003). In the first edition of this chapter, we suggested that the assessment of the relationships within the superfamily requires further exploration based on a wider array of taxa including the type-genus of the family Strigeidae, *Strigea* Abildgaard, 1790; we also marked as important omissions the families Cyathocotylidae Mühling, 1898 and Proterodiplostomidae Dubois, 1936 (Table 2.1).

The sampled taxonomic diversity of the Diplostomoidea has notably increased recently (see Hernández-Mena et al., 2017 and references therein). Of note, Fraija-Fernández et al. (2015) added sequences for *Braunina cordiformis* Wolf, 1903, and confirmed the placement of the Brauninidae Wolf, 1903 within the Diplostomoidea). The broad diversity of taxa sequenced allows hypotheses for the relationships within and among superfamilies of the order Diplostomida to be tested based on nuclear ribosomal RNA genes. Hernández-Mena et al. (2017) generated partial 18S and 28S rRNA gene sequences for 15 and 17 species, respectively, and provided the first molecular phylogenies for the Diplostomida based on the separate and concatenated alignments for the two genes. These included novel sequences for representatives of the Schistosomatoidea (Clinostomidae) and Diplos-tomoidea (Cyathocotylidae, Diplostomidae, Proterodiplostomidae and Strigeidae, notably including the type-genus *Strigea*) and published sequences for representatives of the Brachylaimoidea (Brachylaimidae and Leucochloridiidae), Schistosomatoidea (Clinostomidae, Sanguinicolidae (as Aporocotylidae) and Spirorchiidae Stunkard, 1921) and Diplostomoidea (Brauninidae, Diplostomidae and Strigeidae) plus a sequence for a species of the Liolopidae Odhner, 1912 (see Hernández-Mena et al. 2017 for details). Phylogenetic analyses including 49 species (28S rRNA gene) and 45 species (18S rRNA gene) resulted in congruent hypotheses regarding the relationships among families (and superfamilies) of the order Diplostomida (see Hernández-Mena et al. 2017).

In particular (i) the Diplostomoidea was resolved as monophyletic; (ii) the family Proterodiplostomidae was recovered as the sister taxon to the Diplos-tomidae + Strigeidae within the Diplostomoidea; (iii) the Cyathocotylidae + Brauninidae were found at a basal position within the superfamily; and (iv) the Liolopidae was resolved as basal to the Schistosomatoidea (including the Aporocot-ylidae, Clinostomidae, Spirorchiidae and Schistosomatidae) in individual gene analyses but as a sister taxon to the Diplostomoidea in the combined gene analysis (however, this relationship was not supported.)

Although the Opisthorchioidea and one of its constituent families, the Cryptogonimidae, were resolved in the phylogeny of Olson et al. (2003), the remaining two families were not since the Heterophyidae was recovered as paraphyletic with the Opisthorchiidae nested within it. Seven out of a total of 129 genera of the superfamily were sampled in their study but none of the type-genera of the three families; their re-establishment in a molecular phylogeny is therefore still in the pending state. Recently, Thaenkham et al. (2012) added 18S rDNA sequences for species of three genera, *Haplorchis* Looss, 1899, *Procerovum* Onji & Nishio, 1916 and *Metagonimus* Katsurada, 1912, and examined the relationships within the Opisthorchioidea based on a wider generic representation (including a sequence for a species of *Opisthorchis* Blanchard, 1895, the type-genus of the Opisthorchiidae). However, their analysis also recovered a paraphyletic relationship between the Heterophyidae and Opisthorchiidae, the latter nested within the former.

The diversity of the family Microphallidae, the type of the Microphalloidea, was underrepresented (2 out of 47 genera, i.e. *Microphallus* Ward, 1901 and *Maritrema*) in the study of Olson et al. (2003). These authors found that in some

analyses the representatives of the two subfamilies, the Microphallinae Ward, 1901 and the Maritrematinae Nicoll, 1907, were split among different clades. Tkach et al. (2003) added sequences for more representatives of the two genera and recovered the Microphallidae as monophyletic. Nevertheless, the complex structure and diverse content of the family still awaits exploration of relationships based on a much wider taxon sampling.

2.5 Integrated Approaches to Digenean Diversity, Taxonomy and Systematics

A review of the history of the development of studies on the most comprehensively studied digenean superfamilies (Hemiuroidea, Lepocreadioidea, Plagiorchioidea, Schistosomatoidea and Echinostomatoidea) indicates a framework that would lead to robust estimates of phylogeny: (i) systematic inventory of the group; (ii) detailed understanding of morphology; (iii) taxonomic revision; (iv) classification system; (v) molecular phylogeny; (vi) revised classification. In this section, we shall illustrate the progress within this framework focusing on the superfamilial level.

Historically, the most extensively studied digenean higher-level taxon appears to be the Hemiuroidea, a highly diverse group of parasites found predominantly in marine teleosts but also in freshwater teleosts and elasmobranchs and occasionally in amphibians and reptiles (Gibson 2002c). The knowledge on species diversity accumulated over two centuries, and focused sampling and revisionary work based on material from the North-East Atlantic have largely contributed to an improved classification of this large and heterogeneous group (13 families and 196 genera recognised in the *Keys to the Trematoda*). In a series of monographs, Gibson and Bray provided original descriptions, detailed comments on the morphology and life-cycles, host-parasite records (including larval stages) and identification keys for all of the hemiuroid species recorded from the North-East Atlantic; there included representatives of the families Accacoeliidae, Azygiidae, Hemiuridae, Hirudinellidae Dollfus, 1932, Ptychogonimidae Dollfus, 1937, Sclerodistomidae and Syncoeliidae (see Bray and Gibson 1977; Gibson and Bray 1977, 1986). Gibson and Bray (1979) revised the superfamily and proposed a classification and a hypothesis for the evolution of the Hemiuroidea based on the functional morphology of the adults; based on original data, these authors also provided detailed definitions of hemiuroid structures and analysis on their systematic value and possible function. According to the classification of Gibson and Bray (1979), the Hemiuroidea is divided into 14 families: Accacoeliidae (with two subfamilies); Azygiidae (with two subfamilies); Bathycotylidae Dollfus, 1932; Bunocotylidae Dollfus, 1950 (with four subfamilies); Derogenidae (with three subfamilies); Dictysarcidae Skrjabin & Guschanskaja, 1955 (with three subfamilies); Hemiuridae (with nine subfamilies); Hirudinellidae; Isoparorchiidae Travassos, 1922; Lecithasteridae (with six subfamilies); Ptychogonimidae; Sclerodistomidae (with three subfamilies); Sclerodistomoididae Gibson & Bray, 1979; and Syncoeliidae (with two subfamilies). The studies of Gibson and Bray thus provided a much-needed systematic framework to be evaluated with the aid of molecular evidence.

The first molecular phylogeny of the Hemiouroidea was based on the V4 variable domain of the 18S rRNA gene for 33 species representative of ten hemiuroidean families after the concept of Gibson and Bray (1979) plus the Didymozoidae (see Blair et al. 1998). Analyses of Blair et al. (1998) supported the monophyly of the Hemiuroidea as represented by the taxa sampled and revealed two main groups, one containing all members of the Hemiuridae and lecithasterine lecithasterids and one comprised of the members of Derogenidae, Didymozoidae, Hirudinellidae, Sclerodistomidae, Syncoeliidae and Accacoeliidae, whereas the Isoparorchiidae and the hysterolecithine lecithasterids appeared separately close to the base of the hemiuroid tree and the Azygiidae fell outside the hemiuroid clade. Hemiuroids were well represented although with a lower number of taxa (18 species

belonging to 7 families) in the phylogeny of the Digenea of Olson et al. (2003). Their analyses strongly supported the distinct status of the Hemiurata with Hemiuroidea and Azygioidea as separate superfamilies. Within the Hemiuroidea, the Derogenidae was recovered as polyphyletic, and a paraphyletic relationship of the Hemiuridae and the Lecithasteridae was depicted (as in Blair et al. 1998; Cribb et al. 2001). Consequently, the results of the molecular phylogenies were considered in the *Keys to the Trematoda*: the Azygiidae was recognised at the superfamily level (Gibson 2002b), and the Didymozoidae was included within the Hemiuroidea (see Gibson 2002c).

Pankov et al. (2006) described a new bunocotyline genus *Robinia* Pankov, Webster, Blasco-Costa, Gibson, Littlewood, Balbuena & Kostadinova, 2006, and presented a phylogenetic hypothesis for the Bunocotylinae Dollfus, 1950 and the Hemiuroidea based on sequence data analyses of an increased number of taxa (from 22 species for complete 18S and partial 28S rRNA genes and from 37 species for the V4 domain of the 18S rRNA gene). Both molecular analyses confirmed the monophyly of the Hemiuroidea, its division into two major clades, and the polyphyly of the Derogenidae, as in previous studies (Blair et al. 1998; Cribb et al. 2001; Olson et al. 2003), and suggested that the Gonocercinae Skrjabin & Guschanskaja, 1955 (with two genera, *Gonocerca* Manter, 1925 and *Hemipera* Nicoll, 1913) may require a distinct familial status. Pankov et al. (2006) found poor support for the distinct status of the Lecithasteridae and Hemiuridae, following previous suggestions based on different sequence datasets (Blair et al. 1998; Cribb et al. 2001; Olson et al. 2003). The results of this study also indicated that increased taxon sampling for and analysing the V4 domain of the 18S rRNA gene separately, failed to resolve many monophyletic hemiurid subfamilies thus adding little to the study of Blair et al. (1998). Pankov et al. (2006) suggested that much greater taxon sampling for both 18S and 28S genes is needed in order to test the consistency of the present classification system of the Hemiuroidea with the evolutionary relationships of its members.

The Lepocreadioidea is one of the complex and problematic digenean superfamilies. Ten families and 137 genera are recognised in the *Keys to the Trematoda*, but molecular studies have demonstrated that three of these families (Acanthocolpidae, Apocreadiidae and Brachycladiidae Odhner, 1905) are not closely related to the Lepocreadiidae (see Cribb et al. 2001; Olson et al. 2003; Bray et al. 2005; also see Table 2.1). Bray, Cribb and colleagues devoted a comprehensive series of studies (*c*.50 papers) on the diversity of the Lepocreadioidea in marine teleosts, predominantly in the Indo-West Pacific and the North-East Atlantic, which resulted in detailed descriptions of a vast number of species (including many new), erection of new and/or reassessment of the existing genera and construction of identification keys to species and parasite-host and host-parasite lists (see Bray et al. 2009, for a list of the most inclusive references). These data provided a sound basis for revisory work (Bray and Cribb 1997; Bray 2005a, b, c, d; Hall and Cribb 2005). On the other hand, extensive sampling for molecular studies carried out in parallel with morphological assessments, has supplied an admirable number of sequences for species from a wide range of genera. Bray et al. (2009) assessed the phylogenetic relationships of representative species of the superfamily Lepocreadioidea using partial 28S rDNA and *nad*1 sequences for members of the families Lepocreadiidae (42 species), Enenteridae (6 species), Gyliauchenidae (6 species), and Gorgocephalidae Manter, 1966 (1 species), along with 22 species representing eight other digenean families. The study recovered the Lepocreadioidea as monophyletic, comprised of six groups: three well-recognised families (Enenteridae, Gorgocephalidae and Gyliauchenidae) and three groups resulting from the partitioning of the Lepocreadiidae in the phylogenetic tree. The latter were recognised as families (i.e. Lepocreadiidae Odhner, 1905; Aephnidiogenidae Yamaguti, 1934; and Lepidapedidae Yamaguti, 1958) by Bray and Cribb (2012) who also provided amended family diagnoses. Recently, the new family Gibsonivermidae Bray, Cribb & Cutmore, 2018 was erected for *Gibsonivermis*

Bray, Cribb & Barker 1997 (see Bray et al. 2018), previously considered a genus '*incertae sedis within the superfamily*' Lepocreadioidea (see Bray and Cribb 2012).

A similar increased effort to collect and characterise morphologically and/or molecularly representatives of the members of the Plagiorchioidea and Microphalloidea by Tkach and colleagues (1999, 2000b, 2001b, c) has contributed significantly to our understanding of the relationships and family structures of these large taxa (see above). The results of the molecular phylogenies (Tkach et al. 2000a, 2001a, 2003) are partially reflected in the family level classifications in the *Keys to the Trematoda* (see Tkach 2008a, b, c, d). However, the two superfamilies are far too large and still require sustained systematic research.

Augmented representation of the species/genera of blood flukes has also resulted in advancing the knowledge on the relationships within the superfamily Schistosomatoidea. Snyder and Loker (2000) examined phylogenetic relationships among ten genera (*Austrobilharzia* Johnston, 1917, *Bilharziella* Looss, 1899, *Dendritobilharzia* Skrjabin & Zakharow, 1920, *Gigantobilharzia* Odhner, 1910, *Heterobilharzia* Price, 1929, *Orientobilharzia* Dutt & Srivastava, 1955, *Ornithobilharzia* Odhner, 1912, *Schistosoma* Weinland, 1858, *Schistosomatium* Tanabe, 1923 and *Trichobilharzia* Skrjabin & Zakharow, 1920) of the family Schistosomatidae using 28S rDNA sequences (variable domains D1–D2), and found two major clades, one comprised of the genera *Schistosoma* and *Orientobilharzia* parasitic in mammals and one consisting of predominantly bird parasites. These authors suggested an Asian origin of *Schistosoma.* Snyder (2004) expanded the data on the Schistosomatoidea by generating 18S and 28S rDNA sequences for species belonging to eight genera of the Spirorchiidae. Phylogenetic analyses involving representatives of the order Diplostomida recovered Spirorchiidae as paraphyletic with three genera from marine turtles exhibiting a sister-group relationship with the Schistosomatidae, whereas five genera from freshwater turtles were found to occupy basal positions in the phylogeny of the tetrapod blood flukes. This, coupled with the basal position within the schistosomatid clade of the genera *Austrobilharzia* and *Ornithobilharzia*, both comprising species with marine life-cycles, led to a suggestion that schistosomatids arose after a marine turtle blood fluke ancestor successfully colonised birds (Snyder 2004). Lockyer et al. (2003a) presented the most comprehensive phylogeny of the Schistosomatidae to date, based on the sequences of three genes, complete 18S and 28S rRNA and mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1), for 30 species representing 10 of the 13 known genera and almost all species of *Schistosoma*. The phylogeny provided evidence for the validity of two of the four currently accepted subfamilies (Khalil 2002), the Gigantobilharziinae Mehra, 1940 (comprising the genera *Dendritobilharzia* and *Gigantobilharzia*) and the Schistosomatinae Stiles & Hassall, 1898 (including *Austrobilharzia*, *Heterobilharzia*, *Orientobilharzia*, *Ornithobilharzia*, *Schistosoma* and *Schistosomatium*) but not for the subfamily Bilharzeillinae Price, 1929 since the representatives of *Bilharziella* and *Trichobilharzia* did not form a monophyletic clade. The study of Lockyer et al. (2003a) confirmed an Asian origin for *Schistosoma* and the position of *Orientobilharzia* within the *Schistosoma*. The nomenclatural change has recently been formally justified by Aldhoun and Littlewood (2012) who transferred to *Schistosoma* the four species of *Orientobilharzia* they considered valid [as *Schistosoma bomfordi* Montgomery, 1906, *Schistosoma turkestanicum* Skrjabin, 1913, *Schistosoma dattai* (Dutt & Srivastava, 1952) and *Schistosoma harinasutai* (Kruatrachue, Bhaibulaya & Harinasuta, 1965)] and provided an amended generic diagnosis of *Schistosoma* and a revised key to the subfamily Schistosomatinae.

According to the most recent classification in the *Keys to the Trematoda*, eight families and 83 genera are recognised within the Echinostomatoidea including the Echinostomatidae (with 10 subfamilies and 44 genera), Cathaemasiidae (with two subfamilies and five genera), Fasciolidae (with three subfamilies and six genera), Philophthalmidae (with five subfamilies and 11 genera), Psilostomidae Looss, 1900 (with

six subfamilies and 13 genera) and Rhytidodidae (with two genera) plus the monotypic Calycodidae and Rhopaliidae (see Kostadinova 2005a, b, c; Kostadinova and Jones 2005). Considering the fact that molecular phylogenies of the Digenea (Cribb et al. 2001; Olson et al. 2003) recovered the Cyclocoelidae within the Echinostomatoidea increases the number of subfamilies and genera within the superfamily by 6 and 22, respectively, resulting in 32 subfamilies and 105 genera (see Tkach et al., 2016 for a detailed classification to the generic level).

Recently, Tkach et al. (2016) provided the first comprehensive phylogeny of the Echinostomatoidea and evaluated the consistency of the morphology-based classification system of the superfamily. The phylogeny based on partial 28S rDNA sequences included 80 species, representing eight of the ten families recognised within the Echinostomatoidea including representatives of two of the families indicated above, i.e. the Cathaemasiidae and Rhopaliidae, and five subfamilies of the most complex family of the Echinostomatoidea, the Echinostomatidae. At the generic level, 40 genera representing 17 of the 32 subfamilies recognised prior to the study were sampled. The phylogeny challenged the systematic framework based on comparative morphology at several levels. Tkach et al. (2016) made a number of systematic and nomenclatural changes consistent with the phylogenetic estimates of the generic and supra-generic boundaries after a morphology-based evaluation of the new molecular framework as follows: (i) elevation of the rank of two family-level lineages, the former Himasthlinae and Echinochasminae, to full family status; (ii) erection of the Caballerotrematidae Tkach, Kudlai & Kostadinova, 2016 for *Caballerotrema* Prudhoe, 1960; (iii) revision of the content and diagnosis of the Echinostomatidae (*sensu stricto*) to reflect its phylogeny, resulting in the abolition of the Nephrostominae and Chaunocephalinae as synonyms of the Echinostomatidae (*sensu stricto*); (iv) re-allocation within the Echinostomatidae (*sensu stricto*) of *Artyfechinostomum*, *Cathaemasia*, *Rhopalias* and *Ribeiroia*, resulting in the abolition of the Cathaemasiidae, Rhopaliidae and Ribeiroiinae as synonyms of the Echinostomatidae (*sensu stricto*); and (v) refinements of the generic boundaries within the Echinostomatidae (*sensu stricto*), Psilostomidae and Fasciolidae. Finally, Tkach et al. (2016) provided a phylogeny-based classification of the Echinostomatoidea as follows:

Order Plagiorchiida La Rue, 1957
Suborder Echinostomata La Rue, 1926
Superfamily Echinostomatoidea Looss, 1899 (syn. Cyclocoeloidea Stossich, 1902)
- Family Echinostomatidae Looss, 1899 (syns Rhopaliidae Looss, 1899; Cathaemasiidae Fuhrmann, 1928; Nephrostominae Mendheim, 1943; Ribeiroiinae Travassos, 1951)
- Family Caballerotrematidae Tkach, Kudlai & Kostadinova, 2016
- Family Cyclocoelidae Stossich, 1902 (syn. Typhlocoelidae Harrah, 1922)
- Family Echinochasmidae Odhner, 1910 (syn. Saakotrematidae Odening, 1962)
- Family Fasciolidae Railliet, 1895
- Family Himasthlidae Odhner, 1910
- Family Philophthalmidae Looss, 1899
- Family Psilostomidae Looss, 1900
- ? Family Rhytidodidae Odhner, 1926
- ? Family Calycodidae Dollfus, 1929

2.6 Future Research Prospects

In conclusion, molecular phylogenetics appears key to understanding the evolution of the Digenea. Although there is an agreement that further effort is needed towards achieving an improved representation of digenean taxonomic diversity in molecular phylogenies (Olson et al. 2003; Olson and Tkach 2005; Tkach et al. 2016), challenges in selection of gene loci exist and a direction of efforts appear to have been clarified recently. It is apparent that molecular analyses of digenean relationships at higher taxonomic levels will continue to rely upon the 18S and 28S rDNA sequences because a rich database has already been acquired.

However, evidence has been accumulating recently that promotes the utility of a complete

28S rRNA gene as phylogenetic marker and illustrates the benefits of improved phylogenetic signal when used in combination with 18S rRNA gene at different levels within and between metazoan taxa including platyhelminths (e.g. Mallatt and Winchell 2002; Lockyer et al. 2003a, b). The latter authors examined the utility of this approach in resolving the interrelationships between the major flatworm clades and stressed that Bayesian inference and maximum likelihood appear to give more congruent trees than maximum parsimony with respect to traditional concepts (Lockyer et al. 2003a). Mallatt and colleagues (Mallatt et al. 2004; Mallatt and Giribet 2006) have evaluated the phylogenetic relationships in Ecdysozoa (molting animals) using likelihood-based Bayesian inference on nearly complete 18S + 28S rDNA sequences and suggested that this may prove to be a combination of best genes and a tree-building method for reconstruction of ecdysozoan phylogenies. Waeschenbach et al. (2007) used nearly complete (4047–4593 nt) 28S rDNA sequences in combination with complete 18S rDNA (1940–2228 nt) and Bayesian analyses to resolve cestode interrelationships at the ordinal level. They demonstrated that the addition of domains D4-D12 of the 28S rRNA gene contributes to a substantial improvement of phylogenetic signal resulting in overall better nodal support, topology stability and greater resolution compared with previous molecular estimates of cestode interrelationships based on 18S + partial (domains D1–D3) 28S rRNA genes. With regard to digenean interrelationships, the pioneer study of Lockyer et al. (2003b) is a promising start especially because it is the first phylogeny inferred from a combination of three independent datasets (i.e. for 18S, 28S and *cox*1) (see above).

Mitochondrial genomes may offer a wealth of homologous markers for both systematics and diagnostics, but in contrast to nuclear ribosomal genes, few mitochondrial genes have been tested because of the limited availability of PCR primers and the higher rates of evolution, thus rendering them more suitable for resolving more recent radiations (see e.g. Littlewood 2008 for a review). However, whole mitochondrial genome sequences have been shown to resolve deep-level relationships in many metazoan groups (Timmermans et al. 2010), and the use of mtDNA spanning multiple genes has been considered promising (Littlewood 2008; also see Philippe et al. 2011 for an in-depth focus on the use of genome-scale data in phylogenies). At the less inclusive taxonomic levels, modern genomic approaches may also provide an in-depth understanding of the patterns of speciation and construction of robust phylogenies as illustrated by the recent developments in the genetic research on species of the genus *Schistosoma* (see e.g. Johnston 2006; Littlewood et al. 2006; Le et al. 2001; Zarowiecki et al. 2007; Lawton et al. 2011).

Although the number of digenean mitochondrial genome sequences is increasing steadily (see e.g. references in Le et al. 2016), only a few studies have addressed relationships at the suprageneric level, and the results are typically confirming the previous molecular estimates of digenean interrelationships based on partial (domains D1–D3) 28S rRNA gene. For example, Le et al. (2016) characterised the mitochondrial genome of *Echinochasmus japonicus* Tanabe, 1926, and used concatenated amino acid sequence data for 12 protein-coding genes of 36 species representing 14 digenean families to estimate the relationships within the Echinostomatidae (*sensu lato*) (see Tkach et al. 2016). The resulting phylogenetic tree supported the elevation of the Echinochasmidae to full family rank based on 28S rDNA data, a nomenclatural change already made by Tkach and colleagues (see p. 179 in Tkach et al. 2016) and their phylogeny-based classification of the Echinostomatoidea provided above; this fact was apparently missed by Le et al. (2016).

It is also worth noting that the first genome-wide estimation of the phylogenetic relationships of the order Diplostomida by Brabec et al. (2015) revealed a conflict between nuclear ribosomal and mitochondrial protein phylogeny estimates. Brabec et al. (2015) applied a next-generation sequencing approach and characterised the complete mitochondrial genomes and nuclear rRNA operons of two closely related species, *Diplostomum spathaceum* (Rudolphi, 1819) and *D. pseudospathaceum* Niewiadomska, 1984 (Diplostomoidea:

Diplostomidae) and estimated the phylogenetic position of *Diplostomum* spp. using concatenated amino acid data for 12 protein-coding genes and nucleotide sequence data. The phylogenies included 19 species, representative of the basal (order Diplostomida; Schistosomatidae: seven species), and derived (order Plagiorchiida; 10 species of 6 families: Opisthorchiidae, Dicrocoeliidae, Fasciolidae, Heterophyidae, Paragonimidae and Paramphistomidae) clades of the Digenea in the phylogeny of Olson et al. (2003). Analyses of the mitogenomic data both at the amino acid and nucleotide levels invariably recovered the Diplostomidae as a sister lineage of the Plagiorchiida rather than as a basal lineage of the Diplostomida as inferred in the benchmark phylogeny of the Digenea of Olson et al. (2003) and the rDNA-based phylogenetic analysis for the set of taxa used in the mitogenome-based analysis of Brabec et al. (2015). These authors considered the results of the latter analyses concordant with the finding that the organisation of the mitochondrial genomes of *Diplostomum* spp. closely matches the almost perfectly conserved gene order in mitochondrial genomes of the members of the Plagiorchiida, rather than that of the only members of the order Diplostomida with mitochondrial genomes characterised prior to their study.

Most recently, Locke et al. (2018) significantly enriched the Diplostomida mitogenome database. These authors characterised the complete mitochondrial genomes and nuclear rRNA operons of seven species representative of three families of the Diplostomoidea (i.e. Diplostomidae, four species; Strigeidae, two species; and Cyathocotylidae, one species) and carried out phylogenetic analyses of mitochondrial genomes and ultra-conserved genomic elements. Phylogenetic analyses of the mitogenomic data (12 protein-coding genes) both at the amino acid and nucleotide levels included 34 species (17 species of the Diplostomida and 17 species of the Plagiorchiida) and confirmed the findings of Brabec et al. (2015) (i.e. non-monophyletic Diplostomida) with strong support. However, a maximum likelihood analysis of a much larger dataset of 517 ultra-conserved genomic elements (alignment of 234,783 bp in length), from seven members of the Diplostomoidea and three of the Plagiorchioidea provided unequivocal support for the Diplostomida. Locke et al. (2018) suggested that both mitochondrial and nuclear genome data for representatives of the Diplostomida (Brachylaimoidea, Aporocotylidae, Liolopidae and Spirorchiidae) and of the early divergent lineages of the Plagiorchiida (e.g. suborder Bivesiculata Olson et al. 2003) are needed to address the relationships of the two major lineages of the Digenea.

References

Aldhoun JA, Littlewood DTJ (2012) *Orientobilharzia* Dutt & Srivastava, 1955 (Trematoda: Schistosomatidae), a junior synonym of *Schistosoma* Weinland, 1858. Syst Parasitol 82:81–88

Barker SC, Blair D, Garrett AR, Cribb TH (1993) Utility of the D1 domain of nuclear 28S rRNA for phylogenetic inference in the Digenea. Syst Parasitol 26:181–188

Blair D, Barker S (1993) Affinities of the Glyauchenidae: utility of the 18S rRNA gene for the phylogenetic inference in the Digenea (Platyhelminthes). Int J Parasitol 23:527–532

Blair D, Bray RA, Barker SC (1998) Molecules and morphology in phylogenetic studies of the Hemiuroidea (Digenea: Trematoda: Platyhelminthes). Mol Phylogenet Evol 9:15–25

Brabec J, Kostadinova A, Scholz T, Littlewood DTJ (2015) Complete mitochondrial genomes and nuclear ribosomal RNA operons of two species of *Diplostomum* (Platyhelminthes: Trematoda): a molecular resource for taxonomy and molecular epidemiology of important fish pathogens. Parasit Vectors 8:336

Bray RA (2005a) Family Enenteridae Yamaguti, 1958. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 657–661

Bray RA (2005b) Family Gorgocephalidae Manter, 1966. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 663–664

Bray RA (2005c) Family Lepocreadiidae Odhner, 1905. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 545–602

Bray RA (2005d) Superfamily Lepocreadioidea Odhner, 1905. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 541–543

Bray RA (2008) Introduction and key to superfamilies. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 1–5

Bray RA, Cribb TH (1997) The subfamily Aephnidiogeninae Yamaguti, 1934 (Digenea: Lepocreadiidae), its status and that of the genera *Aephnidiogenes* Nicoll, 1915, *Holorchis* Stossich, 1901, *Austroholorchis* gen. n., *Pseudaephnidiogenes* Yamaguti, 1971, *Pseudoholorchis* Yamaguti, 1958 and *Neolepocreadium* Thomas, 1960. Syst Parasitol 36:47–68

Bray RA, Cribb TH (2003) The digeneans of elasmobranchs—distribution and evolutionary significance. In: Combes C, Jourdane J (eds) Taxonomie, écologie et évolution des métazoaires parasites. (Livre hommage à Louis Euzet). Tome 1. PUP, Perpignan, pp. 67–96

Bray RA, Cribb TH (2012) Reorganisation of the superfamily Lepocreadioidea Odhner, 1905 based on an inferred molecular phylogeny. Syst Parasitol 83:169–177

Bray RA, Gibson DI (1977) The Accacoeliidae (Digenea) of fishes from the north-east Atlantic. Bull Br Museum Nat Hist (Zool) 31:51–99

Bray RA, Webster BL, Bartoli P, Littlewood DTJ (2005) Relationships within the Acanthocolpidae Lühe, 1906 and their place among the Digenea. Acta Parasitol 50:281–291

Bray RA, Gibson DI, Jones A (eds) (2008) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London

Bray RA, Waeschenbach A, Cribb TH, Weedall GD, Dyal P, Littlewood DTJ (2009) The phylogeny of the Lepocreadioidea (Platyhelminthes, Digenea) inferred from nuclear and mitochondrial genes: implications for their systematics and evolution. Acta Parasitol 54:310–329

Bray RA, Cribb TH, Cutmore SC (2018) Lepocreadiidae Odhner, 1905 and Aephnidiogenidae Yamaguti, 1934 (Digenea: Lepocreadioidea) of fishes from Moreton Bay, Queensland, Australia, with the erection of a new family and genus. Syst Parasitol 95:479–498

Brooks DR, McLennan DA (1993) Parascript. Parasites and the language of evolution. Smithsonian Institution Press, Washington

Brooks DR, O'Grady RT, Glen DR (1985) Phylogenetic analysis of the Digenea (Platyhelminthes: Cercomeria) with comments on their adaptive radiation. Can J Zool 63:411–443

Brooks DR, Bandoni SM, MacDonald CA, O'Grady RT (1989) Aspects of the phylogeny of the Trematoda Rudolphi, 1808 (Platyhelminthes: Cercomeria). Can J Zool 67:2609–2624

Cable RM (1974) Phylogeny and taxonomy of trematodes with reference to marine species. In: Vernberg WB (ed) Symbiosis in the sea. University of South California Press, Charleston, pp. 173–193

Cribb TH (2005) Superfamily Allocreadioidea Looss, 1902. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 413–539

Cribb TH, Bray RA, Littlewood DTJ, Pichelin S, Herniou EA (2001) Relationships of the Digenea—evidence from molecules and morphology. In: Littlewood DTJ, Bray RA (eds) Interrelationships of the Platyhelminthes. Taylor & Francis, London, pp. 186–193

Cribb TH, Bray RA, Olson PD, Littlewood DTJ (2003) Life cycle evolution in the Digenea: a new perspective from phylogeny. Adv Parasitol 54:197–254

Deblock S (2008) Family Microphallidae Ward, 1901. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 451–492

Fernandez M, Littlewood DTJ, Latorre A, Raga JA, Rollinson D (1998) Phylogenetic relationships of the family Campulidae (Trematoda) based on 18S rDNA sequences. Parasitology 117:383–391

Font WF, Lotz JM (2008) Family Telorchiidae Looss, 1899. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 425–436

Fraija-Fernández N, Olson PD, Crespo EA, Raga JA, Aznar FJ, Fernández M (2015) Independent host switching events by digenean parasites of cetaceans inferred from ribosomal DNA. Int J Parasitol 45:167–173

Gibson DI (1987) Questions in digenean systematics and evolution. Parasitology 95:429–460

Gibson DI (1996) Trematoda. In: Margolis L, Kabata Z (eds) Guide to the Parasites of Fishes of Canada. Part IV. Canadian Special Publication of Fisheries and Aquatic Sciences No. 124. NRC Press, Ottawa, pp. 1–373

Gibson DI (2002a) Class Trematoda Rudolphi, 1808. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI and The Natural History Museum, Wallingford and London, pp. 1–3

Gibson DI (2002b) Superfamily Azygioidea Lühe, 1909. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI and The Natural History Museum, Wallingford and London, pp. 19–24

Gibson DI (2002c) Superfamily Hemiuroidea Looss, 1899. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI, Wallingford, pp. 299–304

Gibson DI, Bray RA (1977) The Azygiidae, Hirudinellidae, Ptychogonimidae, Sclerodistomidae and Syncoeliidae (Digenea) of fishes from the north-east Atlantic. Bull Br Museum Nat Hist (Zool) 32:167–245

Gibson DI, Bray RA (1979) The Hemiuroidea: terminology, systematics and evolution. Bull Br Museum Nat Hist (Zool) 36:35–146

Gibson DI, Bray RA (1986) The Hemiuridae (Digenea) of fishes from the north-east Atlantic. Bull Br Museum Nat Hist (Zool) 51:1–125

Gibson DI, Bray RA (1994) The evolutionary expansion and host-parasite relationships of the Digenea. Int J Parasitol 24:1213–1226

Gibson DI, Chinabut S (1984) *Rohdella siamensis* gen. et sp. nov. (Aspidogastridae: Rohdeliinae subfam. nov.) from freshwater fishes in Thailand, with a reorganisation of the classification of the subclass Aspidogastrea. Parasitology 88:383–393

Gibson DI, Jones A, Bray RA (eds) (2002) Keys to the Trematoda, vol 1. CABI, Wallingford

Hall KA, Cribb TH (2005) Family Gyliauchenidae Fukui, 1929. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 665–678

Hall KA, Cribb TH, Barker SC (1999) V4 region of small subunit rDNA indicates polyphyly of the Fellodistomidae (Digenea) which is supported by morphology and life-cycle data. Syst Parasitol 43:81–92

Hernández-Mena DI, García-Varela M, Pérez-Ponce de León G (2017) Filling the gaps in the classification of the Digenea Carus, 1863: systematic position of the Proterodiplostomidae Dubois, 1936 within the superfamily Diplostomoidea Poirier, 1886, inferred from nuclear and mitochondrial DNA sequences. Syst Parasitol 94:833–848

Johnston DA (2006) Genomes and genomics of parasitic flatworms. In: Maule AG, Marks NJ (eds) Parasitic flatworms. Molecular biology, biochemistry, immunology and physiology. CABI, Wallingford, pp. 37–80

Jones A (2008) Family Prosthogonimidae Lühe, 1909. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 577–590

Jones A, Bray RA, Gibson DI (eds) (2005) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London

Khalil LF (2002) Family Schistosomatidae Stiles & Hassall, 1898. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI, Wallingford, pp. 419–432

Kostadinova A (2005a) Family Echinostomatidae Looss, 1899. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 9–64

Kostadinova A (2005b) Family Cathaemasiidae Fuhrmann, 1928. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 69–77

Kostadinova A (2005c) Family Psilostomidae Looss, 1900. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 99–118

Kostadinova A, Gibson DI (2002) *Isthmiophora* Lühe, 1909 and *Euparyphium* Dietz, 1909 (Digenea: Echinostomatidae) re-defined, with comments on their nominal species. Syst Parasitol 52:205–217

Kostadinova A, Jones A (2005) Superfamily Echinostomatoidea Looss, 1899. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI and The Natural History Museum, Wallingford and London, pp. 5–8

La Rue GR (1957) The classification of digenetic Trematoda: a review and a new system. Exp Parasitol 6:306–344

Lawton SP, Hirai H, Ironside JE, Johnston D, Rollinson D (2011) Genomes and geography: genomic insights into the evolution and phylogeography of the genus *Schistosoma*. Parasit Vectors 4:131

Le TH, Humair PF, Blair D, Agatsuma T, Littlewood DT, McManus DP (2001) Mitochondrial gene content, arrangement and composition compared in African and Asian schistosomes. Mol Biochem Parasitol 117:61–71

Le TH, Nguyen NTB, Nguyen KT, Doan HTT, Dung DT, Blair D (2016) A complete mitochondrial genome from *Echinochasmus japonicus* supports the elevation of Echinochasminae Odhner, 1910 to family rank (Trematoda: Platyhelminthes). Infect Genet Evol 45:369–377

Littlewood DTJ (2006) The evolution of parasitism in flatworms. In: Maule AG, Marks NJ (eds) Parasitic flatworms. Molecular biology, biochemistry, immunology and physiology. CABI, Wallingford, pp. 1–35

Littlewood DTJ (2008) Platyhelminth systematics and the emergence of new characters. Parasite 15:333–341

Littlewood DTJ, Lockyer AE, Webster BL, Johnston DA, Le TH (2006) The complete mitochondrial genomes of *Schistosoma haematobium* and *Schistosoma spindale* and the evolutionary history of mitochondrial genome changes among parasitic flatworms. Mol Phylogenet Evol 39:452–467

Locke SA, Van Dam AV, Caffara M, Alves Pinto H, López-Hernández D, Blanar CA (2018) Validity of the Diplostomoidea and Diplostomida (Digenea, Platyhelminthes) upheld in phylogenomic analysis. Int J Parasitol 48:1043–1059

Lockyer AE, Olson PD, Littlewood DTJ (2003a) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. Biol J Linn Soc 78:155–171

Lockyer AE, Olson PD, Østergaard P, Rollinson D, Johnston DA, Attwood SW, Southgate VR, Horak P, Snyder SD, Le TH, Agatsuma T, McManus D, Carmichael AC, Naem S, Littlewood DTJ (2003b) The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. Parasitology 126:203–224

Lotz JM, Font WF (2008) Family Pleurogenidae Looss, 1899. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 563–575

Mallatt J, Giribet G (2006) Further use of nearly complete 28S and 18S rRNA genes to classify Ecdysozoa: 37 more arthropods and a kinorhynch. Mol Phylogenet Evol 40:772–794

Mallatt J, Winchell CJ (2002) Testing the new animal phylogeny: first use of combined large-subunit and smallsubunit rRNA gene sequences to classify the protostomes. Mol Biol Evol 19:289–301

Mallatt JM, Garey JR, Shultz JW (2004) Ecdysozoan phylogeny and Bayesian inference: first use of nearly complete 28S and 18S rRNA gene sequences to classify the arthropods and their kin. Mol Phylogenet Evol 31:178–191

Nolan MJ, Cribb TH (2005) The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. Adv Parasitol 60:101–163

Odening K (1961) Historische und moderne Gesichtspunkte beim Aufbau eines natürlichen Systems der digenetischen Trematoden. Biol Beitr 1:73–90

Odening K (1964) Exkretionssystem und systematische stellung einiger Fledermaustrematoden aus Berlin und Umgebung nebst Bemerkungen zum lecithodendrioiden Komplex. Z Parasitenkd 24:453–483

Odening K (1974) Verwandtschaft, system und zyklo-ontogenetische Besonderheiten der Trematoden. Zool Jahrb Systematik 101:345–396

Olson PD, Tkach VV (2005) Advances and trends in the molecular systematics of the parasitic Platyhelminthes. Adv Parasitol 60:165–243

Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int J Parasitol 33:733–755

Pankov P, Webster BL, Blasco MI, Gibson DI, Littlewood DTJ, Kostadinova A (2006) *Robinia aurata* n. g., n. sp. (Digenea: Hemiuridae) from *Liza aurata* (Perciformes: Mugilidae) in the western Mediterranean described from morphology and molecules. Parasitology 133:217–227

Pearson JC (1972) A phylogeny of life-cycle patterns of the Digenea. Adv Parasitol 10:153–189

Pearson JC (1992) On the position of the digenean family Heronimidae: an enquiry into a cladistic classification of the Digenea. Syst Parasitol 21:81–166

Philippe H, Brinkmann H, Lavrov DV, Littlewood DTJ, Manuel M, Wörheide G, Baurain D (2011) Resolving difficult phylogenetic questions: why more sequences are not enough. PLoS Biol 9:e1000602

Poche F (1926) Das System der Platodaria. Arch Naturgesch 91:1–459

Rohde K (1972) The Aspidogastrea, especially *Multicotyle purvisi* Dawes, 1941. Adv Parasitol 10:77–151

Rohde K (1994) The minor groups of parasitic Platyhelminthes. Adv Parasitol 33:145–234

Rohde K (2001) The Aspidogastrea: an archaic group of Platyhelminthes. In: Littlewood DTJ, Bray RA (eds) Interrelationships of the Platyhelminthes. Taylor & Francis, London, pp. 159–166

Rohde K (2002) Subclass Aspidogastrea Faust & Tang, 1936. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI, Wallingford, pp. 5–14

Snyder SD (2004) Phylogeny and paraphyly among tetrapod blood flukes (Digenea: Schistosomatidae and Spirorchiidae). Int J Parasitol 34:1385–1392

Snyder SD, Loker ES (2000) Evolutionary relationships among the Schistosomatidae (Platyhelminthes: Digenea) and an Asian origin for *Schistosoma*. J Parasitol 86:283–288

Thaenkham U, Blair D, Nawa Y, Waikagul J (2012) Families Opisthorchiidae and Heterophyidae: are they distinct? Parasitol Int 61:90–93

Timmermans MJTN, Dodsworth S, Culverwell CL, Bocak L, Ahrens D, Littlewood DTJ, Pons J, Vogler AP (2010) Why barcode? High-throughput multiplex sequencing of mitochondrial genomes for molecular systematics. Nucleic Acids Res 38:e197

Tkach VV (2008a) Family Glypthelminthidae Cheng, 1959. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 357–360

Tkach VV (2008b) Family Haematoloechidae Freitas & Lent, 1939. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 361–366

Tkach VV (2008c) Family Leptophallidae Dayal, 1938. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 367–371

Tkach VV (2008d) Family Plagiorchiidae Lühe, 1901. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CABI and The Natural History Museum, Wallingford and London, pp. 295–325

Tkach VV, Grabda-Kazubska B, Pawlowski J, Świderski Z (1999) Molecular and morphological evidence for close phylogenetic affinities of the genera *Macrodera*, *Leptophallus*, *Metaleptophallus* and *Paralepoderma* (Digenea, Plagiorchiata). Acta Parasitol 44:170–179

Tkach VV, Pawlowski J, Mariaux J (2000a) Phylogenetic analysis of the suborder Plagiorchiata (Platyhelminthes, Digenea) based on partial lsrDNA sequences. Int J Parasitol 30:89–93

Tkach VV, Pawlowski J, Sharpilo VP (2000b) Molecular and morphological differentiation between species of the *Plagiorchis vespertilionis* group (Digenea, Plagiorchiidae) occurring in European bats, with a re-description of *P. vespertilionis* (Müller, 1780). Syst Parasitol 47:9–22

Tkach VV, Pawlowski J, Mariaux J, Swiderski Z (2001a) Molecular phylogeny of the suborder Plagiorchiata and its position in the system of Digenea. In: Littlewood DTJ, Bray RA (eds) Interrelationships of the Platyhelminthes. Taylor & Francis, London, pp. 186–192

Tkach VV, Grabda-Kazubska B, Świderski Z (2001b) Systematic position and phylogenetic relationships of the family Omphalometridae Odening, 1959 (Digenea, Plagiorchiida) inferred from the partial lsrDNA sequences. Int J Parasitol 31:81–85

Tkach VV, Snyder SD, Swiderski Z (2001c) On the phylogenetic relationships of some members of Macroderoididae and Ochetosomatidae (Digenea, Plagiorchioidea). Acta Parasitol 46:267–275

Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM, Swiderski Z (2003) Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Syst Parasitol 56:1–15

Tkach VV, Kudlai O, Kostadinova A (2016) Molecular phylogeny and systematics of the Echinostomatoidea

Looss, 1899 (Platyhelminthes: Digenea). Int J Parasitol 46:171–185

Waeschenbach A, Webster BL, Bray RA, Littlewood DTJ (2007) Added resolution among ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with complete small and large subunit nuclear ribosomal RNA genes. Mol Phylogenet Evol 45:311–325

Zamparo D, Brooks DR (2003) Phylogenetic systematic assessment of the Aspidobothrea (Platyhelminthes, Neodermata, Trematoda). Zool Scr 32:83–93

Zarowiecki MZ, Huyse T, Littlewood DT (2007) Making the most of mitochondrial genomes-markers for phylogeny, molecular ecology and barcodes in *Schistosoma* (Platyhelminthes: Digenea). Int J Parasitol 37:1401–1148

Part II

Trematodes of Interest in Human Health

Schistosomiasis

3

Philip T. LoVerde

3.1 Introduction

In humans, several species of the trematode genus *Schistosoma* cause the disease schistosomiasis, which is classified as a "neglected" tropical disease (NTD, WHO 2010). The disease is also frequently referred to as bilharzia (and bilharziasis) to recognize Theodor Bilharz, the physician who first described the parasite in humans in 1851. Human schistosomiasis is an ancient disease. Reference to *S. haematobium* has been found in Egyptian papyri, and calcified eggs have been discovered in Egyptian mummies from around 1200 B.C. (Ruffer 1910). The eggs of *S. japonicum* were identified in two ancient corpses from the Hunan and Hubei provinces in China have shown that the prevalence of schistosomiasis in China has a history dating more than 2100 years (Wei et al. 1980).

Schistosomiasis is one of the most important parasitic diseases of humans in terms of morbidity and mortality. The World Health Organization (WHO) estimates that schistosomiasis is transmitted in over 78 countries, throughout a wide belt of the tropics and subtropics (WHO 2018). The three major schistosomes infecting humans are *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*. It is difficult to estimate the number of schistosomiasis cases in the world, but the number is considered to be greater than 250 million, mostly in Africa (Hotez 2008). By species, *S. haematobium* causes a urogenital form of disease and is endemic in Africa and the Middle East. Infection with *S. haematobium* also is classified as a group I carcinogen; urogenital schistosomiasis can lead to squamous-cell carcinoma of the bladder (IARC 2012). *S. mansoni* causes a hepato-intestinal schistosomiasis in Africa, the Middle East, South America, and the West Indies. *S. japonicum* causes a hepato-intestinal form of the disease and is endemic in China, the Philippines, Indonesia, and the Mekong Delta. Mortality rates are difficult to assess for schistosomiasis although it has been estimated that schistosomes cause approximately 280,000–500,000 deaths every year. However, mortality from this disease is not the major part of the problem when compared to the years of life that are lost due to the morbidity caused by schistosomiasis. The DALYs index ("Disability-Adjusted Life Years") of schistosomiasis is estimated as 3.3 million per year (Van der Werf et al. 2003; King et al. 2005).

Although schistosomiasis is generally restricted to the tropics and subtropics, a recent outbreak in Corsica demonstrates the potential

P. T. LoVerde (✉)
Department of Biochemistry and Structural Biology, University of Texas Health, San Antonio, TX, USA

Department of Pathology and Laboratory Medicine, University of Texas Health, San Antonio, TX, USA
e-mail: loverde@uthscsa.edu

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_3

for reemergence of this infectious disease in new, economically developed regions in southern Europe (Holtfreter et al. 2014). Overall, around 80–90% of the schistosomiasis cases worldwide occur in sub-Saharan Africa.

In addition to active infections, it is estimated that almost 800 million people worldwide are at risk for schistosomiasis (Steinmann et al. 2006).

Aside from death, schistosome infections can lead to numerous health problems, among them chronic disability, cognitive impairment, chronic anemia, abdominal pain, diarrhea and, for *Schistosoma haematobium* infections, urogenital problems, including bladder dysfunction, hematuria, and correlation with bladder cancer. Schistosomiasis commonly presents itself as a chronic, gradually debilitating illness. Because of this feature and frequent co-infections with other helminths, it has always been difficult to determine the real public health impact of schistosomiasis. For example, diseases such as malaria and HIV/AIDS often have a much higher community profile, and consequently more resources devoted to their control. Schistosomiasis is usually grouped into that category of diseases called the Neglected Tropical Diseases (NTDs), which also include numerous diseases caused by soil-transmitted helminths, protozoa, viruses, and bacteria, (Hotez and Kamath 2009). The NTDs predominantly affect the underserved human populations that live in the poorest conditions, contribute to the maintenance of social inequality and are a strong barrier to country development.

Being a waterborne disease, schistosomiasis is acquired by direct contact with freshwater that contains the infective larvae (cercariae) that emerge from freshwater snail hosts. Where schistosomiasis is endemic, primarily in subsistence agricultural and fishing environments, human contact with parasite-contaminated water is a daily occurrence. As a result of human manipulation of the local environment, such as the development of new irrigation projects or building of dams, the range of the disease can also spread easily by the establishment of new breeding sites for the vector snails. Also, man-made environmental modifications may benefit one schistosome species over that of another. A prime example of this can be seen in the recent history of schistosomiasis in Egypt (Michelson et al. 1993). There, with major water projects and the completion of the Aswan Dam in the mid-twentieth century, schistosomiasis caused by *S. mansoni* largely replaced that caused by *S. haematobium* along major sections of the Nile River, probably reflecting ecological changes favoring those of *Biomphalaria alexandrina* (regional host of *S. mansoni*) over those of the host snails for *S. haematobium* (*Bulinus truncatus*). Another now classic example occurred in Northern Senegal where the construction of the Diama dam (barrage) on the Senegal River basin in 1985 to generate electricity has resulted in marked ecological changes. As a result, there has been a tremendous increase in prevalence and intensity of infection of both intestinal and urogenital schistosomiasis in Richard-Toll (Southgate et al. 2001).

There is a vast research literature devoted to schistosomiasis. These studies amply demonstrate the complexity of the parasite's life cycle, and point to why schistosomiasis is the challenging health problem that it is in developing countries. A single chapter on schistosomiasis cannot cover, in-depth, all aspects of this complex disease, but those areas of particular interest that may be important for future research in the continuing efforts to control this devastating disease have been highlighted.

3.2 Systematics

Schistosomes, usually referred to as blood flukes, are members of the phylum Platyhelminths (flatworms) of the class Trematoda and family Schistosomatidae. The name *Schistosoma* (i.e., split body) refers to the appearance of the adult male worm whose lateral edges fold to form a groove (gynecophoral canal) where the female worm resides. Schistosomes differ from other trematodes in that they are dioecious parasites. Interestingly, at the adult stage there is an obvious sexual dimorphism between male and female worms. Schistosomes and related flukes infect a wide variety of definitive hosts, and currently 21 species are recognized within the genus

Fig. 3.1 Examples of intermediate snail hosts for the three major schistosomes infecting humans. From left to right, *Bulinus truncatus truncatus* (host for *S. haematobium*), *Biomphalaria glabrata* (host for *S. mansoni*), and *Oncomelania hupensis hupensis* (host for *S. japonicum*). (Photo from Biomedical Research Institute)

Schistosoma. Of the five schistosome species most often associated with human infections, the most important clinically and most studied species are *S. mansoni*, *S. haematobium*, and *S. japonicum*. The two others are *S. intercalatum*, which is closely related to *S. haematobium*, and *S. mekongi*, a close relative of *S. japonicum.* For this chapter, we will concentrate on discussions of schistosomiasis caused by *S. mansoni*, *S. haematobium*, and *S. japonicum*.

The geographic distribution of schistosomiasis is limited to the distribution of the species-specific intermediate snail hosts the parasite utilizes. Since so much of the life history of schistosomes involves a mollusc, we should briefly mention the systematics of these snails. Within the phylum Mollusca there are two large families, in the class Gastropoda, that are important for our discussions. These families are the Pomatiopsidae and Planorbidae. The taxonomy of gastropods has been in a state of flux for the last several years, but for the purposes of our discussion *S. japonicum* infects members of the Pomatiopsidae (genus *Oncomelania*). These snails have gills and thus obtain oxygen from the water, many have an operculum, the sexes are separate and they lay one egg at time.

The planorbid snails that serve as intermediate hosts for *S. mansoni* and *S. haematobium* do not have gills nor an operculum but are air breathing and are hermaphroditic. Members of the genus *Biomphalaria* are intermediate hosts for *S. mansoni*, and *Bulinus* spp. are intermediate hosts for *S. haematobium.*

As shown in Fig. 3.1, the shell of the planorbid *Biomphalaria* is planispiral, that is coiled flat like a rope, and the spire of the shell is sunken. Also, like all planorbids, this species has a sinistral shell, that is, the coiling of the shell is left-handed. The shell of *Bulinus* is sinistral. It has a very large body whorl and a small spire. For *Oncomelania* species, the shell is tall spired and can be separated into ribbed- and smooth-shelled morphotypes. *Biomphalaria* and *Bulinus* occupy some of the same ecological niches and are nearly always submerged in water, while *Oncomelania* is an amphibious snail, spending much of its time attached to vegetation, e.g., in rice paddies or on soil above the water surface.

3.3 Biology of the Parasite and Life Cycle

Of the three major human schistosome species, the life cycle of *S. japonicum* was the first to be fully described in 1914, by Miyairi and Suzuki. The life cycle of *S. mansoni* and *S. haematobium* were described in 1918 by Leiper.

The life cycles of the major schistosomes are quite similar—the differences chiefly consist of the intermediate snail species involved, and differences in the tissue distribution within the definitive hosts. A life cycle schematic of schistosomiasis is given in Fig. 3.2. The life cycle stages will be discussed in more depth in the following sections, but a short summary is presented here.

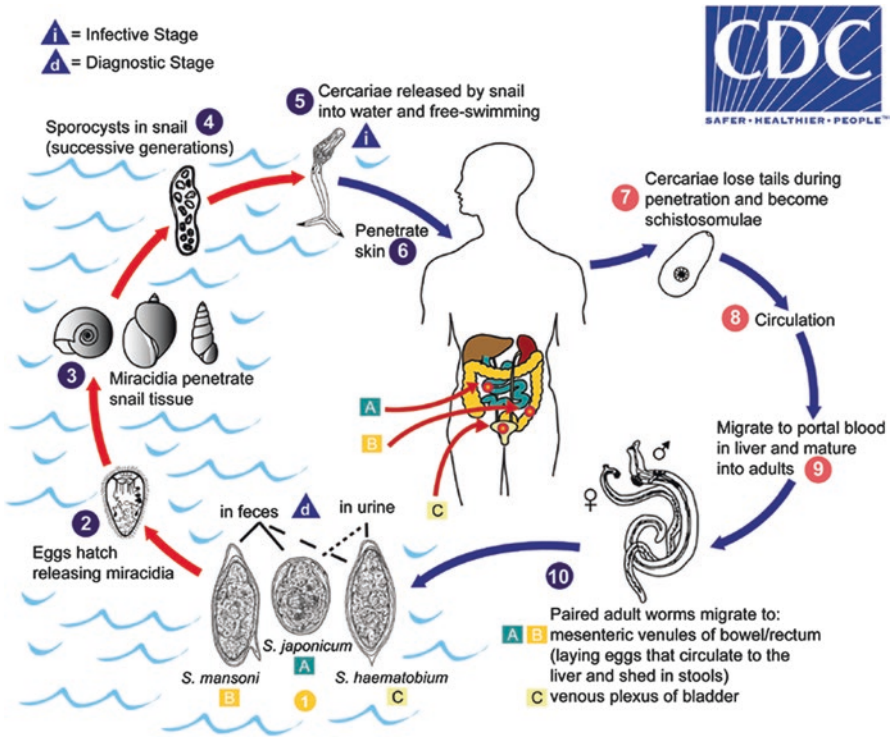

Fig. 3.2 The life cycle of human schistosomes (image courtesy of the Centers for Disease Control and Prevention, DPDx). The figure depicts several life cycle stages that are mentioned in the text. Key stages mentioned in the text include: miracidia, cercariae, schistosomules, adult worms, and eggs

The schistosomes have an infective, free-living non-feeding larval stage (cercaria) that gains entry into the mammalian, definitive host by penetration through unbroken skin. The parasites undergo a migration that involves the lungs and pair in the liver and migrate en copula to either the mesenteric veins draining the intestines (*S. mansoni* and *S. japonicum*) or vesical venous plexus of the urogenital system (*S. haematobium*). Eggs laid by the mature female then pass from the body through feces or urine. Another free-living non-feeding larval stage (miracidium) hatches from the egg once it reaches freshwater and infects the intermediate snail host, whereby asexual reproduction occurs resulting in the infective cercariae. Figure 3.3 shows scanning electron micrographs of different life cycle stages of *S. mansoni and S. haematobium*.

3.3.1 Biology of the Various Life Cycle Stages

3.3.1.1 Cercariae

The schistosome cercaria is a free-living, non-feeding actively swimming stage with a relatively short infectious life span (24–48 h) whose sole purpose is to take the germ line from the snail intermediate host to a suitable definitive host. The mature cercaria has two main segments. The first (body) is the progenitor of the adult worm and is attached to an extremely muscular, bifurcated tail. The tail serves to propel the organism through the water column, and it can serve as a fulcrum to assist the body in gaining entrance into the skin of the definitive host. The length of a *S. mansoni* cercaria is approximately 500 μm, but it contracts and elongates almost continuously.

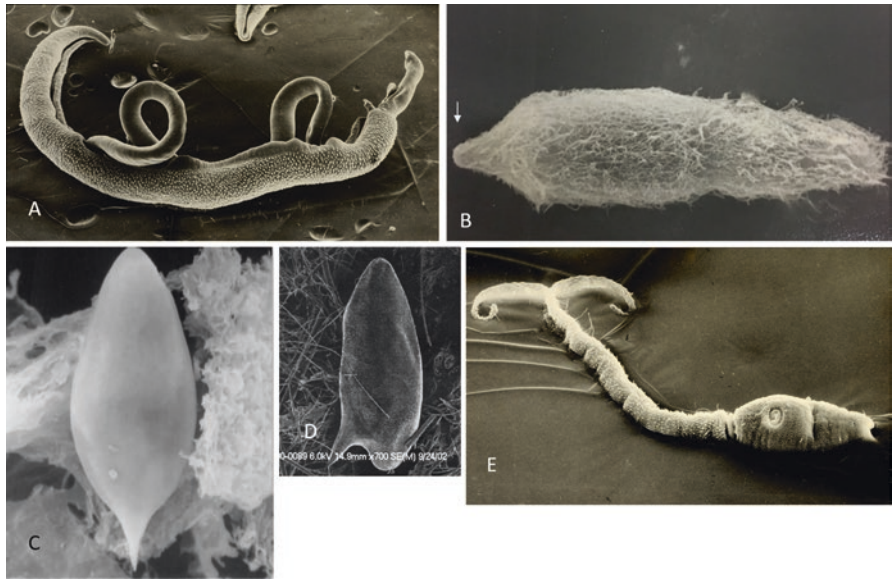

Fig. 3.3 Scanning electron micrographs of *Schistosoma* life stages. (**a**) Scanning electron micrographs of adult male and female *S. mansoni* worms in copula. The female is residing in the gynecophoral canal of the male worm. Length of males ranges from 6 to 13 mm; that of females ranges from 10 to 20 mm. (**b**) Miracidium of *S. haematobium* with epidermal plates covered with cilia. Arrow points to apical papilla important for penetration into the snail host. Dimensions approximately 135 × 55 μm (**c**) Egg of *S. mansoni* with characteristic lateral spine; (**d**) *S. haematobium* with terminal spine. Approximate dimensions are 140 μm (length) by 60 μm (width). (**e**) *S. mansoni* cercariae. Complete cercaria showing body and tail portions. Since both body and tail are contractile, the overall length of this stage varies considerably, usually between 300 and 500 μm. The acetabulum (ventral sucker) is a circular structure in the body portion of the organism. From this position, the pre- and post-acetabular glands are defined. **a** and **e** courtesy of Drs. W.O. Granath and Jim Driver at the University of Montana Electron Microscopy Facility (www.emtrix.org); **b** from LoVerde (1975) **c** and **d** from LoVerde (1976)

Among the many schistosome species, there are usually species-specific morphological and behavioral differences in the cercariae. For example, *S. mansoni* cercariae exhibit a discontinuous swimming pattern throughout the water column, never resting but for more than a few seconds before swimming again. In contrast, *S. japonicum* cercariae may swim to the surface film and remain attached there, quiescent for several minutes at a time, yet still be fully infectious. Development of the intramolluscan stages (miracidium to cercariae) for *S. manoni* and *S. haematobium* is 28–30 days but for *S. japonicum* it is 90 days.

The cercaria of the most studied species (*S. mansoni*) possesses about 1000 cells, with numerous cell types, ranging from sensory cells, muscle cells, nerve cells, support cells, and others (Dorsey et al. 2002). Many of the organ systems found in the adult worm are already formed, in miniature, in the body of the cercaria. The tail is heavily muscular, with an excretory duct running its entire length. This duct forks at the base of the tail furcus and empties out at terminal excretory pores.

A substantial part of the body volume is taken up by the acetabular glands and their contents. These glands (pre- and post-acetabular glands) are so named due to their relative position to the acetabulum (ventral sucker). These glands provide secretions that are involved in host skin penetration and are exhausted soon after penetration, so they do not have counterparts in the adult worm. The morphology and various structures of this stage are well represented in various publications (Dorsey et al. 2002; Stirewalt 1974; Collins et al. 2011).

The cercariae are covered by a glycocalyx which allows it to survive in a water (low osmotic) environment. Upon emergence from a snail, cercariae must find a suitable host. Fatty acids such as linoleic acid and amino acids such as arginine in the skin are important chemo-attractants for the cercariae and form a gradient to attract the cercariae. When a cercaria comes in contact with the human skin surface, it will probe for an entrance site, often finding one at the surface skin irregularities associated with hairs, follicles, or other ridges or wrinkles but is capable of penetrating unbroken skin (Stirewalt 1956, 1966). Droplets of secretion from the post-acetabular glands help serve as adhesive anchors for the body. The cercaria uses strenuous muscle activity to burrow into the crevice, as seen by a series of vigorous tail-thrusts and expansion and contraction of the body. This entry is aided by the secretions of the penetration glands, in which numerous penetration enzymes such as elastase are found (Ingram et al. 2012). The tail detaches as the cercarial body enters the outer layer of skin.

3.3.1.2 Schistosomules

Soon after entering mammalian skin, the body of the cercaria has to adapt from a freshwater environment to one bathed in tissue fluid. In order to do this, it undergoes a series of complex morphological and physiological changes. At this point, it is referred to as a schistosomule (or schistosomulum) (Stirewalt 1974; Stirewalt et al. 1983). Among the more prominent changes is the formation of a double-layered, or heptalaminate membrane, loss of the carbohydrate-rich glycocalyx, and becoming water intolerant. The cercariae are preadapted for life in the vertebrate host. Depending on the species, the schistosomule resides in the skin from one to several days before entering the blood vasculature in the dermis. A percentage can enter the lymphatics usually by day 3 post-exposure, then the bloodstream via the thoracic duct. Once in the bloodstream, it is thought that their migration to the blood capillaries of the lung is entirely passive. In the vasculature of the lungs, it undergoes development for 3–10 days and then migrates from day 10 onward from the venous to the arterial side. At this point, the organism is more elongated and morphologically appears worm-like.

Much of what we know about the schistosomule's early migration pathway is derived from studies in mice. In the early 1980s, radiographic tracking techniques were developed provided a clearer picture of the route to the liver (Georgi 1982; Georgi et al. 1987). About 40% of the cercariae result as adult worms in the mouse, with the remainder probably never reaching the liver, but recirculating in the blood and becoming trapped in other tissues. Whether a similar pattern of migration and percentage of mature worms (from invading cercariae) occurs in humans is a matter of conjecture.

3.3.1.3 Adult Male and Female Worms

In the mouse, the length of time for *S. mansoni* and *S. japonicum* to finally reach the liver differs; *S. japonicum* migrates there several days before those of *S. mansoni.* When they reach the liver, they finish growing to the adult worm stage and mate. Schistosome development and migration is asynchronous.

Once they begin to grow the sexes can be easily distinguished (Fig. 3.3). The male can develop fully in the absence of the female, but the female is stunted and does not achieve sexual maturity in the absence of the male (LoVerde et al. 2004a, b, Fig. 3.4). The male sends a signal independent of sperm transfer that regulates the expression of female-specific genes that results in reproductive development and subsequent egg production. Continuous pairing is necessary for female reproductive development. If a female is removed from a male worm (widowed), she regresses to the immature state. If she is re-mated with a male even after a number of days, reproductive development and egg laying are initiated. Thus continuous pairing is necessary for the physical and reproductive development of the female. In a mature bisexual infection, the male and female lie *en copula*, with the female lying within the gynecophoral canal of the male. Worms pair in the liver before migrating to their final destination, that of the mesenteric veins (*S. mansoni* and *S. japonicum*), or the vesical vein plexus of the urogenital system (*S. haematobium*). The size of

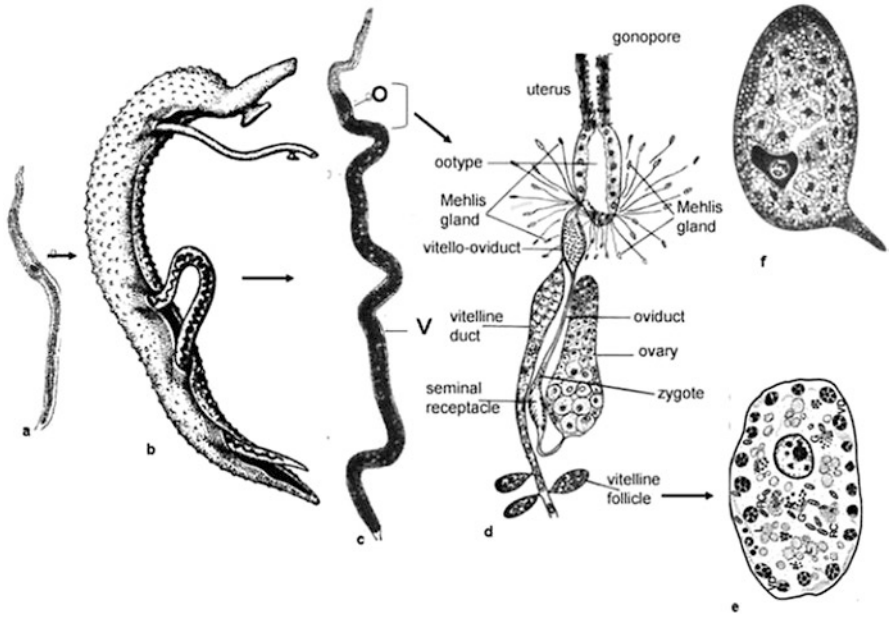

Fig. 3.4 Male–Female interplay promotes female reproductive development. (**a**) Immature *Schistosoma mansoni* female parasite, (**b**) female embedded in the gynecophoric canal of a developed male parasite, (**c**) mature female parasite, where two-thirds of the body consists of vitellaria (V) which develops post pairing, and the female ovary (O). (**d**) Egg production begins with sperm stored in the seminal vesicle, and oocyte is fertilized in the vitelloviduct. The embryo is surrounded by 48 vitelline cells. In the ootype, the eggshell is formed around the egg and one egg is produced at a time. (**e**) Mature vitelline cell containing eggshell precursors and vitelligenin, and (**f**) *S. mansoni* egg, embryo surrounded by vitelline cells. (Modified from LoVerde et al. 2009)

the adult worms varies somewhat between species. Male worms are shorter than the slender and elongated female worms, but they are very muscular and robust, with powerful suckers. This morphology allows the male worm to both carry the female and anchor the pair against the flow of blood at the egg laying site in the venules. Unpaired female worms will not reach the preferred egg-laying destination without being paired with a male worm. Egg laying begins for *S. mansoni* around 32 days; *S. japonicum* approximately 28 days; *S. haematobium* is 90 days.

Adult worms can live in humans for several years. Based on several epidemiologic studies the average life span of the adult worm is thought to be about 5–10 years. However, there have been several case reports demonstrating adult worms living 30 years or more, as shown by continued egg passage from infected individuals who no longer lived in endemic areas (Markel et al. 1978).

3.3.1.4 Eggs

Female schistosomes produce eggs continuously, and it has been reported that each *S. mansoni* mature female worm can produce up to 300 eggs/day. The egg morphology is diagnostic for each species. *S. mansoni* eggs are elongated with a prominent lateral spine near the posterior end (Figs. 3.2 and 3.3). *S. japonicum* worm pairs produce about a 1000 eggs per day. *S. japonicum* eggs are oval and smaller with a nubby spine (Fig. 3.2). *S. haematobium* worm pairs produce about 150 eggs/day. Eggs of *S. haematobium* are elongated and possess a terminal spine at the posterior end (Figs. 3.2 and 3.3).

Sizes of the egg for *S. mansoni* and *S. haematobium* are approximately the same, while *S. japonicum* eggs are smaller. Each egg contains an embryo, the miracidium, which fills nearly the entire interior space of the egg.

For the egg to pass from the body and reach freshwater, it has to gain access from the venous

blood system to the intestinal lumen (*S. mansoni* and *S. japonicum*) or the urogenital tract or bladder (*S. haematobium*). The female worm deposits eggs near the venous wall, and since the eggs are not motile, it is believed that they traverse the tissue walls by a combination of mechanisms. Egg-released enzymes play a large role in their migration through the tissue. This coupled with the action of peristalsis, the host's own inflammatory immune response (granulomas) serves to move this migration along. In the case of *S. haematobium*, it is micturition in combination with the secretion of enzymes and granuloma formation. What happens during this process is the trigger that sets the stage for development of the disease, schistosomiasis. The root of the problem is that not all of the eggs are excreted, and roughly half are trapped in various tissues in the body. For *S. mansoni* and *S. japonicum*, these sites are the intestinal wall and liver. For *S. haematobium*, it is mainly tissues of the urogenital tract. Most, if not all, of the pathology in a schistosome infection results from the deposition of eggs in the tissues and the host's response to them. Later in this chapter we will devote more attention to the egg and the processes of this pathology.

3.3.1.5 Miracidia

Once the egg is eliminated through the feces or urine and reaches freshwater, the egg shell ruptures and the actively motile, multi-ciliated miracidium emerges. What causes egg hatching is not completely defined, but it is believed to be affected by changes in osmotic pressure and other factors such as the release of a hatching enzyme (Jones et al. 2008). The miracidium is one of the two free-living, non-feeding stages of the schistosome (the other being the cercaria). After hatching from an egg, the developmental success of the miracidium (Fig. 3.3) depends on finding a suitable snail host before its glycogen energy stores are depleted, which occurs roughly 12 h after hatching.

The ability of miracidia to infect snails in large bodies of water, sometimes referred to as their "scanning power," has been studied in the lab and the field. Much of the success of the miracidium has to do with the combination of its positive phototaxis, negative geotropism, and the particular dispersion of snails, which, for *Biomphalaria* spp., are often more concentrated near the shallow edges of water bodies. However, there is evidence a chemoattractive response of the miracidium exists when it is in close proximity to the snail. The miracidium will probe the soft tissues of the snail, usually the margins of the headfoot or the tentacles, to search for a site through which it can penetrate most easily. With the aid of enzymes secreted from penetration glands and vigorous burrowing activity, it can gain complete entry into the soft tissues within minutes (for ultrastructure of the miracidium, see Pan 1980).

During entry into the snail, the miracidium loses its multi-ciliated plates (Fig. 3.3), and soon thereafter stops migrating through the tissue. Comparable to the situation in which a cercaria needs to transform into a schistosomule, the miracidium must also undergo changes to adapt to the tissue environment of the snail. In so doing, it begins to transform into the stage referred to as the primary (mother) sporocyst. From this point forward, the reproduction of the schistosome in the snail is asexual. The primary sporocyst stage is essentially a sac-like, breeding chamber within which numerous secondary (daughter) sporocysts begin forming. Around 2 weeks after miracidial penetration, secondary sporocysts escape from the primary sporocyst and migrate to the hepatopancreas and gonads of the snail. Around 2 weeks after settling in these nutrition-rich locations, the secondary sporocysts of *Biomphalaria* and *Bulinus* give rise to subsequent generations of sporocysts and together give rise to thousands of cercariae. In the case of *S. japonicum*, it takes 90 days for intramolluscan development of the parasite. Once cercariae become fully developed, they emerge from the sporocysts, migrate to the anterior end of the snail, and burst into the surrounding water from the margins of the soft tissues, usually the headfoot, mantle collar, and pseudobranch. Under optimum conditions, an infected snail can release hundreds of thousands of cercariae over its lifetime. The astounding productivity of the schistosome in the snail drives home the point that it may only take a small number of

infected snails in a local water body to maintain that area as an active transmission site.

What we know about genetics of the snail and parasite is important here, since their interrelationship has a tremendous impact on the epidemiology of the disease. Most of the research on the development of the parasite in the snail host has been done with the *S. mansoni/B. glabrata* combination. It is during the period of miracidial transformation that a crucial hurdle must be overcome by the parasite for full development to proceed. In the 1950s, Newton (1953) developed the groundwork for looking at the genetic underpinnings of the snail in controlling the parasite's development. His snail crossing studies, using resistant and susceptible *B. glabrata*, and testing the known F1 offspring (determined by pigmentation patterns) for parasite susceptibility clearly showed a genetic control for susceptibility to the parasite. The subsequent, and enormous, contributions by Richards led to the conclusion that the compatibility of host snail and parasite is controlled by a series of genes of both parasite and snail (Richards 1975; Richards and Shade 1987). The interaction ranges from total resistance, or non-susceptibility of the snail, to full compatibility, resulting in a fulminating schistosome infection. The number of genes and multiple alleles involved give testament to the idea that snail and parasite have co-evolved over millennia. Although a great deal is known about the genetic influence of the snail on parasite development, more recently the parasite's genetics in determining host–parasite interactions is being studied (Anderson et al. 2018). The populations of parasites in the field are quite diverse, owing to the multiple alleles that are involved in infectivity. An evaluation of the schistosome population genetics is an important focus in the recently developed schistosomiasis control program in Zanzibar (http://score.uga.edu/).

3.4 Epidemiology

Humans contract schistosomiasis when they come into contact with water sources contaminated with the infectious form of the parasite (cercaria). The degree of human waste contamination to the local water bodies is the driving force that dictates the prevalence of schistosomiasis in a community. The sanitary disposal of human wastes, as seen in most developed countries, is often totally absent in the regions of poverty where schistosomiasis is prevalent.

It is important that control programs take into account the epidemiology of the disease in the local region. Knowing the prevalence of disease, whether transmission is seasonal or year-round, and how effective are the post-treatment evaluations, all play large roles in the direction an effective control program should take.

A great many things contribute to any discussion in the epidemiology of schistosomiasis, and several of these, such as diagnosis, treatment, and control are being discussed more fully in other sections of this chapter.

It is difficult to make sweeping statements about the epidemiology for schistosomiasis, considering the different schistosome species involved. For instance, the fact that *S. japonicum* also uses reservoir hosts complicates the transmission pattern since both *S. mansoni* and *S. haematobium* are primarily transmitted by humans. However, recent evidence indicates that hybridizations and introgressions between parasite species, especially the *haematobium* complex exists. This has profound implications for the control of parasitic diseases, including widening host range, increased transmission potential and altered responses to drug therapy (Borlase et al. 2017; Catalano et al. 2018). That being said, some generally accepted statements about a few components of the epidemiology of schistosomiasis are as follows:

3.4.1 Prevalence

In endemic regions, serosurveys show that almost every long-term resident becomes infected with schistosomes at some point in their life. In regions with typical transmission patterns, 60–80% of school-age children and 20–40% of adults can remain actively infected. Most age-prevalence curves display a peak in schistosomiasis prevalence in school-age and young adult populations,

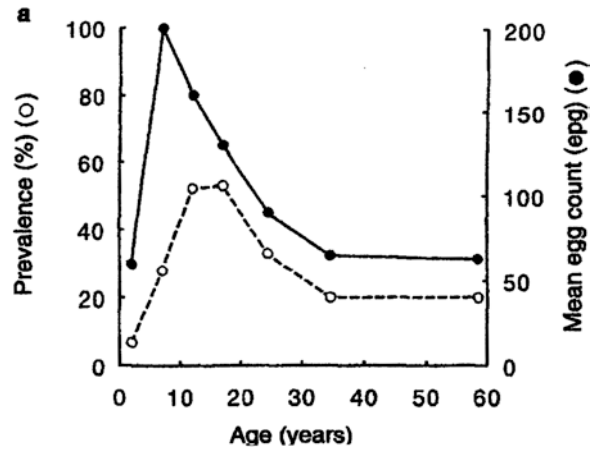

Fig. 3.5 Convex-shaped curve of schistosomiasis prevalence (open circles) and egg excretion (closed circles) by age in a typical population in an endemic area. Infection starts early in life and peaks in the 5–15 year olds, declines and plateaus with age. The latter is taken as evidence of age and exposure-related resistance. Egg excretion follows a similar pattern. (Taken from Gryseels 1994)

with a gradual decline later in life (Fig. 3.5). Not surprisingly, the major contributors to egg contamination to the environment also fall in the school-age/young adult groups. The disease takes a devastating toll on young children (school-aged), who are most at risk because of the high frequency of time spent swimming or bathing in water containing infectious cercariae. What contributes to the decline of prevalence in the older populations may be one or a combination of events: (1) the die-off of the worms after the peak is reached, given the estimate of a mean of around 5–10 years (on average) that the parasite can live in humans; (2) decline in water contact as the population ages and (3) evidence that partial immunity may develop over time (Black et al. 2010).

Schistosomiasis is characterized by focal epidemiology and a skewness, or over dispersion of egg counts within the community. Thus, the majority of eggs being excreted come from a minority of the residents. It may be too simple to attribute this to a direct reflection of worm burdens since there is increasing evidence that a variety of other things may come into play, such as host genes that might contribute to a role in the pattern of egg excretion (Bethony et al. 2002).

In high-transmission areas, some workers have reported schistosomiasis prevalence as high as 100% of the population although lower figures are more commonly reported. Incidence, commonly measured after chemotherapeutic campaigns (e.g., one-year post-treatment), can sometimes rise to near pre-treatment levels within 1–2 years after treatment (termed rebound effect), emphasizing the need for periodic retreatment of the population (Guidi et al. 2010).

3.4.2 Infection Intensity

Intensity of infection is a function of worm burden. Each cercaria gives rise to one adult worm. The adult worms do not multiply in your body. It is the egg stage that is responsible for all the pathogenesis. Therefore, the number of egg-laying worm pairs determines the intensity of infection. On a community-wide basis, there is a rough correlation between intensity of infection and prevalence. For *S. mansoni* and *S. japonicum*, intensities of infection in a community have historically been assessed as eggs/gram of feces. For *S. haematobium* infections, figures of eggs/unit of volume of urine are most often used. Stool and urine examinations are labor intensive and require a certain amount of skill. In addition, usually repeated examinations have to be done to make a diagnosis to account for the discontinuous nature

of egg excretion. Considerable efforts have been made to develop other means of assessing prevalence and intensity, usually based on serum or urine analysis (see Sect. 3.6).

Male inhabitants often tend to have higher worm burdens than females. This may have more to do with social customs than any sex-related factor, for example, male adults and children have a greater degree of occupational and/or recreational water contact than females.

The question of how intensity, egg counts, and worm burdens interrelate in humans was the subject of a series of studies conducted by Cheever and colleagues on autopsies of scores of cadavers in Brazil and Egypt (Cheever and Andrade 1967; Cheever et al. 1977). Among the many significant conclusions from these studies was that, in general, the stool egg counts in *S. mansoni* patients had an almost linear relationship with worm burden. In one study, roughly one egg/gram of feces equated to one worm pair. These autopsies were, for the most part, conducted on individuals who previously presented with significant disease, so we do not know how representative this would be for those with low egg yields. Nevertheless, these and follow-up studies helped cement our knowledge of tissue egg burdens as it correlates with disease in humans.

3.4.3 Snails at Transmission Sites

Surveys of snail populations in endemic areas are essential for defining areas of transmission. It is often found that only a small percentage of snails in an active transmission site are actively shedding cercariae. In addition to examining snails at potential transmission sites, methods have been developed to determine the presence of free-swimming cercariae by mechanical cercariometry devices, or placing sentinel animals (mice) in the water, followed by mouse dissection or hepatic portal perfusion several weeks later, looking for the presence of adult worms.

In some geographic regions, such as northeastern Brazil, transmission sites may dry up for several months of the year. A small percentage of the snails however are known to be able to aestivate that is, withstand these dry conditions by burrowing into the mud and reemerging once the sites contain water again. There have even been reports of snails with pre-patent infections being able to withstand drying, and then liberate cercariae once the transmission site contains water (Barbosa and Coelho 1955).

Transmission does not occur in the USA even though we have many infected immigrants from endemic areas, for a couple of reasons. First, we do not have the snail intermediate hosts, quality of hygiene, and treatment for all diagnosed individuals.

3.5 Clinical Aspects

Depending on the stage of the schistosome infection, a wide range of clinical symptoms may occur, and many of them are hard to distinguish from those of several other diseases. A mild or transient rash may develop within hours or up to a week after cercarial invasion of the skin that can result in itching. However, it usually goes unnoticed and happens most often in travelers who are exposed for the first time.

Sometimes, a febrile illness occurs around 3–6 weeks after cercarial exposure. This acute stage syndrome is commonly referred to as Katayama fever, named after the original location (Katayama district, Hiroshima, Japan) in which this was a prominent feature of the infection. Katayama fever is observed most often in individuals who have had no prior exposure to schistosomes. The etiology is thought to involve circulating immune complexes, and its severity may increase upon oviposition. Aside from fever, there may be headache, myalgia, anorexia, prostration, bloody diarrhea, hepatosplenomegaly, eosinophilia, and elevated IgE levels.

The clinical picture of chronic infections in humans varies somewhat with the schistosome species. A large number of those people infected may be asymptomatic. Those with *S. mansoni* or *S. japonicum* infections though can develop a chronic condition referred to as intestinal schistosomiasis. The clinical manifestations of chronic schistosomiasis are the result of host immune responses to schistosome eggs. Eggs, secreted

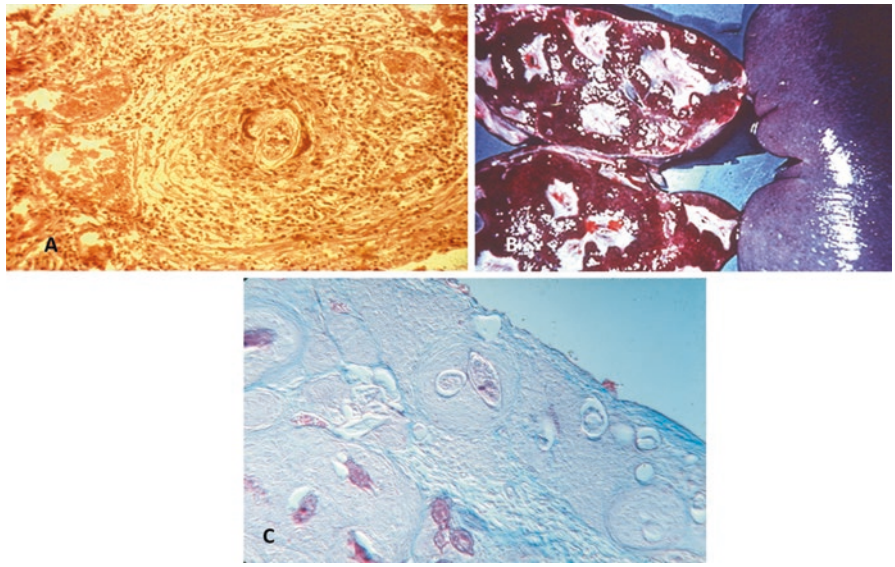

Fig. 3.6 Photomicrographs representing pathology of schistosomiasis. (**a**) Section of a liver exhibiting an acute inflammatory cell (granulomatous) reaction surrounding a live *S. mansoni* egg (center of photograph). The hallmark cell is the eosinophil. Note the granuloma occupies about 100× more space than the egg. (**b**) X-section of a liver (left side of photo) from a *S. mansoni*-infected human (chronic infection). Periportal fibrosis or "clay pipestem fibrosis" is clearly evident as whitish regions. Shown on the right is a portion of the enlarged spleen which weighs 750 g—compared to normal spleen weight of 250 g (**c**) Section through intestine of a hamster experimentally infected with *S. haematobium*. Note the granulomas. The deep red staining eggs are dead eggs that have calcified. (**a** and **b** courtesy of Dr. Alan Cheever; **c** from Dr. LoVerde)

by adult worm pairs living in the bloodstream, become lodged in the capillaries of organs and cause granulomatous inflammatory reactions (Fig. 3.6). *S. mansoni* and *S. japonicum* eggs most commonly lodge in the blood vessels of the liver and intestine and can cause diarrhea, constipation, and blood in the stool. Chronic inflammation can lead to bowel wall ulceration, hyperplasia, and polyposis. Heavy infection can lead to periportal fibrosis of the liver, an increase in portal hypertension and the formation of esophageal and gastric varices in chronic cases (Fig. 3.6). The varices can burst and patients exsanguinate in their second and third decade of life. This is in contrast to urinary or urogenital schistosomiasis caused by *S. haematobium* infections. Blood in the urine (hematuria) is a hallmark of this disease, and in chronic phases infected patients may exhibit a number of urinary tract complications, ranging from bladder calcification, genital lesions, urinary tract obstruction, kidney involvement, hydronephrosis, renal failure, and bladder cancer. The disease is usually classified as urogenital obstructive. Compounding the symptomatology, co-infection with *S. mansoni* and *S. haematobium* is often seen in regions where the two species overlap geographically.

In the case of *S. haematobium* infections, one issue that has recently drawn a great deal of interest is the growing body of evidence supporting an association between urogenital *S. haematobium* infections and an increased risk to HIV/AIDS (Mbabazi et al. 2011). There may be as much as a three- to fourfold increase risk of acquiring HIV in women with a pre-existing *S. haematobium* infection. Several causative factors may be involved in this scenario. Among them are (1) a breach in the integrity of the epithelium of the female genital tract, caused by the parasitic infection, that may facilitate HIV viral entry, and (2) increased susceptibility to the viral infection may result from the predominantly Th2-type immune response with the schistosome infection. There is

also the possibility that inflammation in the genital tract of men with *S. haematobium* infections may increase the risk of male-to-female transmission of the virus.

Apart from the more classical symptoms of schistosomiasis described above, scientists are now beginning to better recognize the role that the disease contributes to chronic anemia, malnutrition in children, growth impairment, reduced memory, learning, and fitness, along with chronic pain, chronic inflammation, and focal organ damage. Since co-infection with other helminths, such as hookworms, often occurs in schistosomiasis-endemic areas, the complication presented by schistosome-induced anemia has been overlooked.

Besides the liver, intestine, and urogenital tract, schistosome eggs have been found in other (ectopic) sites, such as the lung, appendix, and central nervous system. Central nervous system involvement, although rare, can present as transverse myelitis, cerebral lesions, epileptic seizures, and paralysis.

A significant advance in assessing morbidity in the field has been through the use of ultrasound. Protocols for using ultrasound for schistosomiasis-related pathology were developed in the 1990s (Richter et al. 2000). In particular, this can give a better measure of infection intensity in those who may have low egg burdens, but significant disease. It is also useful for assessing possible changes in pathology after drug cure. A good discussion on the use of ultrasound in assessing morbidity in schistosomiasis is given by Carlton et al. (2010), who also describes some of the difficulties of its use with reference to *S. japonicum* infections.

3.6 Diagnosis

The diagnosis of schistosomiasis requires detection of infection; some assays also measure parasite burden. Diagnostic tools include direct assays (demonstration of eggs in the stool or urine via microscopy, or demonstration of schistosome antigen or DNA in the blood, urine, and/or stool) and indirect assays (demonstration of antibody in blood via serology).

The definitive diagnosis for a schistosome infection is the microscopic detection of eggs in stool (for *S. mansoni* and *S. japonicum*) or urine (for *S. haematobium*) samples (Fig. 3.2). The gold standard, at least for *S. mansoni* and *S. japonicum*, has been the Kato-Katz thick smear assay, which uses cellophane and malachite green/glycerin to more easily detect the eggs from prepared fecal specimen. Although relatively quick and inexpensive, like most other direct assays it is complicated by the day-to-day variation in egg passage. With a sensitivity of about 30 eggs/gram feces, active infections may be missed unless multiple samples are taken on subsequent days.

Antigen tests use two proteoglycans, circulating anodic (negatively charged) antigens (CAAs) and circulating cathodic (positively charged) antigens (CCAs) that can be detected in serum and in urine. The levels of antigens correlate well with the intensity of infection. However, CCA may be less suited for diagnosis in areas with low endemicity and in travelers, who are likely to have few worms. There is a commercially available point-of-care assay (Rapid Medical Diagnostics; Pretoria, South Africa) that detects *S. mansoni* CCA in urine. This assay is now used for screening of *S. mansoni*-infected communities as it is as sensitive as a single Kato-Katz test in areas that have a high intensity of infection (Shane et al. 2011; Tchuem-Tchuenté et al. 2012). CAA is detectable early after exposure, more sensitive but more expensive than CCA. It can detect a single worm in an experimentally infected baboon (Corstjens et al. 2014).

Use of genomic tests targeting schistosome DNA sequences enables species-specific diagnosis that is highly sensitive and shows the highest specificity among the diagnostic tests for all schistosome species even in the absence of excreted eggs (Sady et al. 2015).

The source of nucleic acid can be blood, saliva, urine, or stool. DNA can also be detected in vaginal lavage samples for diagnosis of urogenital schistosomiasis.

The frequency of hematuria and proteinuria among *haematobium* infected people has resulted in the use of urine dipsticks for detection of microhematuria. It turns out to be 90% sensitive and thus an inexpensive means for estimating infection prevalence.

Detection of anti-schistosome-specific antibodies with serological assays can be very useful in travelers who present with clinical symptoms and very low or no egg excretion. Production of antibodies against adult worm antigens starts ~4–7 weeks after exposure, and in most cases seroconversion takes place within 3 months. However, serology cannot distinguish between current infection and past exposure in people living in a schistosomiasis-endemic area, where people remain seropositive for several years after treatment.

3.7 Treatment

Early anti-schistosomal drugs were limited by the difficulty in finding chemotherapeutic agents with high efficacy and tolerability. Trivalent antimonial compounds such as tartar emetic was used with severe side effects. The next generation of drugs were new antimony compounds. The side effects caused by these drugs were still intense and serious, causing the death of some patients. The antimonials were all given either intravenously or intramuscularly. The first generation of oral administered drugs such as lucanthone, niridazole, and hycanthone were toxic, mutagenic, and carcinogenic, respectively. Hycanthone was shown to be an effective drug against *S. mansoni* and *S. haematobium*. However, it had severe side effects (liver toxicity and mutagenicity) and its use discontinued. Oxamniquine was developed by Pfizer in 1969. It was shown to be efficacious against *S. mansoni* but ineffective against *S. haematobium and S. japonicum*. It had minimal side effects and could be given as a single oral dose. It was used in Brazil up to the year 2000 when concerns about cost and evidence for drug resistance caused it to be replaced by Praziquantel.

The greatest advance in the field of schistosomiasis chemotherapy came about by the development of Praziquantel (PZQ) in the laboratories of Bayer AG and Merck KGaA in Germany in the mid-1970s. The drug was moved into clinical trials and large-scale use by the mid-1980s. By 1985 the WHO reported that approximately one million people had been treated with PZQ (WHO 1985). PZQ is the drug of choice to treat schistosomiasis and now the major component of schistosomiasis control programs that advocate mass drug administration to affected populations. The WHO recommends that PZQ be used at a single dose of 40 mg/kg (WHO 2006). Since 2007, over 150 million people were treated with praziquantel (IFPMA 2017; WHO 2018). This has taken place through a praziquantel donation program to the World Health Organization in part by companies donating drugs (250 million tablets for each of the next 5 years starting in 2018 to sub-Saharan Africa where over 90% of schistosomiasis cases occur). The goal is to help governments to employ an integrative approach to control and eventually eliminate schistosomiasis. Multiple features have made PZQ the drug of choice for treating schistosomiasis, including: (1) it is effective against all the three major species, *S. mansoni*, *S. haematobium*, and *S. japonicum*, (2) it has good pharmacologic properties (it can be given as a single oral dose and is usually well-tolerated), and (3) its relatively low cost (<$0.10 U.S. per tablet). With the price of praziquantel having dropped the last few years due to the expiration of the patent, it has been made more available for use in numerous mass drug administration campaigns, with considerable success. Studies aimed at determining the effectiveness of PZQ focus on cure rates and/or egg reduction rates. Cure rates of PZQ are generally 60–90%; 100% cure rates are seldom reported (Doenhoff et al. 2009). This is likely due to reinfection in high-transmission areas and other parasite-related factors. PZQ is effective against adult schistosomes, but it is ineffective against juvenile worms. It is theorized that the lack of susceptibility of juvenile worms in high-transmission areas may be related to poor PZQ

cure rates and treatment failures that occur in some areas. As with other drugs, reinfection is still a problem after treatment, and there is a lingering fear in the community that such widespread use of the drug may eventually lead to the development of drug resistance in the parasites. Drug-resistant lines are reported to have been produced in the laboratory (Fallon and Doenhoff 1994; Couto et al. 2011) and some studies have reported reduction in PZQ susceptibility as potential resistance in the field (Ismail et al. 1999; Cioli et al. 2004; Melman et al. 2009). Efforts are underway to determine the mode of drug action and drug resistance as this information would have a huge impact on future discovery of anti-schistosome drugs and control programs.

In terms of deciphering the mode of action of PZQ, early studies demonstrated that PZQ causes damage to the worm tegument, greatly augmented by antibodies directed to tegumental antigens. It also causes muscle paralysis in worms that is associated with an influx of calcium ions. Because of the alteration in ion transport after PZQ treatment, much effort has focused on ion transporters and channels as potential targets of PZQ. Currently, the best candidates for molecular targets of PZQ are calcium ion channels (specifically Beta subunits, Salvador-Recatalà and Greenberg 2012), but there is no consensus agreement. As praziquantel is the only drug used in mass chemotherapy programs, efforts to develop new drugs are underway (e.g., Bais and Greenberg 2018; Cioli et al. 2014). Just as the molecular targets for PZQ remain unclear, potential resistance mechanisms have been elusive. Attempts to discover differences in protein sequences of calcium channels in resistant vs. susceptible isolates were inconclusive. Currently, efforts are underway to discover markers of PZQ resistance in in vitro-selected PZQ-resistant lines and new generation molecular technologies are being applied to dissect transcriptome and genotypic changes in response to PZQ treatment. Significant effort is being devoted to the molecular determination of putative resistance markers in light of the fact that PZQ is being relied on so heavily in control efforts.

Recently, the mode of oxamniquine (OXA) activity and the mechanism for OXA resistance was identified using genetic, molecular, and biochemical approaches (Valentim et al. 2013). OXA is a prodrug that is enzymatically activated in the parasite. OXA binds to a specific schistosome sulfotransferase, known as *Sm*SULT, where it is transiently sulfated. The sulfur group on the released sulfate ester undergoes a nucleophilic attack by schistosome macromolecules. In an S_N2-like reaction, activated OXA binds to DNA and other macromolecules, resulting in killing of the worms. With this information research is underway to identify derivatives of OXA that will kill *S. haematobium* and *S. japonicum* as well as *S. mansoni* (Rugel et al. 2018).

For several years, investigators have also shown that artemisinin drugs also exhibit effectiveness against *Schistosoma* spp. These drugs have potent antimalarial activity and are the part of frontline therapies (artemisinin combination therapies) to treat *Plasmodium falciparum* on a global basis. Artemisinins began to be investigated as schistosomiasis drugs in the 1980s and have been used in patients since the 1990s (Caffrey 2007). Artemisinins are effective against all three major schistosomes infecting humans and they have a marked effect on immature worms, with less activity on adult worms (in contrast to OXA and PZQ). This property makes them good drug candidates for use as prophylaxis in areas of high schistosomiasis transmission (Doenhoff et al. 2008). The co-prevalence of malaria and schistosomiasis in certain areas brings up the possibility of increasing the risk of artemisinin-resistant malaria parasites if schistosomes are being targeted. The emergence of artemisinin-resistant malaria in Southeast Asia has brought much concern to the judicious use of these drugs and may reduce the chance that they can be used for schistosomiasis treatment.

Other drugs that have shown promise against schistosomes include synthetic artemisinin analogs, other antimalarials (mefloquine), metrifonate, and oxadiazoles. Clearly, a larger portfolio of drugs is necessary to preserve the few effective drugs we have and also to combat inevitable

resistant parasites. Studies are underway to identify drugs that could be used in combination with praziquantel (Rugel et al. 2018).

3.8 Control

The World Health Organization has set goals of morbidity control and elimination of schistosomiasis as a public health problem (WHO 2013). Early operational research studies such as the *S. mansoni* control project on the Caribbean Island of St. Lucia in the 1970s–1980s (Jordan 1985) examined the feasibility and cost of three control measures; snail control, chemotherapy, and provision of freshwater on the effect on transmission. The outcome of that 15-year study was that the most cost-effective measure for reducing transmission was the use of chemotherapy.

At the time other field studies also supported the notion that the most efficient mode of attacking the problem of control was mass drug administration (MDA). Mass drug administration aims to lessen morbidity and mortality due to the infection and prevent new infection by limiting transmission through reduction of the overall prevalence in the population. The assumption is that mass drug administration would also lead to reduction in excretion of schistosome eggs, contamination of the environment, and infection of the snail population, which in turn would lead to less source of infection for humans (WHO 2013). Before implementing a large-scale mass drug administration program, epidemiological assessment of the community needed to be done to determine the prevalence and presence of ongoing transmission of infection. The prevalence is the basis for the WHO recommended strategy for preventive chemotherapy. Control programs using MDA target selected subgroups, such as school-aged children in places where the prevalence of schistosomiasis is 50% or higher. As school-age children are most likely to be infected by *Schistosoma* parasites, in part due to age-related water contact behavior, treatment has been specifically focused at this age group to prevent morbidity (Fig. 3.5). Given the high prevalence and intensity of infection typically in school-age children, treating this age group is thought to be more efficient and have a greater impact on reducing transmission to the rest of the community. School-based treatment has been widely used as school-aged children are relatively easy to locate, sample, and therefore reach good coverage levels. This makes school-aged children important in terms of monitoring the impact of MDA treatment. By 2020, WHO aims to increase coverage such that 75% of school-aged children at risk will be regularly treated in endemic countries.

Adults (e.g., fishermen, sand harvesters, shepherds, car washers, irrigation workers, and women performing domestic chores) who have occupations that make them important contributors to transmission in a particular setting in areas of high transmission need treatment to control morbidity and transmission. However, for those who do not comply or are not covered by mass drug administration, such as school-aged children who are not enrolled in school, they may still spread the infection. Likewise, the unavoidable contacts of humans to persistent hot spots of transmission lead to the failure of mass drug administration to stop transmission in high-risk communities. Thus MDA strategies were less effective at reducing prevalence and intensity in the hot spot communities compared to other villages. Persistent hot spots are recognized as having a disproportionate influence on driving transmission in infectious diseases meaning their discovery is very useful for disease control, allowing for a change in the intervention strategy in order to reach control targets (Wiegand et al. 2017).

However, reinfection (especially in children) is a severe problem for effective mass drug administration programs. Therefore, along with mass treatment campaigns, there is a need for parallel operational research to better define the best approach for control, elimination, and verification. In this regard, the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) was supported by the Bill and Melinda Gates Foundation with the goal to inform operational research by undertaking randomized trials of different approaches to mass

drug administration (MDA) in endemic countries in Africa (Shen et al. 2017). They addressed strategic questions to inform efforts to gain control of schistosomiasis in high-prevalence areas, to sustain control and move towards elimination in areas of moderate prevalence, and ultimately to eliminate *Schistosoma* transmission in at-risk communities. They identified markers of *Schistosoma*-associated morbidity that could be used to assess treatment impact. However, it was clear that MDA by itself was not sufficient to eliminate schistosomiasis.

Morbidity control has been achieved in many areas of the world; however, the real problem is to control transmission which eventually would lead to elimination. Switching from a goal of controlling morbidity to interrupting transmission may well be currently feasible in some countries in the Caribbean, some areas in South America, northern Africa, and selected endemic areas in sub-Saharan Africa where there have been improvements in sanitation and access to clean water. However, in most of sub-Saharan Africa, where programmatic interventions still consist solely of annual mass drug administration, such a switch in strategies will rely on operational research to provide the tools on how best to reduce transmission to a point where interruption of transmission may be achievable. An integrated control program that involves a combination of drug treatment, water management, snail control (through habitat modification, irrigation changes, use of molluscicides), treatment of sewage, health education, and behavioral change is desirable (Rollinson et al. 2012). As regards behavioral change, transmission would cease if infected individuals no longer put excreta in the bodies of freshwater where the intermediate host snails live. An example of the development and implementation of a community-designed behavioral intervention occurred in Zanzibar. The interventions that the community developed under the guidance of a social scientist and a trained team resulted in the greatly increased involvement of children in activity-based health education about schistosomiasis and the use of participant-designed and -installed concrete washing platforms and clean water to wash clothes. Communities were encouraged to identify their own problems and solutions. This was attractive with respect to both self-determination and sustainability. For any large control programs, a political will must exist among the government and access to the financial resources be available. The availability of water, sanitation, and hygiene (WASH) in addition to MDA is likely the key intervention for breaking schistosomiasis transmission.

It is critical that monitoring and evaluation programs be used to collect data which are required to assess the impact of current interventions on their progress towards achieving the World Health Organization (WHO) goals of morbidity control and elimination as a public health problem for schistosomiasis.

It is likely that elimination will only be achieved in countries (or areas within countries) with some basal level of a clean water supply and sanitation. In the case of *S. japonicum*, which infects other indigenous animals (e.g., water buffaloes, pigs, and dogs), this potential zoonotic transmission must be taken into account when designing control programs. Likewise, the continued identification of hybrid species will complicate control programs.

3.9 Immune Responses and Pathology

The immunology of schistosomiasis involves two seemingly opposed areas: immunopathology and resistance to reinfection (Wynn et al. 2004; Pearce and MacDonald 2002; Fig. 3.7).

In experimental settings, immune responses to the invading cercariae can be detected as early as 1–2 weeks. In the subsequent 3–5 weeks, during which the host is exposed to migrating immature parasites, the dominant response is T-helper 1 (T_H1)-like. As the parasites mature, mate and begin to produce eggs at weeks 5–6, the response changes such that the T_H1 component decreases and this is associated with the emergence of a strong T_H2 response. This response is induced primarily by egg antigens. This results in a predominant $CD4^+$ Th2 cell response that allows the host, at least in mice, to survive infection. In the absence

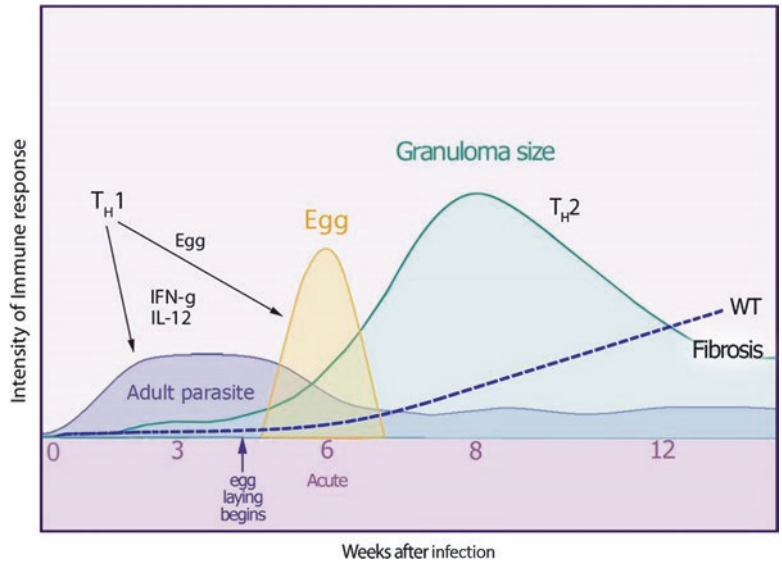

Fig. 3.7 Evolution of the immune response and granuloma formation in a schistosome infection. In experimental settings, immune responses to the invading cercariae can be detected as early as 1–2 weeks. In the subsequent 3–5 weeks, during which the host is exposed to migrating immature parasites, the dominant response is T-helper 1 (T_H1)-like. As the parasites mature, mate, and begin to produce eggs at weeks 5–6, the response alters markedly; the T_H1 (pro-inflammatory) component with the release of INF-γ and IL-12 increases during early egg production (yellow curve) to contribute to granuloma size and then decreases precipitously with the expression of IL-10 and this is associated with the emergence of a strong T_H2 response and an increase in granuloma size (green graph) that leads to fibrosis. The T_H2 response is induced primarily by egg antigens. During the acute phases, the granulomas that form are florid (8 weeks), then with time in the chronic phase of infection, the T_H2 response correlates with the size of the lesions developing around eggs. The T_H2 response is modulated and granulomas that form around newly deposited eggs are smaller than at earlier times during infection (8–12 weeks). WT (dashed blue line) is the increase in fibrosis that occurs during infection. (Drawing by Dr. Tom Wynn)

of a T_H2 response, mice develop hepatotoxicity, endotoxemia, and severe cachexia, which together contribute to the death of the animal.

During this period, patients can present with acute schistosomiasis (Caldas et al. 2008). Acute schistosomiasis occurs most often in travelers or immigrants to schistosome-endemic regions who are exposed to schistosome antigens for the first time. It occurs weeks to months after infection, as a consequence of worm maturation, egg production, release of egg antigen, and the host's florid granulomatous and immune complex responses. This comes about because there are circulating antibodies from the immune response to the juvenile stages and now with egg production all of a sudden, there is antigen excess that results in the symptoms. Acute schistosomiasis is sometimes referred to as Katayama syndrome and the typical clinical presentation is a sudden onset of fever, malaise, myalgia, headache, eosinophilia, fatigue, and abdominal pain lasting 2–10 weeks.

Approximately half of the eggs deposited by the female schistosome get swept up into the circulation and filter out into the periportal tracts in the case of *S. mansoni* and *S. japonicum* where they induce a granulomatous host immune response largely characterized by lymphocytes (which mainly produce T-helper-2 cytokines, e.g., interleukins 4, 5, and 13), eosinophils, and alternatively activated macrophages. These granulomas contain egg proteolytic enzymes to prevent tissue necrosis, but the process of granuloma formation induces chronic inflammation that leads to the disease manifestations of schistosomiasis (Fig. 3.6).

During the chronic phase of infection (infections are long lived, worms can live 30 years, and continue to produce eggs, 300 per day for

Schistosoma mansoni worm pairs, 1000 for each *S. japonicum* worm pair and 150 for each *S. haematobium* worm pair), with time 8–12 weeks in mice the T_H2 response is modulated and granulomas that form around newly deposited eggs are smaller than at earlier florid times during infection. It is thought generally that, during schistosomiasis, immunopathology and immunoregulation are under the control of egg-antigen-specific T_H cells. Two Th2 cytokines, interleukin 13 and interleukin 4 are involved in the granulomatous response and subsequent fibrosis (Figs. 3.6, 3.7). Recent studies in murine schistosomiasis have revealed a critical pathogenic role for interleukin 17 (IL-17)-producing $CD4^+$ cells (T-helper 17 [Th17] cells). These data have also been confirmed in human haematobium infections (Mbow et al. 2013). Regarding regulatory T cells (Tregs), their responses, especially the secretion of IL-10, seem to control pathology. The immunomodulatory effects of helminths are well known. Both parasite-antigen-specific and more generalized levels of immune suppression are well documented in human studies. For example, patients with schistosomiasis have diminished responsiveness to antigens from the infecting parasite. More broadly, helminth infections are associated with down modulated responsiveness in general, in some cases with measurable attenuation of responses to bystander antigens, routine vaccinations or allogeneic tissue transplants. Such "spillover suppression" is thought to be related to the intensity of infection.

Chronic disease is graded according to severity. Infection intensity is one factor that can affect the severity of chronic schistosomiasis, particularly in children. One form of schistosomiasis is the intestinal form of disease. This form of the disease presents as nonspecific intermittent abdominal pain, diarrhea, and rectal bleeding, with the frequency of symptoms often related to the intensity of infection. Such gastrointestinal features are often focal with polyposis. The most serious form is a life-threatening hepatosplenic disease, which is usually accompanied by severe hepatic and periportal fibrosis (also called Symmer's pipestem fibrosis), portal hypertension and portosystemic shunting of venous blood (Fig. 3.6). Patients with periportal fibrosis retain hepatocellular function, differentiating the disease from cirrhosis and other liver diseases. Ascites and hematemesis from esophageal varices as a complication of portal hypertension can rapidly lead to death. The time from initial infection to advanced fibrosis is usually 5–15 years.

By contrast, the defining symptom for urogenital schistosomiasis *(S. haematobium)* is hematuria, often presenting with urinary frequency, burning micturition, and suprapubic discomfort. In endemic regions, hematuria is so widespread that it is thought a natural sign of puberty for boys and is confused with menses in girls. As with severe intestinal schistosomiasis, severe urogenital schistosomiasis results from poor immunoregulation of anti-schistosome-egg responses, leading to chronic fibrosis of the urinary tract presenting as obstructive uropathy (hydroureter and hydronephrosis), which—along with resulting bacterial superinfection and renal dysfunction—can have lethal consequences. Squamous-cell carcinoma of the bladder is also correlated with *S haematobium* infection.

What are the immunological mechanisms that underlie protective immunity and enable the parasite to evade the immune response?

The schistosomule is the target for immune elimination. The immune response is thought to be antibody mediated in which primarily IgE antibodies are involved but so are IgM, IgG, and perhaps IgA. Antibody binds to surface antigens of the larval schistosome and in the presence of effector cells that bind to the antibody Fc receptor effect killing through the release of oxidants such as superoxide anion and glutathione peroxidase. The primary effector cell is thought to be an eosinophil but macrophages, neutrophils, platelets, and complement have been demonstrated in studies in a mouse model to also be involved. Killing takes place sometime in the skin but more often in the vasculature in the lungs. A second mechanism of killing demonstrated in mice has been the sensitization of Th1 cells that release cytokines like INF-γ that activate macrophages to attack the parasite and killing is thought to involve nitric oxide as well as oxidants. Again killing is thought to occur in the schistosomula

stage but often while the parasite is in the lungs. However once the parasite reaches the portal circulation of the liver, it is able to evade the immune response. In fact, the adult parasite can survive in the hostile environment of the vasculature for many years in the face of an ongoing anti-parasite immune response by the infected host. The major immune evasion mechanisms are: reduced surface antigenicity, the development of a tegument intrinsically resistant to immune damage, masking by host-derived molecules (ABH and MHC antigens), loss of exposure of parasite antigens (tegument turnover), fabulation (extracellular proteases that inactivate antibody molecules), locally active immunomodulatory mediators (Tregs), immune suppression, blocking antibody (IgG4), and antioxidant enzymes (SOD, glutathione peroxidase, thioredoxin glutathione reductase) (Mulvenna et al. 2010; Huang et al. 2012; LoVerde et al. 2004a, b).

3.10 Vaccines

A substantial amount of effort has been spent on trying to develop a vaccine for schistosomiasis (Merrifield et al. 2016; Tebeje et al. 2016). It has always been thought that an effective vaccine would reduce the need for repeated drug administration since an effect of the vaccine might be reduction or prevention of reinfection. A vaccine then would give another tool in the arsenal of control. Current vaccine development strategies aim to prevent schistosome infection and/or reduce ova burden through the interruption of parasite egg production. Thus, among the major vaccine targets are the migrating schistosomulum stages as well as adult female reproduction. Efforts have also been made to target the adult schistosomes with a therapeutic vaccine (LoVerde et al. 2004a, b).

There are several types of immune models. The most significant one is the radiation-attenuated cercarial vaccine, which is able to induce consistently high, although not sterilizing, immunity against challenge infection from mice to non-human primates (Coulson 1997). A single exposure to irradiated cercariae induces a T_H1 response, whereas additional boosting leads to a mixed T_H1/T_H2 response. The irradiated cercarial vaccine induces memory and confers 70–90% protection against challenge infection. However, the 10–30% of adult worms that reach the portal system are able to survive the irradiated vaccine response.

When experimental models were first developed for schistosomiasis, in particular in the mouse model, it became apparent that there existed some "immunity" to a cercarial challenge in mice and later in humans that had an existing adult infection. This so-called concomitant immunity, a term borrowed from the tumor immunologists stated that as a result of an active infection, the host had partial immunity to challenge infection. This immunity was directed against the skin and lung-stage worms, leaving the adult worm population unaffected by any immune responses that may have been stimulated. This type of partial immunity to challenge infection makes evolutionary sense as a parasite needs a host to reproduce but if the host is exposed daily and continually acquires parasites, the worm burden will be fatal. Thus, co-evolved mechanisms to control the number of parasites in the host are good for both the parasite and the host.

Epidemiologic studies of human schistosomiasis indicate that age-dependent immunity after multiple exposures is acquired. Evidence is seen in a population by a convex curve that shows a drastic reduction in schistosomiasis prevalence with age (Fig. 3.5). Scientists have argued that repeated treatments with praziquantel may mimic an accelerated form of immunity (termed Drug-Induced Resistance). The concept is that instead of the slow turnover of parasites due to natural untreated infection, regular drug treatment would cause the turnover of parasites more rapidly and provide constant immune stimulation resulting in protection against subsequent challenge infection.

The other models of immunity involve vaccine candidates either as antigens, peptides, or DNA-based. Vaccinated mice exhibit both cellular and humoral immune responses to skin and lung-stage parasites.

Of the several current candidates, two have reached the level of phase I clinical trials; (1) Sm14, a fatty-acid binding protein, and (2) *S. mansoni* tetraspanin (Sm-TSP-2). One has completed phase 2 clinical trials Sh28GST, a glutathione-S-transferase derived from *S. haematobium*. One is undergoing testing in non-human primates, Smp80 (calpain) and shows promise. These vaccines were selected on the basis of their protective immunity in preclinical challenge models, through human immune-epidemiological studies or both. In addition, new schistosome candidate vaccines are being identified through bioinformatics, OMICs approaches, high-throughput screening, and genomic data mining.

3.11 Genomics and Proteomics

Within the last few years, scientists have gathered a wealth of information about the genomes of *S. mansoni, S. japonicum*, and *S. haematobium.* The sequences for both *S. mansoni* and *S. japonicum* were published in 2009 (Berriman et al. 2009; Schistosoma japonicum Genome Sequencing and Functional Analysis Consortium 2009), followed by that for *S. haematobium* in 2012 (Young et al. 2012). The genome of *S. mansoni* was resequenced three more times and it became the reference genome for the Platyhelminths. Schistosomes have 8 chromosome pairs (7 autosomal pairs and 1 pair of sex chromosomes) (Short and Grossman 1981). The females are the heterogametic sex (ZW), and the males homogametic (ZZ). Thus, the female worm determines the sex of the schistosome. Early characterization of schistosome DNA showed that it contains both moderate and highly repeated components (Simpson et al. 1982). From the recent sequence data, repetitive elements comprise about 40% of each of their genomes. About one-fourth of the genome is composed of retrotransposon mobile genetic elements.

The published sequence data show obvious similarities in the overall genomic profiles for all three species. For example, draft genome estimates are 397 Mb for *S. japonicum*, 363 Mb for *S. mansoni*, and 385 Mb for *S. haematobium*. All three species possess a GC content of about 34%. The number of coding genes for all three is also roughly comparable; 13, 469 for *S. japonicum*, 10, 852 for *S. mansoni*, and 13, 073 for *S. haematobium.* Some differences are noted, however, indicating what previous studies have inferred—*S. mansoni* and *S. haematobium* are more closely related to each other than they are to *S. japonicum.* This is perhaps not surprising, considering the geographic distribution of each species and by the vastly different families of snail hosts used by them (planorbids for *S. mansoni* and *S. haematobium*, and prosobranchs for *S. japonicum*).

By mining the genome datasets scientists are hoping to develop new approaches for drug discovery, identifying vaccine candidates, improving diagnostics, developing biomarkers, and discovering other tools in the arsenal for controlling schistosomiasis. This new information may allow us also to gain a better understanding of the mechanisms whereby these organisms are able to live for extended periods of time in tissues of both vertebrate and invertebrate hosts, as well as spending a shorter period of time, as different stages entirely (cercariae and miracidia), as free-living organisms.

A broad range of genomic and proteomic studies are now emerging, taking advantage of the information gained from the sequence information that is now in the public domain. Among them are studies investigating potential drug targets, vaccine candidates, genes important in reproductive biology (Cogswell et al. 2012) and basic knowledge that render schistosomes as successful parasites, in both mammalian and molluscan hosts (Silva et al. 2012; Nahum et al. 2012). These should complement also new approaches for drug screening, such as that being developed in vitro that may enable a more logical and cost-effective approach to the design of anti-schistosomal drugs (Abdulla et al. 2009). For example, a 5 cM genetic map for *S. mansoni* was developed to allow genetic studies to identify genes that control phenotypes (Criscione et al. 2009). Using the genetic map, RNAi and biochemical assays, the gene responsible for oxamniquine (OXA) drug resistance was identified.

This allowed the mode of action to be worked out for OXA (a first for a drug that treats human helminth infections) and the crystal structure of the enzyme to be determined (Valentim et al. 2013; Taylor et al. 2017).

With all this new information, researchers now have a tool box to study biological interactions of the parasite and its hosts and environment, understand genes that control phenotypes by using genetic crosses (Anderson et al. 2018), advanced gene sequencing (exome sequencing, RNA seq, non-coding RNAs), and gene manipulation (RNAi, CRISPR-Cas 9, transfection). The future for schistosomiasis research in uncovering mechanisms of interaction, improved diagnostics, vaccine development, and biomarkers is very promising.

3.12 Concluding Remarks

Schistosomiasis has plagued humanity for thousands of years. However, the last two decades have shown that progress is being made to reduce it in large-scale efforts. While funding for schistosomiasis research is still negligible in comparison to the worldwide problem, it is encouraging that funding is becoming available from a larger number of sources than in the past, and various research consortia have been established with the common goal of reducing the impact schistosomiasis has on those in endemic areas. A large part of reducing morbidity has been conducted through several mass drug administration campaigns. Effort is also being made to fine tune the control approach so that they deliver the "biggest bang for the buck," while incorporating new scientific knowledge to the problems at hand. Praziquantel remains the most effective weapon for schistosomiasis control and its use will only increase in the foreseeable future. However, the over-reliance on PZQ in mass drug administration programs brings the potential for drug resistance to the forefront. Losing the effectiveness of this primary drug would be devastating to schistosomiasis control, along the lines of losing artemisinin effectiveness for treating malaria (which is emerging in Southeast Asia). Because schistosomiasis vaccines may not be formidable weapons to combat the disease in the foreseeable future, there is an urgent need for new effective drugs. Until we see new methods of control or other drugs emerge for treatment, schistosomiasis prevention and control programs must incorporate surveillance and monitoring of mass drug administration programs to detect any sign of emerging resistance. However, control programs will need to remain adaptable to deal with hot spots, hybrids between and among species and zoonotic infections.

In the meantime, it is encouraging to see more research being conducted on the basic biology of the parasite, and comparative studies now being conducted with available genomic information to address issues that have long confronted us. There are many, but among the more compelling are: (1) how does the parasite adapt so quickly to the environment between a molluscan and mammalian host?; (2) what triggers or stimulates the adults to migrate to their organ-specific egg-laying sites in venules?; (3) what is the stimulus that the male worm sends to the female to regulate her gene expression and thus reproductive development? and (4) how can adults live so long in the bloodstream, an obviously hostile place for so many other pathogens?

As we hope to have demonstrated, the research area of schistosomiasis is vast and varied. We hope this chapter gives the reader a starting point to explore the various avenues of this research in more depth.

The author would like to draw the reader's attention to several online resources to gain a better understanding of schistosomiasis and the direction research and control measures are taking. The CDC DPDx website is an excellent resource for general information on schistosomiasis (http://dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm). A good source of information on drug treatment programs is given in the website for the Schistosomiasis Control Initiative (SCI, http://www3.imperial.ac.uk/schisto), as well as the one for Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) at http://score.uga.edu. SchistoDB (http://schistoDB.net/) is a genomic database for *Schistosoma mansoni*,

containing sequences and annotation for *S. mansoni* in a user-friendly database. For lab applications, there are several standard operating procedures available on the NIAID Schistosomiasis Resource Center website at http://www.schisto-resource.org.

Acknowledgments The author acknowledges support from NIH-NIAID (R01 AI115691-01, 1R01AI123434-01) and the Bill and Melinda Gates Foundation (OPP1172792). Electron micrographs (adult worms and cercariae) were provided by Drs. W.O. Granath and Jim Driver at the University of Montana Electron Microscopy Facility (www.emtrix.org) which is supported, in part, by grant P20GM103474 from the National Institute of General Medical Sciences of the National Institutes of Health. We acknowledge Dr. Allen Cheever for our use of some of his pathology images throughout the manuscript and Dr. Tom Wynn for providing the drawing of Fig. 3.7.

References

Abdulla M-H, Ruelas DS, Wolff B, Snedecor J, Lim K-C, Xu F, Renslo AR, Williams J, McKerrow JH, Caffrey CR (2009) Drug discovery for schistosomiasis: hit and lead compounds identified in a library of known drugs by medium-throughput phenotypic screening. PLoS Negl Trop Dis 3:e478. https://doi.org/10.1371/journal.pntd.0000478

Anderson TJC, LoVerde PT, Le Clec'h W, Chevalier F (2018) Genetic crosses and linkage mapping in schistosome parasites. Trends Parasitol 34:982–996. https://doi.org/10.1016/j.pt.2018.08.001

Bais S, Greenberg RM (2018) TRP channels as potential targets for antischistosomals. Int J Parasitol Drugs Drug Resist 8:511–517. https://doi.org/10.1016/j.ijpddr.2018.08.003

Barbosa FS, Coelho MV (1955) Comportamento das formas larvias de Schistosoma mansoni em Australorbis glabratus (Mollusca Planorbidae), sujeitos a estivacao. Publicacoes Avulsas do Instituto Aggeu Magalhaes 4:51–60

Berriman M, Haas BJ, LoVerde PT, Wilson RA, Dillon GP, Cerqueira GC, Mashiyama ST, Al-Lazikani B, Andrade LF, Ashton PD, Aslett MA, Bartholomeu DC, Blandin G, Caffrey CR, Coghlan A, Coulson R, Day TA, Delcher A, DeMarco R, Djikeng A, Eyre T, Gamble JA, Ghedin E, Gu Y, Hertz-Fowler C, Hirai H, Hirai Y, Houston R, Ivens A, Johnston DA, Lacerda D, Macedo CD, McVeigh P, Ning Z, Oliveira G, Overington JP, Parkhill J, Pertea M, Pierce RJ, Protasio AV, Quail MA, Rajandream MA, Rogers J, Sajid M, Salzberg SL, Stanke M, Tivey AR, White O, Williams DL, Wortman J, Wu W, Zamanian M, Zerlotini A, Fraser-Liggett CM, Barrell BG, El-Sayed NM (2009) The genome of the blood fluke *Schistosoma mansoni*. Nature 16:352–358

Bethony J, Williams JT, Blangero J, Kloos H, Gazzinelli A, Soares-Filho B, Coelho L, Alves-Fraga L, Williams-Blangero S, LoVerde PT, Correa-Oliveira R (2002) Additive host genetic factors influence fecal egg excretion rates during *Schistosoma mansoni* infection in a rural area in Brazil. Am J Trop Med Hyg 67:336–343

Black CL, Mwinzi PNM, Muok EMO, Abudho B, Fitzsimmons CM, Dunne DW, Karanja DMS, Secor WE, Colley DG (2010) Influence of exposure history on the immunology and development of resistance to human schistosomiasis mansoni. PLoS Negl Trop Dis 4:e637

Borlase A, Webster JP, Rudge JW (2017) Opportunities and challenges for modelling epidemiological and evolutionary dynamics in a multihost, multiparasite system: zoonotic hybrid schistosomiasis in West Africa. Evol Appl 11:501–515

Caffrey CR (2007) Chemotherapy of schistosomiasis: present and future. Curr Opin Chem Biol 11:433–439

Caldas IR, Campi-Azevedo AC, Oliveira LF, Silveira AM, Oliveira RC, Gazzinelli G (2008) Human schistosomiasis mansoni: immune responses during acute and chronic phases of the infection. Acta Trop 108:109–117

Carlton EJ, Hsiang M, Zhang Y, Johnson S, Hubbard A, Spear RC (2010) The impact of Schistosoma japonicum infection and treatment on ultrasound-detectable morbidity: a five-year cohort study in Southwest China. PLoS Negl Trop Dis 4:e685

Catalano S, Sène M, Diouf ND, Fall CB, Borlase A, Léger E, Bâ K, Webster JP (2018) Rodents as natural hosts of zoonotic schistosoma species and hybrids: an epidemiological and evolutionary perspective from West Africa. J Infect Dis 218:429–433

Cheever AW, Andrade ZA (1967) Pathological lesions associated with *Schistosoma mansoni* infection in man. Trans R Soc Trop Med Hyg 61:626–639

Cheever AW, Kamel IA, Elwi AM, Mosimann JE, Danner R (1977) Schistosoma mansoni and S. haematobium infections in Egypt. II. Quantitative parasitological findings at necropsy. Am J Trop Med Hyg 26:702–716

Cioli D, Botros SS, Wheatcroft-Francklow K, Mbaye A, Southgate V, Tchuem Tchuenté LA, Pica-Mattoccia L, Troiani AR, El-Din SH, Sabra AN, Albin J, Engels D, Doenhoff MJ (2004) Determination of ED50 values for praziquantel in praziquantel-resistant and -susceptible *Schistosoma mansoni* isolates. Int J Parasitol 34:979–987

Cioli D, Pica-Mattoccia L, Basso A, Guidi A (2014) Schistosomiasis control: praziquantel forever? Mol Biochem Parasitol 195:23–29

Cogswell AA, Kommer VP, Williams DL (2012) Transcriptional analysis of a unique set of genes involved in Schistosoma mansoni female reproductive biology. PLoS Negl Trop Dis 6:e1907

Collins JJ III, King RS, Cogswell A, Williams DL, Newmark PA (2011) An atlas for Schistosoma mansoni organs and life-cycle stages using cell type-specific markers and confocal microscopy. PLoS Negl Trop Dis 5:e1009. https://doi.org/10.1371/journal.pntd.0001009

Corstjens PL, De Dood CJ, Kornelis D, Fat EM, Wilson RA, Kariuki TM, Nyakundi RK, Loverde PT, Abrams WR, Tanke HJ, Van Lieshout L, Deelder AM, Van Dam GJ (2014) Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. Parasitology 141:1841–1855

Coulson PS (1997) The radiation-attenuated vaccine against schistosomes in animal models: paradigm for a human vaccine? Adv Parasitol 39:271–336

Couto FF, Coelho PM, Araújo N, Kusel JR, Katz N, Jannotti-Passos LK, Mattos AC (2011) Schistosoma mansoni: a method for inducing resistance to praziquantel using infected *Biomphalaria glabrata* snails. Mem Inst Oswaldo Cruz 106:153–157

Criscione CD, Valentim CLL, Hirai H, LoVerde PT, Anderson TJC (2009) Genomic linkage map of the human blood fluke Schistosoma mansoni. Genome Biol 10:R71

Doenhoff MJ, Cioli D, Utzinger J (2008) Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis 21:659–667

Doenhoff MJ, Hagan P, Cioli D, Southgate V, Pica-Mattoccia L, Botros S, Coles G, Tchuem Tchuenté LA, Mbaye A, Engels D (2009) Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. Parasitology 136:1825–1835

Dorsey CH, Cousin CE, Lewis FA, Stirewalt MA (2002) Ultrastructure of the Schistosoma mansoni cercaria. Micron 33:279–323

Fallon PG, Doenhoff MJ (1994) Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in Schistosoma mansoni in mice is drug specific. Am J Trop Med Hyg 51:83–88

Georgi JR (1982) Schistosoma mansoni: quantification of skin penetration and early migration by differential external radioassay and autoradiography. Parasitology 84:263–281

Georgi JR, Wade SE, Dean DA (1987) Schistosoma mansoni: mechanism of attrition and routes of migration from lungs to hepatic portal system in the laboratory mouse. J Parasitol 73:706–711

Gryseels B (1994) Human resistance to Schistosoma infections: age or experience? Parasitol Today 10:380–384

Guidi A, Andolina C, Makame Ame S, Albonico M, Cioli D, Juma-Haji H (2010) Praziquantel efficacy and long-term appraisal of schistosomiasis control in Pemba Island. Trop Med Int Health 15:614–618

Holtfreter MC, Moné H, Müller-Stöver I, Mouhadi G, Ritcher J (2014) Schistosoma haematobium infections acquired in Corsica, France. August 2013. Euro Surveill 19:20821

Hotez P (ed) (2008) Forgotten people, forgotten diseases: the neglected tropical diseases and their impact on global health and development. ASM, Washington, DC

Hotez PJ, Kamath A (2009) Neglected tropical diseases in sub-Saharan Africa: a review of their prevalence, distribution, and disease burden. PLoS Negl Trop Dis 3:e412

Huang HH, Rigouin C, Williams DL (2012) The redox biology of schistosome parasites and applications for drug development. Curr Pharm Des 8:3595–3611

IARC (2012) A review of human carcinogens: biological agents. Vol. B. International Agency for Research on Cancer. World Health Organization, Geneva

IFPMA (2017) Merck Praziquantel Donation Program. http://partnerships.ifpma.org/partnership/merck-praziquantel-donation-program-mpdp. Accessed 16 Jan 2018

Ingram JR, Rafi SB, Eroy-Reveles AA, Ray M, Lambeth L, Hsieh I, Ruelas D, Lim KC, Sakanari J, Craik CS, Jacobson MP, McKerrow JH (2012) Investigation of the proteolytic functions of an expanded cercarial elastase gene family in Schistosoma mansoni. PLoS Negl Trop Dis 6:e1589

Ismail M, Botros S, Metwally A, William S, Farghally A, Tao LF, Day TA, Bennett JL (1999) Resistance to praziquantel: direct evidence from Schistosoma mansoni isolated from Egyptian villagers. Am J Trop Med Hyg 60:932–935

Jones MK, Bong SH, Green KM, Holmes P, Duke M, Loukas A, McManus DP (2008) Correlative and dynamic imaging of the hatching biology of Schistosoma japonicum from eggs prepared by high pressure freezing. PLoS Negl Trop Dis 2:e334

Jordan PJ (1985) Schistosomiasis: the St. Lucia project. Cambridge University Press, Cambridge

King CH, Dickman K, Tisch DJ (2005) Reassessment of the cost of chronic helminthic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet 365:1561–1569

LoVerde PT (1975) Scanning electron microscope observations on the miracidium of Schistosoma. Int J Parasitol 5:95–97

LoVerde PT (1976) Scanning electron microscopy of the ova of Schistosoma haematobium and Schistosoma mansoni. Egypt J Bilharz 3:69–72

LoVerde PT, Carvalho-Querioz C, Cook R (2004a) Vaccination with antioxidant enzymes confers protective immunity against challenge infection with Schistosoma mansoni. Mem Inst Oswaldo Cruz 99:37–43

LoVerde PT, Niles EG, Osman A, Wu W (2004b) Schistosoma mansoni male–female interactions. Can J Zool 82:357–374

LoVerde PT, Andrade LF, Oliveira G (2009) Signal Transduction Regulates Schistosome Reproductive Biology. Curr Opin Microbiol, 12:422–428

Markel SF, LoVerde PT, Britt EM (1978) Prolonged latent schistosomiasis. JAMA 240:1746–1747

Mbabazi PS, Andan O, Fitzgerald DW, Chitsulo L, Engels D, Downs JA (2011) Examining the relationship between urogenital schistosomiasis and HIV infection. PLoS Negl Trop Dis 5:e1396

Mbow M, Larkin BM, Meurs L, Wammes LJ, de Jong SE, Labuda LA, Camara M, Smits HH, Polman K,

Dieye TN, Mboup S, Stadecker MJ, Yazdanbakhsh M (2013) T-helper 17 cells are associated with pathology in human schistosomiasis. J Infect Dis 207:186–195

Melman SD, Steinauer ML, Cunningham C, Kubatko LS, Mwangi IN, Wynn NB, Mutuku MW, Karanja DM, Colley DG, Black CL, Secor WE, Mkoji GM, Loker ES (2009) Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of Schistosoma mansoni. PLoS Negl Trop Dis 3:e504

Merrifield M, Hotez PJ, Beaumier CM, Gillespie P, Strych U, Hayward T, Bottazzi ME (2016) Advancing a vaccine to prevent human schistosomiasis. Vaccine 34:2988–2991

Michelson MK, Azziz FA, Gamil FM, Wahid AA, Richards FO, Juranek DD, Habib MA, Spencer HC (1993) Recent trends in the prevalence and distribution of schistosomiasis in the Nile delta region. Am J Trop Med Hyg 49:76–87

Mulvenna J, Moertel L, Jones MK, Nawaratna S, Lovas EM, Gobert GN, Colgrave M, Jones A, Loukas A, McManus DP (2010) Exposed proteins of the Schistosoma japonicum tegument. Int J Parasitol 40:543–554

Nahum LA, Mourão MM, Oliveira G (2012) New frontiers in Schistosoma genomics and transcriptomics. J Parasitol Res 2012:849132

Newton WL (1953) The inheritance of susceptibility to infection with Schistosoma mansoni in Australorbis glabratus. Exp Parasitol 2:242–257

Pan SC (1980) The fine structure of the miracidium of Schistosoma mansoni. J Invertebr Pathol 36:307–372

Pearce EJ, MacDonald AS (2002) The immunobiology of schistosomiasis. Nat Rev Immunol 2:499–511

Richards CS (1975) Genetic studies of pathologic conditions and susceptibility to infection in *Biomphalaria glabrata*. Ann N Y Acad Sci 266:394–410

Richards CS, Shade PC (1987) The genetic variation of compatibility in Biomphalaria glabrata and Schistosoma mansoni. J Parasitol 73:1146–1151

Richter J, Hatz C, Campagne G, Bergquist NR, Jenkins JM (2000) Ultrasound in Schistosomiasis: a practical guide to the standard use of ultrasonography for the assessment of schistosomiasis-related morbidity. In: Second International Workshop October 22–26, 1996, Niamey, Niger

Rollinson D, Knopp S, Levitz S, Stothard JR, Tchuem Tchuenté LA, Garba A, Mohammed KA, Schur N, Person B, Colley DG, Utzinger J (2012) Time to set the agenda for schistosomiasis elimination. Acta Trop 128:423–440

Ruffer M (1910) Note on the presence of "Bilharzia haematobia" in Egyptian mummies of the twentieth dynasty. Br Med J 1:16

Rugel A, Tarpley R, Lopez A, Menard T, Guzman MA, Taylor AB, Cao X, Kovalskyy D, Chevalier FD, Anderson TJC, Hart PJ, LoVerde PT, McHardy SF (2018) Design, synthesis, and characterization of novel small molecules as broad range anti-schistosomal agents. ACS Med Chem Lett 9:967–973

Sady H, al-Mekhlafi H, Ngui R, Atroosh W, al-Delaimy A, Nasr N, Dawaki S, Abdulsalam A, Ithoi I, Lim Y, Chua K, Surin J (2015) Detection of Schistosoma mansoni and Schistosoma haematobium by real-time PCR with high resolution melting analysis. Int J Mol Sci 16:16085–16103

Salvador-Recatalà V, Greenberg RM (2012) Calcium channels of schistosomes: unresolved questions and unexpected answers. Wiley Interdiscip Rev Membr Transp Signal 1:85–93

Schistosoma japonicum Genome Sequencing and Functional Analysis Consortium (2009) The Schistosoma japonicum genome reveals features of host-parasite interplay. Nature 16:345–351

Shane HL, Verani JR, Abudho B, Montgomery SP, Blackstock AJ, Mwinzi PN, Butler SE, Karanja DM, Secor WE (2011) Evaluation of urine CCA assays for detection of Schistosoma mansoni infection in Western Kenya. PLoS Negl Trop Dis 5:e951

Shen Y, King CH, Binder S, Zhang F, Whalen CC, Secor WE, Montgomery SP, Mwinzi PN, Olsen A, Magnussen P, Kinung'hi S, Phillips AE, Nalá R, Ferro J, Aurelio HO, Fleming F, Garba A, Hamidou A, Fenwick A, Campbell CH Jr, Colley DG (2017) Protocol and baseline data for a multi-year cohort study of the effects of different mass drug treatment approaches on functional morbidities from schistosomiasis in four African countries. BMC Infect Dis 17:652

Short RB, Grossman AI (1981) Conventional giemsa and C-banded karyotypes of Schistosoma mansoni and S. rodhaini. J Parasitol 67:661–671

Silva LL, Marcet-Houben M, Nahum LA, Zerlotini A, Gabaldón T, Oliveira G (2012) The Schistosoma mansoni phylome: using evolutionary genomics to gain insights into a parasite's biology. BMC Genomics 13:617

Simpson AJ, Sher A, McCutchan TF (1982) The genome of Schistosoma mansoni: isolation of DNA, its size, bases and repetitive sequences. Mol Biochem Parasitol 6:125–137

Southgate VR, Tchuem Tchuenté AL, Sène M, De Clercq D, Théron A, Jourdane J, Webster BL, Rollinson D, Gryseels B, Vercruysse J (2001) Studies on the biology of schistosomiasis with emphasis on the Senegal River basin. Mem Inst Oswaldo Cruz 96:S75–S78

Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis 6:411–425

Stirewalt MA (1956) Penetration of host skin by cercariae of Schistosoma mansoni. I. Observed entry into skin of mouse, hamster, rat, monkey and man. J Parasitol 42:565–580

Stirewalt MA (1966) Skin penetration mechanisms of helminths. In: Soulsby EJL (ed) Biology of parasites. Academic, New York, pp 41–58

Stirewalt MA (1974) Schistosoma mansoni: cercaria to schistosomule. Adv Parasitol 12:115–182

Stirewalt MA, Cousin CE, Dorsey CH (1983) Schistosoma mansoni: stimulus and transformation of cercariae into schistosomules. Exp Parasitol 56:358–368

Taylor AB, Roberts KM, Cao X, Clark NE, Holloway SP, Donati E, Polcaro CM, Pica-Mattoccia L, Tarpley

RS, McHardy SF, Cioli D, LoVerde PT, Fitzpatrick PF, Hart PJ (2017) Structural and enzymatic insights into species-specific resistance to schistosome parasite drug therapy. J Biol Chem 292:11154–11164

Tchuem-Tchuenté L-A, Kueté-Fouodo CJ, Kamwa-Ngassam RI, Sumo L, Dongmo Noumedem C, Kenfack CM, Gipwe NF, Nana ED, Stothard JR, Rollinson D (2012) Evaluation of circulating cathodic antigen (CCA) urine-tests for diagnosis of Schistosoma mansoni infection in Cameroon. PLoS Negl Trop Dis 6:e1758

Tebeje BM, Harvie M, You H, Loukas A, McManus DP (2016) Schistosomiasis vaccines: where do we stand? Parasit Vectors 9:528

Valentim CLL, Cioli D, Chevalier FD, Cao X, Taylor AB, Holloway SP, Pica-Mattoccia L, Guidi A, Basso A, Tsai IJ, Berriman M, Carvalho-Queiroz C, Almeida M, Aguilar H, Frantz DE, Hart PJ, LoVerde PT, Anderson TJC (2013) Genetic and molecular basis of drug resistance and species-1 specific drug action in Schistosome parasites. Science 342:1385–1389

Van der Werf MJ, de Vlas SJ, Brooker S, Looman CWN, Nagelkerke NJD, Habbema JDF, Engels D (2003) Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta Trop 86:125–139

Wei DX, Yang WY, Huang SQ, Lu YF, Su TC, Ma JH, Hu WX, Xie NF (1980) Parasitological studies on the ancient corpse of the Western Han dynasty unearthed from tomb no. 188 on Phoenix Hill at Jiangling County. Acta Acad Med Wuhan 3:1–6

Wiegand RE, Mwinzi PNM, Montgomery SP, Chan YL, Omedo KAM, Muchiri G, Ogutu MO, Rawago F, Odiere MR, Karanja DMS, Secor WE (2017) A persistent hotspot of Schistosoma mansoni infection in a five-year randomized trial of praziquantel preventative chemotherapy strategies. J Infect Dis 216:1425–1433

World Health Organization (1985) The control of schistosomiasis. WHO technical reports series 728. WHO, Geneva

World Health Organization (2006) Preventive chemotherapy in human helminthiasis. Coordinated use of anthelminthic drugs in control interventions, a manual for health professionals and programme managers. http://whqlibdoc.who.int/publications/20/06/9241547103_eng.pdf. Accessed 7 Mar 2001

World Health Organization (2010) First WHO report on neglected tropical diseases. http://www.who.int/neglected_diseases/2010report/en/. Accessed 17 Oct 2016

World Health Organization (2013) Schistosomiasis: progress report 2001–2011, strategic plan 2012–2020. http://apps.who.int/iris/handle/10665/78074. Accessed 17 Oct 2016

World Health Organization (2018) Schistosomiasis fact sheet. https://www.who.int/news-room/fact-sheets/detail/schistosomiasis. Accessed 28 Sept 2018

Wynn TA, Thompson RW, Cheever AW, Mentink-Kane MM (2004) Immunopathogenesis of schistosomiasis. Immunol Rev 201:156–167

Young ND, Jex AR, Li B, Liu S, Yang L, Xiong Z, Li Y, Cantacessi C, Hall RS, Xu X, Chen F, Wu X, Zerlotini A, Oliveira G, Hofmann A, Zhang G, Fang X, Kang Y, Campbell BE, Loukas A, Ranganathan S, Rollinson D, Rinaldi G, Brindley PJ, Yang H, Wang J, Wang J, Gasser RB (2012) Whole-genome sequence of *Schistosoma haematobium*. Nat Genet 44:221–225

Fascioliasis

4

Santiago Mas-Coma, Mª Adela Valero, and Mª Dolores Bargues

4.1 Introduction

Considered a well-known veterinary problem of worldwide distribution, fascioliasis is the vector-borne parasitic disease presenting the widest latitudinal, longitudinal, and altitudinal distribution known at present (Mas-Coma et al. 2003, 2005). In the last two decades, many surveys have shown it to be an important public health problem as well (Chen and Mott 1990; WHO 1995; Mas-Coma et al. 1999a, 2009a), including estimations of 2.4 million, up to 17 million people, or even higher depending on the hitherto unknown situations mainly in several regions of Asia and Africa (Mas-Coma 2004a).

The increasing number of human case reports in many countries of the five continents and the results of studies on pathogenicity and immunity, mainly regarding the chronic period of the disease, are the reasons why it has been decided to no longer consider fascioliasis merely a secondary zoonotic disease, but an important human parasitic disease (Mas-Coma et al. 1999b) and include it as a food-borne trematode disease priority within the agenda of the World Health Organization (WHO 2013).

S. Mas-Coma (✉) · M. A. Valero · M. D. Bargues
Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain
e-mail: S.Mas.Coma@uv.es; Madela.Valero@uv.es; M.D.Bargues@uv.es

4.2 Systematics and Morphology of Causal Agents

Fascioliasis is caused by two species which belong to the subfamily Fasciolinae: *Fasciola hepatica* and *F. gigantica*. This subfamily includes digeneans which infect the liver and more rarely duodenum and lungs of their mammal hosts, are morphologically characterized by branched caeca and dendritic testes, and are transmitted by snails of the family Lymnaeidae (Mas-Coma et al. 2009a).

The adult stage of both fasciolid species has a leaf-shaped body, with a broadly pointed posterior end. The two suckers are relatively small and located close one another in a cone-like anterior extension of the body. The pharynx is well visible. The intestinal caeca are long, reaching the posterior end of the body and presenting a large number of lateral branches. The two branched testes are located in a longitudinal tandem, within the second and third fourth of the body. The cirrus pouch, containing a protrusible spined cirrus, is prominent, preacetabular and opens in a postbifurcal genital pore. The branched ovary is pretesticular and dextral. The vitellaria extend bilaterally up to the hindbody. The short uterus is located between the ovary and the caecal bifurcation. The eggs are operculated, ovoid, yellow, and non-embryonated when laid (Fig. 4.1).

The two species differ in size. The adult stage of *F. hepatica* has a maximum length of 29.0 mm

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_4

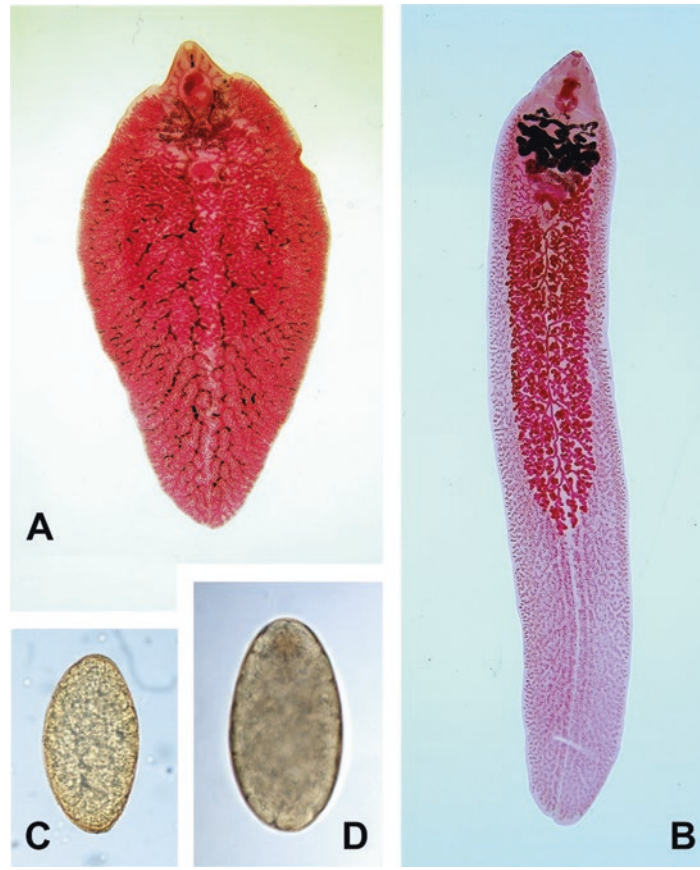

Fig. 4.1 Adults and eggs of fasciolid species: (**a**) adult stage of *Fasciola hepatica* from Bolivia; (**b**) adult stage of *F. gigantica* from Burkina Faso; (**c**) egg of *F. hepatica* found in stools of a human patient from the Bolivian Altiplano endemic area; (**d**) egg of *F. gigantica* found in a faecal sample of a bovine from Bobo Dioulasso, in Burkina Faso. Note almost absence of shoulders and parallel lateral body borders in the adult of *F. gigantica* (**b**). (**a**, **b**) at the same scale; (**c**, **d**) at the same scale. For measurements of adult stages and eggs of both fasciolids see text and Table 4.1 (Orig. S. Mas-Coma)

Table 4.1 Measurements of eggs of *Fasciola hepatica* and *F. gigantica* in different world regions according to the absence or existence of overlap of the two fasciolid species (intermediate hybrid forms have egg size ranges different from pure species)

Endemic areas	Geographical distribution	*Fasciola hepatica*		*Fasciola gigantica*	
		In humans	In animals	In humans	In animals
Areas where *F. gigantica* is absent	The Americas and Europe	100.6–162.2/ 65.9–104.6	73.8–156.8/ 58.1–98.1	–	–
Areas where both fasciolid species are present	Parts of Africa and Asia	106.5–171.5/ 63.9–95.4	120.6–163.9/ 69.2–93.8	150.9–182.2/ 85.1–106.2	130.3–182.8/ 74.0–123.6
Areas where *F. hepatica* is absent	Parts of Africa	–	–	137.2–191.1/ 73.5–120.0	129.6–204.5/ 61.6–112.5

Size given in length/width. All measures in μm
Data from Valero et al. (2009) and Mas-Coma et al. (2014a, b)

and a maximum width of 14.1 mm (Fig. 4.1a), whereas in *F. gigantica* it shows a maximum size reaching 52.3 mm and 11.8 mm (Fig. 4.1b), respectively. Thus, *F. gigantica* is more elongate and narrower, with lateral walls tending to be parallel, and with non-existent or less marked shoulders of the cephalic cone. Moreover, in *F. gigantica* caeca are more branched, mainly those towards the midline of the body, and the branches of the ovary are more numerous and longer. Morphometrically, all the measurements overlap in specimens of "pure" *F. hepatica* and "pure" *F. gigantica*, except the maximum body length, maximum body width, body length–body width ratio, body roundness, and the distance between the ventral sucker and the posterior end

of the body (Periago et al. 2006). These features allow for the phenotypical differentiation between the two species.

However, hybrid specimens may give rise to intermediate forms in those endemic areas where the two species overlap (Mas-Coma et al. 2009a). The presence of such phenotypically intermediate adult and egg forms has been proved in Egypt (Periago et al. 2008), Iran (Ashrafi et al. 2006b), Pakistan (Afshan et al. 2014a), and Bangladesh (Ahasan et al. 2016). Additionally, comparisons of adults and eggs of liver fluke populations from different host species, and adults and eggs experimentally obtained in laboratory rats infected with isolates from different natural hosts revealed that the definitive host species decisively influences the size of adult worms and eggs, and that this influence does not persist in a heterologous host (Valero et al. 2001). Thus, morphometric comparisons of fasciolid populations should always be made inside the same definitive host species.

4.3 Life Cycle

The adult stage of *F. hepatica* and *F. gigantica* parasitizes the large biliary passages and the gallbladder of ruminants, mainly sheep, goats, and cattle, and many other herbivorous domestic and wild animals, including horses, donkeys, mules, and also Old and New World camelids. Buffalo, deer, wild boar, various marsupials, rabbit, hare, and nutria are also susceptible hosts. Grazing domestic pigs may also be infected, but this host usually shows a higher natural resistance against the liver fluke (Mas-Coma and Bargues 1997). Several African wild animals and many rodent species have been found naturally infected, and other species are usually used for experimental purposes (Losos 1986; Mas-Coma et al. 1987, 1988). Humans are susceptible hosts for the infection by both *Fasciola* species (Mas-Coma et al. 2009a).

The life cycle of the two fasciolids takes around 14–23 weeks and follows a similar pattern (Mas-Coma and Bargues 1997; Mas-Coma et al. 2003).

Fasciolid adults produce eggs inside the mammal host. These eggs reach the external milieu by way of bile and intestine. The transit between the definitive mammal host and the intermediate snail host includes the long resistance phase of the egg and the short active phase of miracidium. Eggs shed with the mammal faeces will only continue their development if they reach freshwater of appropriate physico-chemical characteristics. If the climatic conditions are suitable (15–25 °C), the miracidia develop and hatch in about 9–21 days. However, when conditions are unfavourable, they may not mature but may remain viable for several months.

The miracidium hatches under light stimulation and swims rapidly until it contacts an appropriate aquatic or amphibious snail host. The development takes place inside the intermediate snail host and includes miracidium penetration into the snail, sporocyst, redial generations, production of cercariae, and shedding of the latter into water. A maximum of four redial generations have been found although 3 generations are usually produced after monomiracidial infection. The redial generations follow the same developmental pattern in different lymnaeid species. Redial generations follow a complex development (Rondelaud et al. 2009). The stage of cercaria develops within 6–7 weeks at 20–25 °C, its development being delayed at lower temperatures. Thus, the prepatent period is dependent on temperature, higher temperatures reducing it (15 °C: 56–86 days; 25 °C: 38 days).

A short swimming phase of cercaria and a long resistance phase of metacercaria allow for the transit between snail host and mammal host. The shedding process takes place between 9 and 26 °C, independently of light or darkness. Cercariae swim for a short time (1 h) until contacting a solid support, mostly leaves of water plants above or below the water line. They then lose their tails, quickly encyst and become infective within 24 h.

The definitive host is infected by ingestion of metacercariae (Fig. 4.2). Metacercariae excyst in the small intestine within an hour after ingestion, penetrate the host's intestine wall, and appear in the abdominal cavity by about 2 h after ingestion. Most migrating juveniles reach the liver within 6 days after excystment. In the liver they migrate for 5–6 weeks, preferentially feeding directly on

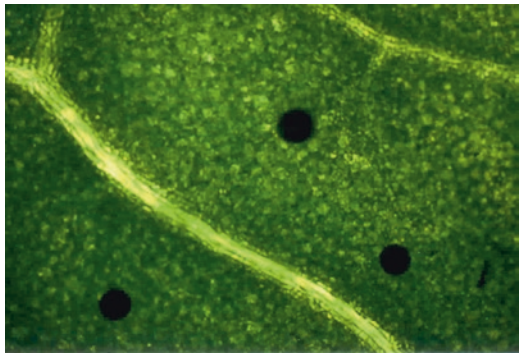

Fig. 4.2 Encysted metacercariae of *Fasciola hepatica* attached to a leaf of a freshwater plant (Orig. S. Mas-Coma)

Fig. 4.3 Main species of lymnaeid vectors of fascioliasis in dorsal view: (**a**) specimen of *Galba truncatula* from Europe; (**b**) specimen of *Radix natalensis* from Africa. Note larger size of the latter (photographs at the same scale) (Orig. S. Mas-Coma)

liver tissue. They finally penetrate into the bile ducts where they become sexually mature.

The prepatent period (from the ingestion of metacercariae to the first appearance of the first eggs in the faeces) is about 2 months (6–13 weeks) in sheep and cattle, varies according to the host, and also depends on the number of the adult flukes in the liver (Valero et al. 2006a). In humans, a period of at least 3–4 months is necessary for the flukes to attain sexual maturity. The lifespan of the parasite in sheep can be as long as 11 years and 9–12 months in cattle. In humans, estimations from several long-term case reports suggest a lifespan of the adult fluke between 9 and 13.5 years.

4.4 Lymnaeid Snail Vectors

The development of fasciolid larval stages is very dependent on the environmental characteristics according to the nature of the free living phases which take place in the external freshwater milieu, and the parasite phase which develops inside the freshwater snail, in its turn also very dependent on the environment. That is why this disease is pronouncedly influenced by climate change (Mas-Coma et al. 2009b). The similarity in the relationships between snails and climate/environment resembles the one known in arthropods participating in the transmission of many infectious diseases and underlies the recent trend of using the term vector also for the intermediate lymnaeid snail hosts transmitting fascioliasis.

Vectors of *Fasciola* are freshwater gastropod snails of the family Lymnaeidae (Fig. 4.3). Different lymnaeid species transmit the two fasciolids, which show a marked and different specificity. There are species of Lymnaeidae which cannot transmit fasciolids, other lymnaeid species which transmit *F. hepatica*, other lymnaeid species which transmit *F. gigantica* and a very few which are able to transmit the two fasciolid species. However, recent molecular studies on lymnaeids have shown that lymnaeid species misclassifications have been usual (Bargues et al. 2001) and additionally hybridization phenomena between the two *Fasciola* species were unknown in the past, so that results of many of the old fasciolid–lymnaeid specificity experiments should be re-assessed (Mas-Coma et al. 2009a).

Molecular studies indicate that *F. hepatica* is mainly transmitted by species of small size belonging to the so-called *Galba* / *Fossaria* group (Bargues et al. 2007, 2011a), including *Galba truncatula* as the main vector and the only one in Europe, but also present in Africa, Asia, and South America (Fig. 4.3a); *Lymnaea humilis*, *L. bulimoides*, and *L. cubensis* in North America, *L. cubensis* in the Caribbean; *L. neotropica*, *L. cousini*, and *L. viator* in South America; and *L. tomentosa* in Australia. The recent discovery of *L. schirazensis*, another species of the same *Galba*/*Fossaria* group which appears to have

been always confused with *G. truncatula* and other similar vector species, in Asia, Europe, Africa, the Caribbean, North America, and South America, has highlighted potential specimen classification problems distorting fasciolid–snail specificity/susceptibility and fascioliasis geographical distribution data. This unexpected finding now recommends the need to review a large body of literature on *G. truncatula* (Bargues et al. 2011a).

The species *Fasciola gigantica* is transmitted by species of the genus *Radix*, mainly *R. natalensis* in Africa (Fig. 4.3b) and varieties of *R. auricularia* and *R. viridis* in Asia (Bargues et al. 2001). *Pseudosuccinea* is a monospecific genus including the species *P. columella* which has colonized all continents and appears to be able to transmit both *Fasciola* species (Bargues et al. 2011c).

A few species among the lymnaeid group of the stagnicolines have proved their capacity to transmit *F. hepatica* under exceptional or local natural conditions in a few areas, such as *L.* (*Stagnicola*) *palustris* and *L.* (*S.*) *fuscus*, and closely related species such as *Omphiscola glabra* (Bargues et al. 2003).

Lymnaeid vectors, with their geographical distribution, define not only the distribution of fascioliasis, but may also explain the distribution of human infection within a country, as has been recently observed in Venezuela (Bargues et al. 2011b) and Chile (Artigas et al. 2011), and within an endemic area, its seasonality or permanent transmission (Mas-Coma et al. 1999c).

4.5 Epidemiology

Despite the restrictions imposed by the necessary climate/environment thresholds, *F. hepatica* has succeeded in expanding from the Near East original geographical area up to actually colonize the five continents. In its turn, *F. gigantica* appears restricted to areas of Africa and Asia where *Radix* vectors allow for their transmission (Mas-Coma et al. 2009a). It should be emphasized, however, that a global analysis of the geographical distribution of human infection shows that the expected correlation between animal and human fascioliasis only appears at a basic level. High prevalences in humans do not seem to be necessarily related to high prevalences in livestock.

Similarly to other water-borne parasitic diseases such as schistosomiasis, within a human endemic area, it has been seen that human and animal infection appears irregularly distributed. The transmission foci are patchily distributed and linked to the presence of appropriate water collections, and human prevalences in school children appear to be related to the distance to water bodies presenting lymnaeids (Mas-Coma et al. 1999c).

4.5.1 Distribution of Human Fascioliasis

In Europe, France is the endemic area where a higher number of human cases have been reported (Anonymous 1988). The first large modern epidemic of human fascioliasis occurred in that country in 1956 (Coudert and Triozon 1958). Between 1950 and 1983, a total of 3297 cases from published reports were catalogued (Gaillet et al. 1983). Most cases were reported from the areas of Lyon, Bretagne Nord—Pas de Calais and Sud-Ouest. More recent reports on Sud-Ouest France refer to more than 300 cases (Giap 1987; Ripert et al. 1987). Reports on 5863 human cases were recorded from only nine hospitals between 1970 and 1982 (Danis et al. 1985), demonstrating that published data were largely underestimating the real situation. The disease is also important in Portugal, with the northern part of the country as a marked endemic area, including 1011 cases diagnosed in Porto between 1970 and 1992 (Sampaio-Silva et al. 1996). In Spain, human fascioliasis appears to be underestimated and mainly distributed in the northern part (Sorribes et al. 1990), with imported cases recently added to autochthonous ones (Turrientes et al. 2004). In other parts of Europe, human infection appears to be sporadic although reported from almost all countries (Esteban et al. 1998).

In Asia, the Near East appears as an important focus of human infection, concerning mainly Iran and Turkey. In Iran, human cases appear above

all concentrated in the province of Gilan, at the Caspian Sea, where several large epidemics, including thousands of human cases, were reported from the end of the 1980s and during the 1990s (Massoud 1990, 1993; Ashrafi et al. 2004). In Mazandaran, fascioliasis has recently shown to be a human health problem too (Moghaddam et al. 2004), and many reports have very recently been published on human cases diagnosed in other provinces. In Turkey, human infection does not seem to be rare. The detection of a 1.8% human prevalence in a village in Eastern Turkey (Yilmaz and Gödekmerdan 2004) suggests that this endemic area may be largely widespread throughout the eastern part of the country.

In southern Asia, whereas only sporadic isolated cases have been diagnosed in India and Afghanistan, a wide human endemic area in the Punjab province and child infection in another area have been recently reported from Pakistan (Qureshi et al. 2016, 2019), and a worrying scenario has been described in Nepal (Sah et al. 2018).

In the Far East, cases in Japan and Korea are sporadic, but recent information on Vietnam becomes bothering (Mas-Coma 2004b). Only occasional cases of human fascioliasis were reported in Vietnam until the 1990s, but over 500 human cases have been diagnosed between 1997 and 2000 (De et al. 2003) and with non-stop increasing numbers thereafter (De et al. 2006; Hung and Dung 2011). A recent report of a 13.8% human prevalence in a village of Laos (Quang et al. 2008) may be interpreted as an epidemiological situation with a broader spread throughout southeastern Asia. Additionally, a human infection focus has been reported from southern China (Chen et al. 2013).

In Africa, numerous human cases have been detected in many governorates of Egypt, mainly children (Fig. 4.4) (Curtale et al. 2000, 2003a, b; Haseeb et al. 2002; Esteban et al. 2003). Initial estimations of 830,000 subjects affected in the Nile Delta region (WHO 1995) probably underestimate the real situation if the high prevalences reaching 18–19% in total population in concrete villages (Esteban et al. 2003) are considered. More recently, human endemic areas have been reported from Ethiopia (Fentie et al. 2013) and Tanzania (Lukambagire et al. 2015), and sporadic human infection has been reported from the Maghreb countries, sub-Saharan countries and even up to South Africa, where infection proved sometimes to be fatal (Black et al. 2013).

Fig. 4.4 Transmission focus of human fascioliasis in the Nile Delta region, in Egypt, with *Galba truncatula*, *Radix natalensis caillaudi* and *Pseudosuccinea columella* as vector species transmitting both *Fasciola hepatica* and *F. gigantica* infecting children (Orig. S. Mas-Coma)

In Latin America, human infection appears mainly in altitude areas of the Andean region. In the Bolivian Altiplano, human prevalences were up to 72% and 100% in coprological and serological surveys, respectively (Hillyer et al. 1992; Bjorland et al. 1995; Esteban et al. 1997a, b, 1999; Mas-Coma et al. 1999c), and intensities reached up to more than 8000 eggs per gram (epg) in children (Mas-Coma et al. 2009a). Similar situations, although with lower intensities, have been described in other altitude areas of Peru, such as in Puno (Esteban et al. 2002), Mantaro valley (Raymundo et al. 2004), and Cajamarca (Gonzalez et al. 2011). Human infection has also been described in altitude areas of Ecuador (Trueba et al. 2000), Colombia (see review in Bargues et al. 2011c), Venezuela (see review in Bargues et al. 2011b), and recently also in Argentina (Carnevale et al. 2013; Bargues et al. 2016). A few human endemic areas have also been described in lowland areas in countries of the Southern Cone, such as Argentina (Mera y Sierra et al. 2011) and Chile (Apt et al. 1993; Artigas et al. 2011), whereas in Uruguay only a relatively low number of cases have been reported (Bargues et al. 2017).

Very recently, a human fascioliasis endemic area has been described for the first time in North America. Children proved to be infected in the state of Puebla, at a mean altitude of 1840 m. Fascioliasis prevalences indicate this area to be mesoendemic with isolated hyperendemic foci, a situation which adds concern about possible human fascioliasis underestimation in other areas of Mexico (Zumaquero-Rios et al., 2013).

In the Caribbean region, human fascioliasis mainly poses problems in Cuba, where the first human cases were already diagnosed in the first half of last century (Kouri and Arenas 1932; Vasquez 1943), many outbreaks have been reported (Esteban et al. 1998) since the first one (Arenas et al. 1948), losses in livestock husbandry due to fascioliasis are very high (Brito Alberto et al. 2010), and patients are continuously diagnosed (Millan et al. 2000; Diaz Fernandez et al. 2011), even in high numbers (Gonzales-Santana et al. 2013). In that island, the disease transmission is assured by two lymnaeid vectors, *L. cubensis* and *Pseudosuccinea columella* (Fig. 4.5). Unfortunately, appropriate field surveys are still lacking (Rojas et al. 2009) and

Fig. 4.5 Typical focus of transmission of animal fascioliasis in Cuba, with *Lymnaea cubensis* as vector species (Orig. S. Mas-Coma)

hence the real situation in the different parts of the island remains unknown. Puerto Rico may still be considered a human infection risky area after the epidemiological situation in the past (Hillyer 1981), and Haiti has recently proved to be also affected by this disease at human level nowadays (Agnamey et al. 2012) although human infection was already detected in Haiti time ago (Clay and Straight 1961).

4.5.2 The Present Epidemiological Baseline

The present baseline on human fascioliasis pronouncedly differs from the knowledge available on human infection two decades ago. Many new concepts have been reached on human fascioliasis from the 1990s up to the present. A list of key aspects may be enumerated (Mas-Coma et al. 2009a):

1. In many areas, there are true human fascioliasis endemic situations, from hypo- to hyperendemics, which is very different of the old concept of humans only becoming infected sporadically in animal endemic areas.
2. In those endemic areas, high prevalences in humans (up to more than 70% by coprology and even reaching 100% by serology) do not appear to be necessarily related to high prevalences in domestic animals.
3. In human endemic areas, fascioliasis mainly affects children and females, with flukes infecting even at very precocious age (1–2-year-old children), usually showing a peak around 9–11 years and declining thereafter, although it may keep high prevalences in adults too (up to 40% in given communities).
4. Worldwide estimations raised from the 2500 reports of 1990 to 2.4 million, 17 million people and may even be higher at present if the almost total lack of knowledge about the situation of this disease in humans in many African and Asian countries is taken into account.
5. Human infection has been reported in 51 different countries from the five continents, showing how geographically expanded the problem might be.
6. The analysis of the distribution of the disease has shown that fascioliasis is the vector-borne parasitic disease showing the widest latitudinal, longitudinal, and altitudinal distribution known.
7. Such a broad distribution including from under sea level (as in the Caspian area) up to the very high altitude (4200 m at the Paso del Condor in Venezuela) is the consequence of the great capacity of both liver flukes and lymnaeid vectors to colonize new areas and their great capacity for adaptation to very different environments, habitats, and climates, even of extreme conditions as the very high altitude regions in Andean areas, where mathematical models well-known for fascioliasis in lowlands of the Northern Hemisphere indicated that the disease could not exist.
8. In human endemic areas, intensities, estimated from amounts of epg of faeces, may reach up to more than 8000 and amounts higher than 400 epg may be frequent in given communities, which markedly differs from the very low burdens (usually from less than 1 to 1–2 epg) reported before the 1990s.
9. Domestic animal species other than the usual sheep and cattle may also play an important role as reservoirs for humans in many different endemic areas, as mainly pigs, donkeys, and buffaloes, depending on the regions.
10. The snail family of lymnaeids shows a systematic-taxonomic chaos which even impedes correct classification of snail specimens by malacology experts, as demonstrated by DNA sequencing methods; classification errors underlie a concept of fasciolid–lymnaeid specificity which must be revisited.
11. Lymnaeid species linked to the disease transmission in many human endemic areas were erroneously classified as local lymnaeid spe-

cies, whereas in fact lymnaeid vector species imported from other continents were involved. This, together with importation/exportation of fasciolid-infected livestock, has given an international dimension to the public health problem in many areas where the disease was previously given local repercussion only.

The above-mentioned issues have given rise to a new platform for the analysis and interpretation of the human disease which is very different from a simple extrapolation from the traditional knowledge of fascioliasis in livestock. Unfortunately, sometimes not sufficient importance is given to this new base or it is not considered at all and consequently incorrect interpretations and erroneous conclusions are increasingly appearing in the recent literature.

4.5.3 Epidemiological Heterogeneity of Human Fascioliasis

After many years of studies on different areas presenting human infection by fasciolid liver flukes in South and Central America, Europe, Africa, and Asia, the classification of epidemiological situations proposed by Mas-Coma et al. (1999a) still appears to be fully valid and useful. This classification includes the following human infection situations:

- *Autochthonous, isolated, non-constant cases*: humans acquire the infection in an area where they live and where animal fascioliasis is also present; these human cases appear sporadically, without any constancy.
- *Imported cases*: human cases diagnosed in a zone lacking the parasite, even in animals, who were infected in an area where transmission occurs.
- *Endemic*: three types of endemic situations can be distinguished according to human prevalences in the total population obtained by coprological diagnosis (data from serological tests may be somewhat higher).
  - *Hypoendemic*: prevalence less than 1%; arithmetic mean intensity less than 50 epg; high epg numbers only in sporadic cases; human participation in transmission through egg shedding may be neglected; hygiene–sanitation characteristics usually including latrines and waste or sewage disposal facilities; outdoor defaecation is not commonly practised.
  - *Mesoendemic*: prevalence between 1 and 10%; 5–15-year-old children may present higher prevalences (holoendemic); arithmetic mean intensity in human communities usually between 50 and 300 epg; individual high epg numbers can be found although intensities over 1000 epg are rare; human subjects may participate in transmission through egg shedding; hygiene–sanitation characteristics may or may not include latrines and waste or sewage disposal facilities; outdoor defaecation may be practised.
  - *Hyperendemic*: prevalence more than 10%; 5–15-year-old children usually present higher prevalences (holoendemic); arithmetic mean intensity in human communities usually more than 300 epg; individual very high epg numbers are encountered, intensities over 1000 epg being relatively frequent; human subjects significantly participate in transmission through egg shedding; hygiene–sanitation characteristics not including the use of latrines; no proper waste or sewage disposal facilities; indiscriminate defaecation is commonly practised.
- *Epidemic*: there are different types of outbreaks according to the endemic/non-endemic situation of the zone.
  - *Epidemics in non-human endemic but animal endemic areas*: outbreaks appearing in zones where previous human reports have always been isolated and sporadic; such outbreaks usually concern a very few subjects infected from the same contamination source (family or small group reports; contaminated wild, home-grown or com-

mercially grown watercress or other metacercariae-carrying vegetables).
- *Epidemics in human endemic areas*: outbreaks appearing in zones presenting human endemics; a more important number of subjects may be concerned; usually related to previous climatic conditions having favoured both the parasite and the snail life cycles; epidemics can take place in hypoendemic, mesoendemic, and hyperendemic areas.

Fascioliasis presents a very wide spectrum of transmission and epidemiological patterns in human hypoendemic to hyperendemic areas. These are related to the large diversity of environments, including different human endemic/epidemic situations, different human demographies, races, diets, habits, traditions and religions, different domestic and wild mammal reservoir species, different lymnaeid transmitting species, zones in both the Northern and Southern hemispheres, altitudes from −27 m up to 4200 m, hot and cold weathers, seasonal and yearly constant temperatures, scarce to pronounced annual rainfall, low and high mean annual potential evapotranspiration, and from lack of dry period to lack of wet period through different dryness/humidity rates. From the landscape point of view, these areas include from altiplanos to valleys, from islands to mainlands, from natural to artificial irrigations, from lakes to lagoons, from large rivers to small streams, and from permanent to temporal water bodies (Mas-Coma et al. 2003).

4.5.4 Transmission Patterns in Human Fascioliasis Areas

A classification of transmission patterns has been proposed (Mas-Coma 2005) and is progressively updated to offer a baseline for future research (Mas-Coma et al. 2009a):

1. A very high altitude pattern related to only *F. hepatica* transmitted by imported *G. truncatula* in Andean countries following transmission throughout the year; within this category, two subpatterns may be distinguished according to physiographic and seasonal characteristics.
   (a) The altiplanic pattern, with transmission throughout the whole year, e.g. in the Northern Bolivian Altiplano and the Puno Altiplano.
   (b) The valley pattern, with seasonality and prevalences and intensities related to altitude, e.g. in the valleys of Cajamarca and Mantaro (Valero et al. 2012c);
2. A Caribbean insular pattern, with reduced but repeated outbreaks in human hypoendemic areas and lymnaeid species other than the main vector species being involved in the transmission, e.g. the Pinar del Rio Province in Cuba.
3. A pattern related to Afro-Mediterranean lowlands, including overlapping *F. hepatica* and *F. gigantica* and several *Galba*/*Fossaria* and *Radix* lymnaeids together with secondary transmitting *Pseudosuccinea*, and where seasonality is typical, e.g. the Behera Governorate in Nile Delta region in Egypt.
4. A pattern related to Caspian surrounding areas, including human hypoendemic areas in which large epidemics occur, occasionally involving up to 10,000 people and with overlapping of *F. hepatica* and *F. gigantica* and several *Galba*/*Fossaria*, *Radix*, and stagnicoline lymnaeids, e.g. the area of Rasht and Bandar-e Anzali in the Gilan province in Iran.
5. A pattern related to lowland areas in Vietnam, which may perhaps be extrapolated to other neighbouring South East Asian countries; this pattern is able to give rise to large human epidemics and is related to only/mainly *F. gigantica* and consequently *Radix* lymnaeids.
6. Another pattern has recently been described in Argentina (Bargues et al. 2016); this pattern is very different from the typical fascioliasis transmission foci because of the desertic-arid/semiarid conditions surrounding the transmission foci in which lymnaeids are confined to lateral river side floodings and small man-made irrigation systems, water availability only depends on the rivers flowing from neighbouring mountains, and all disease transmission

factors are concentrated in small areas where humans and animals go for water supply, vegetable cultures and livestock farming, remembering the schistosomiasis transmission foi in African oases of the Sahara desert.

Human fascioliasis shows a marked heterogeneity of different epidemiological situations and transmission patterns throughout the world. Thus, well-known situations and patterns of fascioliasis may not always explain the disease characteristics in a given area. In other terms, when dealing with an endemic zone not previously studied, the aforementioned situations and patterns of human infection must always be taken into account merely as the starting base. Only once epidemiology and transmission characteristics of the new area are sufficiently assessed, may appropriate control measures be designed for the endemic area in question.

The lymnaeid vector species show a relationship with the transmission pattern. Lymnaeids present pronouncedly different ecological and ethological characteristics depending on the species. Factors such as type of water collection habitats, population dynamics, temperature thresholds, seasonality, or susceptibility regarding liver fluke infection, are crucial for fascioliasis. As in other well-known vector-borne parasitic diseases, lymnaeids constitute excellent markers of the disease characteristics useful for the differentiation between different human fascioliasis situations and patterns, and consequently their assessment is necessary before the appropriate control strategies may be designed.

4.5.5 Seasonality and Long-Term Impacts of Climate and Global Changes

Climatic factors are decisive in the transmission of fascioliasis. The yearly definitive host infection incidence of fascioliasis has been related to air temperature, rainfall, and/or potential evapotranspiration. These factors affect the intermediate snail host population dynamics and the parasite population at the level of both the free living larval stages of egg and metacercaria and the intramolluscan parasitic larval stages of sporocyst, rediae, and cercariae.

Seasonal variation of mainly rainfall and temperature gives rise to different fascioliasis seasonality depending on the areas. In Europe, the transmission of the disease is typically bi-seasonal, due to the activity periods of the lymnaeid vectors in spring and autumn. In the Bolivian Altiplano, however, the transmission takes place throughout the year, lymnaeid vector populations being always present because of inhabiting permanent water bodies instead of temporary ones due to the high evapotranspiration rates at the very high altitude (Mas-Coma et al. 1999c). In other areas, the transmission appears mono-seasonal, due to the existence of only 1-year period with water availability and another period of dryness covering the rest of the year.

Man-made modifications of the environment may also modify the seasonality of fascioliasis in a given endemic area. Thus, artificial field irrigation appears to be sufficient by its own to allow for fascioliasis transmission in Cambodia (Tum et al. 2004, 2007). In the province of Punjab, in Pakistan, a complex transmission model has recently been described, including bi-seasonality with a peak related to rainfall and another peak related to man-made irrigation (Afshan et al. 2014b).

Unfortunately, climate change overlaps other anthropogenic and environmental modifications which are included in the broad term of "global change". Global change refers to many man-made environmental changes such as hydrological changes, e.g. construction of dams, irrigation canals, water reservoirs that establish suitable new environments for the snail vectors that transmit the parasites (Mas-Coma et al. 2009b). Hence, global change factors are able to pronouncedly influence parasitic diseases by their own, so that establishing the causality of disease emergence by climate change is usually not an easy task. However, the aforementioned Pakistani province of Punjab is the first endemic area where the emergence of human infection has been correlated with an increase of fascioliasis transmission risk due to an impact of climate

change throughout a 20-year period by means of an analysis of forecast indices and remote sensing data (Afshan et al. 2014b).

4.5.6 Sources of Human Infection

The ingestion of infective metacercariae by humans may occur by different ways. A very recent worldwide, detailed analysis has clarified all human fascioliasis infection sources, their diversity in the different areas and countries, the related incidence factors, and the methods for the study and analysis of these sources. The high diversity of infection sources and their heterogeneity in different countries underlie the large epidemiological heterogeneity of human fascioliasis throughout (Mas-Coma et al. 2018). The following infection sources have been distinguished:

- Ingestion of freshwater wild plants: main aspects to be considered are the plant markers of transmission foci, watercress, other freshwater wild plants, and wild plants sold in urban markets.
- Ingestion of freshwater cultivated plants, mainly watercress.
- Ingestion of terrestrial cultivated plants needing frequent irrigation.
- Ingestion of terrestrial wild plants: collected in dry habitats but which were submerged in water a few weeks or months before.
- Ingestion of traditional local dishes made with contaminated sylvatic plants.
- Ingestion of raw liver infected with migrating metacercariae which may keep the capacity to restart migration.
- Drinking of contaminated water.
- Drinking of beverages and juices made from local plants.
- Ingestion of dishes and soups made with contaminated water.
- Washing of vegetables, fruits, tubercles, kitchen utensils, or other objects with contaminated water.

Among the incidence factors, disease transmission seasonality, the infectivity of metacercariae under field conditions, as well as several community, familial and social factors in infection risk appear in the forefront. Among the methods to assess human infection sources, the following should be considered: (1) detection of metacercariae attached to plants or floating in freshwater; (2) anamnesis in individual patients, and (3) questionnaire surveys in human endemic areas (Mas-Coma et al. 2018).

Cultural traditions prove to be highly important in given endemic areas. Experimental studies performed with plant-made foods showed the role they may play in human infection in the province of Gilan, Iran (Fig. 4.6) (Ashrafi et al. 2006a).

Fig. 4.6 Freshwater plant usually included in human diet in a focus of fascioliasis transmitted by *Galba truncatula* in Talesh Mountains, province of Gilan, in Iran (Orig. S. Mas-Coma)

In Mexican children, an association between fascioliasis and the habit of eating raw vegetables was identified, including watercress and radish with pronouncedly higher relative risk than lettuce, corncob, spinach, alfalfa juice, and broccoli. The link of fascioliasis risk with consumption of raw vegetables other than watercress should be highlighted, as it suggests contamination when washing terrestrial vegetables with untreated water and/or in plant cultures using natural water for irrigation (Zumaquero-Ríos et al. 2013).

It shall be considered that metacercarial infectivity is dependent upon storage time, being lower when metacercariae are older: the maximum longevity was 31 and 48 weeks using doses of 20 and 150 metacercariae per rat, respectively, although in the latter case only a very low percentage was viable. Moreover, metacercarial viability and infectivity did not show differences between isolates from different reservoir species, demonstrating that flukes from secondary reservoirs as pigs and donkeys involve the same potential risk as those from the main ones sheep and cattle (Valero and Mas-Coma 2000).

4.6 Pathology, Symptomatology, and Clinical Manifestations

Four clinical periods may be distinguished in fascioliasis (Chen and Mott 1990; Mas-Coma and Bargues 1997; Mas-Coma et al. 1999b, 2000). The incubation period includes from the ingestion of metacercariae to the appearance of the first symptoms. In man, this period has not been accurately determined (only "a few" days, 6 weeks, 2–3 months, or even more). The invasive or acute period comprises fluke migration up to the bile ducts. The latent period includes maturation of the parasites and starting of oviposition. This period can last for months or years and the proportion of asymptomatic subjects in this phase is unknown, being often discovered during family screening after a patient is diagnosed (Arjona et al. 1995). Patients may have prominent eosinophilia suggestive of infection, gastrointestinal complaints, or one or more relapses of the acute symptoms. Finally, the biliary, chronic, or obstructive period may develop after months to years of infection. Of these four periods, the second and fourth are the most important because patients are in one or another of these two periods almost always when diagnosed.

4.6.1 Invasive or Acute Period

The symptomatology which appears during this period is due mainly to mechanical destruction of liver tissue and abdominal peritoneum by the migrating larvae causing localized or generalized toxic and allergic reactions lasting 2–4 months. The major symptoms of this phase include fever, abdominal pain usually in the right hypochondrium or below the xyphoid, gastrointestinal disturbances such as loss of appetite, abdominal flatulence, nausea and diarrhoea, respiratory symptoms such as cough, dyspnoea, hemoptysis and chest pain, and also urticaria.

4.6.2 Biliary, Chronic, or Obstructive Period

Once in the bile ducts, adult flukes cause inflammation, hyperplasia of the epithelium, and thickening and dilatation of duct and gall bladder walls. The resulting cholangitis and cholecystitis, combined with the large body of the flukes, are sufficient to cause obstruction. This phase includes biliary colic, epigastric pain, fatty food intolerance, nausea, jaundice, pruritus, and right upper-quadrant abdominal tenderness, among others. Lithiasis of the bile duct or the gall bladder is frequent, whereas cirrhosis does not appear to be so (Marcos et al. 2009). The bile duct and the gall bladder may contain blood mixed with bile (haemobilia), blood clots, and fibrinous plugs. Symptomatology in children from human endemic areas of Peru includes abdominal pain localized in the epigastrium, the Murphy symptom and jaundice as the most frequent clinical biliary characteristics, the rest of the symptoms being non-specific (Marcos-Raymundo et al. 2002).

4.6.3 Clinical Highlights

Little was known about pathogenicity of *F. gigantica* in comparison with *F. hepatica*, mainly due to difficulties in assessing the moment of a patient's infection, the differential diagnosis with *F. hepatica*, and the lack of an animal model similarly susceptible to both fascioliods. However, a long-term, 24-week, experimental study comparing *F. hepatica* and *F. gigantica* has recently been successfully made for the first time in the same animal model host, Guirra sheep (Valero et al. 2016). Serum biochemical parameters of liver damage, serum electrolytes, protein metabolism, plasma proteins, carbohydrate metabolism, hepatic lipid metabolism and inflammation were analysed on a biweekly basis as morbidity indicators. Serum anti-*Fasciola* IgG, coproantigen, and egg shedding were simultaneously followed up. rDNA and mtDNA sequencing and the morphometric study by CIAS showed that fasciolids used fitted standard species characteristics. Results demonstrated that *F. gigantica* is more pathogenic, given its bigger size and biomass. *Fasciola gigantica* proved to follow a delayed development of 1–2 weeks regarding both the biliary phase and the beginning of egg shedding, with respective consequences for biochemical modifications in the acute and chronic periods. The higher *F. gigantica* pathogenicity contrasts with previous studies which only reflected the faster development of *F. hepatica* observed in short-term experiments (Valero et al. 2016).

In a developed country, blood eosinophilia and the ingestion of watercress or any other suggestive freshwater plant in anamnesis are extremely useful in guiding towards a fascioliasis diagnosis. Unfortunately, these two aspects are usually not helpful in human endemic areas of developing countries, where eosinophilia may also be caused by other helminth infections and local food traditions including the ingestion of many uncooked plants may mask liver fluke infection (Mas-Coma et al. 2014a, b).

In human endemic zones, there is usually a decrease of the prevalence from children and young subjects to adult subjects. Despite this, results demonstrate that adult subjects either maintain the parasites acquired when young or can be newly infected as the consequence of inhabiting a zone of high infection risk (Esteban et al. 1999). It must be considered here that the lifespan of the adult fluke in man is between 9 and 13.5 years (Mas-Coma and Bargues 1997). Such a picture suggests that, in those areas, the majority of adult subjects should be in the biliary period, acute lesions by repetitive infections being superimposed on chronic disease with relative frequency (Valero et al. 2017). Thus, the acute period may be prolonged and overlap with both latent and biliary periods.

An association between anaemia and fluke burden (the most important), epg, fluke body area, presence of blood in faeces, IgG1 and eosinophil levels, and percentage of splenic weight was verified in a multivariate analysis. These results lead to the assumption that a high risk of anaemia in subjects with a heavy parasitic burden in human hyperendemic areas is to be expected (Valero et al. 2008). These results are crucial because although there were several reports listing anaemia in patients from endemic areas, results could only be considered with great caution because coinfections were never excluded in those papers and in fact it becomes very difficult, not to say almost impossible, to find subjects from endemic areas only infected by fascioliasis. And among those parasites coinfecting fascioliasis-affected subjects, many are also known to cause anaemia.

The duration of fasciolid infection, intensity of fasciolid infection, and liver damage have been experimentally verified to be associated with bacterobilia by *Escherichia coli* (45% of cases), *Enterococcus faecalis* (45%), and *Klebsiella pneumoniae* (10%). This supports that the obstruction caused by advanced chronic fascioliasis may be related to biliary sepsis. These results lead to a reconsideration of treatment features in human disease, i.e. therapeutic strategies should also consider the possibility of bacterial coinfection (Valero et al. 2006b).

The presence of gallstones was experimentally proved to increase with infection time.

Therefore, the lithogenic induction by infection becomes manifest in situations of advanced chronicity. Gallstone presence was strongly associated with the number of flukes located in the bile duct. The risk of pigment stones appears to depend mainly on factors that favour bile duct obstruction (cholangitis, fluke body development versus time, intensity of infection). Situations of undiagnosed cases, as in subjects presenting undistinguishable symptoms or in those keeping their infection for a long time because of non-treatment or of repetitive reinfections, usually in human endemic areas of developing countries, imply a higher lithiasis risk. Thus, a high gallstone risk may be expected in subjects inhabiting human hyperendemic areas where very high egg outputs detected in humans suggest that liver fluke burdens may also be very high (Valero et al. 2003).

Clinical pictures caused by fasciolids in locations of the human body different from the liver are known as ectopic fascioliasis. Flukes may deviate during migration, enter other organs and cause ectopic fascioliasis. In almost all patients, the causal agent is an immature juvenile, but a reduced number of ectopic cases caused by mature flukes shedding eggs have also been reported (Mas-Coma et al. 2014a, b). In humans, the most frequent ectopic lesions are in the gastrointestinal tract. Other such lesions are in abdominal wall, pancreas, spleen, subcutaneous tissue, heart, blood vessels, the lung and pleural cavity, skeletal muscle, appendix and epididymis (Mas-Coma and Bargues 1997). Pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis.

A wide analysis has shown that neurofascioliasis or intracranial infection by *Fasciola* and ophthalmofascioliasis or direct affection of the eye by migrating flukes may be rare although not sporadic as previously believed. However, manifestations including a very wide range of neurological symptoms, signs, and syndromes, together with meningeal, psychiatric or neuropsychic manifestations, and ocular disorders caused at distance by flukes infecting the liver may be frequent but underestimated due to misdiagnosis, mainly in low-income regions (Mas-Coma et al. 2013, 2014a). The impressive clinical pictures should be highlighted. They include from hemiplegia and paraplegia to disturbances and difficulties of walking capacity, speech disorders, convulsions, epilepsia and coma, amnesia, or visual hallucinations and permanent blindness, only to mention a few, plus the clinical complexity of the puzzling polymorphisms, the disconcerting multifocality of the manifestations, and their changes along the evolution of the disease in a same patient, as well as differences between the clinical pictures shown by different patients. Moreover, these studies emphasize post-treatment sequelae and mortality in neurological patients and the need to consider neurological fascioliasis when estimating the global burden of this disease (Mas-Coma et al. 2013, 2014a).

Very recently, interactions between diverse *Fasciola* infection situations and non-imbalancing fibrinolysis system alterations have been for the first time proposed to explain the complexity, heterogeneity, and timely variations of neurological disorders (Gonzalez-Miguel et al. 2019). Proteomic and mass spectrometry analyses of the *Fasciola hepatica* excretome/secretome identified numerous, several new, plasminogen-binding proteins enhancing plasmin generation. This may underlie blood–brain barrier leakage whether by many simultaneously migrating, small-sized juvenile flukes in the acute phase, or by breakage of encapsulating formations triggered by single worm tracks in the chronic phase. Blood–brain barrier leakages may subsequently occur due to a fibrinolytic system-dependent mechanism involving plasmin-dependent generation of the proinflammatory peptide bradykinin and activation of bradykinin B2 receptors, after different plasminogen-binding protein agglomeration waves. Additionally, inflammation and dilation of blood vessels may be due to contact system-dependent generation bradykinin. This baseline allows for search of indicators to detect neurological risk in fascioliasis patients and experimental work on antifibrinolytic treatments or B2 receptor antagonists for preventing blood–brain barrier leakage (Gonzalez-Miguel et al. 2019).

4.7 Immunobiology and Coinfections

Fasciolid trematodes promote its own survival through several strategies to downregulate the host's immune response during the early phase of infection (Brady et al. 1999). Another study proved that immune response modulation occurs in advanced chronic fascioliasis too. The results indicated that during early chronic infection there was a predominance of a Th2 response, which decreased in the advanced chronic infection characterized by a persistent immune suppression (Girones et al. 2007). Fascioliasis is a potent inducer of Th2 responses which impair the ability to mount any effective Th1 responses against bacteria and other pathogens (Brady et al. 1999; O'Neill et al. 2000; Jaffar et al. 2004).

The rapid and potent ability of fasciolids to suppress the protective arm of the immune response explains why infected hosts do not develop immune resistance. Within 24 h after oral infection, peritoneal macrophages express markers for the Th2-associated phenotype and display a reduced ability to respond to Th1 stimulants. This implies that by the time the newly excysted juveniles have penetrated the intestinal wall and entered the peritoneum, they have already initiated the immune events that will dominate throughout infection. So, these early-stage parasites secrete immunomodulatory molecules that influence the function of innate cells (dendritic cells, macrophages, neutrophils, mast cells, etc.) in the intestinal wall and peritoneal cavity. A systemic antigen-specific Th2 response is firmly established already at 7 days postinfection and is characterized by the secretion of IL-4, IL-5, and IL-13 from splenocytes. As the infection develops (3 weeks), regulatory macrophages (TGF-ß and IL-10 producing) and dendritic cells (IL-10 producing) are recruited to the peritoneum and dendritic cell maturation is inhibited. Mast cells recruited to the site of infection exhibit impaired Th1 promoting abilities. Most CD4∗ T cells in the peritoneum secrete IL-10 but not IL-4 or IFN-gamma. IL-10 secreting Tregs are induced which exert a suppression of both Th1 and Th2 cells that become non-responsive to parasite-specific antigens and mesenteric lymph nodes produce IL-10 and IL-5, but not IFN-gamma and IL-17, in response to stimulation by parasite antigens (Dalton et al. 2013).

The chronic disease is also typified by Th2 responses and suppressed Th1 responses. Serologically, this polarity of immune response is strikingly displayed in the isotype of circulating antibodies. Fluke-infected animals secrete high titres of IgG1 antibodies and virtually no IgG2. Furthermore, blood macrophages are non-responsive to stimulation with endotoxin and exhibit elevated levels of arginase indicative of a phenotype that metabolize L-arginine and are important in promoting Th2 responses and facilitating tissue repair and fibrosis (Dalton et al. 2013).

A consequence of liver fluke infection is the suppression of immune responses directed against concurrent or secondary pathogenic infections. The synergistic capacity of fasciolids in coinfection with other pathogenic agents is well-known, immunological responses to pathogen antigens being markedly suppressed and concomitant infection being exacerbated following fascioliasis infection. The parasitological spectrum of protozoan and helminthic species found in the inhabitants of the human fascioliasis endemic areas, the multiparasitisms, and the associations between liver fluke infection and infection by other pathogenous parasites, all appear to be similar in the different human endemic zones (Esteban et al. 1997a, b, 1999, 2002, 2003). These synergistic associations of fascioliasis with other pathogens are believed to underlie the high morbidity and mortality rates of Aymara children inhabiting the Northern Bolivian Altiplano (Mas-Coma 2004a).

4.8 Genomics and Proteomics

Many different tools known to be useful for intraspecific variability analyses have been applied to fasciolids. Most studies on fasciolid proteins have concentrated on isoenzymes. Only a very few studies considered individual or population-level variation. The same isoenzymes of *F. hepatica*

were detected regardless of the host species (cattle, sheep, goats), but densities of some isoenzyme bands did differ according to host (Blair 1993). Profiles of whole-body proteins and excretory/secretory products obtained with isoelectric focusing differ among worms from different hosts (Lee et al. 1992), and isoelectric focusing is therefore not a good technique. Random amplified polymorphic DNA (RAPD) markers applied to *F. hepatica* showed that the majority of genetic diversity occurred within, rather than between hosts and was also greater within than between populations. Individual cows were infected by numerous genetically different liver flukes, suggesting the influence of mainly migrations and transportation of definitive hosts (Semyenova et al. 2003). Five among six microsatellite markers proved to be polymorphic in *F. hepatica* from Bolivia. No genetic differentiation between sampling sites or between definitive host species (sheep, cattle, pig) was found when applying these microsatellites (Hurtrez-Bousses et al. 2004).

Similarly, the restriction fragment length polymorphism (PCR-RFLP) technique has been applied repeatedly to fasciolids (Marcilla et al. 2002; Huang et al. 2004; Rokni et al. 2010), but unfortunately these assays are only useful for the differentiation of pure species, but not for hybrid forms (Mas-Coma et al. 2009a). Indeed, this was already initially detected three decades ago. Restriction endonuclease maps of rRNA genes were distinct for *F. hepatica* and *F. gigantica*, Japanese *Fasciola* sp. being identical to *F. gigantica*. No intraspecific variations in the maps of *F. hepatica* or of *F. gigantica* were detected, but length heterogeneity was noted in the intergenic spacer, even within individual worms (Blair and McManus 1989).

The aforementioned problems posed by isoenzymes, RAPD, microsatellites, and RFLP techniques explain why these genetic tools have been abandoned or only sporadically used in *Fasciola*. That is why genetic studies on fasciolids mainly rely on DNA sequencing techniques at present.

In an initial approach, a total of six differences were detected between *F. hepatica* from Ipswich and *F. gigantica* from Malaysia in a 28S rRNA gene D1 domain fragment (Barker et al. 1993). However, within the nuclear ribosomal DNA (rDNA) operon. Studies in invertebrates in general have shown that the ITS spacers are the most adequate markers for species differentiation (Mas-Coma and Bargues 2009). The complete sequence of ITS spacers of fasciolids was obtained for the first time at the beginning of this century (Mas-Coma et al. 2001). Nowadays, a large amount of literature is already available about the variability of ITS-1 and ITS-2 in *F. hepatica*, *F. gigantica*, and also hybrid or intermediate forms from throughout. This large information has recently been reviewed, corrected, and a useful standardized nomenclature for DNA markers in fasciolids proposed (Mas-Coma et al. 2009a).

In a large analysis of samples of "pure" *F. hepatica* from numerous countries and continents, only two haplotypes of the ITS-2 differing in only one mutation were found: the most spread FhITS2-H1 and the apparently more geographically restricted FhITS2-H2. On the contrary, the sequence of the other spacer ITS-1 always proved to be identical in "pure" *F. hepatica*: FhITS1-HA. In "pure" *F. gigantica*, only one haplotype in ITS-2 (FgITS2–1) and similarly one in ITS-1 (FgITS1-A) were found. When comparing ITS-2 sequences, the two haplotypes of *F. hepatica* (FhITS2-H1 and H2) with the only one of *F. gigantica* (FgITS2-H1), five polymorphic sites enable the two species to be distinguished. When comparing ITS-1 sequences, the only haplotype of *F. hepatica* (FhITS1-HA) differs from the only haplotype of *F. gigantica* (FgITS1-HA) also in five polymorphic sites (Mas-Coma et al. 2009a).

A comparative study with sequences obtained in countries not included in that countrywide study allowed to reach the conclusion that up to four haplotypes of ITS-2 could be distinguished in *F. hepatica* (FhITS2–1 to 4) and up to five in *F. gigantica* (FgITS2–1 to 5). On the contrary, the ITS-1 appeared to be fully uniform in both *F. hepatica* and *F. gigantica* everywhere (FhITS1-A and FgITS1-A) (Mas-Coma et al. 2009a).

With regard to mitochondrial DNA (mtDNA), the complete genome of *F. hepatica* has been already sequenced, which will be suitable for

studies of variation (Le et al. 2001). Unfortunately, only small fragments of the mtDNA codifying genes *cox*1 and *nad*1 have been used in the numerous local studies, and this becomes a problem due to the biased information gene fragments furnish (Mas-Coma and Bargues 2009).

Only in the aforementioned wide multicountry analysis of samples of "pure" *F. hepatica* and "pure" *F. gigantica* from different continents were these mitochondrial genes analysed in its complete length (Mas-Coma et al. 2009a). "Pure" *F. hepatica* showed a mtDNA *cox*1 codifying gene providing a total of 69 different haplotypes (Fh cox1–1 to 69), including a total of 78 polymorphic sites. A total of 23 different haplotypes of the COX1 protein were found (FhCOX1–1 to 23). In its turn, the mtDNA *nad*1 codifying gene provided a total of 51 different haplotypes (Fh nad1–1 to 51). A total of 15 different haplotypes of the NAD1 protein were found (FhNAD1–1 to 15). "Pure" *F. gigantica* showed a *cox*1 gene providing a total of 11 different haplotypes (Fg cox1–1 to 11). A total of 5 different haplotypes of the COX1 protein were found (FgCOX1–1 to 5). In its turn, the *nad*1 gene provided a total of 15 different haplotypes (Fg nad1–1 to 15). A total of 10 different haplotypes of the NAD1 protein were found (FgNAD1–1 to 10) (Mas-Coma et al. 2009a).

Proteomic tools have provided wide information about the profiles of soluble proteins secreted by fasciolids, mainly on cathepsin L and cathepsin B family of peptidases and their temporal expression as the parasites progresses from tissue to tissue (McVeigh et al. 2012), as well as about the expression and function of several antioxidant molecules, glutathione S-transferases, fatty acid binding proteins and peroxiredoxin which, besides protecting the parasite from damaging reaction, may also have immunoprotective functions (Spithill et al. 2012).

Given the intimate contact between the fluke and host tissues through its migration, antigens associated with the tegument of *Fasciola* also modulate host immune cell function. The tegument of *Fasciola* is a unique syncytial layer that plays the interface between host and parasite. In recent proteomic studies on the adult stage, extracted fractions of *F. hepatica* showed to contain 172–229 proteins, providing valuable insights into the complex protein composition within the tegumental layer as a whole (Dalton et al. 2013). Regarding *Fasciola* juveniles, the enzymatic shaving of peptides from the surface of liver flukes and their subsequent identification, has allowed at least some of the tegumental surface proteome to be identified (De La Torre et al. 2011).

The genome of *F. hepatica* has proved to be among the largest known pathogen genomes at 1.3 Gb, a size which cannot be explained by genome duplication or expansion of a single repeat element. The substantial levels of polymorphism found have tentatively been linked to the evolutionary potential for rapid adaptation to changes in host availability, climate change or to drug or vaccine interventions (Cwiklinski et al. 2015). Intriguingly, the genome of *F. hepatica* isolated from sheep of North America showed a markedly higher repeat content (55.29%) than the aforementioned genome of *F. hepatica* isolated from cattle of United Kingdom (32.0%) (McNulty et al. 2017).

4.9 Diagnosis

Although some suggestive clinical presentation aspects may be useful, mainly in human endemic areas where physicians are aware about liver fluke infection risk in humans, verification needs the use of at least one among the direct parasitological techniques or indirect immunological tests. Other non-invasive diagnostic techniques presently available may be additionally helpful. Non-invasive diagnostic techniques which can be used for human diagnosis are radiology, radioisotope scanning, ultrasound, computed tomography, and magnetic resonance (see reviews in Esteban et al. 1998 and Hillyer 1999).

Stool and blood techniques, the main tools for diagnosis in humans, have been improved in the last two decades. Present availabilities for human diagnosis have recently been reviewed exhaustively, focusing on advantages and weaknesses, sample management, egg differentiation,

qualitative and quantitative diagnosis, antibody and antigen detection, post-treatment monitoring, and post-control surveillance (Mas-Coma et al. 2014b). Main conclusions refer to the pronounced difficulties of diagnosing fascioliasis in humans given the different infection phases and parasite migration capacities, clinical heterogeneity, immunological complexity, different epidemiological situations and transmission patterns, the lack of a diagnostic technique covering all needs and situations, and the advisability for a combined use of different techniques, at least including a stool technique and a blood technique (Mas-Coma et al. 2014b).

4.9.1 Coprological and Other Direct Diagnostic Techniques

Analysis for the detection and identification of fasciolid eggs found in stool sample (Fig. 4.3), duodenal contents or bile continues to be the most appropriate diagnostic strategy for both detection of infection and estimation of intensity. This is even in spite of the recognized lower sensitivity of egg detection in faecal samples and its uselessness for the diagnosis of patients in the acute period, as well as the lack of an accurate relationship between egg counts per gram of faeces and the fluke burden (Valero et al. 2006a, 2009). Identifying fluke adults obtained during an endoscopy of after surgical intervention either by microscopic morphometry (Periago et al. 2006) or molecular tools (Marcilla et al. 2002; Mas-Coma et al. 2009a) may also be performed nowadays although such occasions are evidently not frequent at all. Moreover, the infrastructure for endoscopy or surgery is in general not available in rural endemic areas.

Techniques ranging from a simple direct smear to different concentration methods may be used. Egg concentration has been achieved by flotation and sedimentation techniques. The sedimentation techniques appear to be more accurate and sensitive than flotation techniques (Esteban et al. 1998; Mas-Coma et al. 1999a).

The size of the fluke eggs has always been used for human diagnosis. Based on studies in livestock, the borderlines allowing differentiation between the two species were traditionally considered to be 150 μm in length and 90 μm in width, lower values representing *F. hepatica* and higher values *F. gigantica*. However, large variations were first observed in the size of *F. hepatica* eggs in livestock from different geographical locations (Tinar 1984). Furthermore, it has been experimentally shown that the final host species (sheep, cattle, pig, and donkey) decisively influences the size of the *F. hepatica* eggs even within the same endemic area (Valero et al. 2001). Additionally, the existence of intermediate forms between the two fasciolid species and genetic hybrids of both in overlapping areas increases the problem. The existence of these intermediate forms posed a question mark on whether egg characteristics are suitable as a tool for the differential diagnosis of fascioliasis caused by either species (Valero et al. 2009). A concrete example of this problem was already emphasized in the diagnosis of fascioliasis in humans (Inoue et al. 2007). All in all, the aforementioned problems plus the use of serological tests, all of which unable to differentiate between the two fasciolids, or the lack of a calibrated microscope for measurements with an ocular micrometer explain why subjects diagnosed from areas where both species co-exist, such as in areas of Africa and Asia, are currently referred to as infected by *Fasciola* sp. or simply *Fasciola* (Mas-Coma et al. 2005).

A study of fasciolid eggs from different continents, using a computer image analysis system (CIAS), revealed that eggs shed by humans show morphological traits different from eggs shed by animals. In humans, *F. hepatica* eggs are bigger and *F. gigantica* eggs are smaller than reported to date from livestock, and their measurements overlap when compared. Measurements of *F. hepatica* and *F. gigantica* eggs originating from humans and animals from sympatric areas overlap, and, therefore, they do not allow differential diagnosis when within this overlapping range (Table 4.1) (Valero et al. 2009; Mas-Coma et al. 2014a, b). These new results should aid clinicians since the application of the classic egg size range in human samples may lead to erroneous conclu-

sions. Consequently, fasciolid egg size in human stool samples ought to be corrected in books and monographs as well as in guides of medical parasitology and tropical medicine.

Quantitative coprological analyses become important in epidemiological surveys as well as post-treatment monitoring. Egg burden is also crucial in the moment of deciding the appropriate treatment dose. The 400-epg threshold has been proposed for identifying high intensity infections. To avoid risk of colic, a repeated, timely spaciated mid-dose is recommended in patients shedding more than 400 eggs (WHO 2007; Valero et al. 2012a). The second half of the regimen is administered 24 h later, once the absence of secondary effects verified. The Kato–Katz technique appears to be appropriate because of its simplicity, very low cost, and reproducibility (Mas-Coma et al. 1999b). Its low sensitivity may be solved by repeated application.

Besides eggs in coprological analyses, adults and eggs may also be found elsewhere by means of other invasive techniques: obtaining duodenal fluid, duodenal and biliary aspirates; surgery (laparotomy, cholecystectomy, sphincterotomy); histological examination of liver and/or other organ biopsy materials (Mas-Coma et al. 1999b).

4.9.2 Serological and Other Indirect Diagnostic Techniques

Numerous serological, intradermal, and stool antigen detection tests have been developed. Immunological techniques present the advantages of being applicable during all periods of the disease, but fundamentally during the invasive or acute period, as well as to the other situations in which coprological techniques may present problems. However, immunological techniques offer other types of problems related mainly to sensitivity and specificity. Different serological tests have been used for human diagnosis. Almost all these techniques concern the detection of circulating antibodies and only a very few are designed to detect circulating antigens and immune complexes.

In recent years, efforts have been concentrated in obtaining purified excretory/secretory antigens and/or recombinant molecules to improve serological tests, owing to the problems of the parasitological diagnosis because of the delay in its usefulness in the acute period (coprological examination positive only after 3–4 months postinfection), intermittent egg output dynamics, very low or even absence of egg shedding in cases of only one or a few fluke adults and old, chronic infections, ectopic infections, "false" fascioliasis related to eggs in transit after ingestion of infected liver from domestic animals, or flukes unable to attain maturity in human subjects in non-human endemic areas (Esteban et al. 1998; Mas-Coma et al. 1999b).

Several cysteine proteinases offer highly sensitive and specific markers for human fascioliasis serodiagnosis for *F. hepatica* (Sampaio-Silva et al. 1996; Cordova et al. 1997, 1999; O'Neill et al. 1998, 1999; Strauss et al. 1999; Rokni et al. 2002; Espinoza et al. 2007; Mezo et al. 2003, 2004) as well as for *F. gigantica* infection (Maleewong et al. 1999; Ikeda 1998; Intapan et al. 1998, 2004; Tantrawatpan et al. 2005). *Fasciola hepatica* recombinant cysteine proteinases produced in yeast (O'Neill et al. 1999) or in *Escherichia coli* (Carnevale et al. 2001) have been used in ELISA methods for human infection diagnosis.

Very recent studies in two human hyperendemic areas of Bolivia and Peru have shown that the MM3 coproantigen-detection test allows for high sensitivity and specificity, fast large mass screening capacity, detection in the chronic period, early detection of treatment failure or reinfection in post-treated subjects, and usefulness for surveillance programmes. However, this technique falls short when evaluating the fluke burden on its own (Valero et al. 2012a). The use of a new preservative/diluent CoproGuard™, developed for preservation of *Fasciola* coproantigens, proved to enhance coproantigen extraction without affecting the detection limit of the assay, and the antigenicity of *Fasciola* coproantigens in faecal samples stored at 37 °C was retained throughout the entire observation period (Ubeira et al. 2009). Thus, MM3-COPRO ELISA combined with the use of CoproGuard™ may be a very useful tool for the diagnosis of human fascioliasis.

Another study demonstrated that the commercialized DRG *Fasciola hepatica* IgG (human) ELISA is highly sensitive and specific, has a high negative predictive value but has a low positive predictive value. No correlation between egg output and the *F. hepatica* IgG ELISA test values was observed. It was concluded that this test could be used both as an individual serodiagnostic test for human fascioliasis when backed up by a compatible clinical history together with a second diagnostic technique for other cross-reactive helminth infections and in future large-scale epidemiological studies of human fascioliasis worldwide (Valero et al. 2012b).

A new lateral flow test (SeroFluke) for human diagnosis appears to be a useful step forward (Martinez-Sernandez et al. 2011). This test was constructed with a recombinant cathepsin L1 from *F. hepatica*, and uses protein A and mAb MM3 as detector reagents in the test and control lines, respectively. In comparison with an ELISA test (MM3-SERO), the SeroFluke test showed maximal specificity and sensitivity and the advantage of being applicable to both serum and whole-blood samples. Its simplicity allows it to be used in major hospitals as well as in endemic/hyperendemic regions where point-of-care testing is required.

4.9.3 Fasciolid Species Differentiation by Molecular Tools

Infection by *F. hepatica* and *F. gigantica* cannot be differentiated by clinical, pathological, coprological, or immunological methods. This is a problem in overlapping areas because this differential diagnosis is very important owing to the different pathological transmission and epidemiological characteristics of the two fasciolids, as well as due to intermediate forms in which egg measurements may overlap.

To distinguish between *F. hepatica* and *F. gigantica*, a simple and rapid PCR-RFLP assay, using the common restriction enzymes *Ava*II and *Dra*II, has recently been described. It is based on a 618-bp-long sequence of the 28S rRNA gene recently obtained from populations of South America, Europe, and Africa. This sequence showed no intraspecific variations within each species and a few nucleotide differences between both fasciolids. This assay provides unambiguous results and may be useful for both individual subject diagnosis and epidemiological surveys of humans and animals in endemic regions of sympatry in Africa and Asia (Marcilla et al. 2002). A similar PCR-RFLP assay using restriction endonucleases *Hsp*92II and *Rca*I has been recently applied to differentiate between Chinese liver flukes (Huang et al. 2004). Another such PCR-RFLP method was later developed (Rokni et al. 2010).

Unfortunately, these three aforementioned PCR-RFLP assays are only useful for the differentiation of pure species, but not for hybrid forms (Mas-Coma et al. 2009a). A similar comment may be applied to the recently dev eloped single step duplex PCR for simultaneous detection of both fasciolid species (Le et al. 2012), as well as to the TaqMan real-time PCR-based assay (Alasaad et al. 2011), and other specific PCR-based assays (Ai et al. 2010). None of these methods proves to be able to detect the wide introgression capacity the two fasciolid species have (Mas-Coma et al. 2009a).

Therefore, DNA marker sequencing still remains as the only appropriate method for both haplotyping of the two pure fasciolid species, as well as for the detection of hybridization in intermediate forms. For such a purpose, the complete sequences of the two rDNA spacers ITS-2 and ITS-1 together with those of the mtDNA genes *cox*1 and *nad*1 have so far proved to be the markers of choice, and a complete baseline and nomenclature for these four markers have already been provided (Mas-Coma et al. 2009a).

4.10 Treatment

Many drugs have been used to treat human fascioliasis. Emetine derivatives, the classic drugs, were used widely and still continue to be used today, given intramuscularly or subcutaneously at doses of 1–10 mg/kg a day for 10 days. However, the use of emetine was progressively

abandoned due to their toxic side effects involving heart, liver, and digestive tract. The same occurred with dehydroemetine despite its better tolerability. Dehydroemetine, at a usual dose of 1 mg/kg daily for 10–14 days, was even considered the therapy of choice a few decades ago (Mas-Coma et al. 2014a, b).

Chloroquine was also used to treat *F. hepatica* infection. Although no cidal effects on the flukes were shown, treatment by this aminoquinoline derivative improved the symptoms dramatically when applied in the acute phase. Among xylol derivatives, hexachloro-para-xylol was effectively used, mainly in the old Soviet Union and China. Bithionol, a halogenated phenol derivative, was proposed as the drug of choice for the treatment of *F. hepatica* infection during the last three decades of last century. It was usually applied at a dose of 30–50 mg/kg daily, divided into 3 oral doses on alternate days for 20–30 days. In cases of fascioliasis resistant to emetine and praziquantel treatment, bithionol achieved cure in dosages of 50 mg/kg daily for 10 alternate days or 40 mg/kg daily for 14–15 alternate days. Occasionally, the patients required a second course to obtain a complete cure. The side effects, including diarrhoea, anorexia, nausea, vomiting, pruritus, urticaria and abdominal pain, were usually mild (Chen and Mott 1990; Esteban et al. 1998). Another halogenated phenol derivative such as niclofolan was also assayed for liver fluke treatment in humans but rapidly abandoned due to its wide side effects.

Praziquantel is an isoquinoline-pyrazine derivative which was widely applied for the treatment of human fascioliasis during the 1980s and 1990s, based on the fact that it is the drug of choice for human trematode infections. However, controversial results were found already from the beginning of its application to fascioliasis patients, including many reported praziquantel failures even at high doses. Today, it is generally accepted that *Fasciola* may be the only trematode genus that has practically no response to praziquantel.

Metronidazole and albendazole and sporadically also mebendazole are imidazole derivatives which have been also applied for human fascioliasis treatment with more or less success. But another imidazole derivative as triclabendazole (Egaten®) has become the drug of choice for human fascioliasis caused by both *F. hepatica* and *F. gigantica* at present (Savioli et al. 1999). This drug is better adsorbed if administered after meals (Lecaillon et al. 1998). The recommended dosage is two separate regimens of 10 mg/kg. A cure rate of 79.2% when first used and 100% after a second round of therapy was found in Chile (Apt et al. 1995), and 79.4% and 93.9%, respectively, in Egypt (El-Morshedy et al. 1999). Triclabendazole appears to keep its efficiency at standard regimes in human endemic areas after years (Talaie et al. 2004) although the need for a third dose has been reported in Cuba (Millan et al. 2000).

The risk of appearance of resistance to triclabendazole can neither be forgotten taking into account the veterinary use of triclabendazole (Fasinex®) for livestock treatment in endemic areas since long ago, the tradition of human self-treatments with Fasinex® owing to the very general availability of this drug, and the appearance of triclabendazole resistance in animals in different countries. Triclabendazole resistance was first described in Australia, later in European countries such as Ireland, Scotland, the Netherlands, and Spain (see review in Mas-Coma et al. 2007). Very recently it has also been found in southern Brazil (Oliveira et al. 2008) and Argentina (Olaechea et al. 2011), and thus already in the New World. Up to that moment, triclabendazole resistance only concerned livestock in animal endemic areas, but unfortunately it has very recently been also described (Ortiz et al. 2013) in a human highly endemic area such as the Andean valley of Cajamarca, Peru (Gonzalez et al. 2011). The strategies to minimize the development of resistance include the use of synergistic drug combinations (Fairweather and Boray 1999) although this approach has the risk of building up multiple drug resistance (Gaasenbeek et al. 2001). Additionally, studies suggest that our understanding of the mechanism of resistance to triclabendazole remains far from complete (Fairweather 2005, 2009; Brennan et al. 2007), so that the spreading capacity of triclabendazole resistance remains unknown.

Nitazoxanide is a pyruvate ferredoxin oxidoreductase inhibitor with reported efficacy on a broad parasitological spectrum, such as intestinal protozoans and helminths. It may be considered a good alternative to triclabendazole, at least for the chronic stage of fascioliasis, mainly in those countries where Egaten® is still not registered but nitazoxanide is since several years. Nitazoxanide had demonstrated its efficacy against human fascioliasis in a few trials, in Egypt (Rossignol et al. 1998; Kabil et al. 2000) and Peru (Favennec et al. 2003). Its long 7-day treatment course may nevertheless become a problem. However, its usefulness for the treatment of human cases not responding to triclabendazole (Gargala et al. 2005) is of important additional value. A good nitazoxanide efficacy has recently been reported when applied to liver fluke-infected children in Mexico (Zumaquero-Ríos et al. 2013). However, differences in fasciolid susceptibility to nitazoxanide may exist depending on geographical strains. Thus, no response to nitazoxanide treatment was reported in 24 cases of liver fluke infection in Esmeralda, Camagüey, Cuba (Del Risco Barrios et al. 2001), and a triclabendazole-resistant *F. hepatica*-infected patient not responding to nitazoxanide treatment has recently been reported in the Netherlands (Winkelhagen et al. 2012).

Mirazid® is a drug prepared commercially from myrrh (Arabian or Somali) which is an oleo-gum resin obtained from the stem of thorny trees *Commiphora molmol* and other species of the family Burseraceae. Introduced to the local Egyptian market, it has been highlighted by its efficacy against human fascioliasis in many reports although a recent evaluation proved that it showed only an insignificant activity against the liver fluke (Botros et al. 2009).

Artemisinin derivatives initially showed a high fasciolicidal activity in sheep infection, which was encouraging. Artesunate and artemether, given by the intramuscular route, yielded high egg and worm burden reductions. A study in Vietnam showed that the complete response rate at 3 months was lower than in triclabendazole although those treated with artesunate were significantly more likely to be free of abdominal pain (Hien et al. 2008). Unfortunately, a last study in Egypt demonstrated that artemether, administered at malaria treatment regimens, shows no or only little effect against fascioliasis, and hence does not represent an alternative (Keiser et al. 2011).

4.11 Control

Prevention and control measures recommended for human fascioliasis were traditionally the same to be applied for veterinary fascioliasis, at the levels of domestic animals, snails, and field (Roberts and Suhardono 1996; Torgerson and Claxton 1999; Spithill et al. 1999). However, studies on human endemic areas performed in the last two decades have shown that traditional epidemiological patterns of animal fascioliasis may not always explain the characteristics of human infection in a given area. Therefore, control measures for human fascioliasis should consider the results of the eco-epidemiological studies previously undertaken in the area concerned (Mas-Coma et al. 2009a). This is the reason why the WHO launched a worldwide initiative against this disease including different control strategies depending on the human endemic areas and countries.

The large heterogeneity of the human fascioliasis infection sources underlies a considerable control complexity linked to the many different ways the parasite may follow for successfully accessing the human host. This complexity of the highly heterogenic scenario globally conformed by fascioliasis, that both individual and general prevention measures should face, has deeply been very recently analysed for the first time, including many new concepts, strategies, and even newly proposed specific legislation initiatives (Mas-Coma et al. 2018). Health responsibles, physicians, governmental officers involved, authorities and any other person working on this disease should refer to this freely online available article of the WHO initiative including exhaustive details about measures for the control and fight against human fascioliasis.

In the following, only the traditional frame of control is summarized owing to space restric-

Fig. 4.7 City market showing uncontrolled sale of vegetables involved in the transmission of human fascioliasis in Quy Nhon, Vietnam (Orig. S. Mas-Coma)

tions. With regard to individual measures, the prevention of human infection may be achieved by strict control of the human infection sources in each place, mainly with regard to watercress and other metacercariae-carrying aquatic plants for human consumption, especially in endemic zones. Unfortunately, potassium permanganate, which had been suggested to be the most effective preventive tool for killing metacercariae attached to leaves and vegetables used in salads, has been shown to have no effectivity on metacercarial viability, even at the very high doses (Ashrafi et al. 2006a).

Moreover, it should be considered that infection risks shall not be restricted to only ingestion of freshwater vegetables, as always mentioned. The different human infection sources may be taken into account, mainly in human endemic areas. Drinking of natural freshwater should be avoided in human endemic areas. In many human hyperendemic areas of the Americas, people do not have a history of eating watercress or other freshwater plants (Esteban et al. 2002). In the Nile Delta region, persons living in houses where piped water is present showed to have a higher infection risk (Curtale et al. 2003b).

The problem does not only concern rural areas, as usually believed. The possibility of human infection in urban areas should not be neglected. Thanks to transport of vegetables (both aquatic and terrestrial) from rural endemic zones to cities, plants carrying metacercariae can be sold in non-controlled city markets giving rise to urban infection (Fig. 4.7) (Mas-Coma 2004a).

Within general control measures to be applied in human fascioliasis endemic areas, education should always be included, mainly with regard to the need to let know inhabitants about the human infection sources. The community should be appropriately informed about the disease, its pathogenicity, its transmission, and where to go for diagnosis if suggestive symptoms appear.

The availability of a very effective drug against fascioliasis as triclabendazole prompted the WHO to launch a decisive step forward within its worldwide initiative against human fascioliasis (WHO 2007, 2008) in recent years. This initiative includes action in human fascioliasis endemic areas presenting different epidemiological situations and transmission patterns (Mas-Coma 2005; Mas-Coma et al. 2009a). Pilot schemes were designed to assess the best control strategies according to the different epidemiological situations and transmission patterns in the way to decrease morbidity, mainly in children. Selective patient treatments after passive detection in hospitals was the strategy applied in Vietnam, and infected subject treatment after

active detection in surveys in the Nile Delta high human endemic region the one applied in Egypt. Bolivia and Peru were the other two countries selected for priority intervention due to the very large public health problem posed by this disease. The Northern Bolivian Altiplano was chosen as an example of the Altiplanic pattern, while the Cajamarca valley was chosen as an example of the valley pattern. The respective pilot interventions in the two Andean human endemic areas demonstrated the absence of serious side effects in triclabendazole treatments of schoolchildren (Villegas et al. 2012), which subsequently allowed for the launching of annual campaigns of preventive chemotherapy by mass treatments in these two Andean countries. Many other countries are nowadays receiving yearly triclabendazole donations through WHO for the treatment of their patients, in an expansion of the aforementioned WHO initiative.

In countries where watercress is included in food traditions, such as France, commercial growing of watercress should be carried out under completely controlled conditions, without access for ruminants and snail vectors.

In Egypt, the construction and utilization of the so-called washing units, in which the water was appropriately filtered, gave rise to a marked decrease of human infection in a locality of the Nile Delta region where a high prevalence in humans was initially found (Mas-Coma 2004a).

Regarding veterinary control, previous epidemiological studies may provide for general recommendations on the appropriate time for treatment with effective drugs to achieve economic control, and better information from the livestock farming community. Forecasts of outbreaks may be made based on climatological data and epidemiological models. Recommendations for control measures should be made on a preventive rather than a curative basis, and all measures have to be considered from the point of view of the economy and assessment of local topographical and meteorological conditions. The efficiency of fascioliasis control depends on the correct and integrated application of several measures (Mas-Coma and Bargues 1997): (1) reduction of the parasite load of the animal hosts and pasture contamination by regular strategic use of drugs (preventive treatment in appropriate year periods according to different regions); (2) reduction of the number of snails by physical, chemical, and biological means; (3) reduction of the risks of infection through correct farm management practices (rotational system through fluke-infected and fluke-free paddocks, combined with effective treatment).

Owing to the similarity of the life cycles of the two fasciolid species, prevention and control measures follow the same patterns for both *F. hepatica* and *F. gigantica*. However, the peculiarities of *F. gigantica* should be considered. Thus, in enzootic areas of *F. gigantica*, contraction of the infection by the animals and their contamination of the area with eggs shed with faeces take place when the animals go to drink, rather than when they are grazing in the pasture as is the case in *F. hepatica*. Accordingly, avoiding the watering of the animals from swampy banks of rivers and from bodies of water rich in vegetation would considerably reduce infection chances (Mas-Coma and Bargues 1997).

Lymnaeid vector control has unfortunately not received, by public health officers, the sufficient attention required to definitively eliminate transmission (Chen and Mott 1990). Intensive agricultural methods must be applied to reduce suitable snail habitats. Besides physical methods, there are available control strategies which consist of the use of chemical molluscicides, natural molluscicides of plant origin, biological control (including predators, competitors, the decoy effect and related phenomena, parasitic castration, interspecific trematode antagonism, and pathogens), genetic manipulation, and engineering control. However, the practical application of chemical methods in the control of snails is of doubtful value, requires labour and equipment, and regular yearly strategic molluscicide applications. Moreover, the application of molluscicides in the case of the small *Galba–Fossaria* vector species showing marked amphibious behaviour becomes almost impossible, due to the small size of the water bodies these vectors inhabit.

Acknowledgements Reviews of fascioliasis carried out within Projects SAF2010-20805 of the Ministry of Economy and Competitiveness, Madrid, Spain; by Health Research Project No. PI16/00520, Subprograma Estatal de Generación de Conocimiento de la Acción Estratégica en Salud (AES), Plan Estatal de Investigación Científica y Técnica y de Innovación, ISCIII-MINECO, Madrid, Spain; by Project No. 2017/ACDE/001583 de Innovación para el Desarrollo of the Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Ministry of Foreign Affairs and Cooperation, Madrid, Spain; by the Red de Investigación de Centros de Enfermedades Tropicales – RICET (Project No. RD16/0027/0023 of the PN de I + D + I, ISCIII-Subdirección General de Redes y Centros de Investigación Cooperativa RETICS), Ministry of Health and Consumption, Madrid; by Project No. 2016/099 of the PROMETEO Program, Programa of Ayudas para Grupos de Investigación de Excelencia, Generalitat Valenciana, Valencia, Spain.

References

Afshan K, Valero MA, Qayyum M, Peixoto RV, Magraner A, Mas-Coma S (2014a) Phenotypes of intermediate forms of *Fasciola* and *Fasciola gigantica* in buffaloes from Central Punjab, Pakistan. J Helminthol 88:417–426

Afshan K, Fortes-Lima CA, Artigas P, Valero AM, Qayyum M, Mas-Coma S (2014b) Impact of climate change and man-made irrigation systems on the transmission risk, long-term trend and seasonality of human and animal fascioliasis in Pakistan. Geospat Health 8:317–334

Agnamey P, Fortes-Lopes E, Raccurt CP, Boncy J, Totet A (2012) Cross-sectional serological survey of human fascioliasis in Haiti. J Parasitol Res 2012:751951

Ahasan SA, Valero MA, Chowdhury EH, Islam MT, Islam MR, Hussain MMM, Peixoto RV, Berinde L, Panova M, Mas-Coma S (2016) CIAS detection of *Fasciola hepatica*/*F. gigantica* intermediate forms in bovines from Bangladesh. Acta Parasitol 61:267–277

Ai L, Dong SJ, Zhang WY, Elsheikha HM, Mahmmod YS, Lin RQ, Yuan ZG, Shi YL, Huang WY, Zhu XQ (2010) Specific PCR-based assays for the identification of *Fasciola* species: their development, evaluation and potential usefulness in prevalence surveys. Ann Trop Med Parasitol 104:65–72

Alasaad S, Soriguer RC, Abu-Madi M, El Behairy A, Jowers MJ, Baños PD, Píriz A, Fickel J, Zhu XQ (2011) A TaqMan real-time PCR-based assay for the identification of *Fasciola* spp. Vet Parasitol 179:266–271

Anonymous (1988) Parasitic diseases: hepatic distomiasis caused by *Fasciola hepatica*. Wkly Epidemiol Rec 63:109–111

Apt W, Aguilera X, Vega F, Alacaíno H, Zulantay I, Apt P, González G, Retamal C, Rodríguez J, Sandoval J (1993) Prevalencia de fascioliasis en humanos, caballos, cerdos y conejos silvestres en tres provincias de Chile. Boletín de la Oficina Sanitaria de Panamá 115:405–414

Apt W, Aguilera X, Vega F, Pérez C, Gabor M, Apt P (1995) Treatment of human chronic fascioliasis with triclabendazol: drug efficacy and serologic response. Am J Trop Med Hyg 52:532–535

Arenas R, Espinoza A, Padron E (1948) Fasciolasis hepática con carácter de brote epidémico. Rev Cubana Med Trop 4:92–97

Arjona R, Riancho JA, Aguado JM, Salesa R, González-Macías J (1995) Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. Medicine 74:13–23

Artigas P, Bargues MD, Mera y Sierra R, Agramunt VH, Mas-Coma S (2011) Characterisation of fascioliasis lymnaeid intermediate hosts from Chile by DNA sequencing, with emphasis on *Lymnaea viator* and *Galba truncatula*. Acta Trop 120:245–257

Ashrafi K, Massoud J, Houlakuei K, Mahmoodi M, Joafshani MA, Valero MA, Freire Fuentes MF, Khoubbane M, Artigas P, Mas-Coma S (2004) Evidence suggesting that *Fasciola gigantica* may be the most prevalent causal agent of fascioliasis in the endemic province of Gilan, northern Iran. Iran J Public Health 33:31–37

Ashrafi K, Valero MA, Massoud J, Sobhani A, Solaymani-Mohammadi S, Conde P, Khoubbane M, Bargues MD, Mas-Coma S (2006a) Plant-borne human contamination by fascioliasis. Am J Trop Med Hyg 75:295–302

Ashrafi K, Valero MA, Panov AM, Periago MV, Massoud J, Mas-Coma S (2006b) Phenotypic analysis of adults of *Fasciola hepatica*, *Fasciola gigantica* and intermediate forms from the endemic region of Gilan, Iran. Parasitol Int 55:249–260

Bargues MD, Vigo M, Horak P, Dvorak J, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, Mas-Coma S (2001) European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. Infect Genet Evol 1:85–107

Bargues MD, Horak P, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, Mas-Coma S (2003) Insights into the relationships of Palaearctic and Nearctic lymnaeids (Mollusca: Gastropoda) by rDNA ITS-2 sequencing and phylogeny of stagnicoline intermediate host species of *Fasciola hepatica*. Parasite 10:243–255

Bargues MD, Artigas P, Mera y Sierra RL, Pointier JP, Mas-Coma S (2007) Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and mitochondrial DNA. Ann Trop Med Parasitol 101:621–641

Bargues MD, Artigas P, Khoubbane M, Flores R, Glöer P, Rojas-García R, Ashrafi K, Falkner G, Mas-Coma S (2011a) *Lymnaea schirazensis*, an overlooked snail distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PLoS One 6:e24567

Bargues MD, Gonzalez C, Artigas P, Mas-Coma S (2011b) A new baseline for fascioliasis in Venezuela: lymnaeid vectors ascertained by DNA sequencing and analysis of their relationships with human and animal infection. Parasit Vectors 4:200

Bargues MD, Artigas P, Khoubbane M, Mas-Coma S (2011c) DNA sequence characterisation and phylogeography of *Lymnaea cousini* and related species, vectors of fascioliasis in northern Andean countries, with description of *L. meridensis* n. sp. (Gastropoda: Lymnaeidae). Parasit Vectors 4:132

Bargues MD, Malandrini JB, Artigas P, Soria CC, Velásquez JN, Carnevale S, Mateo L, Khoubbane M, Mas-Coma S (2016) Human fascioliasis endemic areas in Argentina: multigene characterisation of the lymnaeid vectors and climatic-environmental assessment of the transmission pattern. Parasit Vectors 9:306

Bargues MD, Gayo V, Sanchis J, Artigas P, Khoubbane M, Birriel S, Mas-Coma S (2017) DNA multigene characterization of *Fasciola hepatica* and *Lymnaea neotropica* and its fascioliasis transmission capacity in Uruguay, with historical correlation, human report review and infection risk analysis. PLoS Negl Trop Dis 11:e0005352

Barker SC, Blair D, Garret AR, Cribb TH (1993) Utility of the D1 domain of nuclear 28S rRNA for phylogenetic inference in the Digenea. Syst Parasitol 26:181–188

Bjorland J, Bryan RT, Strauss W, Hillyer GV, McAuley JB (1995) An outbreak of acute fascioliasis among Aymara Indians in the Bolivian Altiplano. Clin Infect Dis 21:1228–1233

Black J, Ntusi N, Stead P, Mayosi B, Mendelson M (2013) Human fascioliasis in South Africa. S Afr Med J 103:658–659

Blair D (1993) Molecular variation in fasciolids and *Paragonimus*. Acta Trop 53:277–289

Blair D, McManus DP (1989) Restriction enzyme mapping of ribosomal DNA can distinguish between fasciolid (liver fluke) species. Mol Biochem Parasitol 36:201–208

Botros SS, El-Lakkany NM, Badawy AA, Mahmoud SS, Ebeid FA, Fenwick A (2009) Mirazid shows insignificant activity against ovine fascioliasis. Ann Trop Med Parasitol 103:605–616

Brady MT, O'Nneill SM, Dalton JP, Mills KH (1999) *Fasciola hepatica* suppresses a protective Th1 response against *Bordetella pertussis*. Infect Immun 67:5372–5378

Brennan GP, Fairweather I, Trudgett A, Hoey E, McCoy, McConville M, Meaney M, Robinson M, McFerran N, Ryan L, Lanusse C, Mottier L, Alvarez L, Solana H, Virkel G, Brophy PM (2007) Understanding triclabendazole resistance. Exp Mol Pathol 82:104–109

Brito Alberto E, Hernandez Barreto MA, De La Fe RP, Silveira Pardo EA (2010) Prevalencia, decomisos de hígado y pérdidas económicas por *Fasciola hepatica* en mataderos bovinos de tres provincias de la región central de Cuba. REDVET Revista Electrónica de Veterinaria 11:1–7

Carnevale S, Rodriguez MI, Guarnera EA, Carmona C, Tanos T, Angel SO (2001) Immunodiagnosis of fasciolosis using recombinant procathepsin L cysteine proteinase. Diagn Microbiol Infect Dis 41:43–49

Carnevale S, Cabrera MG, Cucher MA, di Risio CA, Malandrini JB, Kamenetzky L, Alazraqui M, Etchart CB, Pantano ML, Velásquez JN (2013) Direct, immunological and molecular techniques for a fasciolosis survey in a rural area of San Luis, Argentina. J Parasit Dis 37:251–259

Chen MG, Mott KE (1990) Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. Trop Dis Bull 87:R1–R38

Chen JX, Chen MX, Ai L, Xu XN, Jiao JM, Zhu TJ, Su HY, Zang W, Luo JJ, Guo YH, Lv S, Zhou XN (2013) An outbreak of human fascioliasis gigantica in Southwest China. PLoS One 8:e71520

Clay RC, Straight WM (1961) Surgical removal of liver flukes from the common bile duct. JAMA 177:786–788

Cordova M, Herrera P, Nopo L, Bellatin J, Naquira C, Guerra H, Espinoza JR (1997) *Fasciola hepatica* cysteine proteinases: immunodominant antigens in human fascioliasis. Am J Trop Med Hyg 57:660–666

Cordova M, Reategui L, Espinoza JR (1999) Immunodiagnosis of human fascioliasis with *Fasciola hepatica* cysteine proteinases. Trans R Soc Trop Med Hyg 93:54–57

Coudert J, Triozon F (1958) Recherche sur l'épidémiologie de la distomatose humaine à *Fasciola hepatica*. A propos d'une épidémie récente de 500 cas. Rev Hyg Med Soc 6:840–864

Curtale F, Hammoud ES, El Wakeel A, Mas-Coma S, Savioli L (2000) Human fascioliasis, an emerging public health problem in the Nile Delta, Egypt. Res Rev Parasitol 60:129–134

Curtale F, Hassanein YAE, El Wakeel A, Mas-Coma S, Montresore A (2003a) Distribution of human fascioliasis by age and gender among rural population in the Nile Delta, Egypt. J Trop Pediatr 49:264–268

Curtale F, Mas-Coma S, Hassanein YAE, Barduagni P, Pezzotti P, Savioli L (2003b) Clinical signs and household characteristics associated with human fascioliasis among rural population in Egypt: a case-control study. Parassitologia 45:5–11

Cwiklinski K, Dalton JP, Dufresne PJ, La Course J, Williams DJ, Hodgkinson J, Paterson S (2015) The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. Genome Biol 16:71

Dalton JP, Robinson MW, Mulcahy G, O'Neill SM, Donnelly S (2013) Immunomodulatory molecules of *Fasciola hepatica*: candidates for both vaccine and immunotherapeutic development. Vet Parasitol 195:272–285

Danis M, Nozais JP, Chandenier J (1985) La distomatose à *Fasciola hepatica*. II: La fasciolose humaine en France. L'Action Vétérinaire 907:VII–VIII

De La Torre EE, Manzano-Román R, Valero L, Oleaga A, Pérez-Sánchez R, Hernández-González A, Siles-

Lucas M (2011) Comparative proteomic analysis of *Fasciola hepatica* juveniles and *Schistosoma bovis* schistosomula. J Proteomics 74:1534–1544

De NV, Murrell KD, Cong LD, Cam PD, Chaule V, Toan ND, Dalsgaard A (2003) The food-borne trematode zoonoses of Vietnam. Southeast Asian J Trop Med Public Health 34:S12–S34

De NV, Le TH, Waikagul, J (2006) Planborne trematodes and fascioliasis in Vietnam. In: Paper presented at the 5th Seminar on food- and water-borne parasitic zoonoses, Bangkok, 28–30 November 2006

Del Risco Barrios U, Vazquez Drake CT, Garcia Gonzalez G, Sánchez Casas A (2001) Evaluación de la excreción de huevos de *Fasciola hepatica* por tres esquemas terapéuticos. Archivo Médico de Camagüey 5:4

Diaz Fernandez R, Garces Martinez M, Millan Alvarez LM, Perez Lastre J, Millan Marcelo JC (2011) Comportamiento clínico-terapéutico de *Fasciola hepatica* en una serie de 87 pacientes. Rev Cubana Med Trop 63:268–274

El-Morshedy H, Farghaly A, Sharaf S, Abou-Basha L, Barakat R (1999) Triclabendazole in the treatment of human fascioliasis: a community-based study. East Mediterr Health J 5:888–894

Espinoza JR, Maco V, Marcos L, Saez S, Neyra V, Terashima A, Samalvides F, Gotuzzo E, Chavarry E, Huaman MC, Bargues MD, Valero MA, Mas-Coma S (2007) Evaluation of Fas2-ELISA for the serological detection of *Fasciola hepatica* infection in humans. Am J Trop Med Hyg 76:977–982

Esteban JG, Flores A, Aguirre C, Strauss W, Angles R, Mas-Coma S (1997a) Presence of very high prevalence and intensity of infection with *Fasciola hepatica* among Aymara children from the northern Bolivian Altiplano. Acta Trop 66:1–14

Esteban JG, Flores A, Angles R, Strauss W, Aguirre C, Mas-Coma S (1997b) A population-based coprological study of human fascioliasis in a hyperendemic area of the Bolivian Altiplano. Trop Med Int Health 2:695–699

Esteban JG, Bargues MD, Mas-Coma S (1998) Geographical distribution, diagnosis and treatment of human fascioliasis: a review. Res Rev Parasitol 58:13–42

Esteban JG, Flores A, Angles R et al (1999) High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. Trans R Soc Trop Med Hyg 93:151–156

Esteban JG, Gonzalez C, Bargues MD, Mas-Coma S (2002) High fascioliasis infection in children linked to a man-made irrigation zone in Peru. Trop Med Int Health 7:339–348

Esteban JG, Gonzalez C, Curtale F, Muñoz-Antoli C, Valero MA, Bargues MD, El-Sayed M, El-Wakeel AA, Abdel-Wahab Y, Montresor A, Engels D, Savioli L, Mas-Coma S (2003) Hyperendemic fascioliasis associated with schistosomiasis in villages of the Nile Delta, Egypt. Am J Trop Med Hyg 69:429–437

Fairweather I (2005) Triclabendazole: new skills to unravel an old(ish) enigma. J Helminthol 79:227–234

Fairweather I (2009) Triclabendazole progress report, 2005–2009: an advancement of learning? J Helminthol 83:139–150

Fairweather I, Boray JC (1999) Fasciolicides: efficacy, action, resistance and its management. Vet J 158:81–112

Favennec L, Jave Ortiz J, Gargala G, Lopez Chegne N, Ayoub A, Rossignol JF (2003) Double blind, randomized, placebo-controlled study of nitazoxanide in the treatment of fascioliasis in adults and children from northern Peru. Aliment Pharmacol Ther 17:265–270

Fentie T, Ergoiu S, Gedefaw M, Desta A (2013) Epidemiology of human fascioliasis and intestinal parasitosis among schoolchildren in Lake Tana Basin, Northwest Ethiopia. Trans R Soc Trop Med Hyg 107:480–486

Gaasenbeek CPH, Moll L, Cornelissen JBWJ, Vellema P, Borgsteede FH (2001) An experimental study on triclabendazole resistance of *Fasciola hepatica* in sheep. Vet Parasitol 95:37–43

Gaillet P, Liance M, Rivollet D, Houin R (1983) Situation de la fasciolose humaine en France, enquête retrospective portant sur les 30 dernières années. Bulletin de la Société Française de Parasitologie 1:79–82

Gargala G, Abboud P, Borsa-Lebas F (2005) Case report of successful treatment of triclabendazole resistant fascioliasis by nitazoxanide. In: Paper presented at the Medicine and Health in the Tropics (XVIth International Congress for Tropical Medicine and Malaria). Marseille, 11–15 Sept, 2005

Giap LH (1987) Distomatose hépatique à *Fasciola hepatica*. Université de Bordeaux II, Bordeaux, Laboratoire de Parasitologie

Girones N, Valero MA, Garcia-Bodelon MA, Chico-Calero I, Punzón C, Fresno M, Mas-Coma S (2007) Immune suppression in advanced chronic fascioliasis: an experimental study in a rat model. J Infect Dis 195:1504–1512

Gonzales-Santana B, Dalton JP, Vasquez-Camargo F, Parkinson M, Ndao M (2013) The diagnosis of human fascioliasis by enzyme-linked immunosorbent assay (ELISA) using recombinant cathepsin L protease. PLoS Negl Trop Dis 7:e2414

Gonzalez LC, Esteban JG, Bargues MD, Valero MA, Ortiz P, Náquira C, Mas-Coma S (2011) Hyperendemic human fascioliasis in Andean valleys: an altitudinal transect analysis in children of Cajamarca province, Peru. Acta Trop 120:119–129

Gonzalez-Miguel J, Valero MA, Reguera-Gome ZM, Mas-Bargues C, Bargues MD, Simón F, Mas-Coma S (2019) Numerous *Fasciola* plasminogen-binding proteins may underlie blood-brain barrier leakage and explain neurological disorder complexity and heterogeneity in the acute and chronic phases of human fascioliasis. Parasitology 146: 284–298

Haseeb AN, El Shazly AM, Arafa MAS, Morsy AT (2002) A review on fascioliasis in Egypt. J Egypt Soc Parasitol 32:317–335

Hien TT, Truong NT, Minh NH, Dat HD, Dung NT, Hue NT, Dung TK, Tuan PQ, Campbell JI, Farrar JJ, Day

JN (2008) A randomized controlled pilot study of artesunate versus triclabendazole for human fascioliasis in Central Vietnam. Am J Trop Med Hyg 78:388–392
Hillyer GV (1981) Fascioliasis in Puerto Rico: a review. Bol Asoc Med P R 73:94–101
Hillyer GV (1999) Immunodiagnosis of human and animal fasciolosis. In: Dalton JP (ed) Fasciolosis. CAB International, Oxon, pp 435–447
Hillyer GV, Soler de Galanes M, Rodriguez-Perez J, Bjorland J, Silva de Lagrava M, Ramirez-Guzman S, Bryan RT (1992) Use of the falcon assay screening test-enzyme-linked immunosorbent assay (FAST-ELISA) and the enzyme-linked immunoelectrotransfer blot (EITB) to determine the prevalence of human fascioliasis in the Bolivian Altiplano. Am J Trop Med Hyg 46:603–609
Huang WY, He B, Wang CR, Zhu XQ (2004) Characterisation of *Fasciola* species from Mainland China by ITS-2 ribosomal DNA sequence. Vet Parasitol 120:75–83
Hung NM, Dung DT (2011) Controlling helminthiasis period 2006-2010 and study in next period 2011-2015 in Vietnam. Bull Natl Inst Malariol Parasitol Entomol 2:7–15
Hurtrez-Bousses S, Durand P, Jabbour-Zahab R, Guégan JF, Meunier C, Bargues MD, Mas-Coma S, Renaud F (2004) Isolation and characterization of microsatellite markers in the liver fluke (*Fasciola hepatica*). Mol Ecol Notes 4:689–690
Ikeda T (1998) Cystatin capture enzyme-linked immunosorbent assay for immunodiagnosis of human paragonimiasis and fascioliasis. Am J Trop Med Hyg 59:286–290
Inoue K, Kanemasa H, Inoue K, Matsumoto M, Kajita Y, Mitsufuji S, Kataoka K, Okanoue T, Yamada M, Uchikawa R, Tegoshi T, Arizono N (2007) A case of human fasciolosis: discrepancy between egg size and genotype of *Fasciola* sp. Parasitol Res 100:665–667
Intapan PM, Mallewong W, Wongkham C, Tomanakarn K, Ieamviteevanich K, Pipitgool V, Sukolapong V (1998) Excretory-secretory antigen components of adult *Fasciola gigantica* recognized by infected human sera. Southeast Asian J Trop Med Public Health 29:579–583
Intapan PM, Sadee P, Wongkham C, Maleewong W (2004) Development of rapid agglutination test using *Fasciola gigantica* specific antigen for serodiagnosis of human fascioliasis. Southeast Asian J Trop Med Public Health 35:S313–S317
Jaffar Z, Sivakuru T, Roberts K (2004) CD4(+) CD25(+) T cells regulate airway eosinophilic inflammation by modulating the Th2 cell phenotype. J Immunol 172:3842–3849
Kabil SM, El Ashry E, Ashraf NK (2000) An open-label clinical study of nitazoxanide in the treatment of human fascioliasis. Curr Therapeut Res 61:339–345
Keiser J, Sayed H, El-Ghanam M, Sabry H, Anani S, El-Wakeel A, Hatz C, Utzinger J, El-Din SS, El-Maadawy W, Botros S (2011) Efficacy and safety of artemether in the treatment of chronic fascioliasis in Egypt: exploratory phase-2 trials. PLoS Negl Trop Dis 5:e1285
Kouri P, Arenas R (1932) La distomatosis hepática en Cuba. Tomo I, La Propagandista, La Habana, Cuba
Le TH, Blair D, McManus DP (2001) Complete DNA sequence and gene organization of the mitochondrial genome of the liver fluke, *Fasciola hepatica* L. (Plathelminthes; Trematoda). Parasitology 123:609–621
Le TH, Nguyen KT, Nguyen NTB, Doan HT, Le XT, Hoang CT, De NV (2012) Development and evaluation of a single step duplex PCR for simultaneous detection of *Fasciola hepatica* and *Fasciola gigantica* (Fasciolidae; Trematoda; Platyhelminthes). J Clin Microbiol 50:2720–2726
Lecaillon JB, Gobdillon J, Campestrini J (1998) Effect of food on bioavailability of triclabendazole in patients with fascioliasis. Br J Clin Pharmacol 45:601–604
Lee CG, Zimmerman GL, Bishop JK (1992) Host influence on the banding profiles of wholebody protein and excretory-secretory product of *Fasciola hepatica* (Trematoda) by isoelectric focusing. Vet Parasitol 41:57–68
Losos GJ (1986) Infectious tropical diseases of domestic animals. Longman Scientific & Technical, Essex
Lukambagire AS, Mchaile DN, Nyindo M (2015) Diagnosis of human fascioliasis in Arusha region, northern Tanzania by microscopy and clinical manifestations in patients. BMC Infect Dis 15:578
Maleewong W, Wongkham C, Intapan PM, Pipitgool V (1999) *Fasciola gigantica*-specific antigens: purification by a continuous-elution method and its evaluation for the diagnosis of human fascioliasis. Am J Trop Med Hyg 61:648–651
Marcilla A, Bargues MD, Mas-Coma S (2002) A PCR-RFLP assay for the distinction between *Fasciola hepatica* and *F. gigantica*. Mol Cell Probes 16:327–333
Marcos LA, Busalleu A, Terashima A, Espinoza JR (2009) Detection of antibodies against *Fasciola hepatica* in cirrhotic patients from Peru. J Helminthol 83:23–26
Marcos-Raymundo LA, Maco-Flores V, Terashima-Iwashita A, Frine-Samalvides C, Gotuzzo E (2002) Características clínicas de la infección crómica por *Fasciola hepatica* en niños. Rev Gastroenterol Peru 22:228–233
Martinez-Sernandez V, Muiño L, Perteguer MJ, Gárate T, Mezo M, González-Warleta M, Muro A, Correia da Costa JM, Romarís F, Ubeira FM (2011) Development and evaluation of a new lateral flow immunoassay for serodiagnosis of human fasciolosis. PLoS Negl Trop Dis 5:e1376
Mas-Coma S (2004a) Human fascioliasis. In: Cotruvo JA, Dufour A, Rees G, Bartram J, Carr R, Cliver DO, Craun GF, Fayer R, Gannon VPJ (eds) Waterborne zoonoses: identification, causes and control. World Health Organization (WHO), London, pp 305–322
Mas-Coma S (2004b) Human fascioliasis: epidemiological patterns in human endemic areas of South America, Africa and Asia. Southeast Asian J Trop Med Public Health 35:1–11

Mas-Coma S (2005) Epidemiology of fascioliasis in human endemic areas. J Helminthol 79:207–216

Mas-Coma S, Bargues MD (1997) Human liver flukes: a review. Res Rev Parasitol 57:145–218

Mas-Coma S, Bargues MD (2009) Populations, hybrids and the systematic concepts of species and subspecies in Chagas disease triatomine vectors inferred from nuclear ribosomal and mitochondrial DNA. Acta Trop 110:112–136

Mas-Coma S, Fons R, Feliu C, Bargues MD, Valero MA, Galan-Puchades MT (1987) Conséquences des phènomènes liés à l'insularité dans les maladies parasitaires. La grande douve du foie (*Fasciola hepatica*) et les Muridés en Corse. Bulletin de la Société Neuchâteloise des Sciences Naturelles 110:57–62

Mas-Coma S, Fons R, Feliu C, Bargues MD, Valero MA, Galan-Puchades MT (1988) Small mammals as natural definitive hosts of the liver fluke, *Fasciola hepatica* Linnaeus, 1758 (Trematoda: Fasciolidae): a review and two new records of epidemiologic interest on the island of Corsica. Riv Parassitol 5:73–78

Mas-Coma S, Esteban JG, Bargues MD (1999a) Epidemiology of human fascioliasis: a review and proposed new classification. Bull World Health Organ 77:340–346

Mas-Coma S, Bargues MD, Esteban JG (1999b) Human fasciolosis. In: Dalton JP (ed) Fasciolosis. CAB International, Oxon, pp 411–434

Mas-Coma S, Angles R, Esteban JG, Bargues MD, Buchon P, Franken M, Strauss W (1999c) The northern Bolivian Altiplano: a region highly endemic for human fascioliasis. Trop Med Int Health 4:454–467

Mas-Coma S, Bargues MD, Marty AM, Marty AM, Neafie RC (2000) Hepatic trematodiases. In: Meyers WM, Neafie AM, Marty AM, Wear DJ (eds) Pathology of infectious diseases, Helminthiases, vol 1. Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC, pp 69–92

Mas-Coma S, Funatsu IR, Bargues MD (2001) *Fasciola hepatica* and lymnaeid snails occurring at very high altitude in South America. Parasitology 123:S115–S127

Mas-Coma S, Bargues MD, Valero MA, Fuentes MV (2003) Adaptation capacities of *Fasciola hepatica* and their relationships with human fascioliasis: from below sea level up to the very high altitude. In: Combes C, Jourdane J (eds) Taxonomy, ecology and evolution of metazoan parasites, vol 2. Presses Universitaires de Perpignan, Perpignan, pp 81–123

Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278

Mas-Coma S, Bargues MD, Valero MA (2007) Plantborne trematode zoonoses: fascioliasis and fasciolopsiasis. In: Murrell D, Fried B (eds) World class parasites, Food-borne parasites, fish and plant-borne parasites, vol 11. Springer, New York

Mas-Coma S, Valero MA, Bargues MD (2009a) *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol 69:41–146

Mas-Coma S, Valero MA, Bargues MD (2009b) Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. Vet Parasitol 163:264–280

Mas-Coma S, Agramunt VH, Valero MA (2013) Direct and indirect affection of the central nervous system by *Fasciola* infection. Handb Clin Neurol 114:297–310

Mas-Coma S, Agramunt VH, Valero MA (2014a) Neurological and ocular fascioliasis in humans. Adv Parasitol 84:27–149

Mas-Coma S, Bargues MD, Valero MA (2014b) Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitology 141:S1918–S1946

Mas-Coma S, Bargues MD, Valero MA (2018) Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. Parasitology 145:S1665–S1699

Massoud J (1990) Fascioliasis outbreak of man and drug test (Triclabendazol) in Caspian littoral, northern part of Iran, 1989. Bulletin de la Société Française de Parasitologie 8:S438

Massoud, J (1993) Present status of human fascioliasis in Iran. In: Food-borne trematodes, World Health Organization, WHO Manual, Manila, Mimeogr. Rep. Sch/SG/93 w:19

McNulty SN, Tort JF, Rinaldi G, Fischer K, Rosa BA, Smircich P, Fontenla S, Choi YJ, Tyagi R, Hallsworth-Pepin K, Mann VH, Kammili L, Latham PS, Dell'Oca N, Dominguez F, Carmona C, Fischer PU, Brindley PJ, Mitreva M (2017) Genomes of *Fasciola hepatica* from the Americas reveal colonization with Neorickettsia Endobacteria related to the agents of Potomac horse and human Sennetsu fevers. PLoS Genet 13:e1006537

McVeigh P, Maule AG, Dalton JP, Robinson MW (2012) *Fasciola hepatica* virulence-associated cysteine peptidases: a systems biology perspective. Microbes Infect 14:301–310

Mera y Sierra R, Agramunt VH, Cuervo P, Mas-Coma S (2011) Human fascioliasis in Argentina: retrospective overview, critical analysis and baseline for future research. Parasit Vectors 4:104

Mezo M, Gonzalez-Warleta M, Ubeira FM (2003) Optimized serodiagnosis of sheep fascioliasis by fast-D protein liquid chromatography fractionation of *F. hepatica* excretory-secretory antigens. J Parasitol 89:843–849

Mezo M, Gonzalez-Warleta M, Carro C, Ubeira FM (2004) An ultrasensitive capture ELISA for detection of *Fasciola hepatica* coproantigens in sheep and cattle using a new monoclonal antibody (MM3). J Parasitol 90:845–852

Millan JC, Mull R, Freise S, Richter J (2000) The efficacy and tolerability of triclabendazole in Cuban patients with latent and chronic *Fasciola hepatica* infection. Am J Trop Med Hyg 63:264–269

Moghaddam AS, Massoud J, Mahmoodi M, Mahvi AH, Periago MV, Artigas P, Fuentes MV, Bargues MD,

Mas-Coma S (2004) Human and animal fascioliasis in Mazandaran province, northern Iran. Parasitol Res 94:61–69

O'Neill SM, Parkinson SM, Strauss W et al (1998) Immunodiagnosis of *Fasciola hepatica* (Fascioliasis) in a human population in the Bolivian Altiplano using purifi ed cathepsin L cysteine proteinase. Am J Trop Med Hyg 58:417–423

O'Neill SM, Parkinson SM, Dowd AJ, Strauss W, Angles R, Dalton JP (1999) Immunodiagnosis of human fascioliasis using recombinant *Fasciola hepatica* cathepsin L1 cysteine proteinase. Am J Trop Med Hyg 60:749–751

O'Neill SM, Brady MT, Callanan JJ, Mulcahy G, Joyce P, Mills KH, Dalton JP (2000) *Fasciola hepatica* infection downregulates Th1 responses in mice. Parasite Immunol 22:147–155

Olaechea F, Lovera V, Larroza M, Raffo F, Cabrera R (2011) Resistance of *Fasciola hepatica* against triclabendazole in cattle in Patagonia (Argentina). Vet Parasitol 178:364–366

Oliveira DR, Ferreira DM, Stival CC, Romero F, Cavagnolli F, Kloss A, Araújo FB, Molento MB (2008) Triclabendazole resistance involving *Fasciola hepatica* in sheep and goats during an outbreak in Almirante Tamandare, Parana, Brazil. Rev Bras Parasitol Vet 17:S149–S153

Ortiz P, Scarcella S, Cerna C, Rosales C, Cabrera M, Guzmán M, Lamenza P, Solana H (2013) Resistance of *Fasciola hepatica* against triclabendazole in cattle in Cajamarca (Peru): a clinical trial and in vive efficacy test in sheep. Vet Parasitol 195:118–121

Periago MV, Valero MA, Panova M, Mas-Coma S (2006) Phenotypic comparison of allopatric populations of *Fasciola hepatica* and *Fasciola gigantica* from European and African bovines using a computer image analysis system (CIAS). Parasitol Res 99:368–378

Periago MV, Valero MA, El Sayed M, Ashrafi K, El Wakeel A, Mohamed MY, Desquesnes M, Curtale F, Mas-Coma S (2008) First phenotypic description of *Fasciola hepatica* / *Fasciola gigantica* intermediate forms from the human endemic area of the Nile Delta, Egypt. Infect Genet Evol 8:51–58

Quang TD, Duong TH, Richard-Lenoble D, Odermatt P, Khammanivong K (2008) Emergence chez l'homme de fasciolose à *Fasciola gigantica* et de distomatose intestinale à *Fasciolopsis buski* au Laos. Cahiers Santé 18:119–124

Qureshi AW, Tanveer A, Mas-Coma S (2016) Epidemiological analysis of human fascioliasis in northeastern Punjab, Pakistan. Acta Trop 156:157–164

Qureshi AW, Zeb A, Mansoor A, Hayat A, Mas-Coma S (2019) *Fasciola hepatica* infection in children actively detected in a survey in rural areas of Mardan district, Khyber Pakhtunkhawa province, northern Pakistan. Parasitol Int 69:39–46

Raymundo LA, Maco Flores V, Terashima A, Samalvides F, Miranda E, Tantalean M, Espinoza JR, Gotuzzo E (2004) Hiperendemicidad de Fasciolosis humana en el Valle del Mantaro, Perú: factores de riesgo de la infeccion por *Fasciola hepatica*. Rev Gastroenterol Peru 24:158–164

Ripert C, Tribouley J, Luang Dinh Giap G, Combe A, Laborde M (1987) Epidémiologie de la fasciolose humaine dans le sud ouest de la France. Bulletin de la Société Française de Parasitologie 5:227–230

Roberts JA, Suhardono (1996) Approaches to the control of fasciolosis in ruminants. Int J Parasitol 26:971–981

Rojas L, Vazquez A, Domenech I, Robertson LJ (2009) Fascioliasis: can Cuba conquer this emerging parasitosis? Trends Parasitol 26:26–34

Rokni MB, Massoud J, O'Neill SM, Parkinson M, Dalton JP (2002) Diagnosis of human fasciolosis in the Gilan province of northern Iran: application of cathepsin L-ELISA. Diagn Microbiol Infect Dis 44:175–179

Rokni MB, Mirhendi H, Mizani A, Mohebali M, Sharbatkhori M, Kia EB, Abdoli H, Izadi S (2010) Identification and differentiation of *Fasciola hepatica* and *Fasciola gigantica* using a simple PCR-restriction enzyme method. Exp Parasitol 124:209–213

Rondelaud D, Belfaiza M, Vignoles P, Moncef M, Dreyfuss G (2009) Redial generations of *Fasciola hepatica*: a review. J Helminthol 83:245–254

Rossignol JF, Abaza H, Friedman H (1998) Successful treatment of human fascioliasis with nitazoxanide. Trans R Soc Trop Med Hyg 92:103–104

Sah R, Khadka S, Lakhey PJ, Pradhan S, Shah NP, Singh YP, Mas-Coma S (2018) Human case of *Fasciola gigantica-like* infection, review of human fascioliasis reports in Nepal, and epidemiological analysis within the south Central Asia. Acta Parasitol 63:435–443

Sampaio-Silva ML, Correia da Costa JM, Viana da Costa AM, Pires MA, Lopes SA, Castro AM, Monjour L (1996) Antigenic components of excretory-secretory products of adult *Fasciola hepatica* recognized in human infections. Am J Trop Med Hyg 54:146–148

Savioli L, Chistulo L, Montresor A (1999) New opportunities for the control of fascioliasis. Bull World Health Organ 77:300

Semyenova SK, Morozova EV, Chrisanfova GG (2003) RAPD variability and genetic diversity in two populations of liver fluke, *Fasciola hepatica*. Acta Parasitol 48:125–130

Sorribes J, Aubary MJ, Bargues MD et al (1990) Revisión de los casos humanos de Fascioliasis en España entre los años 1970 y 1989. In: Paper presented at the Congreso de Zoonosis, 1° Interautonómico, Reunión de la International Zoonoses Association (IZA), Valencia, Spain

Spithill TW, Smooker PM, Copeman DB (1999) *Fasciola gigantica*: epidemiology, control, immunology and molecular biology. In: Dalton JP (ed) Fasciolosis. CAB International, Oxon, pp 465–525

Spithill TW, Carmona C, Piedrafita D, Smooker PM (2012) Prospects for immunoprophylaxis against *Fasciola hepatica* (liver fluke). In: Caffrey CR, Selzer M (eds) Parasitic helminths: targets, screens, drugs and vaccines. Wiley, Weinheim, pp 465–484

Strauss W, O'Neill SM, Parkinson M, Angles R, Dalton JP (1999) Diagnosis of human fascioliasis: detection of

anti-cathepsin L antibodies in blood samples collected on filter paper. Am J Trop Med Hyg 60:746–748

Talaie H, Emami H, Yadegarinia D, Nava-Ocampo AA, Massoud J, Azmoudeh M, Mas-Coma S (2004) Randomized trial of a single, double and triple dose of 10 mg/kg of a human formulation of triclabendazole in patients with fascioliasis. Clin Exp Pharmacol Physiol 31:777–782

Tantrawatpan C, Maleewong W, Wongkham C, Wongkham S, Intapan PM, Nakashima K (2005) Serodiagnosis of human fascioliasis by cystatin capture enzyme-linked immunosorbent assay with recombinant *Fasciola gigantica* capthespsin L antigen. Am J Trop Med Hyg 72:82–86

Tinar R (1984) Yumurta boyutlarina gore *Fasciola gigantica* ile *Fasciola hepatica*' nin ayirimi uzerinde arastirmalar. Ankara Üniversitesi Veteriner Fakültesi 31:207–229

Torgerson P, Claxton J (1999) Epidemiology and control. In: Dalton JP (ed) Fasciolosis. CAB International, Oxon, pp 113–149

Trueba G, Guerrero T, Fornasini M, Casariego I, Zapata S, Ontaneda S, Vasco L (2000) Detection of *Fasciola hepatica* infection in a community located in the Ecuadorian Andes. Am J Trop Med Hyg 62:518

Tum S, Puotinen ML, Copeman DB (2004) A geographic information system model for mapping risk of fasciolosis in cattle and buffaloes in Cambodia. Vet Parasitol 122:141–149

Tum S, Puotinen ML, Skerratt LF, Chan B, Sothoeun S (2007) Validation of a geographic information system model for mapping the risk of fasciolosis in cattle and buffaloes in Cambodia. Vet Parasitol 143:364–367

Turrientes MC, Saenz de Santamaria A, Ceballos E, Díaz M, Barreno M, Muro A, Pardo J, López-Vélez R (2004) Fasciolosis importada y autóctona. Enfermedades Emergentes 6:188

Ubeira FM, Muiño L, Valero MA, Periago MV, Pérez-Crespo I, Mezo M, González-Warleta M, Romarís F, Paniagua E, Cortizo S, Llovo J, Más-Coma S (2009) MM3-ELISA detection of *Fasciola hepatica* coproantigens in preserved human stool samples. Am J Trop Med Hyg 81:156–162

Valero MA, Mas-Coma S (2000) Comparative infectivity of *Fasciola hepatica* metacercariae from isolates of the main and secondary reservoir animal host species in the Bolivian Altiplano high human endemic region. Folia Parasitol 47:17–22

Valero MA, Darce NA, Panova M, Mas-Coma S (2001) Relationships between host species and morphometric patterns in *Fasciola hepatica* adults and eggs from the northern Bolivian Altiplano hyperendemic region. Vet Parasitol 102:85–100

Valero MA, Santana M, Morales M, Hernandez JL, Mas-Coma S (2003) Risk of gallstone disease in advanced chronic phase of fascioliasis: an experimental study in a rat model. J Infect Dis 188:787–793

Valero MA, De Renzi M, Panova M, Garcia-Bodelon MA, Periago MV, Ordoñez D, Mas-Coma S (2006a) Crowding effect on adult growth, pre-patent period and egg shedding of *Fasciola hepatica*. Parasitology 133:453–463

Valero MA, Navarro M, Garcia-Bodelon MA, Marcilla A, Morales M, Hernandez JL, Mengual P, Mas-Coma S (2006b) High risk of bacterobilia in advanced experimental chronic fasciolosis. Acta Trop 100:17–23

Valero MA, Girones N, Garcia-Bodelon MA, Periago MV, Chico-Calero I, Khoubbane M, Fresno M, Mas-Coma S (2008) Anaemia in advanced chronic fasciolosis. Acta Trop 108:35–43

Valero MA, Perez-Crespo I, Periago MV, Khoubbane M, Mas-Coma S (2009) Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. Acta Trop 111:150–159

Valero MA, Periago MV, Perez-Crespo I, Angles R, Villegas F, Aguirre C, Strauss W, Espinoza JR, Herrera P, Terashima A, Tamayo H, Engels D, Gabrielli AF, Mas-Coma S (2012a) Field evaluation of a coproantigen detection test for fascioliasis diagnosis and surveillance in human hyperendemic areas of Andean countries. PLoS Negl Trop Dis 6:e1812

Valero MA, Periago MV, Perez-Crespo I, Rodríguez E, Perteguer MJ, Gárate T, González-Barberá EM, Mas-Coma S (2012b) Assessing the validity of an ELISA test for the serological diagnosis of human fascioliasis in different epidemiological situations. Trop Med Int Health 17:630–636

Valero MA, Perez-Crespo I, Khoubbane M, Artigas P, Panova M, Ortiz P, Maco V, Espinoza JR, Mas-Coma S (2012c) *Fasciola hepatica* phenotypic characterisation in Andean human endemic areas: valley versus altiplanic patterns analysed in liver flukes from sheep from Cajamarca and Mantaro, Peru. Infect Genet Evol 12:403–410

Valero MA, Bargues MD, Khoubbane M, Artigas P, Quesada C, Berinde L, Ubeira FM, Mezo M, Hernandez JL, Agramunt VH, Mas-Coma S (2016) Higher physiopathogenicity by *Fasciola gigantica* than by the genetically close *F. hepatica*: experimental long-term follow-up of biochemical markers. Trans R Soc Trop Med Hyg 110:55–66

Valero MA, Perez-Crespo I, Chillon-Marinas C, Khoubbane M, Quesada C, Reguera-Gomez M, Mas-Coma S, Fresno M, Gironès N (2017) *Fasciola hepatica* reinfection potentiates a mixed Th1/Th2/Th17/Treg response and correlates with the clinical phenotypes of anemia. PLoS One 12:e0173456

Vasquez P (1943) *Fasciola hepatica*, Reporte del primer caso observado en Cuba, en un niño. Arch Med Intern 12:163

Villegas F, Angles R, Barrientos R, Barrios G, Valero MA, Hamed K, Grueninger H, Ault SK, Montresor A, Engels D, Mas-Coma S, Gabrielli AF (2012) Administration of triclabendazole is safe and effective in controlling fascioliasis in an endemic community of the Bolivian Altiplano. PLoS Negl Trop Dis 6:e1720

WHO (1995) Control of foodborne trematode infections, WHO technical report series, vol 849. World Health Organization, Geneva

WHO (2007) Report of the WHO informal meeting on use of triclabendazole in fascioliasis control. World Health Organization, Geneva

WHO (2008) Fact sheet on fascioliasis. In: World Health Organization (ed) Action against worms. World Health Organization, Geneva, pp 1–8

WHO (2013) Sustaining the drive to overcome the global impact of neglected tropical diseases. World Health Organization, Geneva

Winkelhagen AJ, Mank T, de Vries PJ, Soetekouw R (2012) Apparent triclabendazole-resistant human *Fasciola* hepatica infection, the Netherlands. Emerg Infect Dis 18:1028–1029

Yilmaz H, Gödekmerdan A (2004) Human fasciolosis in Van province, Turkey. Acta Trop 92:161–162

Zumaquero-Ríos JL, Sarracent-Pérez J, Rojas-García R, Rojas-Rivero L, Martínez-Tovilla Y, Valero MA, Mas-Coma S (2013) Fascioliasis and intestinal parasitoses affecting schoolchildren in Atlixco, Puebla state, Mexico: epidemiology and treatment with nitazoxanide. PLoS Negl Trop Dis 7:e2553

Paragonimiasis

5

David Blair

5.1 Introduction

Paragonimiasis is a zoonotic disease caused by lung flukes of the genus *Paragonimus*. Humans usually become infected by eating freshwater crabs or crayfish containing encysted metacercariae of these worms. However, an alternative route of infection exists: ingestion of raw meat from a mammalian paratenic host. Adult worms normally occur in pairs in cysts in the lungs from which they void their eggs via air passages. The pulmonary form is typical in cases of human infection due to *P. westermani*, *P. heterotremus*, and a few other species (Table 5.1). Worms may occupy other sites in the body, notably the brain, but lung flukes have made their presence felt in almost every organ. Ectopic paragonimiasis is particularly common when infection is due to members of the *P. skrjabini* complex (Table 5.1). Human paragonimiasis occurs primarily in the tropics and subtropics of Asia, Africa, and the Americas, with different species being responsible in different areas (Table 5.1).

As agents of a "neglected tropical disease" (Utzinger et al. 2012), there is a tendency for people to regard lung flukes as unimportant and imposing a decreasing and trivial burden on human populations, having been eliminated in many formerly endemic areas. This is dangerous and misleading: paragonimiasis is a major and continuing problem. In 2005, about 292 million people were at risk and about 23 million people in 48 countries (mostly in China) infected (Fürst et al. 2012b; Utzinger et al. 2012). Of this total, over five million likely had heavy (i.e., symptomatic) infections, and about 166,000 had cerebral involvement. Supplementary tables in a later meta-analysis suggested only slight global declines between 2005 and 2015 (Global Burden of Disease 2015 2016).

Even in the twenty-first century, surveys of newly discovered endemic foci, or new examination of known foci, uncover prevalences of paragonimiasis that, for more fashionable diseases, would lead to great alarm. For example, paragonimiasis occupied fourth place on the list of causes of morbidity in the Municipality of Roxas, Philippines, in 2003 (Belizario et al. 2007) and 27.2% of over 900 registered tuberculosis patients had *Paragonimus* eggs in their sputum in 2002 (de Leon and Piad 2005). Prevalence in the same area was lower, but still high, in a study conducted in 2011–2013 (Belizario et al. 2014). A survey in NE India found local prevalence (based on serology) of 51.7% in children under 15 years of age and 18.7% in adults (Devi et al. 2007). Of these, 20.9% of children and 4.1% of adults had eggs in their sputum. In SE Nigeria, 13.2% of a population surveyed were egg-positive for paragonimiasis

D. Blair (✉)
College of Science and Engineering, James Cook University, Townsville, QLD, Australia

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_5

Table 5.1 The major species of lung flukes responsible for human disease

Species	Geographical distribution	Type of paragonimiasis in humans	Natural definitive hosts[a]	Notes
P. westermani complex	E Asia (China and Taiwan, Korea, Japan, SE Siberia); SE Asia (Philippines, Malaysia, Thailand, Cambodia, Laos, Vietnam), S Asia (Sri Lanka, India), possibly also Nepal[b], Pakistan and Papua New Guinea	Usually pulmonary; pleural disease not uncommon and other forms sometimes	Cercopithecids, canids, felids, herpestids, viverrids, mustelids, murids	A species complex exhibiting considerable geographical genetic variation. Apparently infective to humans only in E Asia and the Philippines
P. skrjabini and subspecies such as *P. s. miyazakii*	E Asia (China, Japan), Thailand; may extend westwards into NE India	Pulmonary forms rare; pleural and ectopic forms usual	Canids, felids, mustelids, viverrids, murids, hystricids	A species complex, but more restricted in distribution than *P. westermani*. Humans not usually suitable definitive host
P. heterotremus complex	S and SW China, Vietnam, Laos, Thailand, NE India, likely in Myanmar2	Usually pulmonary; ectopic cases reported (Singh et al. 2012a, b)	Felids, sciurids, murids	Likely also a species complex: one human case reported of *P. pseudoheterotremus*
P. africanus	W Africa: Equatorial Guinea, Cameroon, Nigeria, possibly Ivory Coast	Usually pulmonary	Lorids, cercopithecids, herpestids, viverrids	
P. uterobilateralis	W Africa: Gabon, Cameroon, Nigeria, Liberia	Usually pulmonary	Canids, herpestids, mustelids, viverrids	
P. kellicotti	N America: Mississippi Basin and Atlantic coast of the USA; Ontario and Quebec in Canada	Usually pleural and pulmonary	Didelphids, canids, felids, mustelids, procyonids, suids, bovids, murids	Rare in humans, but humans are permissive hosts
P. mexicanus	Central and S America: Mexico, Costa Rica, Panama, Guatemala, Ecuador, Peru; probably other countries in the region	Usually pulmonary	Didelphids, cebids, canids, felids, mustelids, procyonids, suids	Possibly a species complex

Additional species may occasionally infect humans

[a]Omits experimental hosts, paratenic hosts, and humans

[b]Pulmonary paragonimiasis is known from Myanmar and Nepal, but the causative species has not been identified with confidence: it could be either *P. heterotremus* or *P. westermani*

(Uttah 2013). It is important not to extrapolate too far from these focal data. They represent prevalences in particular communities or small areas and not national levels in any country.

In keeping with the persisting view that paragonimiasis is a rather quaint and unusual condition, medical journals sometimes feature quizzes and commentaries in which readers are invited to arrive at a diagnosis (Wilgus et al. 2017). Veterinary journals in N America, where infections of domestic pets with *P. kellicotti* sometimes occur, also quiz their readers on this topic (Palić et al. 2011). It would be interesting to know how many reach the correct diagnosis of paragonimiasis without being prompted.

Lung fluke disease is not a recent affliction of human societies. Evidence of paragonimiasis has been found in mummies and other archaeological

material in Japan, Korea, and pre-Columbian S. America (Sianto et al. 2009). Eggs of *Paragonimus* species have been found at a Third Century AD archaeological site in Japan (Matsui et al. 2003). In Korea (Shin et al. 2012), DNA sequences from eggs in tissues of a 400-year-old mummy confirmed the presence of *P. westermani*.

Despite its antiquity, paragonimiasis has spread its wings in the modern world. Cheap travel allows adventurous tourists the opportunity to try food that is new to them (and to take parasitic souvenirs home). However, even the stay-at-homes in non-endemic countries can sample parasites readily (Fried and Abruzzi 2010). Crabs and crayfish, incidentally containing lung fluke metacercariae, are now regularly shipped around the world, taking this and other diseases virtually anywhere (Robertson et al. 2014). There are many papers reporting paragonimiasis cases in returned travelers, or in people who have eaten imported foods (Diaz 2013; Parikh et al. 2016). Given the jet-age mobility of *Paragonimus*-infected crustaceans, lung flukes have been included in the European priority list of food-borne parasites for surveillance (Bouwknegt et al. 2018).

Recent publications focusing on the situation and trends in particular countries or regions include the following: China (Yang et al. 2000; Liu et al. 2008; Lv et al. 2013; Wang et al. 2016), Japan (Nakamura-Uchiyama et al. 2003; Nishida and Shibahara 2003; Otsuji 2003; Nawa and Nakamura-Uchiyama 2005; Nagayasu et al. 2015), Korea (Choi 1990; Cho et al. 1997; Shin et al. 2008; Choi et al. 2010), Vietnam (Doanh et al. 2005; Doanh et al. 2013b), Laos (Ratsavong et al. 2017), Thailand (Waikagul and Yoonuan 2005), The Philippines (de Leon and Piad 2005; Belizario et al. 2007), India (Singh et al. 2012b; Tandon et al. 2015), Nepal (Sah and Khadka 2017), Brazil (Tuon et al. 2008), Ecuador (Calvopiña et al. 2014), Colombia (Vélez et al. 2000), Peru (Ibáñez-Herrera 2012), USA (Procop 2009; Fischer and Weil 2015), Africa (Aka et al. 2008; Cumberlidge et al. 2018), South Africa (Appleton 2014), Cameroon (Nkouawa et al. 2009), and Nigeria (Okoro et al. 2013; Uttah 2013).

Some recent general reviews are available (Miyazaki 1991; Blair et al. 1999, 2007; Sugiyama et al. 2012; Chai 2013a; Nawa et al. 2014; Kong et al. 2015; Adams 2018).

5.2 Life Cycle of *Paragonimus* Species

Like other trematodes, *Paragonimus* species have complex life cycles (Fig. 5.1). Hermaphroditic adult worms, resembling small coffee beans in size and shape in life, typically occur in the lung parenchyma of mammals, encapsulated in (usually) pairs in a fibrous cyst about 10–15 mm in diameter (Sogandares-Bernal and Seed 1973). The cysts can communicate with bronchioles, allowing exit of eggs and cyst debris. Eggs, voided in sputum, or in feces if swallowed, hatch in freshwater to yield motile miracidia. These penetrate the tissues of particular species of freshwater snails, where a process of asexual multiplication produces many motile microcercous cercariae. Cercariae leaving the snail enter the tissues of a crustacean, usually a crab or crayfish (Shibahara 1991; Gyoten 2000), or possibly crabs may also be infected by eating infected snails (Shibahara 1991). Here, each cercaria develops and grows into a metacercaria (usually in the gills, heart, digestive gland and other viscera, or the muscles), a "sit-and-wait" stage that is infective to mammals. Freshwater crabs and crayfish are prized food for many mammal species, including humans. Ingestion of inadequately prepared crabs or crayfish leads to infection, with metacercariae emerging from their cysts in the stomach or intestine and migrating through tissues towards the pleural spaces and lungs (Miyazaki 1991). Worms can persist for many years in the lungs, thus obscuring their geographic origin if the patient has moved between countries. For example, a man was still expectorating eggs at least twenty years after leaving an endemic area (Yokogawa et al. 1960).

If infected crustaceans are eaten by mammals in which worms are unable to mature, the juvenile worms may remain quiescent in the tissues (Habe et al. 1996). If a suitable host eats such a

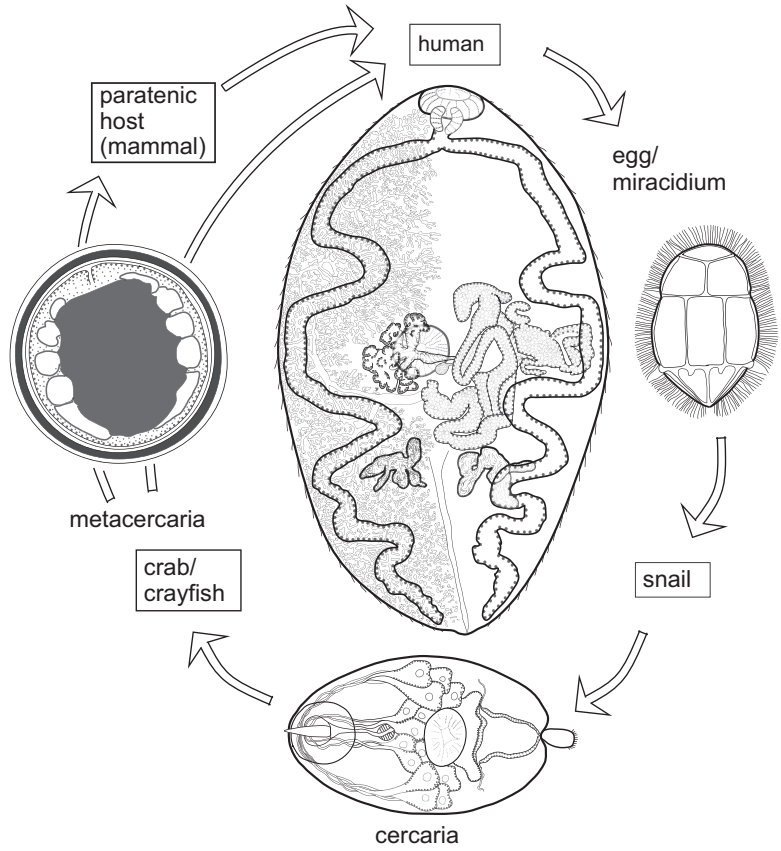

Fig. 5.1 Generalized life cycle of *Paragonimus* species. A miracidium hatching from the voided egg swims to find a suitable snail host. Within the hemocele of the snail, cycles of asexual reproduction produce numerous cercariae which leave the snail and penetrate a crab or crayfish. Here, they grow and transform into metacercariae. If eaten by a suitable mammal definitive host, metacercariae migrate to the lungs, where they mature. In some unsuitable mammal species, metacercariae burrow through the gut wall and remain as small juvenile worms within the musculature. If such paratenic hosts are eaten by a suitable definitive host, the juvenile worms can mature in their new home. See the main text for more details. The drawing of the adult in the center of the figure is a dorsal view based on a photograph of a strongly compressed specimen of *P. westermani* from Vietnam, supplied by Dr. P. N. Doanh. Vitellaria are indicated on the left side of the body only

paratenic host, worms from the latter may mature normally in their new home. Occurrence of paragonimiasis in top predators such as tigers, which are unlikely to eat small crabs directly, occurs via a range of paratenic hosts (Habe et al. 1996). By such a route, human hunters and their hunting dogs in Japan can become infected if they eat undercooked meat of wild boars (Nakano et al. 2009; Irie et al. 2017) or of deer (Yoshida et al. 2016).

5.3 Taxonomy of *Paragonimus* Species

Paragonimus Braun 1899 is the sole genus in the family Paragonimidae (Blair 2008). There have been two recent major taxonomic treatments of the family (Chen 1985; Kurochkin 1987). The earliest record of a lung fluke was the description by Diesing in 1850 of *Distomum rude*, based on

specimens found in the lungs of a giant otter in Brazil by Natterer in 1828. This was later placed in the genus *Paragonimus* (Stiles and Hassall 1900). Both the original description (Diesing 1850) and a redescription by Braun (1901) failed to reveal some features vital for unambiguous identification. The type specimens are in poor condition (Voelker et al. 1981) and no additional specimens have been found despite recent surveys of the original locality (Voelker et al. 1981; Tongu et al. 1993). The earliest described species that can be assigned to *Paragonimus* without doubt is *P. compactus* (Cobbold 1859) from an Indian mongoose (Cobbold 1859). The first human cases reported were from Taiwan and date to 1879 (Manson 1880). Since then, >50 names have been applied to members of the genus, the majority of these in eastern and southern Asia (Blair et al. 1999). Even within the last few years, new species have been proposed and described (Doanh et al. 2007; Waikagul 2007; Shan et al. 2009; Bayssade-Dufour et al. 2014, 2015).

Paragonimus arguably presents more taxonomic problems than does any other genus of food-borne trematodes. It will be important to resolve some of these because species (Miyazaki 1991) and even different populations within a nominal species (see below) can differ in their biology and pathological effects. It is still not possible to say how many species of *Paragonimus* exist. Critical reviews of the literature and recent molecular work suggest that many names ought to fall as synonyms (Blair et al. 1999, 2005). On the other hand, *P. caliensis*, frequently considered a synonym of *P. mexicanus*, was recently shown to be valid (Hernández-Chea et al. 2017).

Differentiation between species of lung flukes has long been based on some reasonably obvious morphological features (Miyazaki 1991; Blair et al. 1999; Singh et al. 2012b). For adults (see Fig. 5.1 for a typical example), these include arrangement of spines on the tegument, degree of branching of ovary and testes, body length/width ratios, and relative sizes of the suckers. Metacercarial cysts were long assumed to be morphologically conservative within a species, and many species have been proposed based only on the distinctive appearance of particular metacercariae. Characters of metacercariae that have been used include details of cyst walls (presence or absence, number and thickness), relative diameters of suckers, the anterior extent of the excretory bladder, presence, and length of a stylet on the oral sucker, presence of colored granules in the body, number of flame cells, body spination, and arrangement of papillae around suckers (Miyazaki 1991; Blair et al. 1999; Narain et al. 2009). In the case of eggs, there can be specific differences in size, shape, and shell sculpturing (Blair et al. 1999).

Until the application of molecular techniques to the taxonomy of the group, the stability and reliability of these morphological characters to distinguish among ~50 species could not be assessed. DNA sequence data (usually from portions of the mitochondrial genome and the nuclear ribosomal genes and spacers) have now shown that many morphological features of metacercariae in particular are uninformative or misleading (Blair et al. 1997, 2005; Iwagami et al. 2008; Doanh et al. 2015b, 2016a). While tending to be morphologically rather conservative, features of adults can also be misleading. The arrangement of surface spines (singly, or in comb-like clusters) has long been regarded as a major taxonomic marker. However, very closely related species, that in some cases can hybridize, may differ with respect to this feature (Blair et al. 1998; Nawa and Doanh 2009; Doanh et al. 2013a).

Molecular data have also indicated something else that was unexpected. Several "species" appear to represent species complexes. This is most apparent in the type species of the genus, *Paragonimus westermani* (Kerbert 1878) Braun, 1899, described from the lungs of a Bengal tiger that had died in Amsterdam Zoo in 1877 (Kerbert 1878). It turns out that "*P. westermani*" is very widespread, occurring in eastern and southern Asia, from China (and adjacent parts of SE Russia (Esaulova and Seryodkin 2012)), Japan, and Korea west to India (Devi et al. 2010) and south to Sri Lanka (Iwagami et al. 2000), the Philippines (Sato et al. 2003), and possibly Papua

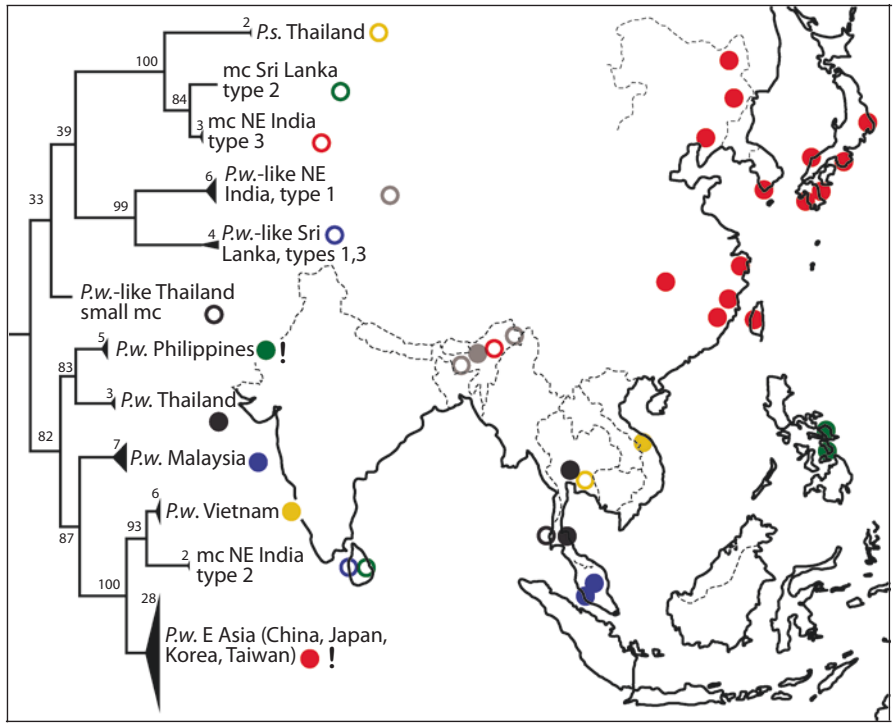

Fig. 5.2 Phylogenetic tree (based on partial mitochondrial *cox1* DNA sequences (382 bp)) of members of the *P. westermani* complex, with color symbols to show locations of populations on a map of eastern and southern Asia. The tree was inferred using MrBayes and the substitution model GTR+I+G. Posterior support values are shown at nodes. The tree was rooted by outgroup (not shown). Most available sequences from GenBank were used. Subtrees containing multiple sequences have been collapsed to a triangle, the number of sequences represented is indicated to the top left of the triangle. In some cases, sequences were derived from metacercariae alone (abbreviated as "mc") and adults were not obtained. "**!**" indicates human pathogen (there has also been one human case from NE India). See the main text for more details. "*P.w.*"—*P. westermani*. "*P.s.*"—*P. siamensis*

New Guinea (Wang et al. 2011). (Note that reports of endemic paragonimiasis caused by *Paragonimus westermani* in other parts of the world are likely to be erroneous, often based on the assumption that human cases are always due to this species). Note also that human cases due to *P. westermani* are likely only caused by populations of the complex from China, Japan, Korea, Taiwan, far SE Russia, the Philippines and, rarely, India.

Morphologically, adult "*P. westermani*" are very similar across this range, with spines spaced singly on the tegument, and testes and ovary possessing few, relatively simple, lobes. Hence, the application of the name *P. westermani* has not been controversial until recently. Another nominal species, *P. siamensis*, has been shown by molecular tools to nest within the *P. westermani* complex (Blair et al. 1998; Devi et al. 2013a) (Fig. 5.2). Adults of the former species differ from those of *P. westermani* mainly in having spines arranged in comb-like clusters.

Molecular and morphological uniformity of adults and metacercariae is greatest in eastern Asia (China, especially the east, NE, and south, Taiwan, Japan, Korea, and adjacent parts of the Russian Federation). This is also the region from which most human cases originate. In the western part of the range (Vietnam to India and Sri Lanka), there is more variation between populations: various metacercarial morphologies have been noted that often correspond with molecular differences yet remain within the *P. westermani* complex (Iwagami et al. 2003; Devi et al. 2010,

2013a). There are three morphologies in NE India (two phylogenetically close to *P. westermani sensu lato* and one close to *P. siamensis*) (Devi et al. 2013a), two metacercarial types in Thailand that segregate according to molecular data in different parts of a phylogenetic tree, yet yield indistinguishable adults of the *P. westermani* type (Binchai et al. 2007; Sugiyama et al. 2007) and at least two types in Sri Lanka (Iwagami et al. 2003, 2008). In Vietnam, numerous morphological variants of metacercariae can co-occur, but are not distinguishable in their DNA sequences (Doanh et al. 2016a). There is only one confirmed record of human infection with *P. westermani* in the region from Vietnam to the Indian subcontinent (Singh et al. 2015).

Individuals across most of the range of the *P. westermani* complex appear to be diploid, producing gametes by meiosis. However, in NE China, Japan, and Korea, triploid worms are common and often sympatric with diploids (Agatsuma et al. 2003). Triploids are unable to produce normal sperm (Fujino and Ishii 1982). However, they do produce eggs parthenogenetically. Thus, whereas diploid, sexually reproducing worms require partners for exchange of sperm and for cyst formation in the lungs of a mammal, each triploid worm is capable of cyst formation and egg production by itself (although more than one worm may occupy a cyst in heavy infections) (Miyazaki et al. 1981a, b). Triploid adult worms, their metacercariae and eggs are all larger than is the case for diploids. Tetraploid forms, which might produce viable gametes, have been found in China (Terasaki et al. 1996). Research on relationships and origins of the polyploid forms is likely to continue for some time (Bae et al. 2008; Saijuntha et al. 2016).

In addition to the genetic and ploidy differences within the *P. westermani* complex, there are marked biological differences in different places. Populations differ in their pre-patent period (Devi et al. 2010). Populations also differ in their preferred mammal hosts and in their snail hosts. For example, worm populations in the Philippines (and at least one member of the complex from India) mature readily in rats whereas those in Malaysia and Japan can utilize rats only as paratenic hosts (Habe et al. 1996; Devi et al. 2010). Geographical variation in apparent infectivity to humans has been mentioned earlier. Most strikingly, human cases attributable to members of the *P. westermani* complex are almost entirely restricted to the eastern part of the range (with the possible inclusion of Papua New Guinea) (Wang et al. 2011). This is probably not due to differing dietary habits: *P. heterotremus* is the principal or only species infecting humans in many areas where crabs containing metacercariae of *P. westermani* are also found (e.g., NE India, Laos, Vietnam), indicating unsafe consumption of crabs in those places. Knowledge of snail hosts in different regions is very limited and taxonomy of the relevant taxa is often confused (Attwood 2010; Adema et al. 2012; Doanh et al. 2018). However, different snail families are known to be used by members of the *P. westermani* complex in different countries (Blair et al. 2007; Iwagami et al. 2009).

All of this genetic and biological diversity has been detected so far in a single nominal species! Genetic distances, based on DNA sequences, between some members of the *P. westermani* complex are as great as between very distinct species of *Paragonimus* (Devi et al. 2013a). If future taxonomists decide to recognize several different species within the *P. westermani* complex, there will be some difficult nomenclatural problems to deal with (Blair et al. 2007).

Other species complexes exist within the genus. The *P. skrjabini* complex (Blair et al. 2005; Doanh et al. 2012) of eastern and southern Asia is the most important of these in terms of public health. On the basis of both morphological and molecular data, it has been proposed that *P. miyazakii* from Japan and *P. skrjabini* from China are only distinct from one another at the level of subspecies (Blair et al. 2005). Neither of these nominal taxa commonly matures in humans (Yatera et al. 2015), but they are a frequent cause of severe ectopic paragonimiasis (Table 5.1). Also in Asia, *P. bangkokensis* and *P. harinasutai* belong to a distinct complex, the membership of which has yet to be fully established (Doanh et al. 2009; Nawa and Doanh 2009). These two species are able to hybridize and may co-occur in the same individual

crab, yet have generally retained their separate identities (Doanh et al. 2013a). In the Americas, it has recently been proposed that the human pathogen *P. mexicanus* constitutes a number of cryptic species (López-Caballero et al. 2013).

Paragonimus heterotremus is emerging as an important cause of paragonimiasis spanning the region from India to southern China and Vietnam. Molecular and other variation is now being discovered within this species, and it may eventually be regarded as constituting another complex. In 2007, *P. pseudoheterotremus* was proposed as a sister species to *P. heterotremus* in Thailand, based on both morphological (primarily of the metacercarial stage) and molecular differences (Waikagul 2007; Thaenkham and Waikagul 2008). Later studies in Thailand and Vietnam showed that the situation is not clear-cut (Sanpool et al. 2013; Doanh et al. 2015b). Although phylogenies based on a mitochondrial gene identified several distinct geographic clusters within this putative species complex, all Vietnamese samples fell into a single cluster regardless of their metacercarial morphology (Doanh et al. 2015b). Biological differences relating to mammalian hosts have also been reported between *P. heterotremus* and *P. pseudoheterotremus*, but these might only reflect differences between local populations of a single widespread species (Doanh et al. 2015a).

5.4 Evolution

Very distinct species of *Paragonimus* live in Asia, Africa, and the Americas. These lie far apart in molecular phylogenies and, where known, snail hosts are also distinct between continents. Given this, the presence of *Paragonimus* species in the Americas likely long pre-dates the arrival of humans. This is unlike the situation for *Schistosoma mansoni*, introduced to S. America during the slave trade period (Morgan et al. 2005). Blair et al. (2001) proposed a Gondwanan origin for *Paragonimus*, with vicariance the explanation for its modern distribution whereas Attwood (2010) preferred an Asian origin with subsequent dispersal to other continents. Whichever is the case, by far the greatest diversity in the genus is in eastern Asia. Attwood has speculated that this diversity in Asia, in contrast with the few endemic *Schistosoma* species there, could be due to transmission ecology: *Schistosoma* species have largely adopted very vagile final hosts (often humans), aiding dispersal over large areas and inhibiting local speciation. *Paragonimus* species, on the other hand, tend to utilize definitive hosts that are less vagile and more likely to establish local transmission cycles, facilitating speciation (Attwood 2010).

Molecular phylogenies have been used in support of biogeographic and evolutionary scenarios for the *P. westermani* complex. It used to be thought that the complex first appeared in southern Asia, perhaps in what is now Sri Lanka. From there, populations radiated towards the north and east, evolving as they went and adopting new intermediate and final hosts (Blair et al. 2001; Iwagami et al. 2003). However, the recent findings of pairs (or trios) of relatively distantly related (based on molecular data) populations in both NE India and S Thailand (see above) defy such simple interpretation. Worse, one of the NE Indian forms is phylogenetically moderately close to populations in E Asia (Japan, Korea, E China) that were regarded as the most derived under earlier scenarios. Much more work will be needed to reach an understanding of the evolution of the *P. westermani* complex. Identities of snail hosts, very poorly known outside E Asia, may be illuminating in this regard.

5.5 Epidemiology and Control Measures

5.5.1 Prevalence and Changes

Comparisons of the situation in 2005 and 2015 in the "Global Burden of Disease Study 2015" suggest only slight declines in prevalence of paragonimiasis globally and slight to moderate declines in the number of years lived with disability (Table 5.2) (Global Burden of Disease 2015 2016). High levels of uncertainty in the data were indicated. Critical evaluation of estimates

Table 5.2 Estimates of prevalence of paragonimiasis globally and of years lived with disability (YLD) in 2005 and 2015

Severity and type of paragonimiasis	Prevalence in thousands (95% CI)		YLDs in thousands (95% CI)	
	2005	2015	2005	2015
Asymptomatic paragonimiasis	23,333.4 (13,242.2–30,251.8)	22,771.7 (12,990.5–29,225.4)	<0.05 (0.0 to <0.05)	<0.05 (0.0 to <0.05)
Cerebral paragonimiasis	222.3 (64.1–449.5)	181.8 (59.4–353.0)	34.6 (9.5–71.4)	22.6 (6.8–45.9)
Mild paragonimiasis	4,861.0 (677.2–11,657.1)	4,745.4 (646.0–11,394.6)	90.8 (12.3–230.8)	88.4 (11.8–222.4)
Moderate paragonimiasis	1,141.1 (156.1–2732.2)	1,113.9 (150.4–2642.8)	243.1 (32.8–631.5)	236.4 (31.7–611.0)
Severe paragonimiasis	1,383.4 (194.0–3270.3)	1,350.5 (189.4–3207.8)	530.2 (74.4–1391.1)	515.5 (71.3–1351.0)

Source: Supplementary material in Global Burden of Disease 2015 (2016)

of disability weight for paragonimiasis was the subject of a later study (Feng et al. 2018).

Published prevalence figures disguise a dynamic situation. In many places, prevalence has dropped to a very low level. On the other hand, prevalence in some known endemic areas has proved to be higher than previously thought and new foci have been discovered in recent years (see Sect. 5.5.1). In Nigeria, paragonimiasis was a major problem during the Biafran War (1967–1970). It then largely disappeared from many areas and was forgotten until recent surveys revealed startlingly high prevalence in the SE of the country (Umoh and Useh 2009; Eke et al. 2013; Uttah 2013). New foci have recently been found in Cameroon (Moyou-Somo et al. 2014), NE India (Singh et al. 2009, 2012b; Devi et al. 2013b), Vietnam (Doanh et al. 2005), and Laos (Odermatt et al. 2007, 2009; Ratsavong et al. 2017). It seems highly likely that many more foci will be found, especially in Africa and the Americas, where reported investigations are few (Culquichicón et al. 2017; Cumberlidge et al. 2018).

In contrast to the situation in many countries, Japan, Taiwan, and S Korea have been successful in reducing incidence to negligible levels. This has been done by mass-screening campaigns followed by chemotherapy and education as to the causes of the disease.

In S Korea, in excess of one million people were infected in the 1950s (Walton and Chyu 1959). Infection rates were between 7.4 and 52.8% in different parts of the country (Oh 1969). Huge reductions occurred from the 1970s to the 1990s (Cho et al. 1997) and the disease is nowadays regarded as rare, even a curiosity (Koh et al. 2012). In Taiwan, prevalence of paragonimiasis (based on findings of eggs in sputum samples) used to be as high as 24% in some areas (Cross 1984). Nowadays, the disease has virtually disappeared (Huang and Liu 2003).

China has a well-developed public-health system that includes surveillance and control of parasitic diseases (Yang et al. 2014; Wang et al. 2016). There have been targeted interventions to reduce prevalence of paragonimiasis (Zhong et al. 1981; Xu 1991). Despite this, the disease is still present in many areas, with an estimated overall seroprevalence in 8 endemic provinces in 2001–2004 of 1.7% (China, Coordinating Office of the National Survey on the Important Human Parasitic Diseases 2005). The extrapolation to an estimate of ~22 million infected persons nationally (Fürst et al. 2012b) might represent an overestimate since paragonimiasis is not uniformly present across China. Little can be said about trends in many other countries of the eastern/SE Asian region. In Thailand, reduction in prevalence has been noted (Yoonuan et al. 2008), whereas in endemic areas of the Philippines, prevalence remains high (Belizario et al. 2006).

In some hyperendemic areas, humans were probably the principal definitive hosts in the past (Kim 1969). However, crabs or crayfish infected with metacercariae can still be found in areas

where human prevalence is nowadays negligible (Song et al. 2017a), indicating that the parasites are still maintained in reservoir hosts such as peri-domestic cats and dogs (Cho et al. 1997; Shin and Min 1999; Liu et al. 2008; Zhou et al. 2008; Chen et al. 2012a). It is also certain that in many areas there is a true sylvatic cycle (wild mammals are the principal definitive hosts), a cycle that is very hard to break. *Paragonimus kellicotti* in parts of N America is maintained in a sylvatic cycle (Fischer et al. 2011) and the same likely applies to *P. mexicanus* and to African species.

5.5.2 Environmental Change and Pollution

Although frequently alluded to in the literature, it is hard to find quantitative information on the reduction of paragonimiasis that is due to environmental change and degradation (Cross 1984; Cho et al. 1997; Lou et al. 2011). Low prevalence in one Chinese village in an otherwise highly endemic area was a consequence of death of local stream animals due to mercury and cyanide contamination from a gold mine (Wang et al. 1998). Construction of the Three-Gorges Dam on the Yangtze River in China has raised concerns about the possible effects of this massive project on transmission of a number of parasite species (Jackson and Sleigh 2000). It is not clear the extent to which the dam has impacted on the upstream prevalence of *P. skrjabini* (Zhang et al. 2014; Peng et al. 2018).

5.5.3 Cultural and Dietary Habits Aiding Transmission

As a food-borne disease, host diet is obviously central in maintenance of paragonimiasis in a human population. Diet, in turn, is frequently culturally determined, making it hard to eliminate risky habits (Doanh et al. 2011). Freshwater crabs and crayfish are widely regarded as tasty snacks (Cho et al. 1997) or even as an important source of protein (Belizario et al. 2006). There are numerous accounts of appetizing local dishes that include undercooked crabs or crayfish (Yogore et al. 1958; Cabrera 1984; Singh et al. 1993; Song et al. 2008; Procop 2009). Crabs and crayfish have also been used for medicinal purposes. Juice filtered from crushed crayfish was used in the past to treat measles in Korea (Yun 1960; Choi 1990) and a correlation was noted between prevalence of measles and that of paragonimiasis (Park 1962). A similar use of crab juice persists in Laos (Song et al. 2008). In some parts of China and Africa, women eat crabs to enhance fertility (Wang et al. 1998; Moyou-Somo and Tagni-Zukam 2003). Infection also seems possible when simply handling infected crustaceans and utensils used in preparing them for the table (Choo et al. 2003), and there have been suggestions that infection can be acquired by drinking untreated water (Zhou et al. 2008; Kim 2014).

Changing dietary patterns are having a profound effect on epidemiology of paragonimiasis. In Japan during the 1950s, highest prevalences were seen in children (Uchiyama et al. 1999). Today, paragonimiasis, although rare, is mainly seen in middle-aged males (Nawa and Nakamura-Uchiyama 2005). This is because these men like to eat raw meat of wild boars or deer, common paratenic hosts of *P. westermani* there. Many of the dogs used for boar hunting are also infected (Kirino et al. 2009; Nakano et al. 2009; Irie et al. 2017): some deer and wild boars were seropositive for *P. westermani* (Nakano et al. 2009; Yoshida et al. 2016). In Korea, economic development, convenience food, and technological toys have lured people indoors and changed their diets (Cho et al. 1997). In Thailand and Nigeria, younger people are becoming less fond of traditional food, leaving older age-classes to carry the burden of paragonimiasis without them (Yoonuan et al. 2008; Uttah 2013).

Diet is also influenced by age, gender, and role in society. Thus in some areas where paragonimiasis is (or was) largely uncontrolled, it is (or was) a disease of children, as mentioned above for Japan. More recently, studies in NE India (Devi et al. 2007), China (Wang et al. 1998; Zhang et al. 2012; Peng et al. 2018), Cameroon (Moyou-Somo et al. 2014), and SE Nigeria (Ibanga and Eyo 2001; Umoh and Useh

2009) have all found highest prevalence in children. In SW Cameroon, the number of paragonimiasis cases has declined among adults but prevalence is still high in children under 10 years of age (Nkouawa et al. 2011). However, in some cultures, prevalence is highest in adults (Song et al. 2008; Uttah et al. 2013).

Gender biases have been noted in many reports, with male patients generally outnumbering females (Sadun and Buck 1960). One meta-analysis estimated a global male/female ratio of 1.267 in numbers of paragonimiasis cases but did acknowledge that ratios vary considerably from place to place (Fürst et al. 2012b). In China, boys greatly outnumber girls among paragonimiasis patients (Xu et al. 2012; Gong et al. 2017; Peng et al. 2018), perhaps suggesting that boys are more adventurous in catching and eating raw crabs. The bias towards infection in adult male Koreans in the past might have been because they were more likely than females to drink alcohol, which was accompanied by ingestion of freshwater crabs (Sadun and Buck 1960). In Laos, prevalence is higher among males, possibly because they were more likely to work outdoors where they could find and eat crabs (Song et al. 2008). Some reports from NE India noted that almost all patients were male (Singh et al. 1986), but this could be due to failure of females to seek medical attention for cultural reasons. Surveys in different parts of NE India did not detect a gender bias (Devi et al. 2007; Sunanda et al. 2016). In one Nigerian study, women had a relatively high prevalence because they were more likely to eat crabs (Uttah 2013), perhaps while handling and preparing food, but prevalence was also high among fishermen working in local rivers (Uttah et al. 2013). Cases of paragonimiasis due to *P. kellicotti* in the USA are nowadays mostly seen in male campers and canoeists who eat raw crayfish to show off to their peers (Lane et al. 2012).

Nor are urban dwellers and tourists safe. Metacercariae are still found in crabs sold in markets in large Japanese cities such as Tokyo and Fukuoka (Sugiyama et al. 2009). A recent national survey for parasitic diseases in China found the highest prevalences of paragonimiasis in the municipalities of Shanghai and Chongqing (China, Coordinating Office of the National Survey on the Important Human Parasitic Diseases 2005; Zhou et al. 2008). Presumably this is because urban dwellers have a relatively high disposable income and can afford to eat luxuries such as crabs or cultured crayfish imported from rural areas (Feng et al. 2018; Peng et al. 2018). Thus, urban paragonimiasis in China is a disease of affluence rather than of poverty.

Infected crabs may also cross oceans (Fürst et al. 2012a; Diaz 2013) to be served in Asian restaurants in the USA where they have caused paragonimiasis in American patrons (Boland et al. 2011; Wright et al. 2011; Parikh et al. 2016). Increased globalization of the food supply is likely to lead to many more cases confounding medical practitioners in non-endemic areas (Robertson et al. 2013). Not surprisingly, immigrants arriving in non-endemic areas often bring their diseases with them (Fried and Abruzzi 2010). And of course, tourists might return from tropical holidays with more souvenirs than they bargained for (Malvy et al. 2006).

5.5.4 Control Measures

The World Health Organization has set out a goal that, by 2020, 75% of the population at risk of food-borne trematode infections (including paragonimiasis) should be reached by preventive chemotherapy so that morbidity due to these infections is controlled in all endemic countries (World Health Organization 2015).

Low-cost provision of relevant education is one component of intervention which has the benefit of lacking clinical side effects and ethical issues. Awareness of the disease and how it can be prevented, both among medical professionals and the general public, are key to efforts to reduce prevalence of paragonimiasis. However, many countries are now paying less attention to parasites and parasitology in their medical curricula and universities (Peng et al. 2012; Song et al. 2017b). There has been some progress: in China, a new program has been established in Chongqing to train medical workers about *P. skrjabini* (Zhang et al. 2013), and "information, education and communication" strategies are proposed as part of the drive to eliminate neglected tropical diseases

(Yang et al. 2014). Localized awareness campaigns have been mounted recently in the USA (targeting campers and canoeists who might be tempted to eat crayfish during their adventures) (Patrick et al. 2010) and Colombia (targeting school children) (Arias et al. 2011).

The importance of combining treatment with education about paragonimiasis has been demonstrated in a remote part of NE India. Infected individuals were treated during a visit by a health team in 2005 (Devi et al. 2007), and provided with information about the disease and how to avoid it (Narain et al. 2015). Six years later, the area was revisited and a highly significant ($P<0.001$) decline in prevalence of paragonimiasis was found, both in terms of serological tests and egg-positivity. For example, in 2005 51.7% of children ($n = 263$) were seropositive, but only 15.9% ($n = 305$) in 2011 (Narain et al. 2015). In the Philippines, surveys, followed by treatment, were accompanied by health education material (Belizario et al. 2014). In targeted regions of northern Vietnam, prevalence of paragonimiasis fell greatly after a combination of health education and mass drug administration (World Health Organization 2017).

The possibility of developing a vaccine against paragonimiasis has been mentioned a few times in the literature and candidate molecules have been suggested (Zhao et al. 2007; Kasny et al. 2009). Given the difficulties involved in producing vaccines against any helminth species (MacDonald et al. 2002; Haçarız and Sayers 2016) and the logistical difficulties of distribution, storage, and mass administration, it does not seem practical to pursue the idea of a vaccine for paragonimiasis. For a more upbeat assessment of the future of vaccines against metazoan parasites, see Stutzer et al. (2018).

5.6 Genomics and Proteomics

Proteomic and genomic data are providing new insights into molecular mechanisms used by parasites in all areas of their physiology, but especially those involved with pathogenesis and immune evasion, and those that might be drug targets or useful for diagnosis (Blair et al. 2016; Haçarız and Sayers 2016). Research on the "omics" of *Paragonimus* species has lagged behind that on the genus *Schistosoma*. Nevertheless, work to produce draft genomes of several *Paragonimus* species is well underway (Martin et al. 2018). Analysis of transcriptome data for *P. kellicotti*, produced as part of this sequencing initiative, predicted many peptides, of which over 2000 were verified by mass spectrometry (McNulty et al. 2014). Immunoaffinity purification of proteins from adult worm homogenate, followed by mass spectrometry, identified 321 transcripts that were immunoreactive. Among these were cysteine proteases and a putative myoglobin (McNulty et al. 2014). A later study analyzed the transcriptomes of adult *P. westermani* and *P. skrjabini* (Li et al. 2016), and found these to be broadly similar to that of *P. kellicotti*. However, some species-specific transcripts were noted. A myoglobin isoform, first noted as immunogenic and highly represented in *P. kellicotti* (McNulty et al. 2014), showed strong sequence conservation with the other two species sequenced, but was not conserved widely across the trematodes. The authors speculated that this might have utility as a pan-*Paragonimus* diagnostic molecule (Li et al. 2016).

MicroRNAs have been reported from *P. westermani* (Ai et al. 2012). Small RNA molecules were sequenced using high-throughput technology on a Solexa machine. This class of molecule might be used for diagnosis and as targets for therapeutic intervention (Cai et al. 2016).

5.7 Immunobiology

In their mammalian host, lung flukes need to be able to move through tissues, feed, and void wastes and eggs. They also need to evade and subvert, for months or years, the effects of the host immune system that might react against their bodies or products (Schwartz et al. 2018). All this they often do without causing overt disease in the host: overt disease is likely to have a strong immunopathological component (McSorley and Maizels 2012). Compared to the range of immunological studies on *Schistosoma*

species, relatively little work has been done specifically on *Paragonimus* species. However, all helminths elicit rather similar immune responses in their hosts (Maizels et al. 2004), so it is likely that their methods of immune evasion have features in common. To quote Maizels et alia (Maizels et al. 2004) directly: "Helminths do not simply ward off immune attack; rather, they influence and direct immune responses away from the modes most damaging to them, regulating the host immune response to create niches that optimize successful feeding and reproduction". The main players most often mentioned on the host's team are the cells and molecules involved in the T_H2 response, including effector cells such as eosinophils (Behm and Ovington 2000). Eosinophilia is a very frequent finding in paragonimiasis. Many functions and properties have been attributed to eosinophils, including exocytic attack on helminths via released substances, and pro-inflammatory effects as well as mechanisms to modulate immune processes (Rosenberg et al. 2013). Although *in vitro* destruction of parasites by eosinophils has been demonstrated, the situation *in vivo* is far from simple and will be an important focus for future research (Anthony et al. 2007). The literature on *Paragonimus* infections has tended to emphasize the interplay between eosinophils and the parasite. Lung fluke species deploy a wide range of substances against the immune system, notably proteases and other excretory-secretory products (ESPs). Cysteine proteases (CPs), of which there are at least 15 different types (Lee et al. 2006), are heavily represented among ESPs and genes encoding them are among the most abundantly transcribed in the adult worm genome (Kim et al. 2006; Ahn et al. 2015). CPs are involved in metacercarial excystment (Chung et al. 1995), lysis of host tissue to allow migration of larvae (Na et al. 2006; Caffrey et al. 2018) and interaction with the immune system in various ways (Chung et al. 2008). In the last class of activities, CPs have been demonstrated to break down host IgG (Chung et al. 1997) thus having a suppressive effect on eosinophil function (Shin et al. 2001). CPs may also stimulate eosinophils to degranulate and produce superoxides (Chung et al. 2008; Shin et al. 2009).

The roles of protease inhibitors, produced by the parasites, are less well understood. Such inhibitors may break down host proteases, thus protecting the parasite from attack, and may also act to modulate the effects of proteases produced by the parasites themselves (Ranasinghe and McManus 2017).

Many ESP components remain poorly characterized: over 140 protein spots could be seen after two-dimensional gel electrophoresis of ESP material (Lee et al. 2006). As-yet unidentified components of ESP, in relatively high concentrations, increase the rate of apoptosis in eosinophils, likely favoring worm survival (Min et al. 2004). Substances in ESP can also regulate IL-8 production in human eosinophils (Shin and Lee 2000). Other molecules produced by *P. westermani* can act to detoxify reactive oxygen species produced by cells of the immune system (Li et al. 2005; Kim et al. 2007c, 2009).

5.8 Clinical Manifestations and Pathology

Many useful overviews of clinical aspects have appeared since the year 2000 (Marty and Neafie 2000; Jeon et al. 2005; Procop 2009; Singh et al. 2012b; Chai 2013a).

It is not always appreciated that clinical manifestations can differ according to the species of *Paragonimus* involved (Table 5.3), yet many case reports do not identify the species. Feng et al. (2018), in a major analysis of disability weight as applied to paragonimiasis, extracted information from 80 papers from an initial pool of 1378 papers. Among the 80 articles, 64 (mostly from China) did not name the species causing paragonimiasis: the researchers inferred the species responsible based on the geographical location of cases (Feng et al. 2018). This could impact on the accuracy of data in Table 5.3.

5.8.1 Early Infection

After ingestion, metacercariae excyst in the small intestine and migrate through the wall of the gastrointestinal tract. From the abdominal cavity,

Table 5.3 Comparisons of outcomes of infection with *P. westermani* (*Pw*) and *P. skrjabini* (*Ps*) in China

Outcome	Percent *Pw* cases with particular outcome (*n*)	Percent *Ps* cases with particular outcome (*n*)
Lung outcomes	26.0 (578)	14.6 (748)
Pleural outcomes	14.5 (321)	20.3 (1039)
Pericardial outcomes	1.4 (31)	4.9 (252)
Headache	11.3 (251)	8.0 (411)
Epilepsy	0.5 (12)	1.5 (79)
Motor loss	0.3 (7)	1.5 (79)
Vision impairment	0.0 (1)	0.4 (18)
Diarrhea	6.9 (154)	2.0 (104)
Abdominal pain	13.7 (304)	10.4 (530)
Hepatomegaly	13.1 (290)	7.9 (404)
Skin rash	3.3 (73)	1.6 (83)
Subcutaneous mass	8.9 (197)	26.8 (1371)

Source: meta-analysis by Feng et al. (2018), their supplementary material Table 1

Note that in most cases the identity of the causative species was inferred from the geographical location of the case (Feng et al. 2018). This may have led to some inaccuracies

juvenile worms generally migrate to the pleural cavity, sometimes via the liver. This is the acute stage of the disease that lasts for less than a month (Zhong et al. 1981; Procop 2009). Hepatic injury may occur in early infection, especially in children (Chen et al. 2001). Signs and symptoms are often few during this phase except in heavy infections. However, if present, these may include fever, abdominal discomfort and diarrhea, which can appear in as little as 2–4 days after ingestion of metacercariae (Zhong et al. 1981). Eosinophilia and elevated IgE levels are typical laboratory findings in the early stages (Cho et al. 2011).

5.8.2 Pleural Manifestations

Pleural manifestations may appear prior to parenchymal/pulmonary ones (Procop 2009) and may persist for long periods, even after apparent cure (Vidamaly et al. 2009). Activities of worms in the pleural spaces can cause pleural effusion, pneumothorax, and thickening of the pleura. Such effects may be particularly common in light infections of diploid *P. westermani* in which an individual, unable to find a mate, continues to wander in the pleural spaces eliciting a response from the host. Such unmated individuals can produce unviable eggs (Miyazaki et al. 1981b). Light infections, producing pleural symptoms, are typically seen in Japan nowadays (Nakamura-Uchiyama et al. 2002), but such symptoms appear to be less common in Korea (Jeon et al. 2005) and Laos (Kanpittaya et al. 2010). Eggs of North American *P. kellicotti* seem to elicit a particularly strong inflammatory response and pleural symptoms and effusion are usual (Procop 2009). Since cysts containing adult worms tend to be peripheral in the lungs, often close to pleural surfaces (Im et al. 1997), it is not surprising that some cysts discharge eggs into pleural spaces (Procop 2009).

Symptoms related to pleural manifestations include chest pains and those related to the presence of pleural effusion. Laboratory findings might include peripheral blood eosinophilia and elevated IgE levels. Pleural effusion can be variable in appearance (Otsuji 2003) but is frequently eosinophilic, with low pH, low glucose, and high lactate dehydrogenase and protein levels (Romeo and Pollock 1986; Taniguchi et al. 2001; Jeon et al. 2005; Light 2011; Hwang et al. 2015) and is often a good source of anti-*Paragonimus* antibodies (Cho et al. 2011). It may also contain fluke eggs (Thewjitcharoen and Poopitaya 2006; Wright et al. 2011; Singh et al. 2014), or even adult flukes (Heath and Marshall 1997; Vidamaly et al. 2009). Pleural manifestations are common and pulmonary symptoms rare in infections due to *P. skrjabini* subspecies (Otsuji 2003; Zhang et al. 2012).

5.8.3 Pulmonary Manifestations

Although they are hermaphroditic, adults of *Paragonimus* species do not self-fertilize (Miyazaki et al. 1981b). Diploid worms need to exchange sperm with another individual and move into a location from which they can void their eggs to the environment. Worms encounter

others in the pleural spaces then move into the lung parenchyma where pairs become encapsulated in a cyst that becomes increasingly fibrotic and typically up to 2 cm in diameter (Lumsden and Sogandares-Bernal 1970; Choi 1990). Within this, worms exchange sperm and produce eggs, sometimes for many years. This stage is reached not less than one month after initial infection, but can take much longer (Blair et al. 1999), and signals the starts of the chronic phase. The cysts rupture into bronchi or bronchioles, permitting escape of eggs and other cyst contents. Eggs may be expectorated in sputum or swallowed and voided via feces. Presence of worms encapsulated in the lungs produces classic pulmonary paragonimiasis, of which symptoms are cough (which may be worsened by exertion), hemoptysis, dyspnea, chest pains, fatigue, fever, and loss of appetite (Kagawa 1997; Chen et al. 2001). The first four symptoms in this list are the most commonly seen. "Rusty" sputum, containing parasite eggs, blood, necrotic material, and Charcot-Leyden crystals, is reported in a proportion of patients and is the classic sign of paragonimiasis. Symptoms may be more pronounced in heavy infections. A proportion of individuals, even those with heavy pulmonary infections, remain symptom-free (Im et al. 1997).

5.8.4 Ectopic Paragonimiasis

Although pulmonary paragonimiasis is the best-known form of the disease in humans, ectopic paragonimiasis is also common. Cerebral, cutaneous, abdominal (Kim et al. 2007a; Cho et al. 2011; Lee et al. 2012), and hepatic (Kim et al. 2004; Cheng et al. 2010; Li et al. 2012; Ye et al. 2017) sites are the most commonly mentioned in the literature, but lesions due to the activities of worms have been found in almost every part of the body, including a finger-tip (Sim et al. 2010), eyelids (Than et al. 1994), and genitalia (Jiang et al. 2015; Liang et al. 2018). Hepatic paragonimiasis is commonly reported from Sichuan Province in China and is likely caused by *P. skrjabini* (Lu et al. 2012). Common symptoms are chronic abdominal or back pain, low-grade fever, and nausea (Lu et al. 2012).

Not surprisingly, cerebral paragonimiasis has received much attention because it can have lethal consequences (causing an estimated 244 deaths in 2005) (Fürst et al. 2012b) and is the most commonly reported form of ectopic paragonimiasis (Yokogawa 1965).

Cerebral paragonimiasis due to *P. westermani* was regarded as the commonest form of "brain tumor" in Korea in the 1960s, with an estimated 5000 cases living in Korea in January 1966 (Oh 1969). The condition was seen predominantly in adolescent and young adult males. Today, its rarity in Korea is such that medical practitioners encountering it there are likely to misdiagnose it (Koh et al. 2012). At least in the case of infection due to *P. westermani*, cerebral paragonimiasis is more likely in individuals with an established pulmonary infection, and about 0.8% of those with active pulmonary paragonimiasis in Korea develop the cerebral form (Oh 1969). The cerebral form is much more prevalent among hospitalized paragonimiasis patients (Oh 1969; Lv et al. 2010). A recent estimate is that 0.72% of all paragonimiasis cases have cerebral involvement (Fürst et al. 2012b) The proportion of people infected with members of the *P. skrjabini* complex who develop cerebral symptoms is likely to be relatively high (Lv et al. 2010) and lung lesions are much less common (Zhang et al. 2012; Chen et al. 2013). Sixteen percent of 213 cases of paragonimiasis due to *P. skrjabini* in China had symptoms suggestive of neurological involvement (severe headache and vomiting) (Zhang et al. 2012). Reports of cerebral paragonimiasis caused by other species are few (Procop 2009; Singh et al. 2011).

Worms possibly reach the brain by traveling via tissue through the jugular foramen into the interior of the skull (Kusner and King 1993), penetrating the meninges and burrowing into the brain (Cha et al. 1994). This is the "active" phase, during which worms are alive and moving in tissues, secreting a range of biologically active compounds, and frequently expelling eggs. Worms and eggs become surrounded by granulomatous lesions that can be cystic or solid, the former type

containing caseous necrotic material (Cha et al. 1994). These lesions eventually become calcified. Adult worms may not persist for long periods, but numerous eggs may be found in the lesions they have produced (Choo et al. 2003), even when many years have probably elapsed since the death of the adult worms (Huang and Liu 2003; Amaro et al. 2016).

Many neurological signs and symptoms of cerebral paragonimiasis have been reported (Kusner and King 1993). The most common are seizures, headache, vomiting, nausea, and fever and localized weakness (Kusner and King 1993; Im et al. 1997). Decline in cognitive function and visual disturbances are also frequent (Lv et al. 2010). Many of these are most pronounced in the early stages while worms are actively moving around, and most deaths occur during this stage. Epileptic seizures are often associated with cerebral paragonimiasis (Wagner and Newton 2009).

Subcutaneous nodules, often migratory, are a common manifestation of ectopic paragonimiasis. These most frequently appear on the abdomen and chest (Zhong et al. 1981). On surgical removal, such nodules sometimes contain worms (Miyazaki and Harinasuta 1966) or eggs (Kim et al. 2007b; Lee et al. 2012). This form of ectopic infection is relatively uncommon when *P. westermani* is the causative species, but cases are reported from time to time (Ashitani et al. 2000; Chen et al. 2001; Dainichi et al. 2003; Kim et al. 2007b; Lee et al. 2012; Xu et al. 2012). The first reported case of paragonimiasis due to *P. heterotremus* presented in Thailand with subcutaneous nodules (Miyazaki and Harinasuta 1966) and cases from India (Singh et al. 2012a) are likely also to be due to this species. Such nodules are particularly common in paragonimiasis caused by members of the *P. skrjabini* complex that rarely mature in humans (Xu 1991). In China, three different studies reported that 56%, 42%, and 20.4% of patients, respectively, exhibited subcutaneous nodules (Jiang et al. 2000; Zhang et al. 2012; Peng et al. 2018), which were often migratory. A single nodule was typical, but a few patients had multiple nodules (Zhang et al. 2012).

5.9 Diagnosis

As is the case in infections due to many species of food-borne trematodes, symptoms of paragonimiasis are often rather vague and non-specific, hence creating diagnostic difficulties. In many cases, there are no overt signs or symptoms at all (Nakamura-Uchiyama et al. 2002; Peng et al. 2018). Diagnostic confusion with pulmonary tuberculosis, lung cancer, and other diseases remains very common (Toscano et al. 1995; Devi et al. 2007; Song et al. 2011; Calvopiña et al. 2016). Hence, unless paragonimiasis is actually suspected, correct diagnosis is delayed, and the patient might be subjected to expensive and ineffective treatments. Paragonimiasis appears to be more common than pulmonary tuberculosis in some endemic areas such as NE India (Narain et al. 2004; Devi et al. 2007), parts of Laos (Odermatt et al. 2007), and parts of SE Nigeria (Umoh and Useh 2009). Very often, reports note that failure of the patient to respond to treatment for tuberculosis, or the unexpected finding of lung fluke eggs (Koh et al. 2012) or worms (Osaki et al. 2007) during surgery, prompt clinicians to enquire about past eating habits of their patients, and the way to a diagnosis of paragonimiasis is clear. Enlightened health workers are now promoting screening for paragonimiasis in endemic areas alongside TB surveys (Belizario et al. 2016; Das et al. 2016; Ratsavong et al. 2017).

The four classes of diagnostic approaches are serology/immunology, parasitology, radiology/medical imaging, and molecular DNA-based approaches. Demonstration of lung fluke eggs in sputum, feces, bronchial washings, or surgical specimens provides a definitive diagnosis. However, eggs can be hard to find, even in some active pulmonary cases, and especially in cases of ectopic paragonimiasis. Consequently, immunologically based tests are regarded as more sensitive for diagnosis (Chen et al. 2001; Devi et al. 2007; Doanh et al. 2011; Appleton 2014) and are discussed first.

5.9.1 Serology/Immunology

Several recent reviews include immunological methods for diagnosis of paragonimiasis (Blair et al. 2007; Narain et al. 2009; Procop 2009; Sripa et al. 2010; Sugiyama et al. 2012; Chai 2013a; Esteban et al. 2014). Here, only tests that are most often mentioned in recent literature, or are of particular historic interest, will be discussed. These include the intradermal test, ELISA, multiple-dot assay, immunoblotting, and the dot immune-gold filtration assay. Of these, ELISA and immunoblotting are the main methods in current use. Both are useful in a laboratory setting, but less easy to apply in field situations.

5.9.1.1 Commonly Used Immunological Methods

The earliest immunological method to be used widely was the intradermal test (Yokogawa et al. 1960). A small amount of diluted worm antigen is injected into the skin of the forearm and the size of the resulting wheal compared after a few minutes with that elicited by a control injection. The test is simple, inexpensive, and quite sensitive. But there are some drawbacks, mainly (1) a high proportion of false positives (Cheng et al. 2005); (2) cross-reactions with other trematodiases, especially if the antigen used is not purified (Yokogawa et al. 1960; Sawada et al. 1968); and (3) a positive reaction can persist long after the infection has been cleared (Yokogawa et al. 1960). The intradermal test has often been used as the first step in mass-screening programs in Japan (Yokogawa 1965), China (Liu et al. 2008), and Korea (Kim 1969; Choi 1990), generally supplemented by more specific serological tests and/or examination of sputum for eggs. Huge numbers of people have been screened in this way (Otsuji 2003). Its use continues in a number of countries including Colombia (Vélez et al. 2000), Laos (Song et al. 2008), India (Singh et al. 1993), and China (Zhang et al. 2012).

Many variants of the enzyme-linked immunosorbent assay (ELISA) have been described in the literature (Blair et al. 2007), and these are among the most widely used of tests today (Lee et al. 2010). In the commonest formats, antigens from the parasite are bound to a surface, typically in a microtiter plate, for ease of handling. Antibodies in host sera are allowed to react with these. The quantity of complexed antigen/antibody is determined using a secondary antibody, which is generally commercially purchased, specific for particular classes or subclasses of host antibody, and conjugated with a reporter enzyme that produces a color change in a substrate. Other variants have been successfully tried. For example, immunoglobulin-binding proteins of bacterial origin, such as Protein A, can be used instead of parasite antigen (Ikeda 2001) and peroxidase-conjugated Protein G can be used instead of secondary antibody (Nkouawa et al. 2009). Urine shows promise as an alternative to host serum as a source of antibodies (Qiu et al. 2016).

Lung fluke antigens that have been used include crude antigen extracts, excretory-secretory products (Narain et al. 2005), recombinant peptides (Lee et al. 2007; Pothong et al. 2018), and various purified or partially purified antigens including cysteine proteases (CPs) (Ikeda et al. 1996). There is increasing interest in the use of recombinant CPs for diagnosis. Advantages are that well-defined antigens can be produced *in vitro* in quantity and with consistent quality: there is no need to raise parasites in laboratory hosts or to deal with batches of parasite material that might vary in quality (Ahn et al. 2015; Yoonuan et al. 2016; Yu et al. 2017).

A comparison of somatic with excretory-secretory antigens from local *P. heterotremus* in NE India, done during development of an ELISA test for paragonimiasis, found that both preparations of antigens had 100% sensitivity but that the latter was more specific (100% as opposed to 91.3%) (Narain et al. 2005).

Multiple dot-ELISA is used in Japan and elsewhere to screen for several parasites simultaneously. Antigens from a range of parasite species are dotted onto a nitrocellulose membrane, the membrane is dried to bind the antigens and then flooded with patient serum to permit detection of antibodies against all the parasites assayed at the same time, following essentially the same steps

used in microplate ELISA (Itoh and Sato 1990; Maruyama et al. 1996; Ikehara et al. 2010; Doanh et al. 2011; Sugiyama et al. 2012). In a conceptually similar approach, also aimed at simultaneous screening for multiple parasites, antigens from parasites were partially purified using SDS-PAGE and antigen bands recovered from the gel, added to protein microarrays and screened as for ELISA (Chen et al. 2012b). In this case, a 35 kDa band was the target antigen for paragonimiasis.

Methods based on the use of colloidal gold as a reporter have been developed. Colloidal gold is conjugated either with Protein A (which binds immunoglobulins) or with rabbit anti-human IgG. The dot immunogold filtration assay (DIGFA) is said to be better than ELISA because it is easier, faster, and cheaper, but exhibits comparable sensitivity and specificity (Qian and Sugiyama 2007; Feng et al. 2010; Singh et al. 2012a; Sugiyama et al. 2012). A colloidal gold immunochromatographic strip for detection of infection due to *P. skrjabini* has been described (Wang et al. 2014). In practice, these methods have been little used for the diagnosis of paragonimiasis.

There may be cross-reactions when sera from patients with other parasites (schistosomes, liver flukes, etc.) are tested for paragonimiasis, especially when crude extracts of parasite antigen are used (Itoh and Sato 1990; Fischer et al. 2013). In immunoblotting, parasite antigens are separated electrophoretically before western blotting and probing with sera. In this way, immune reactions with diagnostic antigens of particular molecular masses can be identified (Slemenda et al. 1988). This approach has been used for diagnosis of paragonimiasis by the Centers for Disease Control, Atlanta, Georgia in the USA, since 1988, the antigen generally used being a crude extract from *P. westermani*. However, extracts from *P. kellicotti* are better for diagnosis of that species (Fischer et al. 2013).

It remains difficult to distinguish between infections due to different species of *Paragonimus* using ELISA or immunoblot (Nkouawa et al. 2009; Fischer et al. 2013). ELISA inhibition tests can go some way to dealing with this problem (Yoshino et al. 1998) but their use is not widespread. The multiple dot-ELISA (above) was developed partly for this reason and can provide a stronger reaction with the homologous species when antigens from *P. westermani* and *P. s. miyazakii* are both included (Itoh and Sato 1990; Ono et al. 1992). Ouchterlony's double diffusion test (Yoshino et al. 1998) and the DIGFA method (Qian and Sugiyama 2007) can also help in distinguishing between *Paragonimus* species.

5.9.1.2 Immunological Indication of Cure After Treatment

Intradermal tests can be positive long after cure of paragonimiasis. Similarly, IgG ELISA tests may yield positive results for long periods after treatment, and antibody levels may even exhibit a transient rise after chemotherapy, probably in response to material released from dead worms (Knobloch et al. 1984; Cho et al. 1989; Obara et al. 2004). Hence, a positive immunological test does not always indicate that an active infection is present. IgG levels return to normal at from four to eighteen months (Cho et al. 1989; Maleewong et al. 1992) after cure, or even longer (Cho et al. 2000). The variation is possibly related to intensity and duration of infection. The presence of worm eggs in tissues may partly explain the slow decline of specific antibody levels (Cho et al. 1989, 2000).

5.9.2 Parasitological Diagnosis

Direct parasitological diagnosis, by finding eggs in sputum or feces, is likely to be the only option available in clinics in poorly equipped rural areas. If "rusty" colored sputum containing blood, parasite eggs, and Charcot-Leyden crystals is found, then a definitive diagnosis of pulmonary paragonimiasis can be reached easily. However, such diagnostic manifestations are not always seen, even when mature worms are present in the lungs, and never during pre-patent early infections or ectopic paragonimiasis.

If possible, multiple sputum samples should be examined from each patient (Belizario et al. 2014). The record seems to be 27 for the number of sputum specimens examined before eggs were found (Sadun and Buck 1960). Eggs are rarely

found in feces, but this most commonly in children, who tend to swallow rather than spitting (Toscano et al. 1995). Eggs may also be found in bronchial washings and brushings (Mukae et al. 2001), pleural effusion, and surgical specimens such as lung biopsies (Horn et al. 2016).

Paragonimiasis and tuberculosis co-occur in many places. The Ziehl–Neelsen staining (ZNS) technique, used to stain mycobacteria in sputum, had long been assumed to destroy lung fluke eggs (Slesak et al. 2011). Thus, diagnosticians trying to exclude infections with either of these pathogens have tested sputum samples separately for each. Handling and microscopic examination of fresh sputum for *Paragonimus* eggs carries some risk of tuberculosis transmission to laboratory staff. Recent modifications to the ZNS technique prevent destruction of fluke eggs, thus providing a low-cost method for distinguishing between active pulmonary paragonimiasis and tuberculosis (Slesak et al. 2011). Furthermore, the ZNS slides can be kept for further evaluation and archiving although quality decreases with time and storage conditions (Ratsavong et al. 2017).

Diagnosticians need to be aware that not all operculate trematode eggs of a certain size are of *Paragonimus* species (Tantrawatpan et al. 2016). Eggs of many families of trematodes resemble those of *Paragonimus* in size, shape, and color (e.g., echinostomes, fasciolids, paramphistomes, and strigeids). Reports of *Paragonimus* eggs in fecal samples in unusual hosts and locations need to be treated with caution. For example, (Müller-Graf et al. 1996) tentatively assigned to *Paragonimus* eggs from the feces of baboons in Tanzania. Children in a slum area of Dhaka, Bangladesh, were reported as shedding *Paragonimus* eggs in their stool (Hosna et al. 2018).

5.9.3 Molecular Diagnosis by DNA Detection or Sequencing

Several molecular approaches to diagnosis have been reported. However, there has been little application of these to date in clinical practice. Eggs, either in sputum or feces, have generally been the targets of these efforts. It proved possible to PCR-amplify and sequence the nuclear ribosomal ITS2 region from as few as 3–5 eggs of *P. westermani* from sputum (Chang et al. 2000), and from a single egg of *P. heterotremus* (Doanh et al. 2011). This same genomic region has been the target for other studies using PCR on eggs from patients (Le et al. 2006; Devi et al. 2007; Singh et al. 2007, 2009; Yahiro et al. 2008; Nkouawa et al. 2009; Fischer et al. 2011; Intapan et al. 2012). Only one study has detected DNA from eggs in human fecal samples (Nkouawa et al. 2009) and it was suggested that molecular detection of *Paragonimus* DNA in feces might be more efficient than attempting a physical search for eggs in feces. Others have detected DNA from feces of experimental or natural animal hosts (Intapan et al. 2005; Xiong et al. 2011; Doanh et al. 2016b). Coupling of melting-curve analysis with fluorescence resonance energy transfer real-time PCR makes it possible to detect and distinguish DNA from several trematode species in a single reaction (Tantrawatpan et al. 2016).

In one interesting development, it proved possible to amplify and sequence ITS2 from a paraffin-embedded section of lung tissue from a suspected case of paragonimiasis due to *P. kellicotti* (Fischer et al. 2011). Such approaches might make it possible to obtain data from archived pathological specimens.

A loop-mediated isothermal amplification (LAMP) protocol has been established for *P. westermani* (Chen et al. 2011). Using purified DNA from this species, the detection limit was 10^{-8} ng/μL of DNA (Chen et al. 2011). LAMP is said to be far more sensitive than conventional PCR and can possibly be applied to, e.g., pleural fluid. The approach uses four species-specific primers that recognize six regions in the target DNA. The reaction can be completed in an hour at a single temperature (60°C) and a simple in-tube visualization of successful amplification is possible (Zhao et al. 2012). No complicated equipment is needed. This method may be suitable for field labs and small hospitals. It could also be applied to field identification of metacercariae and eggs, provided a species-specific set of primers is available.

5.9.4 Radiology/Medical Imaging

Several recent reviews include material on medical imaging as it relates to paragonimiasis (Martinez et al. 2005; Restrepo et al. 2007; Procop 2009; Henry et al. 2012; Chai 2013a; Henry and Cummings 2013; Jones and Mazal 2016). Imaging methods include conventional chest X-ray, X-ray, and computed tomography, MRI and ultrasonography. Paragonimiasis is often not suspected when imaging procedures are requested by clinicians, and findings often lead to an initial diagnosis of other conditions, such as tuberculosis or cancer (Kim et al. 2011a; Barennes et al. 2014).

Chest X-rays have been used for very many years for diagnosis of paragonimiasis (Yokogawa et al. 1960) and increasingly are coupled with computed tomography (CT) for more detailed anatomical observations (Im et al. 1992, 1997; Kim et al. 2005; Kuroki et al. 2005; Henry et al. 2012; Henry and Cummings 2013). Abnormalities are not always apparent, even in cases of active pulmonary paragonimiasis (Singh et al. 1986; Devi et al. 2007). Common observations in early paragonimiasis include pneumothorax, pleural effusion, airspace consolidation, and linear opacities (Im et al. 1997; Jeon et al. 2005). The last of these probably represent migration tracks of worms from the pleural spaces into the lung parenchyma (Im et al. 1997; Kim et al. 2005) and might provide strong support for a diagnosis of paragonimiasis (Henry et al. 2012; Akaba et al. 2016). Distinct cystic lesions and nodules less than 3–4 cm in diameter and bronchiectasis tend to be seen in established infections (Im et al. 1997; Jeon et al. 2005). Cysts may appear as ring shadows, within which worms may be detected in some cases (Im et al. 1997). Pleural thickening may be present adjacent to worm nodules (Kim et al. 2005).

Radiographic abnormalities can be slow to resolve—months to years—after treatment (Moyou-Somo and Tagni-Zukam 2003; Vidamaly et al. 2009). Occasionally, nodules may resolve as small calcifications in the lungs (Im et al. 1997).

CT imaging results from series of hepatic paragonimiasis (Li et al. 2012) and abdominal paragonimiasis cases (Shim et al. 2012) have recently been reported. Zhang et al. (2017) used MDCT to differentiate hepatic paragonimiasis from small hepatocellular carcinoma, pathological conditions that can be very difficult to tell apart. Lesions of hepatic paragonimiasis are primarily subcapsular, usually in the right lobe and appear as tunnels and/or cystic masses that contain eosinophils, necrotic material, and Charcot-Leyden crystals on pathology examination (Lu et al. 2012; Ye et al. 2017; Zhang et al. 2017).

Ultrasonography provides relatively little anatomical detail. However, it can supplement other imaging techniques (Park et al. 2013). Furthermore, it is capable of real-time imaging of moving structures and can be used to guide biopsy procedures to obtain diagnostic material (inflammatory material, eggs, fragments of worms) from nodules in abdominal and thoracic organs (Shim et al. 2012; Lu et al. 2013; Park et al. 2013; Oh et al. 2015).

CT methods and magnetic resonance imaging (MRI) are useful for imaging cerebral paragonimiasis (Cha et al. 1994; Im et al. 1997; Xia et al. 2014). In early "active" infection, characteristic ring-like granulomatous lesions are commonly seen using CT scans. These may be solitary or resemble clusters of grapes, each usually 1–3 cm in diameter and each surrounded by edematous areas (Im et al. 1997). Later these become calcified, resembling "soap bubbles" or egg shells with surrounding parenchymal damage (Im et al. 1997).

MRI methods do not involve the use of ionizing radiation such as X-rays and are good for visualizing lesions in soft tissues such as the brain. T1- and T2-weighted images differ in the ways they show fat and water in tissues. Descriptions of brain lesions seen using MRI are generally similar to those based on CT images. In the earliest stages of cerebral paragonimiasis, especially in children, hemorrhage might be present, seen as high signal intensity in T1-weighted images or as high or low intensity on T2-weighted images (Zhang et al. 2006). As granulomatous lesions form, these may be seen as "soap bubbles" or a "grape cluster" of ring-like structures with surrounding edema, each ring being up to

3 cm in diameter (Kim et al. 2011b). Following eventual calcification, the lesions may resemble egg shells with central content of varying intensities (Nomura et al. 1999; Kim et al. 2011b). MRI has also been used to image lesions caused by spinal paragonimiasis (Kim et al. 2011b; Qin and Cai 2012; Wang et al. 2018).

5.10 Treatment

5.10.1 Chemotherapy

Two drugs are generally recommended for treatment of pulmonary paragonimiasis: praziquantel (PZQ) and triclabendazole (TCL) (Fürst et al. 2012c; Chai 2013b). PZQ has a long history of use in paragonimiasis (Chai 2013b). The recommended course is 25 mg/kg body weight taken three times daily with meals for 2–3 days (Hong 2018). Cure rates are generally very high (Keiser and Utzinger 2010). However, repeat rounds of treatment are sometimes necessary (Chen et al. 2001; Hu et al. 2016; Gong et al. 2017), especially when pleural effusion is present (Sumitani et al. 2005; Vidamaly et al. 2009; Cho et al. 2011; Oh et al. 2011). PZQ has also been used for treatment of cerebral paragonimiasis, with good results reported (Chen et al. 2013; Wu et al. 2013). However, PZQ is only likely to alleviate symptoms in "active" cases, when worms are still present: mechanical insult and release of eggs and bioactive compounds will cease when the worms die. Of course, PZQ treatment can eliminate any pulmonary infection that might be a source for later cerebral invasion (Lv et al. 2010). Co-administration of anti-inflammatories is sometimes recommended for cerebral paragonimiasis because of the risk of reaction against substances released from dying worms (Kusner and King 1993). The extent to which this is a problem remains unclear (Chen et al. 2013).

Side effects of PZQ administration are generally mild (Hong 2018). Patients may report mild and transient insomnia, nausea, headache, dizziness, vomiting, and abdominal pain; less common effects include rash and hypotension (Fürst et al. 2012c). A very few patients exhibit an allergic response after administration of PZQ (Kyung et al. 2011; Chai 2013b). A strong inflammatory reaction was observed in one Lao patient after PZQ administration (Clyti et al. 2006). There is no indication yet of resistance to PZQ by *Paragonimus* species although there is evidence of resistance in *Schistosoma mansoni* (Wang et al. 2012) and the possibility of resistance developing in other trematode species remains a cause for concern (Keiser and Utzinger 2010; Prichard et al. 2012), especially if the drug is used for mass administration. Caution is suggested in administering PZQ to pregnant or breastfeeding women, people with ectopic infections, and children under 4 years of age. The main contraindications are hypersensitivity and cysticercosis (World Health Organization 1995; Fürst et al. 2012c).

Triclabendazole is also effective against paragonimiasis (World Health Organization 1995; Fürst et al. 2012c) and might have some advantages, given that only one or two doses are required (Calvopiña et al. 2003) and that dose rates are lower than for PZQ. The recommended regimen is 10 mg/kg body weight in a single dose (which may be repeated after 12–24 h in heavy infections) (Calvopiña et al. 2003) or 20 mg/kg of body weight, in two separate doses of 10 mg/kg, administered on the same day (World Health Organization 2011). TCL might be better tolerated by patients than PZQ (Belizario et al. 2007). It is also the better option for mass drug administration, in part because the one or two doses required can be given under supervision, avoiding compliance problems that might arise if subjects are given tablets to take at home over several days. In the Philippines, a single dose of TCL (10 mg/kg body weight) was found to be as effective as the three-dose regimen of PZQ (Belizario et al. 2007). One case of suspected triclabendazole resistance has been reported (Kyung et al. 2011).

Common side effects are similar to those seen after PZQ administration and include mild and transient abdominal and epigastric pain, sweating, and eosinophilia; less common effects include nausea, vomiting, headache, dizziness, cough, fever, urticaria, pruritus, and skin rash.

Again, caution is suggested for treatment of pregnant or breastfeeding women, people with ectopic infections, and children <6 years (Fürst et al. 2012c).

Although newer drugs, and combinations of drugs, are being explored for treatment of foodborne diseases, including trematodes (Keiser and Utzinger 2010), no alternatives suitable for treatment of paragonimiasis are apparent as yet (Xue et al. 2008).

5.10.2 Surgical Intervention

Often surgeons discover underlying paragonimiasis when they were expecting some other condition (Huang and Liu 2003; Lee et al. 2012). Surgery is also commonly reported in cases of hepatic paragonimiasis, especially when no clear diagnosis has been made (Ye et al. 2017). However, surgery is not normally appropriate for uncomplicated pulmonary paragonimiasis: treatment with praziquantel or triclabendazole should suffice. Invasive interventions are more common in pleural cases, especially when voluminous pleural effusions are present that have not resolved after drug treatment. Draining of effusion is a common procedure which may need to be repeated (Vidamaly et al. 2009). Surgical decortication of the pleura is also sometimes required to remove fibrous material (Yun 1962; DeFrain and Hooker 2002). Surgery is also appropriate for uncomplicated ectopic cases, especially subcutaneous (Ashitani et al. 2000; Sim et al. 2010).

Surgery was the only intervention available in cerebral paragonimiasis until the drug bithionol was introduced around 1961 (Oh 1969). Since then, PZQ and TCL have superseded bithionol (Rhee et al. 1987). Drug treatment is the treatment of choice in active early cerebral cases (Oh 1969; Cha et al. 1994), but is of little value in chronic "inactive" cases, except as a means of treating any concomitant pulmonary infection. In one study in China, 73 of 88 patients with cerebral paragonimiasis showed improvement when treated with PZQ (Chen et al. 2013): surgery was done only on those with relatively superficial lesions that were accessible and easy to remove.

References

Adams AM (2018) Foodborne trematodes: *Paragonimus* and *Fasciola*. In: Ortega YR, Sterling CR (eds) Foodborne parasites. Springer, Cham, pp 293–324

Adema CM, Bayne CJ, Bridger JM, Knight M, Loker ES, Yoshino TP, Zhang SM (2012) Will all scientists working on snails and the diseases they transmit please stand up? PLoS Negl Trop Dis 6:e1835

Agatsuma T, Iwagami M, Sato Y, Iwashita J, Hong SJ, Kang SY, Ho LY, Su KE, Kawashima K, Abe T (2003) The origin of the triploid in *Paragonimus westermani* on the basis of variable regions in the mitochondrial DNA. J Helminthol 77:279–285

Ahn CS, Na BK, Chung D, Kim JG, Kim JT, Kong Y (2015) Expression characteristics and specific antibody reactivity of diverse cathepsin F members of *Paragonimus westermani*. Parasitol Int 64:37–42

Ai L, Chen MX, Chen SH, Zhang YN, Li H, Cai YC, Lu Y, Tian LG, Zhou XN, Chen JX (2012) Characterization of microRNAs in *Paragonimus westermani* by Solexa deep sequencing and bioinformatics analysis. J Anim Vet Adv 11:3469–3473

Aka NA, Adoubryn K, Rondelaud D, Dreyfuss G (2008) Human paragonimiasis in Africa. Ann Afr Med 7:153–162

Akaba T, Takeyama K, Toriyama M, Kubo A, Mizobuchi R, Yamada T, Tagaya E, Kondo M, Sakai S, Tamaoki J (2016) Pulmonary paragonimiasis: the detection of a worm migration track as a diagnostic clue for uncertain eosinophilic pleural effusion. Intern Med 55:503–506

Amaro DE, Cowell A, Tuohy MJ, Procop GW, Morhaime J, Reed SL (2016) Cerebral paragonimiasis presenting with sudden death. Am J Trop Med Hyg 95:1424–1427

Anthony RM, Rutitzky LI, Urban JFJ, Stadecker MJ, Gause WC (2007) Protective immune mechanisms in helminth infection. Nat Rev Immunol 7:975–987

Appleton CC (2014) Paragonimiasis in KwaZulu-Natal Province, South Africa. J Helminthol 88:123–128

Arias SM, Salazar LM, Casas E, Henao A, Velásquez LE (2011) *Paragonimus* sp. en cangrejos y sensibilización de la comunidad educativa hacia los ecosistemas acuáticos de La Miel y La Clara, Caldas, Antioquia. Biomedica 31:209–215

Ashitani J, Kumamoto K, Matsukura S (2000) Paragonimiasis westermani with multifocal lesions in lungs and skin. Intern Med 39:433–436

Attwood SW (2010) Studies on the parasitology, phylogeography and the evolution of host-parasite interactions for the snail intermediate hosts of medically important trematode genera in Southeast Asia. Adv Parasitol 73:405–440

Bae YA, Ahn JS, Kim SH, Rhyu MG, Kong Y, Cho SY (2008) PwRn1, a novel Ty3/gypsy-like retrotransposon of *Paragonimus westermani*: molecular characters and its differentially preserved mobile potential according to host chromosomal polyploidy. BMC Genomics 9:482

Barennes H, Slesak G, Buisson Y, Odermatt P (2014) Paragonimiasis as an important alternative misdiagnosed disease for suspected acid-fast bacilli sputum smear-negative tuberculosis. Am J Trop Med Hyg 90:384–385

Bayssade-Dufour C, Chermette R, Šundić D, Radujković BM (2014) *Paragonimus gondwanensis* n. sp. (Digenea, Paragonimidae), parasite of mammals (humans and carnivores) in Cameroon. Ecol Montenegrina 1:256–267

Bayssade-Dufour C, Chermette R, Šundić D, Radujković BM (2015) *Paragonimus kerberti* n. sp. (Digenea, Paragonimidae), parasite of carnivores in Cameroon. Ecol Montenegrina 2:271–277

Behm CA, Ovington KS (2000) The role of eosinophils in parasitic helminth infections: insights from genetically modified mice. Parasitol Today 16:202–209

Belizario V Jr, Totanes FI, Asuncion CA, de Leon W, Jorge M, Ang C, Naig JR (2014) Integrated surveillance of pulmonary tuberculosis and paragonimiasis in Zamboanga del Norte, the Philippines. Pathog Glob Health 108:95–102

Belizario VY, de Leon WV, Bugayong MPG, de Guzman AD, Valderama MT (2006) An assessment of reinfection rates and treatment outcomes of patients with pulmonary paragonimiasis. Natl Res Council Philippines Res J 8-9:10–19

Belizario VY, Mallari AO, de Leon WU, Lucero AC (2007) Assessment of the efficacy, safety and tolerability of praziquantel and triclabendazole in the treatment of paragonimiasis. Southeast Asian J Trop Med Public Health 38:97–105

Belizario VYJ, Jorge MCI, Ng JV, Mistica MS, Lam HY, Rivera LJJ, Anino MRC, delos Trinos JPCR (2016) A model for integrating paragonimiasis surveillance and control with tuberculosis control program. Southeast Asian J Trop Med Public Health 47:625–637

Binchai S, Rangsiruji A, Ketudat P, Morishima Y, Sugiyama H (2007) Molecular systematics of a new form of *Paragonimus westermani* discovered in Thailand. Southeast Asian J Trop Med Public Health 38:92–96

Blair D (2008) Family Paragonimidae Dollfus, 1939. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda. CAB International and Natural History Museum, London, pp 271–273

Blair D, Agatsuma T, Watanobe T (1997) Molecular evidence for the synonymy of three species of *Paragonimus*, *P. ohirai* Miyazaki, 1939, *P. iloktsuenensis* Chen, 1940 and *P. sadoensis* Miyazaki et al., 1968. J Helminthol 71:305–310

Blair D, Waikagul J, Honzako Y, Agatsuma T (1998) Phylogenetic relationships among the Thai species of *Paragonimus* inferred from DNA sequences. In: Tada I, Kojima S, Tsuji M (eds) Proceedings, IX International Congress of Parasitology, Chiba, Japan. Monduzzi Editore Sp. A, Bologna, pp 643–647

Blair D, Xu ZB, Agatsuma T (1999) Paragonimiasis and the genus *Paragonimus*. Adv Parasitol 42:113–222

Blair D, Davis GM, Wu B (2001) Evolutionary relationships between trematodes and snails emphasizing schistosomes and paragonimids. Parasitology 123:S229–S243

Blair D, Chang ZS, Chen MG, Cui AL, Wu B, Agatsuma T, Iwagami M, Corlis D, Fu CB, Zhan XM (2005) *Paragonimus skrjabini* Chen, 1959 (Digenea: Paragonimidae) and related species in eastern Asia: a combined molecular and morphological approach to identification and taxonomy. Syst Parasitol 60:1–21

Blair D, Agatsuma T, Wang W (2007) Paragonimiasis. In: Murrell KA, Fried B (eds) World class parasites. Springer, New York, pp 117–150

Blair D, Nawa Y, Mitreva M, Doanh PN (2016) Gene diversity and genetic variation in lung flukes (genus *Paragonimus*). Trans R Soc Trop Med Hyg 110:6–12

Boland JM, Vaszar LT, Jones JL, Mathison BA, Rovzar MA, Colby TV, Leslie KO, Tazelaar HD (2011) Pleuropulmonary infection by *Paragonimus westermani* in the United States: a rare cause of eosinophilic pneumonia after ingestion of live crabs. Am J Surg Pathol 35:707–713

Bouwknegt M, Devleesschauwer B, Graham H, Robertson LJ, van der Giessen JW (2018) The Euro-FBP workshop participants. Prioritisation of food-borne parasites in Europe, 2016. Eurosurveillance 23:17–00161

Braun M (1901) Zur Kenntniss der Trematoden der Saugethiere. Zoologische Jahrbücher Abtheilung für Systematik, Geographie und Biologie der Thiere 14:311–384

Cabrera BD (1984) Paragonimiasis in the Philippines: current status. Arzneimittel Forschung 34:1188–1192

Caffrey CR, Goupil L, Rebello KM, Dalton JP, Smith D (2018) Cysteine proteases as digestive enzymes in parasitic helminths. PLoS Negl Trop Dis 12:e0005840

Cai P, Gobert GN, McManus DP (2016) MicroRNAs in parasitic helminthiases: current status and future perspectives. Trends Parasitol 32:71–86

Calvopiña M, Guderian RH, Paredes W, Cooper PJ (2003) Comparison of two single-day regimens of triclabendazole for the treatment of human pulmonary paragonimiasis. Trans R Soc Trop Med Hyg 97:451–454

Calvopiña M, Romero D, Castañeda B, Hashiguchi Y, Sugiyama H (2014) Current status of *Paragonimus* and paragonimiasis in Ecuador. Mem Inst Oswaldo Cruz 109:849–855

Calvopiña M, Romero-Alvarez D, Macias R, Sugiyama H (2016) Severe pleuropulmonary paragonimiasis caused by *Paragonimus mexicanus* treated as tuberculosis in Ecuador. Am J Trop Med Hyg 96:97–99

Cha SH, Chang KH, Cho SY, Han MH, Kong Y, Suh DC, Choi CG, Kang HK, Kim MS (1994) Cerebral paragonimiasis in early active stage: CT and MR features. Am J Roentgenol 162:141–145

Chai JY (2013a) Paragonimiasis. Handb Clin Neurol 114:273–286

Chai JY (2013b) Praziquantel treatment in trematode and cestode infections: an update. Infect Chemother 45:32–43

Chang ZS, Wu B, Blair D, Zhang Y, Hu L, Chen SH, Chen M, Feng Z, Davis G (2000) Gene sequences for identification of *Paragonimus* eggs from a human case. Chin J Parasitol Parasit Dis 18:213–215

Chen HT (1985) Paragonimidae Dollfus, 1939. Science Press, Beijing

Chen MG, Chang ZS, Shao XY, Liu MD, Blair D, Chen SH, Zhang YN, Hong JL, Shen BG, Feng Z (2001) Paragonimiasis in Yongjia County, Zhejiang Province, China: clinical, parasitological and karyotypic studies on *Paragonimus westermani*. Southeast Asian J Trop Med Public Health 32:760–769

Chen MX, Ai L, Zhang RL, Xia JJ, Wang K, Chen SH, Zhang YN, Xu MJ, Li X, Zhu XQ, Chen JX (2011) Sensitive and rapid detection of *Paragonimus westermani* infection in humans and animals by loop-mediated isothermal amplification (LAMP). Parasitol Res 108:1193–1198

Chen J, Xu MJ, Zhou DH, Song HQ, Wang CR, Zhu XQ (2012a) Canine and feline parasitic zoonoses in China. Parasit Vectors 5:e152

Chen JX, Chen M, Ai L, Chen J, Chen S, Zhang Y, Cai Y, Zhu X, Zhou X (2012b) A protein microarray for the rapid screening of patients suspected of infection with various food-borne helminthiases. PLoS Negl Trop Dis 6:e1899

Chen JY, Chen Z, Lin JK, Zhu G, Meng H, Cui GY, Wu N, Hu R, Pan JX, Zou YJ, Feng H (2013) Cerebral paragonimiasis: a retrospective analysis of 89 cases. Clin Neurol Neurosurg 115:546–551

Cheng YZ, Xu LS, Chen BJ, Li LS, Zhang RY, Lin CX, Lin JX, Li YS, Li YR, Fang YY, Lin KQ, Zheng GB (2005) Survey on the current status of important human parasitic infections in Fujian Province. Chin J Parasitol Parasit Dis 23:283–287

Cheng WY, Li F, Zhuang HM, Zhong DR, Wu CX, Zhu ZH (2010) Hepatic paragonimiasis revealed by FDG PET/CT. Clin Nucl Med 35:726–728

China, Coordinating Office of the National Survey on the Important Human Parasitic Diseases (2005) A national survey on current status of the important parasitic diseases in human population. Chin J Parasitol Parasit Dis 23:332–340. In Chinese, English abstract

Cho SY, Kim SI, Kang SY, Kong Y, Han SK, Shim YS, Han YC (1989) Antibody changes in paragonimiasis patients after praziquantel treatment as observed by ELISA and immunoblot. Korean J Parasitol 27:15–21

Cho SY, Kong Y, Kang SY (1997) Epidemiology of paragonimiasis in Korea. Southeast Asian J Trop Med Public Health 28:32–36

Cho SY, Kong Y, Yun DH, Kang SY, Kim LS, Chung YB, Yang HJ (2000) Persisting antibody reaction in paragonimiasis after praziquantel treatment is elicited mainly by egg antigens. Korean J Parasitol 38:75–84

Cho AR, Lee HR, Lee KS, Lee SE, Lee SY (2011) A case of pulmonary paragonimiasis with involvement of the abdominal muscle in a 9-year-old girl. Korean J Parasitol 49:409–412

Choi DW (1990) *Paragonimus* and paragonimiasis in Korea. Korean J Parasitol 28(Suppl):79–102

Choi WH, Chu JP, Jiang MH, Lee YS, Kim BS, Kim DG, Park YK (2010) Analysis of parasitic diseases diagnosed by tissue biopsy specimens at KyungHee Medical Center (1984-2005) in Seoul, Korea. Korean J Parasitol 48:85–88

Choo JD, Suh BS, Lee HS, Lee JS, Song CJ, Shin DW, Lee YH (2003) Chronic cerebral paragonimiasis combined with aneurysmal subarachnoid hemorrhage. Am J Trop Med Hyg 69:466–469

Chung YB, Kong Y, Joo IJ, Cho SY, Kang SY (1995) Excystment of *Paragonimus westermani* metacercariae by endogenous cysteine protease. J Parasitol 81:137–142

Chung YB, Yang HJ, Kang SY, Kong Y, Cho SY (1997) Activities of different cysteine proteases of *Paragonimus westermani* in cleaving human IgG. Korean J Parasitol 35:139–142

Chung YB, Kita H, Shin MH (2008) A 27 kDa cysteine protease secreted by newly excysted *Paragonimus westermani* metacercariae induces superoxide anion production and degranulation of human eosinophils. Korean J Parasitol 46:95–99

Clyti E, Khéosang P, Huerre M, Sayasone S, Martinez-Aussel B, Strobel M (2006) Cutaneous paragonimiasis with flare up after treatment: a clinical case from Laos. Int J Dermatol 45:1110–1112

Cobbold T (1859) On some new forms of Entozoa. Trans Linn Soc Lond 22:363–366

Cross JH (1984) Changing patterns of some trematode infections in Asia. Arzneimittel Forschung 34:1224–1226

Culquichicón C, Hernández-Pacherres A, Labán-Seminario LM, Cardona-Ospina JA, Rodríguez-Morales AJ (2017) Where are we after 60 years of paragonimiasis research? A bibliometric assessment. Infez Med 25:142–149

Cumberlidge N, Rollinson D, Vercruysse J, Tchuenté L-AT, Webster B, Clark PF (2018) *Paragonimus* and paragonimiasis in West and Central Africa: unresolved questions. Parasitology 145:1748–1757

Dainichi T, Nakahara T, Moroi Y, Urabe K, Koga T, Tanaka M, Nawa Y, Furue M (2003) A case of cutaneous paragonimiasis with pleural effusion. Int J Dermatol 42:699–702

Das M, Doleckova K, Shenoy R, Mahanta J, Narain K, Devi KR, Konyak T, Mansoor H, Isaakidis P (2016) Paragonimiasis in tuberculosis patients in Nagaland, India. Glob Health Action 9:32387

de Leon WU, Piad JNC (2005) Paragonimiasis in the Philippines. In: Arizono N, Chai JY, Nawa Y, Takahashi Y (eds) Asian parasitology, vol. 1: food-borne helminthiasis in Asia. FAP Journal, Japan, pp 133–137

DeFrain M, Hooker R (2002) North American paragonimiasis—case report of a severe clinical infection. Chest 121:1368–1372

Devi K, Narain K, Bhattacharya S, Negmu K, Agatsuma T, Blair D, Wickramashinghe S, Mahanta J (2007) Pleuropulmonary paragonimiasis due to *Paragonimus heterotremus*: molecular diagnosis, prevalence of infection and clinicoradiological features in an

endemic area of northeastern India. Trans R Soc Trop Med Hyg 101:786–792

Devi KR, Narain K, Agatsuma T, Blair D, Nagataki M, Wickramasinghe S, Yatawara L, Mahanta J (2010) Morphological and molecular characterization of *Paragonimus westermani* in northeastern India. Acta Trop 116:31–38

Devi KR, Narain K, Mahanta J, Nirmolia T, Blair D, Saika SP, Agatsuma T (2013a) Presence of three distinct genotypes within the *Paragonimus westermani* complex in northeastern India. Parasitology 140:76–86

Devi RK, Narain K, Mahanta J, Deori R, Lego K, Goswami D, Kumar RS, Agatsuma T (2013b) Active detection of tuberculosis and paragonimiasis in the remote areas in North-Eastern India using cough as a simple indicator. Pathog Glob Health 107:153–156

Diaz JH (2013) Paragonimiasis acquired in the United States: native and nonnative species. Clin Microbiol Rev 26:493–504

Diesing CM (1850) Systema helminthum. Wilhelmum Braumüller, Vindobonae (Vienna)

Doanh PN, Le NT, The DT (2005) *Paragonimus* and paragonimiasis in Vietnam. In: Arizono N, Chai JY, Nawa Y, Takahashi Y (eds) Asian parasitology, vol 1: food-borne helminthiasis in Asia. FAP Journal, Japan, pp 149–153

Doanh PN, Shinohara A, Horii Y, Habe S, Nawa Y, Le NT (2007) Description of a new lung fluke species, *Paragonimus vietnamensis* sp. nov. (Trematoda, Paragonimidae), found in northern Vietnam. Parasitol Res 101:1495–1501

Doanh PN, Shinohara A, Horii Y, Yahiro S, Habe S, Vannavong N, Strobel M, Nakamura S, Nawa Y (2009) Morphological differences and molecular similarities between *Paragonimus bangkokensis* and *P. harinasutai*. Parasitol Res 105:429–439

Doanh PN, Dung DT, Thach DTC, Horii Y, Shinohara A, Nawa Y (2011) Human paragonimiasis in Viet Nam: epidemiological survey and identification of the responsible species by DNA sequencing of eggs in patients' sputum. Parasitol Int 60:534–537

Doanh PN, Hien HV, Nonaka N, Horii Y, Nawa Y (2012) Discovery of *Paragonimus skrjabini* in Vietnam and its phylogenetic status in the *Paragonimus skrjabini* complex. J Helminthol 87:450–456

Doanh PN, Guo ZH, Nonaka N, Horii Y, Nawa Y (2013a) Natural hybridization between *Paragonimus bangkokensis* and *Paragonimus harinasutai*. Parasitol Int 62:240–245

Doanh PN, Horii Y, Nawa Y (2013b) *Paragonimus* and Paragonimiasis in Vietnam: an update. Korean J Parasitol 51:621–627

Doanh PN, Hien HV, An PT, Tu LA (2015a) Development of lung fluke, *Paragonimus heterotremus*, in rat and mice, and the role of paratenic host in its life cycle. Tap Chi Sinh Hoc 37:265–271

Doanh PN, Thaenkham U, An PT, Hien HV, Horii Y, Nawa Y (2015b) Metacercarial polymorphism and genetic variation of *Paragonimus heterotremus* (Digenea: Paragonimidae), and a re-appraisal of the taxonomic status of *Paragonimus pseudoheterotremus*. J Helminthol 89:182–188

Doanh NP, Tu AL, Bui TD, Loan TH, Nonaka N, Horii Y, Blair D, Nawa Y (2016a) Molecular and morphological variation of *Paragonimus westermani* in Vietnam with records of new second intermediate crab hosts and a new locality in a northern province. Parasitology 143:1639–1646

Doanh P, Hien H, Tu L, Nonaka N, Horii Y, Nawa Y (2016b) Molecular identification of the trematode *Paragonimus* in faecal samples from the wild cat *Prionailurus bengalensis* in the Da Krong Nature Reserve, Vietnam. J Helminthol 90:658–662

Doanh PN, Tu LA, Van Hien H, Van Duc N, Horii Y, Blair D, Nawa Y (2018) First intermediate hosts of *Paragonimus* spp. in Vietnam and identification of intramolluscan stages of different *Paragonimus* species. Parasit Vectors 11:328

Eke RA, Nwosu UM, Enwereji EE, Emerole CV (2013) Paragonimiasis reemergence in Nigeria: predisposing factors and recommendations for early intervention and everlasting eradication. ISRN Infect Dis 2013:257810

Esaulova NV, Seryodkin IV (2012) Paragonimiasis of Amur tiger (*Panthera tigris altaica*) in Primorsky Krai. Russ Vet J 2012:15–17

Esteban JG, Muñoz-Antoli C, Toledo R, Ash LR (2014) Diagnosis of human trematode infections. In: Toledo R, Fried B (eds) Digenetic trematodes. Springer, New York, pp 293–327

Feng X, Wen H, Zhang Z, Chen X, Ma X, Zhang J, Qi X, Bradshaw H, Vuitton D, Craig PS (2010) Dot immunogold filtration assay (DIGFA) with multiple native antigens for rapid serodiagnosis of human cystic and alveolar echinococcosis. Acta Trop 113:114–120

Feng Y, Fürst T, Liu L, Yang GJ (2018) Estimation of disability weight for paragonimiasis: a systematic analysis. Infect Dis Poverty 7:e110

Fischer PU, Weil GJ (2015) North American paragonimiasis: epidemiology and diagnostic strategies. Expert Rev Anti Infect Ther 13:779–786

Fischer PU, Curtis KC, Marcos LA, Weil GJ (2011) Molecular characterization of the North American lung fluke *Paragonimus kellicotti* in Missouri and its development in mongolian gerbils. Am J Trop Med Hyg 84:1005–1011

Fischer PU, Curtis KC, Folk SM, Wilkins PP, Marcos LA, Weil GJ (2013) Serological diagnosis of North American paragonimiasis by western blot using *Paragonimus kellicotti* adult worm antigen. Am J Trop Med Hyg 88:1035–1040

Fried B, Abruzzi A (2010) Food-borne trematode infections of humans in the United States of America. Parasitol Res 106:1263–1280

Fujino T, Ishii Y (1982) Ultrastructural studies on spermatogenesis in a parthenogenetic type of *Paragonimus westermani* (Kerbert 1878) proposed as *P. pulmonalis* (Baelz 1880). J Parasitol 68:433–441

Fürst T, Duthaler U, Sripa B, Utzinger J, Keiser J (2012a) Trematode infections: liver and lung flukes. Infect Dis Clin North Am 26:399–419

Fürst T, Keiser J, Utzinger J (2012b) Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect Dis 12:210–221

Fürst T, Sayasone S, Odermatt P, Keiser J, Utzinger J (2012c) Manifestation, diagnosis, and management of foodborne trematodiasis. BMJ 344:e4093

Global Burden of Disease 2015, Disease and Injury Incidence and Prevalence Collaborators (2016) Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388:1545–1602

Gong Z, Miao R, Shu M, Zhu Y, Wen Y, Guo Q, Liao Q, Wan C (2017) Paragonimiasis in children in Southwest China: a retrospective case reports review from 2005 to 2016. Medicine 96:e7265

Gyoten J (2000) Infection by *Paragonimus miyazakii* cercariae of their crab hosts, *Geothelphusa dehaani*, by percutaneous penetration. J Parasitol 86:1342–1345

Habe S, Lai KP, Agatsuma T, Ow-Yang CK, Kawashima K (1996) Growth of Malaysian *Paragonimus westermani* in mammals and the mode of transmission of the fluke among mammals. Jpn J Trop Med Hyg 24:225–232

Haçarız O, Sayers GP (2016) The omic approach to parasitic trematode research—a review of techniques and developments within the past 5 years. Parasitol Res 115:2523–2543

Heath HW, Marshall SG (1997) Pleural paragonimiasis in a Laotian child. Pediatr Infect Dis J 16:1182–1185

Henry TS, Cummings KW (2013) Role of imaging in the diagnosis and management of parasitic infections. Curr Opin Pulm Med 19:310–317

Henry TS, Lane MA, Weil GJ, Bailey TC, Bhalla S (2012) Chest CT features of North American paragonimiasis. Am J Roentgenol 198:1076–1083

Hernández-Chea R, Jiménez-Rocha A, Castro R, Blair D, Dolz G (2017) Morphological and molecular characterization of the metacercaria of *Paragonimus caliensis*, as a separate species from *P. mexicanus* in Costa Rica. Parasitol Int 66:126–133

Hong S-T (2018) Albendazole and praziquantel: review and safety monitoring in Korea. Infect Chemother 50:1–10

Horn CB, Patel NR, Hawasli JA, Edwards MA (2016) *Paragonimus kellicotti* presenting with hemoptysis and a left upper lobe mass. Ann Thorac Surg 102:e393–e395

Hosna A, Nahar A, Khanum H (2018) Occurrence of intestinal parasites among the slum children. Biores Commun 4:470–475

Hu Y, Qian J, Yang D, Zheng X (2016) Pleuropulmonary paragonimiasis with migrated lesions cured by multiple therapies. Indian J Pathol Microbiol 59:56

Huang PH, Liu CL (2003) A man with chronic cerebral paragonimiasis found in Taiwan. J Med Sci 23:167–170

Hwang K-E, Song H-Y, Jung J-W, Oh S-J, Yoon K-H, Park D-S, Jeong E-T, Kim H-R (2015) Pleural fluid characteristics of pleuropulmonary paragonimiasis masquerading as pleural tuberculosis. Korean J Intern Med 30:56–61

Ibáñez-Herrera N (2012) Paragonimiasis: an important endemic disease in Perú. SCIÉNDO 15:89–96

Ibanga ES, Eyo VM (2001) Pulmonary paragonimiasis in Oban community in Akamkpa Local Government Area, Cross River State, Nigeria: prevalence and intensity of infection. Trans R Soc Trop Med Hyg 95:159–160

Ikeda T (2001) Protein A immunocapture assay detecting antibodies to fluke cysteine proteinases for immunodiagnosis of human paragonimiasis and fascioliasis. J Helminthol 75:245–249

Ikeda T, Oikawa Y, Nishiyama T (1996) Enzyme-linked immunosorbent assay using cysteine proteinase antigens for immunodiagnosis of human paragonimiasis. Am J Trop Med Hyg 55:434–437

Ikehara M, Komase Y, Morita A, Yamaguchi H, Yamamoto T (2010) Paragonimiasis in a person whose symptoms were shown 22 years after emigrating to Japan from Laos. J Infect Chemother 16:49–52

Im JG, Whang HY, Kim WS, Han MC, Shim YS, Cho SY (1992) Pleuropulmonary paragonimiasis: radiologic findings in 71 patients. Am J Roentgenol 159:39–43

Im JG, Chang KH, Reeder MM (1997) Current diagnostic imaging of pulmonary and cerebral paragonimiasis, with pathological correlation. Semin Roentgenol 32:301–324

Intapan PM, Wongkham C, Imtawil KJ, Pumidonming W, Prasongdee TK, Miwa M, Maleewong W (2005) Detection of *Paragonimus heterotremus* eggs in experimentally infected cats by a polymerase chain reaction-based method. J Parasitol 91:195–198

Intapan PM, Sanpool O, Thanchomnang T, Imtawil K, Pongchaiyakul C, Nawa Y, Maleewong W (2012) Molecular identification of a case of *Paragonimus pseudoheterotremus* infection in Thailand. Am J Trop Med Hyg 87:706–709

Irie T, Yamaguchi Y, Doanh PN, Guo ZH, Habe S, Horii Y, Nonaka N (2017) Infection with *Paragonimus westermani* of boar-hunting dogs in Western Japan maintained via artificial feeding with wild boar meat by hunters. J Vet Med Sci 79:1419–1425

Itoh M, Sato S (1990) Multi-dot enzyme-linked immunosorbent assay for serodiagnosis of trematodiasis. Southeast Asian J Trop Med Public Health 21:471–474

Iwagami M, Ho LY, Su K, Lai PF, Fukushima M, Nakano M, Blair D, Kawashima K, Agatsuma T (2000) Molecular phylogeographic studies on *Paragonimus westermani* in Asia. J Helminthol 74:315–322

Iwagami M, Rajapakse RPVJ, Paranagama W, Agatsuma T (2003) Identities of two *Paragonimus* species from Sri Lanka inferred from molecular sequences. J Helminthol 77:239–245

Iwagami M, Rajapakse RPVJ, Paranagama W, Okada T, Kano S, Agatsuma T (2008) Ancient divergence of

Paragonimus westermani in Sri Lanka. Parasitol Res 102:845–852

Iwagami M, Rajapakse R, Yatawara L, Kano S, Agatsuma T (2009) The first intermediate host of *Paragonimus westermani* in Sri Lanka. Acta Trop 109:27–29

Jackson S, Sleigh A (2000) Resettlement for China's Three Gorges Dam: socio-economic impact and institutional tensions. Communis Post-Commun 33:223–241

Jeon K, Koh WJ, Kim H, Kwon OJ, Kim TS, Lee KS, Han J (2005) Clinical features of recently diagnosed pulmonary paragonimiasis in Korea. Chest 128:1423–1430

Jiang X, Fan Z, Wang X, Du Y (2000) Clinical analysis of 258 cases with *Pagumogonimus skrjabini* in west of Hubei Province. Endemic Dis Bull 15:88–90

Jiang A, Ge L, You Y, Shen Z (2015) The diagnosis of cutaneous paragonimiasis after the external migration of the parasite from a punch biopsy site. JAAD Case Rep 1:239–240

Jones P, Mazal J (2016) Medical imaging of neglected tropical diseases of the Americas. Radiol Technol 87:393–419

Kagawa FT (1997) Pulmonary paragonimiasis. Semin Respir Infect 12:149–158

Kanpittaya J, Sawanyawisuth K, Vannavong A, Intapan PM, Maleewong W, Zhang W, Strobel M (2010) Different chest radiographic findings of pulmonary paragonimiasis in two endemic countries. Am J Trop Med Hyg 83:924–926

Kasny M, Mikes L, Hampl V, Dvorak J, Caffrey CR, Dalton JP, Horak P (2009) Peptidases of trematodes. Adv Parasitol 69:205–297

Keiser J, Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. Adv Parasitol 73:197–230

Kerbert C (1878) Zur Trematoden-Kenntnis. Zool Anz 1:271–273

Kim JS (1969) Mass chemotherapy in the control of paragonimiasis. Korean J Parasitol 7:6–14

Kim HJ (2014) Pneumothorax induced by pulmonary paragonimiasis: two cases report. Korean J Thorac Cardiovasc Surg 47:310–312

Kim EA, Juhng SK, Kim HW, Kim GD, Lee YW, Cho HJ, Won JJ (2004) Imaging findings of hepatic paragonimiasis: a case report. J Korean Med Sci 19:759–762

Kim TS, Han J, Shim SS, Jeon K, Koh WJ (2005) Pleuropulmonary paragonimiasis: CT findings in 31 patients. Am J Roentgenol 185:616–621

Kim TS, de Guzman JV, Kong HH, Chung DI (2006) Comparison of gene representation between diploid and triploid *Paragonimus westermani* by expressed sequence tag analysis. J Parasitol 92:803–816

Kim JY, Kang CM, Choi GH, Yang WI, Sim SB, Kwon JE, Kim KS, Choi JS, Lee WJ, Kim BR (2007a) Laparoscopic excision of intra-abdominal paragonimiasis. Surg Laparosc Endosc Percutan Tech 17:556–558

Kim SH, Min KU, Lee JH, Kim TH, Son JW, Yoon HJ et al (2007b) Two cases of cutaneous paragonimiasis initially presenting with respiratory symptoms. Resp Med Extra 3:52–56

Kim TY, Lee JY, Kim TI, Moon KH, Kang SY, Hong SJ (2007c) Molecular cloning and enzymatic characterization of a class mu glutathione S-transferase of *Paragonimus westermani*. Parasitol Res 101:1225–1231

Kim SH, Cai GB, Bae YA, Lee EG, Lee YS, Kong Y (2009) Two novel phospholipid hydroperoxide glutathione peroxidase genes of *Paragonimus westermani* induced by oxidative stress. Parasitology 136:553–565

Kim KU, Lee K, Park HK, Jeong YJ, Yu HS, Lee MK (2011a) A pulmonary paragonimiasis case mimicking metastatic pulmonary tumor. Korean J Parasitol 49:69–72

Kim MK, Cho BM, Yoon DY, Nam ES (2011b) Imaging features of intradural spinal paragonimiasis: a case report. Br J Radiol 84:E72–E74

Kirino Y, Nakano N, Doanh PN, Nawa Y, Horii Y (2009) A seroepidemiological survey for paragonimosis among boar-hunting dogs in central and southern Kyushu, Japan. Vet Parasitol 161:335–338

Knobloch J, Paz G, Feldmeier H, Wegner D, Voelker J (1984) Serum antibody levels in human paragonimiasis before and after therapy with praziquantel. Trans R Soc Trop Med Hyg 78:835–836

Koh EJ, Kim SK, Wang KC, Chai JY, Chong S, Park SH, Cheon JE, Phi JH (2012) The return of an old worm: cerebral paragonimiasis presenting with intracerebral hemorrhage. J Korean Med Sci 27:1428–1432

Kong Y, Doanh PN, Nawa Y (2015) Paragonimus. In: Xiao L, Ryan U, Feng YY (eds) Biology of foodborne parasites. CRC, Boca Raton, FL, pp 445–462

Kurochkin YV (1987) Trematodes of the fauna of the SSSR. Paragonimidae, Nauka, Moscow

Kuroki M, Hatabu H, Nakata H, Hashiguchi N, Shimizu T, Uchino N, Tamura S (2005) High-resolution computed tomography findings of *P. westermani*. J Thorac Imaging 20:210–213

Kusner DJ, King CH (1993) Cerebral paragonimiasis. Semin Neurol 13:201–208

Kyung SY, Cho YK, Kim YJ, Park JW, Jeong SH, Lee JI, Sung YM, Lee SP (2011) A paragonimiasis patient with allergic reaction to praziquantel and resistance to triclabendazole: successful treatment after desensitization to praziquantel. Korean J Parasitol 49:73–77

Lane MA, Marcos LA, Onen NF, Demertzis LM, Hayes EV, Davila SZ, Nurutdinova DR, Bailey TC, Weil GJ (2012) *Paragonimus kellicotti* fluke infections in Missouri, USA. Emerg Infect Dis 18:1263–1267

Le TH, De NV, Blair D, McManus DP, Kino H, Agatsuma T (2006) *Paragonimus heterotremus* Chen and Hsia (1964), in Vietnam: a molecular identification and relationships of isolates from different hosts and geographical origins. Acta Trop 98:25–33

Lee EG, Na BK, Bae YA, Kim SH, Je EY, Ju JW, Cho SH, Kim TS, Kang SY, Cho SY, Kong Y (2006) Identification of immunodominant excretory-secretory cysteine proteases of adult *Paragonimus westermani* by proteome analysis. Proteomics 6:1290–1300

Lee J, Lee J, Kim S, Yong T (2007) Molecular cloning and characterization of a major egg antigen in

Paragonimus westermani and its use in ELISA for the immunodiagnosis of paragonimiasis. Parasitol Res 100:677–681

Lee MK, Hong SJ, Kim HR (2010) Seroprevalence of tissue invading parasitic infections diagnosed by ELISA in Korea. J Korean Med Sci 25:1272–1276

Lee CH, Kim JH, Moon WS, Lee MR (2012) Paragonimiasis in the abdominal cavity and subcutaneous tissue: report of 3 cases. Korean J Parasitol 50:345–347

Li AH, Na BK, Kong Y, Cho SH, Zhao QP, Kim TS (2005) Molecular cloning and characterization of copper/zinc-superoxide dismutase of *Paragonimus westermani*. J Parasitol 91:293–299

Li X, Yu J, Yang Z, Chu Z, Peng L, Sudarshan K (2012) Correlations between MDCT features and clinicopathological findings of hepatic paragonimiasis. Eur J Radiol 81:e421–e425

Li B-W, McNulty SN, Rosa BA, Tyagi R, Zeng QR, Gu K-Z, Weil GJ, Mitreva M (2016) Conservation and diversification of the transcriptomes of adult *Paragonimus westermani* and *P. skrjabini*. Parasit Vectors 9:497

Liang T, Liang G, Du Y, Wang X, Wang Y, Chen A, Chen Z, Du J, Li H, Yu L (2018) Scrotal paragonimiasis in adults: two case reports and review of literature. Medicine 97:e0328

Light RW (2011) Pleural effusions. Med Clin North Am 95:1055–1070

Liu Q, Wei F, Liu WS, Yang ST, Zhang XC (2008) Paragonimiasis: an important food-borne zoonosis in China. Trends Parasitol 24:318–323

López-Caballero J, Oceguera-Figueroa A, León-Règagnon V (2013) Detection of multiple species of human *Paragonimus* from Mexico using morphological data and molecular barcodes. Mol Ecol Resour 13:1125–1136

Lou HQ, Hu Y, Zhang L, Zhang YJ, Tu PG, Yu XT, Jin YJ, Wang L (2011) Epidemiological survey and phylogenetic analysis of *Paragonimus westermani* isolates in Jinhua, China. Afr J Biotechnol 10:14286–14292

Lu C-Y, Hu Y-J, Chen W-X (2012) Characteristic MR and CT imaging findings of hepatobiliary paragonimiasis and their pathologic correlations. Acta Radiol 53:481–484

Lu Q, Ling W, Ma L, Huang Z, Lu C, Luo Y (2013) Contrast-enhanced ultrasonographic findings of hepatic paragonimiasis. World J Gastroenterol 19:2087–2091

Lumsden RD, Sogandares-Bernal F (1970) Ultrastructural manifestations of pulmonary paragonimiasis. J Parasitol 56:1095–1109

Lv S, Zhang Y, Steinmann P, Zhou XN, Utzinger J (2010) Helminth infections of the central nervous system occurring in southeast Asia and the far East. Adv Parasitol 72:351–408

Lv S, Tian LG, Liu Q, Qian MB, Fu Q, Steinmann P, Chen JX, Yang GJ, Yang K, Zhou XN (2013) Water-related parasitic diseases in China. Int J Environ Res Public Health 10:1977–2016

MacDonald AS, Araujo MI, Pearce EJ (2002) Immunology of parasitic helminth infections. Infect Immun 70:427–433

Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE (2004) Helminth parasites—masters of regulation. Immunol Rev 201:89–116

Maleewong W, Wongkham C, Pariyanonda S, Intapan P (1992) Analysis of antibody-levels before and after praziquantel treatment in human paragonimiasis-heterotremus. Asian Pac J Allergy Immunol 10:69–72

Malvy D, Ezzedine KH, Receveur MC, Pistone T, Mercie P, Longy-Boursier M (2006) Extra-pulmonary paragonimiasis with unusual arthritis and cutaneous features among a tourist returning from Gabon. Travel Med Infect Dis 4:340–342

Manson P (1880) Further observations on microfilariae, with description of new species. J Quekett Microsc Club 6:138–140

Martin J, Tyagi R, Rosa BA, Mitreva M (2018) A multi-omics database for parasitic nematodes and trematodes. In: Kollmar M (ed) Eukaryotic genomic databases. Humana Press, New York, pp 371–397

Martinez S, Restrepo CS, Carrillo JA, Betancourt SL, Franquet T, Varon C, Ojeda P, Gimenez A (2005) Thoracic manifestations of tropical parasitic infections: a pictorial review. Radiographics 25:135–155

Marty AM, Neafie RC (2000) Paragonimiasis. In: Meyers WM, Neafie RC, Marty AM, Wear DJ (eds) Pathology of infectious diseases: volume I—helminthiases. Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC, pp 49–67

Maruyama H, Noda S, Nawa Y (1996) Emerging problems of parasitic diseases in southern Kyushu, Japan. Jpn J Parasitol 45:192–200

Matsui A, Kanehara M, Kanehara M (2003) Palaeoparasitology in Japan—discovery of toilet features. Mem Inst Oswaldo Cruz 98:127–136

McNulty SN, Fischer PU, Townsend RR, Curtis KC, Weil GJ, Mitreva M (2014) Systems biology studies of adult *Paragonimus* lung flukes facilitate the identification of immunodominant parasite antigens. PLoS Negl Trop Dis 8:e3242

McSorley HJ, Maizels RM (2012) Helminth infections and host immune regulation. Clin Microbiol Rev 25:585–608

Min DY, Lee YA, Ryu JS, Ahn MH, Chung YB, Sim S, Shin MH (2004) Caspase-3-mediated apoptosis of human eosinophils by the tissue-invading helminth *Paragonimus westermani*. Int Arch Allergy Immunol 133:357–364

Miyazaki I (1991) An illustrated book of helminthic zoonoses. International Medical Foundation of Japan, Tokyo

Miyazaki I, Harinasuta T (1966) The first case of human paragonimiasis caused by *Paragonimus heterotremus* Chen et Hsia, 1964. Ann Trop Med Parasitol 60:509–514

Miyazaki I, Habe S, Terasaki K (1981a) On the pairing of adult lung flukes. Med Bull Fukuoka Univ 8:159–167

Miyazaki I, Terasaki K, Habe S (1981b) Comparison of single worm infection to cats between *Paragonimus westermani* and *P. pulmonalis*. Med Bull Fukuoka Univ 8:405–416

Morgan JAT, Dejong RJ, Adeoye GO, Ansa EDO, Barbosa CS, Bremond P, Cesari IM, Charbonnel N, Correa LR, Coulibaly G, D'Andrea PS, De Souza CP, Doenhoff MJ, File S, Idris MA, Incani RN, Jarne P, Karanja DMS, Kazibwe F, Kpikpi J, Lwambo NJS, Mabaye A, Magalhaes LA, Makundi A, Mone H, Mouahid G, Muchemi GM, Mungai BN, Sene M, Southgate V, Tchuente LAT, Theron A, Yousif F, Magalhaes EMZ, Mkoji GM, Loker ES (2005) Origin and diversification of the human parasite *Schistosoma mansoni*. Mol Ecol 14:3889–3902

Moyou-Somo R, Tagni-Zukam D (2003) La paragonimose au Cameroun: tableaux radio-cliniques et evolution sous traitment. Médicine Tropicale 63:163–167

Moyou-Somo R, Mfouapong-Ewane HB, Nkoa T, Etaluka-Mungo B, Kum-Kan W, Kefie-Arrey C (2014) A new focus of pleuro-pulmonary paragonimiasis in Manjo Health District, littoral region of Cameroon. Int J Trop Dis Health 4:963–972

Mukae H, Taniguchi H, Matsumoto N, Iiboshi H, Ashitani JI, Matsukura S, Nawa Y (2001) Clinicoradiologic features of pleuropulmonary *Paragonimus westermani* on Kyusyu Island, Japan. Chest 120:514–520

Müller-Graf C, Collins D, Woolhouse M (1996) Intestinal parasite burden in five troops of olive baboons (*Papio cynocephalus anubis*) in Gombe Stream National Park, Tanzania. Parasitology 112:489–497

Na BK, Kim SH, Lee EG, Kim TS, Bae YA, Kang I, Yu JR, Sohn WM, Cho SY, Kong Y (2006) Critical roles for excretory-secretory cysteine proteases during tissue invasion of *Paragonimus westermani* newly excysted metacercariae. Cell Microbiol 8:1034–1046

Nagayasu E, Yoshida A, Hombu A, Horii Y, Maruyama H (2015) Paragonimiasis in Japan: a twelve-year retrospective case review (2001-2012). Intern Med 54:179–186

Nakamura-Uchiyama F, Mukae H, Nawa Y (2002) Paragonimiasis: a Japanese perspective. Clin Chest Med 23:409–420

Nakamura-Uchiyama F, Hiromatsu K, Ishiwata K, Sakamoto Y, Nawa Y (2003) The current status of parasitic diseases in Japan. Intern Med 42:222–236

Nakano N, Kirino Y, Uchida K, Nakamura-Uchiyama F, Nawa Y, Horii Y (2009) Large-group infection of boar-hunting dogs with *Paragonimus westermani* in Miyazaki Prefecture, Japan, with special reference to a case of sudden death due to bilateral pneumothorax. J Vet Med Sci 71:657–660

Narain K, Devi KR, Mahanta J (2004) Pulmonary paragonimiasis and smear-negative pulmonary tuberculosis: a diagnostic dilemma. Int J Tuberc Lung Dis 8:621–622

Narain K, Devi KR, Mahanta J (2005) Development of enzyme-linked immunosorbent assay for serodiagnosis of human paragonimiasis. Indian J Med Res 121:739–746

Narain K, Agatsuma T, Blair D (2009) Paragonimus. In: Liu D (ed) Molecular detection of foodborne pathogens. Taylor & Francis, Boca Raton, FL, pp 827–837

Narain K, Devi KR, Bhattacharya S, Negmu K, Rajguru S, Mahanta J (2015) Declining prevalence of pulmonary paragonimiasis following treatment & community education in a remote tribal population of Arunachal Pradesh, India. Indian J Med Res 141:648

Nawa Y, Doanh PN (2009) *Paragonimus bangkokensis* and *P. harinasutai*: reappraisal of phylogenetic status. J Trop Med Parasitol 32:87–96

Nawa Y, Nakamura-Uchiyama F (2005) *Paragonimus* and paragonimiasis in Japan. In: Arizono N, Chai JY, Nawa Y, Takahashi Y (eds) Asian parasitology, foodborne helminthiasis in Asia, pp 125–131

Nawa Y, Thaenkham U, Doanh PN, Blair D (2014) *Paragonimus westermani* and other *Paragonimus* species. In: Motarjemi Y (ed) Encyclopedia of food safety. Elsevier, Amsterdam, pp 179–188

Nishida H, Shibahara T (2003) Epidemiology of paragonimiasis. In: Otsuru M, Kamegai S, Hayashi S (eds) Progress of medical parasitology in Japan, pp 201–217

Nkouawa A, Okamoto M, Mabou AK, Edinga E, Yamasaki H, Sako Y, Nakao M, Nakaya K, Blair D, Agatsuma T, Enyong P, Shibahara T, Moyou-Somo R, Ito A (2009) Paragonimiasis in Cameroon: molecular identification, serodiagnosis and clinical manifestations. Trans R Soc Trop Med Hyg 103:255–261

Nkouawa A, Sako Y, Moyou-Somo R, Ito A (2011) Serological and molecular tools to detect neurologic parasitic zoonoses in rural Cameroon. Southeast Asian J Trop Med Public Health 42:1365–1374

Nomura M, Nitta H, Nakada M, Yamashima T, Yamashita J (1999) MRI findings of cerebral paragonimiasis in chronic stage. Clin Radiol 54:622–624

Obara A, Nakamura-Uchiyama F, Hiromatsu K, Nawa Y (2004) Paragonimiasis cases recently found among immigrants in Japan. Intern Med 43:388–392

Odermatt P, Habe S, Manichanh S, Tran DS, Duong V, Zhang W, Phommanthet K, Nakamuira S, Barennes H, Strobel M, Dreyfuss G (2007) Paragonimiasis and its intermediate hosts in a transmission focus in Lao People's Democratic Republic. Acta Trop 103:108–115

Odermatt P, Veasna D, Zhang W, Vannavong N, Phrommala S, Habe S, Barennes H, Strobel M (2009) Rapid identification of paragonimiasis foci by lay informants in Lao People's Democratic Republic. PLoS Negl Trop Dis 3:e521

Oh SJ (1969) Cerebral paragonimiasis. J Neurol Sci 8:27–48

Oh IJ, Kim YI, Chi SY, Ban HJ, Kwon YS, Kim KS, Kim YC, Kim YH, Seon HJ, Lim SC, Shin HY, Kim SO (2011) Can pleuropulmonary paragonimiasis be cured by only the 1st set of chemotherapy? treatment outcome and clinical features of recently developed pleuropulmonary paragonimiasis. Intern Med 50:1365–1370

Oh CR, Kim M-J, Lee KH (2015) A case of intra-abdominal paragonimiasis mimicking metastasis of

lung cancer diagnosed by endoscopic ultrasound-guided fine needle aspiration. Korean J Gastroenterol 66:41–45

Okoro N, Onyeagba R, Anyim C, Eda O, Okoli C, Orji I, Okonkwo E, Ekuma U, Agah M (2013) Prevalence of *Paragonimus* infection. Am J Infect Dis 9:17–23

Ono SI, Nagamachi S, Kusumoto S, Watanabe K, Imai JI, Nawa Y (1992) A case of paragonimiasis miyazakii with cavitating lesions in the lung: the first case found in Miyazaki Prefecture. Jpn J Parasitol 41:279–282

Osaki T, Takama T, Nakagawa M, Oyama T (2007) Pulmonary *Paragonimus westermani* with false-positive fluorodeoxyglucose positron emission tomography mimicking primary lung cancer. Gen Thorac Cardiovasc Surg 55:470–472

Otsuji Y (2003) Paragonimiasis. In: Otsuru M, Kamegai S, Hayashi S (eds) Progress of medical parasitology in Japan, pp 183–200

Palić J, Hostetter SJ, Riedesel E, Richardson-Bill R, Jarvinen JA (2011) What is your diagnosis? Aspirate of a lung nodule in a dog. Vet Clin Pathol 40:99–100

Parikh MS, Seeley E, Krishna G (2016) Electromagnetic navigational bronchoscopy spares a drunken crab from the surgeon's knife. J Bronchology Interv Pulmonol 24:241–243

Park CM (1962) Epidemiological aspects of paragonimiasis in Korea. Yonsei Med J 3:85–92

Park YS, Lee CH, Kim KA, Park CM (2013) Imaging findings of splenic paragonimiasis: using contrast-enhanced ultrasonography. J Med Ultrasound 21:36–38

Patrick SL, Turabelidze G, Marx H, Grim A, Weil CJ, Bailey TC, Onen NF, Demertzis LM, Tuteur PG, Hayes EV, Davila SZ, Folk SM, Mitihem RE, Kammerer E, Fannon LP Jr, Jones JL, Wilkins PP, Lo YC (2010) Human paragonimiasis after eating raw or undercooked crayfish—Missouri, July 2006-September 2010. Morb Mortal Wkly Rep 59:1573–1576

Peng H-J, Zhang C, Wang C-M, Chen X-G (2012) Current status and challenge of human parasitology teaching in China. Pathog Glob Health 106:386–390

Peng X, Zhang J, Zhang J, Wang Y, Zhang X (2018) Incidence of paragonimiasis in Chongqing China: a 6-year retrospective case review. Parasitology 145:792–796

Pothong K, Komalamisra C, Kalambaheti T, Watthanakulpanich D, Yoshino TP, Dekumyoy P (2018) ELISA based on a recombinant *Paragonimus heterotremus* protein for serodiagnosis of human paragonimiasis in Thailand. Parasit Vectors 11:322

Prichard RK, Basanez MG, Boatin BA, McCarthy JS, Garcia HH, Yang GJ, Sripa B, Lustigman S (2012) A research agenda for helminth diseases of humans: intervention for control and elimination. PLoS Negl Trop Dis 6:e1549

Procop GW (2009) North American paragonimiasis (caused by *Paragonimus kellicotti*) in the context of global paragonimiasis. Clin Microbiol Rev 22:415–446

Qian BZ, Sugiyama H (2007) Development of an immunological diagnostic kit for paragonimiasis and its use for an epidemiological survey of zoonotic paragonimiasis in Mainland China, 1. Multi-antigen dot immuno-gold assay (multi-DIGFA) for serodiagnosis of paragonimiasis. Final report of JHSF project. The Japan Health Sciences Foundation, Tokyo

Qin Y, Cai JH (2012) MRI findings of intraspinal extradural paragonimiasis granuloma in a child. Pediatr Radiol 42:1250–1253

Qiu XG, Nakamura-Uchiyama F, Nawa Y, Itoh M (2016) A tool for mass-screening of paragonimiasis: an enzyme-linked immunosorbent assay with urine samples. Trop Med Health 44:1

Ranasinghe SL, McManus DP (2017) Protease inhibitors of parasitic flukes: emerging roles in parasite survival and immune defence. Trends Parasitol 33:400–413

Ratsavong K, Quet F, Nzabintwali F, Diendéré J, Sebert J, Strobel M, Buisson Y (2017) Usefulness and limits of Ziehl-Neelsen staining to detect paragonimiasis in highly endemic tuberculosis areas. Parasit Epidemiol Control 2:1–7

Restrepo CS, Raut AA, Riascos R, Martinez S, Carrillo J, Prasad SR (2007) Imaging manifestations of tropical parasitic infections. Semin Roentgenol 42:37–48

Rhee CH, Chung CK, Jung HW, Cho SY, Son HJ (1987) Cerebral paragonimiasis treated with praziquantel: case report. J Korean Neurosurg Soc 16:853–857

Robertson LJ, van der Giessen JWB, Batz MB, Kojima M, Cahill S (2013) Have foodborne parasites finally become a global concern? Trends Parasitol 29:101–103

Robertson LJ, Sprong H, Ortega YR, van der Giessen JW, Fayer R (2014) Impacts of globalisation on foodborne parasites. Trends Parasitol 30:37–52

Romeo DP, Pollock JJ (1986) Pulmonary paragonimiasis: diagnostic value of pleural fluid analysis. South Med J 79:241–243

Rosenberg HF, Dyer KD, Foster PS (2013) Eosinophils: changing perspectives in health and disease. Nat Rev Immunol 13:9–22

Sadun EH, Buck AA (1960) Paragonimiasis in South Korea—immunodiagnostic, epidemiologic, clinical roentgenologic and therapeutic studies. Am J Trop Med Hyg 9:562–599

Sah R, Khadka S (2017) Case series of paragonimiasis from Nepal. Oxf Med Case Rep 2017(11):omx083

Saijuntha W, Tantrawatpan C, Jarilla BR, Agatsuma T, Andrews RH, Petney TN (2016) Intron sequence of the taurocyamine kinase gene as a marker to investigate genetic variation of *Paragonimus* species in Japan and the origins of triploidy in *P. westermani*. Trans R Soc Trop Med Hyg 110:67–73

Sanpool O, Intapan PM, Thanchomnang T, Janwan P, Nawa Y, Blair D, Maleewong W (2013) Molecular variation in the *Paragonimus heterotremus* complex in Thailand and Myanmar. Korean J Parasitol 51:677–681

Sato Y, Iwagami M, Iwashita J, Abe T, Yukawa M, Blas BL, Kawashima K, Agatsuma T (2003) Phylogenetic

status of a lung fluke in the Philippines based on mitochondrial genome. Jpn J Trop Med Hyg 31:1–3
Sawada T, Takei K, Sato S, Matsuyama S (1968) Studies on the immunodiagnosis of paragonimiasis. III. Intradermal skin tests with fractionated antigens. J Infect Dis 188:235–239
Schwartz C, Hams E, Fallon PG (2018) Helminth modulation of lung inflammation. Trends Parasitol 34:388–403
Shan X, Lin C, Li Y, Hu Y, Shen X, Lou H (2009) A new species of *Paragonimus sheni*—with a key to the species adult worm and metacercariae of the genus *Paragonimus* in China. Chin J Zoonoses 25:1143–1148
Shibahara T (1991) The route of infection of *Paragonimus westermani* (diploid type) cercariae in the freshwater crab, *Geothelphusa dehaani*. J Helminthol 65:38–42
Shim SS, Kim Y, Lee JK, Lee JH, Song DE (2012) Pleuropulmonary and abdominal paragonimiasis: CT and ultrasound findings. Br J Radiol 85:403–410
Shin MH, Lee SY (2000) Proteolytic activity of cysteine protease in excretory-secretory product of *Paragonimus westermani* newly excysted metacercariae pivotally regulates IL-8 production of human eosinophils. Parasite Immunol 22:529–533
Shin M, Min D (1999) Infection status of *Paragonimus westermani* metacercariae in crayfish (*Cambaroides similis*) collected from Bogildo (Islet), Wando-gun, Chollanam-do, Korea. Korean J Parasitol 37:55–57
Shin MH, Kita H, Park HY, Seoh JY (2001) Cysteine protease secreted by *Paragonimus westemani* attenuates effector functions of human eosinophils stimulated with immunoglobulin G. Infect Immun 69:1599–1604
Shin EH, Giuk SM, Kim HJ, Lee SH, Chai JY (2008) Trends in parasitic diseases in the Republic of Korea. Trends Parasitol 24:143–150
Shin MH, Lee YA, Min DY (2009) Eosinophil-mediated tissue inflammatory responses in helminth infection. Korean J Parasitol 47:S125–S131
Shin DH, Oh CS, Lee SJ, Lee EJ, Yim SG, Kim MJ, Kim YS, Lee SD, Lee YS, Lee HJ, Seo M (2012) Ectopic paragonimiasis from 400-year-old female mummy of Korea. J Archaeol Sci 39:1103–1110
Sianto L, Chame M, Silva CSP, Gonçalves MLC, Reinhard K, Fugassa M, Araújo A (2009) Animal helminths in human archaeological remains: a review of zoonoses in the past. Rev Inst Med Trop Sao Paulo 51:119–130
Sim YS, Lee JH, Hong SC, Chang JH, Kang SR, Yang HJ, Sung SH (2010) *Paragonimus westermani* found in the tip of a little finger. Intern Med 49:1645–1648
Singh TS, Mutum SS, Razaque MA (1986) Pulmonary paragonimiasis: clinical features, diagnosis and treatment of 39 cases in Manipur. Trans R Soc Trop Med Hyg 80:967–971
Singh TS, Mutum S, Razaque MA, Singh YI, Singh EY (1993) Paragonimiasis in Manipur. Indian J Med Res 97:247–252
Singh TS, Sugiyama H, Rangsiruji A, Devi KR (2007) Morphological and molecular characterizations of *Paragonimus heterotremus*, the causative agent of human paragonimiasis in India. Southeast Asian J Trop Med Public Health 38:82–86
Singh TS, Sugiyama H, Umehara A, Hiese S, Khalo K (2009) *Paragonimus heterotremus* infection in Nagaland: a new focus of paragonimiasis in India. Indian J Med Microbiol 27:123–127
Singh TS, Khamo V, Sugiyama H (2011) Cerebral paragonimiasis mimicking tuberculoma: first case report in India. Trop Parasitol 1:39–41
Singh TS, Devi KHR, Singh SR, Sugiyama H (2012a) A case of cutaneous paragonimiasis presented with minimal pleuritis. Trop Parasitol 2:142–144
Singh TS, Sugiyama H, Rangsiruji A (2012b) *Paragonimus* and paragonimiasis in India. Indian J Med Res 136:192–204
Singh T, Sugiyama H, Lepcha C, Khanna S (2014) Massive pleural effusion due to paragonimiasis: biochemical, cytological, and parasitological findings. Indian J Pathol Microbiol 57:492
Singh T, Hiromu S, Devi K, Singh W (2015) First case of *Paragonimus westermani* infection in a female patient in India. Indian J Med Microbiol 33(Suppl):156–159
Slemenda SB, Maddison SE, Jong EC, Moore DD (1988) Diagnosis of paragonimiasis by immunoblot. Am J Trop Med Hyg 39:469–471
Slesak G, Inthalad S, Basy P, Keomanivong D, Phoutsavath O, Khampoui S, Grosrenaud A, Amstutz V, Barennes H, Buisson Y, Odermatt P (2011) Ziehl-Neelsen staining technique can diagnose paragonimiasis. PLoS Negl Trop Dis 5:e1048
Sogandares-Bernal F, Seed JR (1973) American paragonimiasis. In: Cheng TC (ed) Current topics in comparative pathobiology, 2nd edn. Academic, New York, pp 1–56
Song H, Min D, Rim H, Youthanavanh V, Daluny B, Sengdara V, Virasack B, Bounlay P (2008) Skin test for paragonimiasis among schoolchildren and villagers in Namback District, Luangprabang Province, Lao PDR. Korean J Parasitol 46:179–182
Song JU, Um SW, Koh WJ, Suh GY, Chung MP, Kim H, Kwon OJ, Jeon K (2011) Pulmonary paragonimiasis mimicking lung cancer in a tertiary referral centre in Korea. Int J Tuberc Lung Dis 15:674–679
Song J-H, Dai F, Bai X, Kim T-I, Yang H-J, Kim T-S, Cho S-H, Hong S-J (2017a) Recent incidence of *Paragonimus westermani* metacercariae in freshwater crayfish, *Cambaroides similis*, from two enzootic sites in Jeollanam-do, Korea. Korean J Parasitol 55:347
Song LG, Zheng XY, Lin DT, Wang GX, Wu ZD (2017b) Parasitology should not be abandoned: data from outpatient parasitological testing in Guangdong, China. Infect Dis Poverty 6:e119
Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ (2010) Food-borne trematodiases in Southeast Asia: epidemiology, pathology, clinical manifestation and control. Adv Parasitol 72:305–350
Stiles CW, Hassall A (1900) Notes on parasites. 51. The lung fluke (*Paragonimus westermanii*) in swine and its relation to parasitic haemoptysis in man. Annu Rep Bureau Anim Indus US Dept Agric 16:560–611

Stutzer C, Richards SA, Ferreira M, Baron S, Maritz-Olivier C (2018) Metazoan parasite vaccines: present status and future prospects. Front Cell Infect Microbiol 8:67

Sugiyama H, Morishima Y, Binchai S, Rangsiruji A, Ketudat P (2007) New form of *Paragonimus westermani* discovered in Thailand: morphological characteristics and host susceptibility. Southeast Asian J Trop Med Public Health 38:87–91

Sugiyama H, Umehara A, Morishima Y, Yamasaki H, Kawanaka M (2009) Detection of *Paragonimus* metacercariae in the Japanese freshwater crab, *Geothelphusa dehaani*, bought at retail fish markets in Japan. Jpn J Infect Dis 62:324–325

Sugiyama H, Singh TS, Rangsiruji A (2012) Paragonimiasis. In: Liu DY (ed) Molecular detection of human parasitic pathogens. CRC, Boca Raton, pp 423–435

Sumitani M, Mikawa T, Miki Y, Nisida K, Tochino Y, Kamimori T, Fujiwara H, Fujikawa T, Nakamura F (2005) A case of chronic pleuritis by *Paragonimus westermani* infection resistant to standard chemotherapy and cured by three additional cycles of chemotherapy. Nihon Kokyuki Gakkai Zasshi 43:427–431. In Japanese, English abstract

Sunanda H, Shivalingaiah B, Paley T, Asoka W (2016) Demographic characteristic and analysis of pulmonary paragonimiasis in patients attending RIMS, Manipur. Lung India 33:140

Tandon V, Shylla JA, Ghatani S, Athokpam VD, Sahu R (2015) Neglected tropical diseases: Trematodiases—the Indian scenario. Proc Natl Acad Sci India Sect B Biol Sci 85:901–907

Taniguchi H, Mukae H, Matsumoto N, Tokojima M, Katoh S, Matsukura S, Ogawa K, Kohno S, Nawa Y (2001) Elevated IL-5 levels in pleural fluid of patients with paragonimiasis westermani. Clin Exp Immunol 123:94–98

Tantrawatpan C, Saijuntha W, Manochantr S, Kheolamai P, Thanchomnang T, Sadaow L, Intapan PM, Maleewong W (2016) A singleplex real-time fluorescence resonance energy transfer PCR with melting curve analysis for the differential detection of *Paragonimus heterotremus*, *Echinostoma malayanum* and *Fasciola gigantica* eggs in faeces. Trans R Soc Trop Med Hyg 110:74–83

Terasaki K, Shibahara T, Noda Y, Kayano H (1996) The oocyte of triploid fluke receiving intrusion of the sperm from diploid fluke—evidence for the origin of tetraploids in *Paragonimus westermani*. J Parasitol 82:947–950

Thaenkham U, Waikagul J (2008) Molecular phylogenetic relationship of *Paragonimus pseudoheterotremus*. Southeast Asian J Trop Med Public Health 39:217–221

Than M, Soe Z, Boey R, Pye S (1994) Paragonimiasis—pulmonary and extra pulmonary. J Myanmar Military Med 3:34–36

Thewjitcharoen Y, Poopitaya S (2006) Paragonimiasis presenting with unilateral pseudochylothorax: case report and literature review. Scand J Infect Dis 38:386–388

Tongu Y, Iwanaga Y, Hata H, Yokogawa M, Tsuji M, Plata A (1993) A survey on *Paragonimus rudis* in the Rio Guapore, Brazil. Jpn J Parasitol 42:438–439

Toscano C, Yu SH, Nunn P, Mott KE (1995) Paragonimiasis and tuberculosis, diagnostic confusion: a review of the literature. Trop Dis Bull 92:R1–R26

Tuon FF, Amato VS, Amato Neto V (2008) Paragonimiasis in Brazil. Braz J Infect Dis 12:1

Uchiyama F, Morimoto Y, Nawa Y (1999) Re-emergence of paragonimiasis in Kyushu, Japan. Southeast Asian J Trop Med Public Health 30:686–691

Umoh NO, Useh MF (2009) Epidemiology of paragonimiasis in Oban community of Cross River State, Nigeria. Mary Slessor J Med 9:1–10

Uttah EC (2013) Paragonimiasis and renewed crab-eating behavior in six communities from two ethnocultural clusters in Southeastern Nigeria. ISRN Infect Dis. https://doi.org/10.5402/2013/569485

Uttah EC, Etim SE, Ibe DC (2013) Familial and occupational clustering of paragonimiasis in a riverine community in eastern Nigeria. Transnat J Sci Technol 3:25–35

Utzinger J, Becker SL, Knopp S, Blum J, Neumayr AL, Keiser J, Hatz CF (2012) Neglected tropical diseases: diagnosis, clinical management, treatment and control. Swiss Med Wkly 142:w13727

Vélez ID, Ortega J, Hurtado MI, Salazar AL, Robledo SM, Jimenez JN, Velásquez LE (2000) Epidemiology of paragonimiasis in Colombia. Trans R Soc Trop Med Hyg 94:661–663

Vidamaly S, Choumlivong K, Keolouangkhot V, Vannavong N, Kanpittaya J, Strobel M (2009) Paragonimiasis: a common cause of persistent pleural effusion in Lao PDR. Trans R Soc Trop Med Hyg 103:1019–1023

Voelker J, Muller G, Prata A (1981) What is *Paragonimus rudis* (Diesing, 1850)? Report on a field study in Mato Grosso, Brasil. Mem Inst Oswaldo Cruz 76:409–414

Wagner RG, Newton CR (2009) Do helminths cause epilepsy? Parasite Immunol 31:697–705

Waikagul J (2007) A new species of *Paragonimus* (Trematoda: Troglotrematidae) from a cat infected with metacercariae from mountain crabs *Larnaudia larnaudii*. J Parasitol 93:1496–1500

Waikagul J, Yoonuan T (2005) *Paragonimus* and paragonimiasis in Thailand. In: Arizono N, Chai JY, Nawa Y, Takahashi Y (eds) Asian parasitology, vol 1: foodborne helminthiasis in Asia. FAP Journal, Tokyo, pp 139–148

Walton BC, Chyu I (1959) Clonorchiasis and paragonimiasis in the Republic of Korea. Bull World Health Organ 21:721–726

Wang ZQ, Cui J, Mao FR, Ling XM, Jin XX, Wu F (1998) Epidemiological study of paragonimosis in Central China. Helminthologia 35:117–121

Wang W, Blair D, Min T, Li F, Wang D (2011) *Paragonimus* worm from a New Guinea native in 1926. Asian Pac J Trop Med 4:76–78

Wang W, Wang L, Liang YS (2012) Susceptibility or resistance of praziquantel in human schistosomiasis: a review. Parasitol Res 111:1871–1877

Wang Y, Wang L, Zhang J, Wang G, Chen W (2014) Preparation of colloidal gold immunochromatographic strip for detection of paragonimiasis skrjabini. PLoS One 9:e92034

Wang J-L, Li T-T, Huang S-Y, Cong W, Zhu X-Q (2016) Major parasitic diseases of poverty in mainland China: perspectives for better control. Infect Dis Poverty 5:e67

Wang M, Ju Y, Liu X, Lv L, Wang Z, Jiang S (2018) Hemorrhagic intraspinal paragonimiasis. Neurology 90:237–238

Wilgus NC, Williams P, Bolesta ES, Chamberland RR (2017) Photo quiz: a 46-year-old man with a lung mass and history of hemoptysis. J Clin Microbiol 55:1603

World Health Organization (1995) Control of foodborne trematode infections, WHO Technical Report Series number 849. WHO, Geneva

World Health Organization (2011) Report of the WHO expert consultation on foodborne trematode infections and taeniasis/cysticercosis, held in Vientiane, Lao People's Democratic Republic 12-16 October 2009. Document number WHO/HTM/NTD/PCT/2011.3. WHO, Geneva

World Health Organization (2015) Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected tropical diseases 2015. WHO, Geneva

World Health Organization (2017) Expert consultation to accelerate control of foodborne trematode infections, taeniasis and cysticercosis, Seoul, Republic of Korea, 17-19 May 2017: meeting report. WHO Regional Office for the Western Pacific, Manila

Wright RS, Jean M, Rochelle K, Fisk D (2011) Chylothorax caused by *Paragonimus westermani* in a native Californian. Chest 140:1064–1066

Wu JY, Zhang BR, Zhao GH (2013) Cerebral infarction and cranial venous sinus thrombosis caused by paragonimiasis. CNS Neurosci Therapeut 19:734–736

Xia Y, Chen J, Ju Y, You C (2014) Characteristic CT and MR imaging findings of cerebral paragonimiasis. J Neuroradiol 43:200–206

Xiong TQ, Yang BB, Cheng YJ, Liu N, Zhou BJ (2011) Sequence analysis of egg DNA to identify the paragonimiasis caused by *Paragonimus skrjabini*. J Trop Med 11:1248–1251

Xu ZB (1991) Studies on clinical manifestations, diagnosis and control of paragonimiasis in China. Southeast Asian J Trop Med Public Health 22(Suppl):345–348

Xu HZ, Tang LF, Zheng XP, Chen ZM (2012) Paragonimiasis in Chinese children: 58 cases analysis. Iran J Pediatrics 22:505–511

Xue J, Utzinger J, Zhang YN, Tanner M, Keiser J, Xiao SH (2008) Artemether and tribendimidine lack activity in experimental treatment of *Paragonimus westermani* in the dog. Parasitol Res 102:537–540

Yahiro S, Habe S, Duong V, Odermatt P, Barennes H, Strobel M, Nakamura S (2008) Identification of the human paragonimiasis causative agent in Lao People's Democratic Republic. J Parasitol 94:1176–1177

Yang JS, Chen MG, Feng Z, Blair D (2000) *Paragonimus* and paragonimiasis in China: a review of the literature. Chin J Parasitol Parasit Dis Spec issue 2000:1–78

Yang G-J, Liu L, Zhu H-R, Griffiths SM, Tanner M, Bergquist R, Utzinger J, Zhou X-N (2014) China's sustained drive to eliminate neglected tropical diseases. Lancet Infect Dis 14:881–892

Yatera K, Hanaka M, Hanaka T, Yamasaki K, Nishida C, Kawanami T, Kawanami Y, Ishimoto H, Kanazawa T, Mukae H (2015) A rare case of paragonimiasis miyazakii with lung involvement diagnosed seven years after infection: a case report and literature review. Parasitol Int 64:274–280

Ye X, Xiong X, Cheng N, Lu J, Lin Y (2017) Hepatic paragonimiasis: a single-center retrospective analysis of 32 cases in Mainland China. Gastroenterol Rep 5:282–287

Yogore MG Jr, Cabrera BD, Noble GA (1958) Studies on paragonimiasis, IV. A survey in Casiguran, Sorsogon, Philippines. Phillipine J Sci 87:197–215

Yokogawa M (1965) *Paragonimus* and paragonimiasis. Adv Parasitol 3:99–158

Yokogawa S, Cort W, Yokogawa M (1960) *Paragonimus* and paragonimiasis. Exp Parasitol 10(81-137):139–205

Yoonuan T, Vanvanitchai Y, Dekumyoy P, Komalamisra C, Kojima S, Waikagul J (2008) Paragonimiasis prevalences in Saraburi Province, Thailand, measured 20 years apart. Southeast Asian J Trop Med Public Health 39:593–600

Yoonuan T, Nuamtanong S, Dekumyoy P, Phuphisut O, Adisakwattana P (2016) Molecular and immunological characterization of cathepsin L-like cysteine protease of *Paragonimus pseudoheterotremus*. Parasitol Res 115:4457–4470

Yoshida A, Matsuo K, Moribe J, Tanaka R, Kikuchi T, Nagayasu E, Misawa N, Maruyama H (2016) Venison, another source of *Paragonimus westermani* infection. Parasitol Int 65:607–612

Yoshino I, Nawa Y, Yano T, Ichinose Y (1998) Paragonimiasis westermani presenting as an asymptomatic nodular lesion in the lung: report of a case. Surg Today 28:108–110

Yu S, Zhang X, Chen W, Zheng H, Ai G, Ye N, Wang Y (2017) Development of an immunodiagnosis method using recombinant PsCP for detection of *Paragonimus skrjabini* infection in human. Parasitol Res 116:377–385

Yun D (1960) Paragonimiasis in children in Korea. Related to the custom of ingesting raw crayfish for the treatment of measles. J Pediatr 56:736–751

Yun DJ (1962) Clinical aspects of paragonimiasis. Yonsei Med J 3:93–101

Zhang JS, Huan Y, Sun LJ, Zhang GY, Ge YL, Zhao HT (2006) MRI features of pediatric cerebral paragonimiasis in the active stage. J Magn Reson Imaging 23:569–573

Zhang XL, Wang L, Wang GX, Chen WB, He X, Niu H, Li ZL, Chen L, Wang LF (2012) Distribution

and clinical features of *Paragonimiasis skrjabini* in Three Gorges Reservoir Region. Parasitol Int 61:645–649

Zhang J, Zhang XL, Huang FS, Xu WY (2013) Systematic teaching method to enhance the effectiveness of training for paragonimiasis. Pathog Glob Health 107: 11–14

Zhang X, Peng L, Liu W, Fan L, Zhang Q, Chen G, He P, Wu R, Liu A, Yang Y (2014) Response of primary vectors and related diseases to impoundment by the Three Gorges Dam. Trop Med Int Health 19: 440–449

Zhang S, Xie S-M, Chen Y-H, Liu X-B, Mai G (2017) Distinct MDCT imaging features to differential diagnosis of hepatic paragonimiasis and small hepatocellular carcinoma. Oncotarget 8:37291

Zhao QP, Moon SU, Na BK, Kim SH, Cho SH, Lee HW, Kong Y, Sohn WM, Jiang MS, Kim TS (2007) *Paragonimus westermani*: biochemical and immunological characterizations of paramyosin. Exp Parasitol 115:9–18

Zhao GH, Li J, Blair D, Li XY, Elsheikha HM, Lin RQ, Zou FC, Zhu XQ (2012) Biotechnological advances in the diagnosis, species differentiation and phylogenetic analysis of *Schistosoma* species. Biotechnol Adv 30:1381–1389

Zhong HL, He LY, Xu ZB, Cao WJ (1981) Recent progress in studies of *Paragonimus* and paragonimiasis control in China. Chin Med J 94:483–494

Zhou P, Chen N, Zhang RL, Lin RQ, Zhu XQ (2008) Food-borne parasitic zoonoses in China: perspective for control. Trends Parasitol 24:190–196

Liver Flukes: *Clonorchis* and *Opisthorchis*

6

Weerachai Saijuntha, Paiboon Sithithaworn, Nadda Kiatsopit, Ross H. Andrews, and Trevor N. Petney

6.1 History and Taxonomy

Opisthorchiid flukes most commonly occur in the bile duct, gall bladder, and liver of their mammalian and avian hosts (Scholz 2008). They are small to medium sized with 33 recognized genera in the family Opisthorchiidae. These are divided into 13 subfamilies (King and Scholz 2001; Scholz 2008).

W. Saijuntha
Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham, Thailand
e-mail: weerachai.s@msu.ac.th

P. Sithithaworn (✉) · N. Kiatsopit
Department of Parasitology, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand
e-mail: paib_sit@kku.ac.th

R. H. Andrews
CASCAP, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Faculty of Medicine, St Mary's Campus, Imperial College London, London, UK

T. N. Petney
CASCAP, Faculty of Medicine, Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen, Thailand

Department of Paleontology and Evolution, State Museum of Natural History, Karlsruhe, Germany
e-mail: trevor.petney@smnk.de

Both of the genera *Clonorchis* and *Opisthorchis* fall within the subfamily Opisthorchiinae. *Clonorchis sinensis* from East Asia and *Opisthorchis viverrini* from the Lower Mekong Basin are currently recognized as the most important human pathogens. Both are involved in the development of human cholangiocarcinoma and have been classified as group one carcinogens by the International Agency for Research on Cancer, a part of the World Health Organization (IARC 2012). The presence of *C. sinensis* and *O. viverrini* in East and continental Southeast Asia, respectively, is strongly correlated with the incidence of cholangiocarcinoma, particularly in northeast Thailand which has the highest incidence worldwide (IARC 2012). Although *Opisthorchis felineus*, which occurs from Europe across to eastern Siberia, has not yet been recognized as a carcinogen there is evidence suggesting that this is the case (Erhardt et al. 1962). It is, nevertheless, a pathogen of considerable significance in its own right in terms of hepatobiliary diseases originated from biliary fibrosis (Pozio et al. 2013).

Opisthorchis lobatus, a new species recently found in freshwater fish in Lao PDR, may also cause zoonosis but its role in humans is not known and it will not be dealt with here (Thaenkham et al. 2011). Nor will the avian species including *O. cheelis*, *O. longissimus*, and *O. parageminus* also reported from Southeast Asia (Nawa et al. 2015; Doanh and Nawa 2016; Dao et al. 2017). Dao et al. (2016) reported the

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_6

sympatric distribution of duck and human genotypes of "*O. viverrini*". The discovery of several species in the genus *Opisthorchis* in addition to the species complex of *O. viverrini* reflects complicated host and parasite interaction and their co-evolution.

Clonorchis sinensis was first described by JFP McConnell in the August 21st issue of the Lancet in 1875 from postmortem specimens collected from the bile duct of a Chinese seaman who died in Calcutta, India. On September 18th of the same year, TS Cobbold wrote a short letter, also published in the Lancet, indicating that from McConnell's description of the trematodes it was undoubtedly a new species which he then named *Distoma sinense*. This name was later revised by Looss (1907), as *Clonorchis sinensis*, the type species for the genus. At the time of its discovery, McConnell noted "The morbid anatomy of the liver in this case seems unequivocally to point to the presence of the flukes in its biliary ducts as the exciting cause of the acute and extensive structural degeneration of the proper structure of that organ, and of that cholemic condition induced by the obstruction of the biliary channels which appears to have been the immediate cause of death." clearly pointing out the pathological significance of this species.

The discovery of *Opisthorchis viverrini* initially follows a quite different path. Poirier (1886) was the first to discover and describe this species which had been retrieved from the biliary ducts of a fishing cat (*Prionailurus viverrinus*) which had been kept in the Zoological Gardens attached to the Paris Natural History Museum. It was only over 20 years later that Leiper (1915) described the first specimens from humans supplied by WFJ Kerr from Chiang Mai in the north of Thailand. In 1916, Kerr published a parasitologically more detailed paper listing 17% of the 230 adult male prisoners examined to be infected. Interestingly, 74% of those examined were Laotians who had lived in the Chiang Mai area for their whole lives—there were very few Thais in the sample group. In this paper, Kerr originally identified the species as *Opisthorchis felineus*, but in a postscript indicates that they were identified by Leiper as *O. viverrini*. About a decade after Kerr's paper, Prommas (1927) reported the first case of infection (again as *O. felineus*) from the northeast of Thailand at Roi-et while Bedier and Chesneau (1929) reported high prevalences of 25% and 15% from Thakhek and Vientiane, respectively, in Lao PDR.

Gurlt was the first to describe and illustrate *O. felineus* (subfamily Opisthorchiinae) although he confused it with *Distomum conus* Creplin 1825 (Erhardt et al. 1962). It was only in 1885 that *O. felineus* which had been isolated from cats were described as a valid species by Rivolta as *Distomum felineum* (Rivolta 1884). It was later moved from the genus *Distomum*, *Opisthorchis*, created by Blanchard in 1895. This is the type genus for the family Opisthorchiidae Looss, 1899. Opisthorchiasis in humans caused by *O. felineus* was first described by Vinogradoff (1892) from Siberia.

6.2 Current Status and Geographical Distribution

Human populations show high levels of infection with all three liver fluke species within each of their distributional ranges. Up to 680 million people worldwide are at risk of infection (Keiser and Utzinger 2005). Recent estimates indicate that 45 million people living in Asia and Europe are infected, with approximately 35 million *C. sinensis* cases, ten million *O. viverrini* cases, and 1.2 million cases of *O. felineus* (WHO 1995; Sithithaworn et al. 2012a).

Clonorchis sinensis is the most frequent human parasite of the three with 600 million people at risk of infection (Keiser and Utzinger 2005) in East Asia from mid-Vietnam through much of China, including Taiwan, into Korea and the far east of Russia (Rim 2005; Hong and Fang 2012). Although *C. sinensis* was previously endemic in Japan, the last human case was in 1991 and no autochthonous case has been reported since (Lun et al. 2005). Doanh and Nawa (2016) suggest that previous identifications of *C. sinensis* and *O. viverrini* from Vietnam may have been flawed leading to overestimates in the prevalences of both species in this country. The second most common species is *O. viverrini* which is found along the

Lower Mekong and its tributaries in the north and northeast of Thailand, Lao PDR, Cambodia, and southern Vietnam, with recent reports from Myanmar (Aung et al. 2017; Sanpool et al. 2018). Data for the last three countries are sparse and for *O. viverrini* most of our information comes from the north and northeast of Thailand with an increasing list of publications from Lao PDR (Sithithaworn and Haswell-Elkins 2003; Andrews et al. 2008; Petney et al. 2013). Movement of people within the Mekong area is probably, at least in part, responsible for the presence of *O. viverrini* infection in other parts of Thailand (Buathong et al. 2017). The information which is available suggests that as many as 67 million people may be at risk of infection (Keiser and Utzinger 2005).

Although *O. felineus*, the European liver fluke, is the most poorly studied of the three species it has been reported from continental European countries except Finland, Norway, and Sweden. It does not occur in the UK. Animal hosts are wild and domestic carnivores (Erhardt et al. 1962) but humans probably play a significant role in parasite transmission (Petney et al. 2013). Human cases have been reported to occur in Belarus, Germany, Greece, Italy, Poland, Romania, Russia, Spain, the Ukraine, the Baltic countries, Moldova, and Kazakhstan, but records from some of these countries are over 50 years old (Erhardt et al. 1962; Mordvinov et al. 2012; Pozio et al. 2013). *O. felineus* represents a significant health problem in certain areas of Siberia where evidence indicates that its prevalence in both humans and in animals increases from west to east (Mordvinov et al. 2012). In the Ob-Irtysh basin, where the prevalence of infection peaks, it is of particular medical significance (Mordvinov et al. 2012; Fedorova et al. 2017).

Although clonorchiasis is endemic to East Asia and opisthorchiasis to the Mekong area of Southeast Asia and parts of eastern Russia all can occasionally be found in non-endemic areas having been introduced by infected tourists, refuges, or workers, who have moved from endemic to non-endemic areas (Hira et al. 1987; Molina et al. 1988; WHO 1995; Saksirisampant et al. 2002; Fried and Abruzzi 2010). The flukes have, however, not currently become endemic in these areas due to the lack of suitable intermediate hosts.

A predictive future climate model for Thailand suggests that the distribution of *O. viverrini* will be significantly affected by anticipated changes in precipitation and temperature with the northeast becoming increasingly unsuitable (Suwannatrai et al. 2017).

6.3 Biology and Life Cycle

Liver flukes are hermaphroditic trematodes which are dorso-ventrally flattened. The body has an oral sucker situated anteriorly and a ventral sucker at mid-body. The differentiation of the species is based on morphology. The adult worms differ in the shape and position of their testes and the arrangement of the vitelline glands. *C. sinensis* can be separated from the other two species by the presence of branched testes in a tandem position and the continuously distributed vitelline glands (Fig. 6.1). Although *O. viverrini* is

Fig. 6.1 Adult worms of the liver flukes. (**a**) *Opisthorchis viverrini* (**b**) *Clonorchis sinensis*

Fig. 6.2 A general life cycle of the opisthorchiid liver flukes

similar to *O. felineus* in having lobed testes and a cluster vitelline gland, it differs by having deeper lobulation of and greater extremity of the testes and also lacks transversely compressed patterns of vitelline follicles. The size of the adult flukes depends on the species involved. *O. viverrini* is the smallest, measuring 5.5–10 × 0.77–1.65 mm. *O. felineus* is somewhat larger measuring 7–12 × 2–3 mm (Kaewkes 2003; Pozio et al. 2013) while *C. sinensis* is the largest measuring 10–25 × 3–5 mm. Variation in the size of the adults is density dependent, with individuals being smaller the higher the infestation, and also dependent on the diameter of the bile duct they inhabit (Flavell et al. 1983).

The eggs of *C. sinensis*, *O. felineus*, and *O. viverrini* are morphologically similar making them difficult to distinguish from one another. The operculum of each species has a distinct shoulder while a small knob or comma shape appendage is found at the abopercular end (Kaewkes 2003). The surface of the egg shell is rough and irregular having been described as having a "musk-melon pattern" by scanning electron microscopy (Tesana et al. 1991).

The transmission cycle of all three liver fluke species goes through three phases: (1) the infection of the snail first intermediate hosts via host feces, (2) cercarial release and finding fish second intermediate hosts for development of the infective metacercariae stage, and (3) ingestion of metacercariae in raw or partially cooked fish by humans. Petney et al. (2013) argue that the three species differ in terms of the relative significance of the zoonotic and anthroponotic components of the epidemiological cycle with *O. viverrini* having mainly human final hosts, *O. felineus* in Europe mainly wild carnivore hosts and *C. sinensis* and *O. felineus* in its Asian range a mixture of the two. This has considerable epidemiological significance, particularly in control and prevention programs.

The life cycles (Fig. 6.2) of all three species are very similar with a snail first intermediate host with usually low prevalences of infection, fish second intermediate hosts with substantially higher levels of infection, and usually a carnivorous mammal as final host (Schuster 2002; Zhang et al. 2007). The low prevalences of infection in the snail first intermediate hosts is, at least in part, compensated for by the often very high prevalences in fish, the infective phase for humans.

The distribution of each species is closely related to that of their hosts. This is particularly true of the snail first intermediate hosts that tend to be more restricted in the number of species used (Hong and Fang 2012; Kiatsopit et al. 2012; Petney et al. 2012). For *O. viverrini*, the presence

of snails of *Bithynia siamensis goniomphalos* in nearby freshwater sources is a prerequisite for the presence of the parasite in the human population (Petney et al. 2012; Wang et al. 2013).

The importance of fecal contamination of freshwater sources inhabited by intermediate snail hosts by wild and domestic animal hosts (i.e., the zoonotic cycle) varies greatly between the three fluke species. In its European range fecal matter containing *O. felineus* eggs is almost exclusively found in wild and domestic carnivores. In areas with widespread human infection, fecal contamination of freshwater by infected animals is certainly reduced and in the case of *O. viverrini* probably only plays a minor role in infecting snails.

Clonorchis sinensis is a species with a relatively broad range of hosts sharing anthroponotic and zoonotic components in its epidemiological cycle (Petney et al. 2013). *C. sinensis* is known to use 8 main snail species as intermediate hosts. These come from 5 different families (Assimineidae, Bithyniidae, Hydrobiidae, Melaniidae, Thiaridae) (Lun et al. 2005). There is some geographic differentiation in the distribution and prevalence of *C. sinensis* in the different snail species. Prevalences vary locally and may be as high as 27% for *Alocinma longicornis* in parts of Guangdong and 8% for *Bithynia fuchsianus* in parts of Guangxi although most values are substantially lower (Lun et al. 2005).

Once the snail has ingested the embryonated eggs, which are passed in the feces of the final host, the eggs hatch to release miracidia which then undergo development to sporocysts then rediae and finally cercariae in the snail. The tailed cercarial stage escapes from the snail host and actively swims to find a suitable fish second intermediate host which it then penetrates, losing its tail and encysting to become a metacercaria embedded either in the muscles of the fish or under the scales.

C. sinensis utilizes predominantly fish in 11 families, with 46 genera and 132 species of which 32 genera and 71 species belong to the family Cyprinidae, but also several crustacean second intermediate hosts (Lun et al. 2005). The fish include a number of species which are commonly used in aquaculture fish, including the common carp (*Cyprinus carpio*), the grass carp (*Ctenopharyngodon idellus*), the silver carp (*Hypophthalmichthys molitrix*), as well as tilapia (*Oreochromis mossambicus*) (Lowe et al. 2000; Naylor et al. 2000; Lun et al. 2005; Gozlan et al. 2010). Wild fish species can also have high prevalences and intensities of infection (Bui et al. 2016).

The mammalian final hosts are infected when they eat raw or partially cooked fish containing the metacercariae of *C. sinensis*. This fluke has an unusually broad final host spectrum that includes human associated species such as cats and dogs, stock animals such as pigs and the brown rat (*Rattus norvegicus*), all of which are effective reservoir hosts, as well as a wide variety of wild fish-eating carnivores and occasionally birds (Mas-Coma and Bargues 1997; Lun et al. 2005). Nevertheless, humans are considered to be the most important final host (Lun et al. 2005).

After being eaten, the metacercariae excyst in the host's duodenum and then move through the hepatopancreatic ampulla into the biliary ducts and towards the liver. Here they attach to the mucosal lining and develop into hermaphrodite adults. These begin reproduction after about 3–4 weeks and may remain viable for years (Rim 2005). Attwood and Chou (1978) reported that parasites survive up to 26 years in an infected human. In the case of humans, infection occurs through the deliberate ingestion of raw, partially cooked or fermented fish containing the infective metacercariae as part of tradition a food culture (Grundy-Warr et al. 2012; Onsurathum et al. 2016a, b).

Although there are varying estimates of the number of humans infected with *C. sinensis*, it appears that this number is increasing (WHO 1995). This is particularly the case in China where prevalence was estimated to be 4.7 million in the early 1990s to almost 13 million today (WHO 1995; Fang et al. 2008). In the Republic of Korea, the picture is variable with estimates of 4.6% in 1971, 1.4% in 1997, and 2.4% in 2004 (Kim et al. 2009b). Unfortunately, little long-term records are available from Vietnam.

In addition to its direct influence on human health, *Clonorchis* infection in the second intermediate host can also reach substantial prevalences in aquaculture fish potentially limiting profitability in the aquaculture industry (Keiser and Utzinger 2005). Chen et al. (2010) found that freshwater fish in aquaculture had a 37.09% prevalence of infection with a mean number of 10.7 cercariae/fish, while 3.07% of shrimps from freshwater ponds carried on average a single metacercaria.

Molecular methods will probably extend our knowledge of the distribution of opisthorchid parasites. Traub et al. (2009), using PCR-based technology, detected *C. sinensis* eggs in 23% (5/22) of human feces which tested positive by microscopy as well as PCR for "*Opisthorchis*" like eggs. The samples came from a rural community in Eastern Thailand (Chachoengsao Province) extending the known range of *C. sinensis* substantially to the south and east of its recognized distribution in an area where it is sympatric with *O. viverrini*. Whether *C. sinensis* overlaps elsewhere with *O. viverrini* or *O. felineus* is currently unknown. *C. sinensis* is known to infect a variety of mammalian hosts including domestic dogs, cats, and pigs (Rim 2005; Lai et al. 2016). Dogs and cats can have high prevalences of infection (0.8–48.5% in dogs, 0–64.1% in cats), which, however, varies, considerably between endemic areas in China (Hong and Fang 2012). The higher prevalence in cats, as for *O. viverrini*, is probably due to their preference for eating fish. Pigs, which are omnivorous, had a prevalence of infection of 27% in southern China (Lin et al. 2005).

Opisthorchis viverrini is known only from three currently recognized taxa within a single snail genus, *Bithynia funiculata*, *B. siamensis gomiomphalos*, and *B. s. siamensis*, from Southeast Asia (Petney et al. 2012). In Thailand, all three taxa of *Bithynia* are found, namely *B. funiculata* in the north, *B. s. siamensis* in the center, and *B. s. goniomphalos* in the northeast (Sithithaworn et al. 2007b). However, a recent study based on combined morphological and molecular identification methods found that in addition to *B. funiculata*, *B. s. siamensis* and *B. s. goniomphalos* were also distributed in the north of Thailand (Naruemon et al., unpublished). No regional separation of *Bithynia* snails has been reported in other parts of Southeast Asia, probably due to insufficient surveys (Kiatsopit et al. 2013).

The prevalence of *O. viverrini* infection in *Bithynia* snails is variable, with numerous collections being parasite free. If the parasite is present, cercarial release commonly occurs from about 0.1–2% of individuals, but some collections have infection rates of 6–9% (Table 6.1) (Kiatsopit et al. 2012; Petney et al. 2012). Snail population density is strongly seasonal, being highly abundant later in the rainy season, when reproduction occurs. At this time, the *Bithynia* are extensively

Table 6.1 List of first intermediate hosts in Southeast Asia infected by cercariae of *O. viverrini*

Country	Snail	Sample size	% Prevalence	References
Thailand	*Bithynia funiculata*	352	0.30	Ngern-klun et al. (2006)
	B. siamensis siamensis	2800	1.60	Upatham and Sukhapanth (1980)
	B. s. goniomphalos	5729	0.45	Prasopdee et al. (2015)
	B. s. goniomphalos	1382	3.04	Kiatsopit et al. (2012)
	B. s. goniomphalos	537	0.37	Kaewkes et al. (2012a)
	Bithynia snails	18,078	0.13	Kaewkes et al. (2012b)
	B. s. goniomphalos	4874	0.61–1.30	Sri-Aroon et al. (2005)
	B. s. goniomphalos	N/A	0.14	Lohachit (2004-2005)
	B. s. goniomphalos	6150	0.05	Adam et al. (1993)
	B. s. goniomphalos	48,327	0.07	Brockelman et al. (1986)
Cambodia	*B. s. siamensis*	406	0.25	Miyamoto et al. (2014)
Lao PDR	*B. s. goniomphalos*	3142	2.01	Kiatsopit et al. (2012)
	B. s. goniomphalos	81	2.47	Sri-Aroon et al. (2011)
	B. s. goniomphalos	3913	0.60	Giboda et al. (1991)
	B. s. goniomphalos	2000	0.95	Ditrich et al. (1990)

distributed in shallow water and rice fields. They can be found at a depth of at least 3 m, albeit in a much lower density (Suwannatrai et al. 2011). During the dry season, the population density crashes and the snails which survive are often found buried in the mud for seasonal aestivation (Brockelman et al. 1986; Petney et al. 2012).

The snails are infected by ingesting the embryonated eggs of the parasite which are excreted with fecal matter. Indeed, human fecal bacterial contamination of freshwater bodies can act as an indicator for the seasonal transmission of *O. viverrini* eggs to snail intermediate hosts (Kaewkes et al. 2012b). Once ingested, the eggs hatch to a miracidium which in turn develops to a sporocyst (Kaewkes 2003). Peak hatching occurred at temperatures between 24 and 28 °C and could be induced with porcine leucine aminopeptidase (Khampoosa et al. 2018). By way of contrast, Prasopdee et al. (2015) found that a temperature of 34 °C gave the highest rate of infection of 44%, and that the likelihood of infection in small snails was significantly higher than for medium-sized snails. Once the sporocyst has developed within its snail host, it gives rise to numerous rediae that in turn produce numerous pleurolophocerous cercariae. The factors determining the time of cercarial release and the number released appear to vary dependent on season, location, and snail size although the pattern is not consistent (Namsanor et al. 2015; Laoprom et al. 2016). After released, they actively seek an appropriate fish second intermediate host, in the case of *O. viverrini* these belong to the family Cyprinidae (Sithithaworn et al. 2007b; Zhang et al. 2007; Mordvinov et al. 2012).

The process of host finding by cercariae is complex. Free swimming cercariae are very efficient at actively locating the appropriate species of fish in a large volume of water (Haas et al. 1990). The intensity of infection in fish varies by season, species, individuals, and types of water bodies. Most metacercariae are distributed throughout the body of fish with some being found in the head. For *O. viverrini*, metacercarial burdens peak in winter (October–February) and become low in the rainy season and summer; thus, transmission of the parasite from fish to humans is probably seasonal. Donthaisong et al. (2014) found over 80% of metacercariae in the body of the fish and that cercarial infection dosage, and age and size of fish were important determinants for a successful infection.

Many species of cyprinid fish have been reported as potential hosts for *O. viverrini* (WHO 1995; Petney et al. 2013, 2018). In Thailand, Lao PDR and Cambodia, at least 40 species of fish from 18 genera have been reported to serve as intermediate hosts of *O. viverrini*. Of these, the genera *Cyclocheilichthys*, *Puntius*, and *Hampala* are considered to be the most important (Pitaksakulrat et al. 2013). For *O. viverrini*, the prevalence of infection in the fish second intermediate host is very much higher than in the *Bithynia* snail first intermediate hosts (Table 6.2). This is, however, species of fish and locality dependent, and ranges from 2.1 to 100% (Waikagul 1998). For example, 30.4–97.1% prevalence was found in *C. apogon* (Touch et al. 2009), 43.1–100% in *C. armatus* (Rim et al. 2008; Manivong et al. 2009), 69.9–93.7% in *P. leiacanthus* (Vichasri et al. 1982), and 33.3–74.1% in *H. dispar* (Rim et al. 2008; Manivong et al. 2009). The average number of metacercariae infecting fish varies from one to thousands with the highest intensity (average 1989.8/fish) in *C. armatus* from Savannakhet, Lao PDR (Rim et al. 2008).

Humans are the dominant hosts for *O. viverrini*, while other domestic mammals, for instance dogs and cats, can act as reservoir hosts (Sithithaworn et al. 2007b; Aunpromma et al. 2012, 2016). A mathematical model of *O. viverrini* transmission by Bürli et al. (2018b) indicates that humans are necessary for the maintenance of the transmission cycle and can sustain this cycle without additional reservoir hosts. Domestic cats have been found to have a relatively high prevalence in the northeast of Thailand, making them potentially significant zoonotic sources of the disease during human-based control programs (Aunpromma et al. 2012). Dogs usually have a much lower prevalence (Aunpromma et al. 2012) although Prakobwong et al. (2017) found the reverse with dogs having 18% prevalence compared to cats with 11%. Hamsters, rabbits, and guinea pigs are experimentally highly susceptible to infection (WHO 1995). There is no current information for native mammals and other fish-eating animals which may also be infected.

Table 6.2 List of second intermediate hosts in Southeast Asia infected by metacercariae of *O. viverrini*

Family	Fish	% Prevalence	Country	References
Cyprinidae	*Labiobarbus siamensis*	51.3–100	Thailand, Cambodia	Phalee et al. (2008), Touch et al. (2013) and Miyamoto et al. (2014)
	Cyclocheilichthys armatus	19.16–100	Thailand, Cambodia, Lao PDR	Wykoff et al. (1965), Sithithaworn et al. (1997), Awiruttapanich (2004), Phalee et al. (2008), Rim et al. (2008, 2013), Manivong et al. (2009), Kaewpitoon et al. (2012), Pinlaor et al. (2013), Pitaksakulrat et al. (2013) and Touch et al. (2013)
	Hampala macrolepidota	2.6–100	Thailand, Cambodia, Lao PDR	Ditrich et al. (1990), Giboda et al. (1991), Scholz et al. (1991), Sukontason et al. (1999), Sayasone et al. (2007), Touch et al. (2009) and Kaewpitoon et al. (2012)
	Amblyrhynchichthys truncates	100	Cambodia	Touch et al. (2013)
	Neolissochilus stracheyi	100	Lao PDR	Rim et al. (2013)
	Lobocheilos melanotaenia	100	Lao PDR	Sayasone et al. (2007)
	Puntius partipentazona	100	Thailand	Vichasri et al. (1982)
	Cyclocheilichthys apogon	25.0–97.1	Thailand, Cambodia, Lao PDR	Vichasri et al. (1982), Sithithaworn et al. (2006), Touch et al. (2009, 2013) and Rim et al. (2013)
	Hampala dispar	6.49–94.80	Thailand, Cambodia, Lao PDR	Wykoff et al. (1965), Vichasri et al. (1982), Ditrich et al. (1990), Giboda et al. (1991), Scholz et al. (1991), Sithithaworn et al. (1997, 2006), Rim et al. (2008, 2013), Manivong et al. (2009), Touch et al. (2009, 2013), Ngoen-klan et al. (2010), Kaewpitoon et al. (2012), Pinlaor et al. (2013) and Miyamoto et al. (2014)
	Puntius brevis (Puntius leiacanthus)	14.0–93.7	Thailand, Cambodia, Lao PDR, Vietnam	Vichasri et al. (1982), Giboda et al. (1991), Sithithaworn et al. (1997), Sayasone et al. (2007), Rim et al. (2008, 2013), Touch et al. (2009, 2013), Dung et al. (2014) and Miyamoto et al. 2014)
	Poropuntius laoensis	90.0	Lao PDR	Sayasone et al. (2007)
	Puntius orphoides	31.0–90.0	Thailand, Cambodia, Lao PDR	Wykoff et al. (1965), Sithithaworn et al. (2006), Touch et al. (2009, 2013), Ngoen-Klan et al. (2010), Sohn et al. (2012) and Pinlaor et al. (2013)
	Cyclocheilichthys repasson	10.0–80.0	Thailand, Cambodia, Lao PDR	Ditrich et al. (1990), Giboda et al. (1991), Scholz et al. 1991; Rim et al. (2008), Manivong et al. (2009), Kaewpitoon et al. (2012) and Miyamoto et al. (2014)
	Cyclocheilichthys enoplos	2.1–80.0	Cambodia, Lao PDR	Sayasone et al. (2007), Manivong et al. (2009), Touch et al. (2009) and Rim et al. (2013)
	Esomus metallicus	10.0–75.0	Thailand, Lao PDR	Wykoff et al. (1965) and Rim et al. (2008)
	Labiobarbus lineatus	3.0–69.6	Thailand, Lao PDR	Wykoff et al. (1965) and Manivong et al. (2009)
	Barbonymus altus	30.0–66.7	Cambodia	Touch et al. (2009, 2013) and Ngoen-Klan et al. (2010)
	Barbonymus schwanenfeldii	66.0	Cambodia	Touch et al. (2013)
	Puntioplites proctozystron	2.0–60.0	Thailand, Cambodia, Lao PDR	Wykoff et al. (1965), Manivong et al. (2009), Touch et al. (2009, 2013), Ngoen-Klan et al. (2010), Kaewpitoon et al. (2012), Sohn et al. (2012), Pinlaor et al. (2013) and Rim et al. (2013)

	Thynnichthys thynnoides	3.7–59.7	Thailand, Cambodia,	Sukontason et al. (1999) and Touch et al. (2009, 2013)
	Cyclocheilichthys lagleri	58.2	Cambodia	Touch et al. (2013)
	Hypsibarbus lagleri	50.0	Lao PDR	Rim et al. (2008)
	Mystacoleucus marginatus	50.0	Lao PDR	Rim et al. (2008)
	Onychostoma elongatum	44.4	Lao PDR	Rim et al. (2008)
	Henicorhynchus lineatus	42.9	Lao PDR	Manivong et al. (2009)
	Labeo chrysophekadion	40.0	Cambodia	Sohn et al. (2012) and Miyamoto et al. (2014)
	Oreichthys parvus	40.0	Lao PDR	Sayasone et al. (2007)
	Henicorhynchus lobatus	33.3	Cambodia	Touch et al. (2013)
	Hypsibarbus pierrei	33.3	Lao PDR	Rim et al. (2013)
	Hypsibarbus wetmorei	33.3	Lao PDR	Rim et al. (2013)
	Poropuntius deauratus	33.3	Lao PDR	Rim et al. (2013)
	Puntioplites falcifer	33.3	Lao PDR	Rim et al. (2008)
	Rasbora ourotacniatiran	33.3	Lao PDR	Sayasone et al. (2007)
	Osteochilus waandersii	30.5	Lao PDR	Manivong et al. (2009)
	Carassius auratus	28.1	Vietnam	Dung et al. (2014)
	Cyclocheilichthys furcatus	25.0	Cambodia, Lao PDR	Ngoen-Klan et al. (2010) and Rim et al. (2013)
	Puntius viehoever	22.0	Thailand	Wykoff et al. (1965)
	Osteochilus hasselti	6.1–20.0	Thailand, Cambodia, Lao PDR	Rim et al. (2008), Ngoen-Klan et al. (2010), Pinlaor et al. (2013), Touch et al. (2013) and Miyamoto et al. (2014)
	Puntius stoliczkanus	16.67	Thailand	Wongsawad et al. (2013)
	Barbonymus gonionotus	2.0–16.1	Thailand, Cambodia, Lao PDR	Wykoff et al. (1965), Ditrich et al. (1990), Giboda et al. (1991), Scholz et al. (1991) Sukontason et al. (1999) Pitaksakulrat et al. (2013), Touch et al. (2013) and Miyamoto et al. (2014)
	Onychostoma fusiforme	14.3	Lao PDR	Rim et al. (2013)
	Henicorhynchus siamensis	4.3–10.9	Thailand, Cambodia	Touch et al. (2009, 2013) and Pinlaor et al. (2013)
	Paralaubuca barroni	10.0	Lao PDR	Rim et al. (2013)
	Crossocheilus reticulatus	5.6	Cambodia	Touch et al. (2013)
	Rasbora spp.	4.3	Vietnam	Dung et al. (2014)
	Osteochilus sp.	4.0	Thailand	Wykoff et al. (1965)

For *O. viverrini*, when fecal contamination from humans is reduced or eliminated by mass treatment and/or improved sanitation the prevalence of infection can be substantially reduced (Saowakontha et al. 1993; Jongsuksuntigul and Imsomboon 2003). However, complete elimination of infection may not be possible if domestic cat and dog reservoir hosts maintain the source of parasite and hence play a critical role in maintaining the life cycle. Based on the pattern of age-intensity profiles, *O. viverrini* may survive for 10–20 years (Sithithaworn and Haswell-Elkins 2003).

In the case of *C. sinensis* or *O. viverrini*, which are both known to cause significant health problems (Sithithaworn et al. 2007b), considerable epidemiological information is available, e.g., prevalence and intensity of infection increase with age and males tend to have a higher prevalence of infection than females (Sithithaworn et al. 2007b). Thus, hepatobiliary morbidity is more frequent in older individuals and males than in younger people and females. In addition, as is usual with many parasite species, the distribution in the host population is neither random nor uniform, but overdispersed with a few individuals harboring most of the worms (Sithithaworn et al. 1991). Interestingly, some individuals appear to be predisposed to a heavy infection, with the intensity of infection returning to pretreatment levels after treatment (Upatham et al. 1988). Such epidemiological characteristics must be incorporated into the models on which control and eradication programs are based.

The infective metacercariae of all species discussed here are found only in their specific (mostly cyprinid) fish second intermediate hosts. These fish, when eaten raw, fermented or undercooked, act as the source of infection to humans and animal hosts (Grundy-Warr et al. 2012). Traditional dishes based on raw fish are the main sources of infection of *C. sinensis* throughout its range, for example, goi ca mai (raw fish salad) and slices of raw silver carp in Vietnam, yusheng, a raw fish salad in China and sushi, sliced raw fish, in Korea and Japan (Rim 2005). The length of time which the metacercariae remain viable depends on the method of preparing the food. In areas where less infected fish dishes are eaten, being replaced by beef or pork, infection prevalence and intensity can be reduced (Feldmeier et al. 2016).

In Southeast Asia, particularly in Thailand and Lao PDR, the raw or partially cooked fish dishes which act as the source of the liver fluke infection can be grouped into 3 categories. Fresh raw fish dishes without heating are called "koi pla." These pose a high risk of infection. A moderate risk is presented by quickly fermented dish (1–2 days) known as "pla som." The last dish is fermented fish known as "pla ra" which normally requires long-term fermentation but short-term and variable ingredients may provide favorable environments for metacercarial survival. Pla ra is a common ingredient for cooking in Southeast Asia, for example, in papaya salad (som tum) (Grundy-Warr et al. 2012). In Lao PDR, the fermented fish is known as "pla dak," In other countries such as Cambodia raw fish are prepared as "pla hoc" which is similar to pla som and this may serve as a source of infection. The usual sources of *O. felineus* infection in Russia are dried or salted fish. Other dishes include sliced raw fish (stroganina), which is popular among native Siberians, and fish pickled in vinegar.

The number of *Bithynia* species present in the Eurasian area from a single species, *Bithynia leachi,* to four morphologically similar species, *B. inflata*, *B. leachi*, *B. troscheli*, and *B. sibirica* (Lazutkina et al. 2009) of which the first three of these can act as intermediate hosts for *O. felineus* (Mordvinov et al. 2012). As with *O. viverrini*, cyprinid fish also act as the exclusive second intermediate hosts for *O. felineus* (Erhardt et al. 1962). These include the ide (*Leuciscus idus*), roach (*Rutilus rutilus*), European dace (*Leuciscus leuciscus*), tench (*Tinca tinca*), verhovka (*Leuciscus delineatus*), and silver crucian carp (*Carassius auratus gibelio*) (Mordvinov et al. 2012). After an outbreak of *O. felineus* infection in Italy, 83.1% of tench from Lake Bolsena were found to be infected (Armignacco et al. 2008). Apart from this outbreak, people are seldom infected in Europe probably because raw fish is not common in the human diet in this area

(Pozio et al. 2013). However, prevalences can be very high in people in the Asian distributional area of this species where raw fish are more commonly consumed (Ogorodova et al. 2007).

In Europe, domestic cats and dogs can act as hosts (Erhardt et al. 1962; Hering-Hagenbeck and Schuster 1996) in addition to a wide range of wild carnivores. These include several fox species, the raccoon dog and wolves, as well as *Martes* and *Mustela* spp., badger, otter and wolverine (Erhardt et al. 1962; Shimalov and Shimalov 2003). Unlike *C. sinensis* and *O. viverrini*, *O. felineus* has also been reported from the Pinnipedia: the Caspian seal (*Pusa caspica*) from the brackish Caspian Sea, the bearded seal (*Erignathus barbatus*) from the Arctic and the gray seal (*Halichoerus grypus*) (Erhardt et al. 1962; Mordvinov et al. 2012). Non-carnivore and therefore presumably accidental hosts include chipmunks (*Eutamias sibiricus*) (Mordvinov et al. 2012), beaver (*Castor fiber*), European water vole (*Arvicola terrestris*), the brown rat (*Rattus norvegicus*), rabbit (*Oryctolagus cuniculus*), and wild and domestic pig (*Sus scrofa*) (Erhardt et al. 1962; Mordvinov et al. 2012).

6.4 Molecular Biology, Genetics, and Evolution

Advances in molecular biology have provided opportunities for increasing our understanding of the developmental and reproductive biology, as well as genetic diversity and evolution, of medically important trematodes including *Opisthorchis* and *Clonorchis*. They have also helped us to understand host–parasite interactions and the pathogenesis of the diseases caused by these flukes. This has aided in the improvement of diagnostic methods, new drug treatments, and potentially vaccine development. In addition, it is likely that new molecular data will help in the examining theories on the molecular basis of chronic clonorchiasis and opisthorchiasis-induced cholangiocarcinoma. Below we will also discuss the cytogenetics, genomics, transcriptomics, and proteomics of these species.

6.4.1 Cytogenetic Analysis

The cytogenetic study of the liver flukes focuses on the study of the structure and function of the chromosomes. It includes the analysis of G-banded chromosomes, other cytogenetic banding techniques, as well as molecular cytogenetics, such as fluorescent *in situ* hybridization (FISH) and comparative genomic hybridization (CGH). The karyotypes of *C. sinensis* are reported to be either of 2n = 14 (samples from the far east of Russia) (Zadesenets et al. 2012b) and 2n = 56 (samples from Korea and China) (Park et al. 2000a). The $2n = 56$ karyotype was described according to the number and morphology of chromosomes, probably being the octaploid form of typical opisthorchiid karyotype with $2n = 14$ (Zadesenets et al. 2012b). The karyotypes from Russia consisted of pairs of large meta- and submetacentric chromosomes and five pairs of small chromosomes (Zadesenets et al. 2012b). Those from Korea and China can be divided into two groups based on their sizes, with 8 pairs of large and 20 pairs of small chromosomes. They have the same number of 16 submetacentric and 8 subtelomeric pairs, but the Korean isolates have 3 metacentric and 1 meta-/submetacentric pairs, whereas the Chinese isolates have 2 and 2 pairs. In addition, the mean total length of the diploid complements of the specimens collected in China is slightly longer than that of those collected in the Korea (Park et al. 2000a). The question of the phylogenetic relationship between *C. sinensis* from Russia, China, and Korea therefore remains open. The liver fluke described in China and Korea could be an octaploid form of normal *C. sinensis* or possibly distinct species (Zadesenets et al. 2012b).

The karyotype of *O. felineus* collected from West Siberia was $2n = 14$ and consisted of two pairs of large submetacentrics and five pairs of small chromosomes. There are 3 pairs of metacentric and 4 pairs of submetacentric chromosomes (Polyakov et al. 2010). A comparison of the relative length and centromere indices of the chromosomes of these *O. felineus* did not reveal significant differences (Polyakov et al. 2010).

In contrast, the karyotype of *O. viverrini* is $2n = 12$ and includes two pairs of large submetacentrics, one pair of medium-sized submetacentrics, one pair of small-sized subtelocentrics or acrocentrics and one pair of small-sized acrocentric chromosomes (Komalamisra 1999). The medium-sized submetacentric chromosome of *O. viverrini* is probably the result of the fusion of two chromosomes from ancestral karyotypes (Zadesenets et al. 2012b). However, the comparative analysis of mitotic and meiotic chromosomes by heterologous FISH revealed six pairs of chromosomes in the karyotype of *O. viverrini*, indicating a relatively recent chromosomal fusion event which took place in the formation of the modern karyotype (Zadesenets et al. 2012b). However, none of the *O. viverrini* chromosomes have shown any interstitial telomere sequences (ITSs) after FISH by telomeric DNA probe or PNA telomere probe (Zadesenets et al. 2012a). More recently, the chromosome number, length, and nomenclature of each chromosome were determined by scanning electron microscopy. The six chromosomes consist of one large metacentric, one medium-sized metacentric, two small-sized metacentric, one small-sized submetacentric, and one small-sized acrocentric chromosomes (Kaewkong et al. 2012a). Moreover, the repetitive sequences show that despite the small size of opisthorchis genomes, a large amount of interspersed repetitive DNA sequence is distributed along the euchromatic regions (Zadesenets et al. 2012c).

6.4.2 Genome

There are some reports on the genomic features characterizing *C. sinensis,* but very few on *O. viverrini* and *O. felineus*. The assembled genome of *C. sinensis* has a total size of 516 Mb with approximately 16,000 reliable protein-coding gene models. Genes for the complete pathways for glycolysis, the Krebs cycle and fatty acid metabolism were found, but key genes involved in fatty acid biosynthesis are missing from the genome, reflecting the fact that the liver fluke receives lipids from the bile of its host. Moreover, genes encoding proteases, kinases, and phosphatase enzymes, tegument and excretory-secretory products, host-binding proteins and receptors were also discovered. In addition, 53 genes related to sex determination, sex differentiation, and sexual reproduction were identified (Wang et al. 2011). The genome of *C. sinensis* contained more than 100 copies of a long terminal-repeat retrotransposon (*CsRn1*) which belongs to the Ty3/gypsy-like long terminal-repeat transposon family. The functional domains of Gag, proteinase, reverse transcriptase, Rnase H, and subdomains of integrase are strongly conserved in *CsRn1*, which has been predicted to be mobile element based on structural considerations and from the presence of mRNA transcripts (Bae et al. 2001). Insertions of *CsRn1* appear preferentially at repetitive and agenic chromosomal regions. Furthermore, *CsRn1* was reported to induce variations in the genome that may influence the evolution of *C. sinensis* (Bae and Kong 2003). The finding of such genomic characters of *C. sinensis* reveal the evolutionary interplay between parasite and host, which may be valuable for understanding host and parasite interactions (Wang et al. 2011). In the case of *O. viverrini*, the estimated genomes size reported by real-time PCR was 75.95 Mb (Kaewkong et al. 2012b). However, whole genome sequencing of these liver flukes has not been reported.

The complete mitochondrial genomes of all three liver flukes have been successfully characterized. The mtDNA sequences of *O. viverrini*, *O. felineus*, and *C. sinensis* were variable and ranged between 13,510 and 14,277 bp and comprised 36 genes (Shekhovtsov et al. 2010; Cai et al. 2012). Of these, 12 genes encoded for proteins, i.e., cytochrome c oxidase subunit 1 (CO1), CO2, CO3, NADH dehydrogenase subunit 1 (ND1), ND2, ND3, ND4, ND4L, ND5, ND6, cytochrome b and ATPase subunit 6. Two genes encoded for ribosomal RNA, i.e., small subunit rRNA (*rrn*S) and large subunit rRNA (*rrn*L). The number of tRNA encoding genes varied between the different species, i.e., 20 genes for *O. viverrini* and 22 genes for *O. felineus* and

C. sinensis (Shekhovtsov et al. 2010; Cai et al. 2012). The gene content and arrangement were almost identical between species. There were two non-coding regions, the long non-coding region (LNR) and the short non-coding region (SNR). For the *C. sinensis*, there is a lack of tandem repeat (Cai et al. 2012), whereas there was tandem repeat, interrupted in LNR region, in *O. felineus*. Moreover, when comparing the length of non-coding region of the mtDNA of *C. sinensis* from Russia and Vietnam there were significant differences between species (Shekhovtsov et al. 2010).

6.4.3 Transcriptome

Expressed sequence tags (ESTs) for adult *C. sinensis* and *O. viverrini* have been reported with at least 3000 and 4194 ESTs, respectively, and have been registered in public dbEST databases (Mulvenna et al. 2010; Huang et al. 2012). The most abundant genes in adult *C. sinensis* include cysteine proteases and mitochondrial genes, which may support biliary epithelia destruction by adult liver flukes to evade host immune attack (Kang et al. 2004; Nagano et al. 2004). The second most abundant gene transcripts were proteins constituting muscular tissues, which enable adult flukes to abrade and feed on the biliary epithelium (Kwon et al. 2005). Vitelline precursor protein was the third most abundantly expressed gene product. It is responsible for hardening the eggshell encasing the germ cell and surrounding yolk cells (Tang et al. 2005).

The ESTs of the metacercarial stage of *C. sinensis* could be assembled into 322 genes. Those expressed most abundantly were for proteases and metabolic, transcription and translation housekeeping proteins (Huang et al. 2012). To obtain an insight in the developmental gene expression and regulation of *C. sinensis*, the adult and metacercariae ESTs of *C. sinensis* were compared. It was found that genes encoding structural and cytoskeletal proteins, transcription and translation machinery proteins, and energy metabolism-related proteins were highly expressed in *C. sinensis* metacercariae, while the other genes were highly expressed in the adult stage. These data may explain, to some extent at least, that *C. sinensis* metacercariae in fish hosts have a quite different physiology and metabolism compared with adult *C. sinensis* in mammals (Huang et al. 2012).

The most abundant genes in adult *O. viverrini* encoded for myoglobin, vitelline precursors, egg shell proteins and glutathione S-transferase. The other abundantly expressed genes encoded proteins involved in host-parasite relationships and included proteases, saposin-like proteins and dynein light chains (Laha et al. 2007). Homologues of some of the most abundantly represented proteins in *C. sinensis* and *O. viverrini* ESTs are cysteine protease, myoglobin and vitelline B precursors, whereas others were over-expressed in each species (Laha et al. 2007). The open reading frame (ORF) region in ESTs was also used to predict the expressed proteins in proteomic analysis. Such ORFs were generated from 4194 available EST sequences of *O. viverrini* and subsequently analyzed for secretory signal sequence and the transmembrane domain. A total of 897 potential ORFs were identified, of which 78 were predicted to contain a secretory signal sequence and 42 to contained two or more transmembrane domains (Mulvenna et al. 2010). Proteases were highly presented in the *O. viverrini* transcripts encoding secreted proteins, with five different cathepsins, a legumain and an S1 type serine protease all predicted to contain a signal sequence (Mulvenna et al. 2010). In addition, more than 50% of the predicted protein sequences of *C. sinensis* and *O. viverrini* were inferred to be homologues, reflecting their relatively close biological and physiological relationships (Young et al. 2010). Comparison of the predicted proteins of liver flukes and other trematodes, *S. japonicum*, *S. mansoni*, *F. hepatica* found that 29–31% protein sequences were homologous (Young et al. 2010). However, this prediction of expressed proteins may not be satisfactory as some ORF encoded sequences might not express or express a low protein level. Thus, additional proteomic analysis is needed to provide more informative data on the proteins expressed in these liver flukes.

6.4.4 Proteome

Proteomic analyses should provide information on potential new and specific targets for treatment the infection. Moreover, the identification of parasitic-specific proteins could clearly facilitate the design of new tools for rapid and cheap diagnosis, which in turn could help breaking the transmission cycle of the parasite, as well as help in the identification of potential targets for vaccination, one of the best ways to control these parasite infections (Toledo et al. 2011).

The adult stage of all three flukes dwells in the bile duct which provides an anaerobic environment where a large amount of exogenous glucose is used as a carbon source for energy metabolism. Thus, the most important endogenous proteins examined in the liver flukes are glycolytic enzymes which play an important role in the glycolysis pathway. Phosphoglycerate kinase (PGK), a glycolytic enzyme, was found extensively, localized in the muscular tissues of the oral and ventral suckers, ovary, testes, tegument and intrauterine eggs of *C. sinensis* (Hong et al. 2000, 2003a). The inhibitors of several glycolytic enzymes of *C. sinensis* have also been reported, e.g., vanadate can inhibit phosphoglycerate mutase (PGM) (Song et al. 2007), whereas lactase dehydrogenase (LDH) was inhibited by Cu^{+2}, Fe^{+2} and Zn^{+2} (Yang et al. 2006). The cytosolic and mitochondrial malate dehydrogenases (cMDH and mMDH) of *C. sinensis* share low amino acid sequence homology (22%) and these enzymes are differentially inhibited by 4.4'-bis-dimethylamino diphenylcarbol. cMDH is more stable against heat and acidity than mMDH. Moreover, cMDH plays a pivotal role on the cytosolic side of the malate-aspartate shuttle. mMDH is a key enzyme in the tricarboxylic acid cycle and in the malate-aspartate shuttle. Thus these glycolytic enzymes are required for the survival and pathogenesis of these liver flukes (Zheng et al. 2005, 2006, 2008).

The mechanism of pathogenesis due to liver fluke infection mainly involves the interaction between parasite antigens and the host immune response (Sripa et al. 2007). Therefore, the excretory-secretory (ES) proteins and tegumental proteins of the liver fluke play crucial roles in host-parasite interactions, pathogenesis and disease outcomes. Myoglobin is an abundant protein in the ES products of *C. sinensis*. It may play an oxygen-capturing role and then slowly release this oxygen to metabolic pathways in bile duct. Recombinant myoglobin reacted with the sera of *C. sinensis*-infected rabbits and clonorchiasis patients. Lysophosphatidic acid phosphatase (LPAP), belonging to the acid phosphatase family, has been identified as an ES-antigen in adult *C. sinensis*. It shows high sensitivity and specificity in the serodiagnosis of human clonorchiasis (Kim et al. 2009a). The proteomic analyses by the 2-D proteome mapping of *C. sinensis* ES products identified 62 protein spots including thioredoxin peroxidase, myoglobin and a number cysteine proteases that were expressed abundantly (Ju et al. 2009). More recently, Zheng et al. (2011) reported a proteome analysis of ES products of *C. sinensis* using LC-MS/MS analysis and found 110 proteins including 39 known functional proteins and 71 unknown proteins. The enzyme fructose 1,6-bisphosphate (Cs-FPBase) within the ES product was a potential causative agent of hepatic fibrosis (Zheng et al. 2011).

A comparative proteomic analysis of the developmental stages from juvenile to adults of *O. viverrini* was made by 2-D gel electrophoresis (Boonmee et al. 2003). The total number of protein spots varied between 210 and 239 according to the age of the worm (1–4 weeks). Only small differences in the pattern of protein spots were found during parasite maturation (Boonmee et al. 2003). The secreted and surface-exposed proteomes of *O. viverrini* has also been reported (Mulvenna et al. 2010). The secretory proteins were analyzed using peptide OFFGEL electrophoresis (OGE) and (multiple reaction monitoring) MRM. A total of 25 proteins, 13 from MS/MS analysis of OGE and 12 from MRM identifications, were positively identified as constituents of Day 1 ES (*in vitro* culture of worms), whereas the remaining 18 proteins identified in Day 17 ES are a likely consequence of the culturing process (Mulvenna et al. 2010). Proteases were abundant, but proteolytic enzymes were under-represented

in the ES of *O. viverrini.* However, the prediction of the secretory proteins from a signal sequence or based on a transmembrane domain in the ESTs found 26 known proteins and 39 unknown proteins to be secretory proteins. Of these, only five, i.e., cathepsin F-like cysteine protease, cathepsin D, venom allergen-like protein 8, cystatin and granulin were detected by proteomic identification (Mulvenna et al. 2010). Granulin, which is a homologue of human granulin, is a potent growth factor involved in cell proliferation and wound healing. A granulin of *O. viverrini* (*Ov*-GRN-1) was examined and found to be expressed in most parasite tissue, particularly the gut and tegument. *Ov*-GRN-1 is probably the major growth factor protein in the ES products secreted by *O. viverrini* that can induce the proliferation of host cells which may ultimately manifest in cholangiocarcinoma (Smout et al. 2009). The *O. viverrini* proteome and host-parasite interaction has been recently reviewed (Suttiprapa et al. 2018).

The tegumental syncytium, which is the outermost surface of liver flukes, is considered to be very important for host response and parasite survival. Thus, it is generally seen as the most susceptible target for vaccines and drugs. Several tegumental proteins of the liver flukes have been characterized and identified. The tegumental protein of *C. sinensis,* CsTP31.8, has been proven to be an antigenic protein (Huang et al. 2007). CsTP20.8 is expressed in adult worms and metacercariae but not at the egg stage. However, CsTP20.8 protein is considered to have limited value for the serodiagnosis of clonorchiasis because it shows only moderate sensitivity and although it has high specificity (Zhou et al. 2007). Another tegumental protein CsTP21.1 was identified from adult *C. sinensis* by bioinformatics analysis. It is localized in the tegument of adult worms (Chen et al. 2011). Interestingly, CsTP21.1 is considered a trematode-nematode pan-specific antigen that could be useful for the development of a universal immunodiagnostic kit for human infection with trematodes and nematodes (Chen et al. 2011).

Membrane-spanning proteins of *O. viverrini* are predicted to include 28 known proteins belonging to the transporters/channels, protease/hydrolytic enzymes, structural/membrane organization proteins and other miscellaneous proteins, as well 8 unknown proteins based on transmembrane domains. However, proteomic identification found only four membrane proteins, i.e., ATP-ADP antiporter, Sm-TSP-2, succinate dehydrogenase and succinate dehydrogenase complex, subunit C expressed in *O. viverrini* (Mulvenna et al. 2010).

6.4.5 Vaccine Development

It is clear that a vaccine against any of the major human pathogens within the Opisthorchiidae would greatly aid control measures although progress in research in this direction, which has been under way for some time has been limited (Sripa et al. 2012). The goal for the development of a vaccine against *O. viverrini* and/or *C. sinensis* is not only to limit the pathologic sequels due to acute and chronic infections, but, as is the case with other carcinogenic pathogens such as human papilloma virus, it could also protect against cancer (Frazer et al. 2011). Genomic studies on both *O. viverrini* and *C. sinensis* may well facilitate the development of vaccines with time. As indicated above, the tegument is generally viewed as the most susceptible target for vaccines and drugs in liver flukes because it is a dynamic host-interactive layer with roles in nutrition, immune evasion and modulation, pathogenesis, excretion and signal transduction (Jones et al. 2004; Van Hellemond et al. 2006). Large proteins such as multifunctional secreted proteases and tegumental proteins have been identified as potential targets for the development of drugs and vaccines (Wang et al. 2011).

DNA vaccines against *C. sinensis* were produced by encoding cysteine proteinase and a fatty acid-binding protein and tested in a rat model. They showed 31.5% and 40.9% protection efficacy, respectively (Kim et al. 2006). In addition, an oral vaccine using *C. sinensis* tegumental protein 22.3 kDa fused with the *Bacillus subtilis* spore coat CotC showed 44.7% protection in the rat model (Zhou et al. 2008). An *O. viverrini*-crude somatic antigen (CSAg) administered with

complete Freund's adjuvant or alum was used to stimulate immune responses in *O. viverrini*-primed hamsters. The greatest protection was 48.4% and elevated TGF-β induced by *O. viverrini* may play an important role in parasite survival (Jittimanee et al. 2012). The reported protection rate of the vaccine candidate molecules against these liver flukes is not satisfactory and more studies are required.

6.4.6 Phylogenetics, Systematics, and Genetic Diversity

A variety of molecular markers/techniques has been used to examine the genetic diversity of *Clonorchis* and *Opisthorchis* species at the inter- and intra-species levels. Although there are considerable similarities between *C. sinensis*, *O. viverrini*, and *O. felineus*, there is also a great deal of regional variation both within and between species. Adaptation to differences in the environment and host selection process, as well as limited gene flow between different water sheds can all play a considerable role in determining the genetic constitution of each isolated species and population (Sithithaworn et al. 2012b). Although all three species are closely related, their systematic position remains controversial (Petney et al. 2018). Some reports indicate that *O. viverrini* is more closely related to *C. sinensis* than *O. felineus* when examined using 12 mitochondrial protein-coding genes (Shekhovtsov et al. 2009) and the ninth intron region of the paramyosin gene (Cai et al. 2012). By contrast, it has also been suggested that *C. sinensis* is more closely related to *O. felineus* than to *O. viverrini* based on ITS2 and mitochondrial DNA (Katokhin et al. 2008; Saijuntha et al. 2009; Liu et al. 2012), or even that *C. sinensis* and *O. viverrini* are more closely related to one another than to *O. felineus* when examined by ITS and CO1 sequences (Kang et al. 2008). Thus the situation is far from clear and more powerful genetic markers together with greater sample sizes and more geographical isolates need to be studied for a comprehensive phylogenetic analysis among these liver flukes.

Considerable genetic diversity has been observed in *C. sinensis*, based on its geographic distribution within China, Korea and the Russian Federation, as well as among different reservoir hosts including people. More details of genetic variation exploring of this fluke has been recently reviewed (Wang et al. 2018). Isoenzyme markers can be used to differentiate *C. sinensis* into the two populations from two different geographical isolates from Korea and China (Park et al. 2000b; Park and Yong 2001). However, the DNA regions of ribosomal DNA and mitochondrial DNA sequences were strongly conserved and nearly identical between different isolates (Park and Yong 2001; Lee and Huh 2004; Park 2007). In another study based on ITS1 sequencing, two levels of intra-specific variation, i.e., inter- and intra-individual, were observed and these showed a "northern" and a "southern" genetic group of *C. sinensis* according to their distribution in China, Korea and the Russian Federation (Tatonova et al. 2012). Moreover, the eggs of *C. sinensis* collected from a well preserved Chinese body which had been buried in 167 BC revealed differences in the ITS1 sequence at 15 nucleotide positions compared to the present samples, suggesting sequence divergence through time (Liu et al. 2007). More recently the genetic variation and phylogeography of *C. sinensis* was studied from two geographical localities in southern far east Russian and compared to the other geographic localities from China, Korea, Japan and Vietnam by CO1 sequence. A total of 18 haplotypes were observed. Of these 4 were common to Russian and Chinese isolates, and the other two were common to Russian and the other isolates. The Russian isolates differed from those of the other localities in haplotype frequencies (Tatonova et al. 2013).

The role which animal reservoir hosts play in genetic variation of *C. sinensis* is currently being investigated. Both RAPD and MGE-PCR was used to examine the genetic variation among individual adult *C. sinensis* collected from cats and dogs in two geographical areas, Guangdong province in the South and Heilongjiang province in the North of China (Lai et al. 2008; Liu et al. 2012). Both revealed genetic polymorphisms

among *C. sinensis* individuals from these hosts in each location. In a recent study from different geographical localities in Korea, as well as in China, using mitochondrial genes sequences, the genetic variation present in *C. sinensis* from naturally infected cats, dogs, rabbits and humans was examined. Intra-specific nucleotide variation of the Korean population ranged between 0 and 1.6% (Liu et al. 2012), whereas 0–1.7% was found in the Chinese population (Xiao et al. 2013). Recently the microsatellite marker of *C. sinensis* has been characterized and found that 24 of 40 loci showed potential to differentiate between *C. sinensis* and *O. viverrini*. Of these, seven loci revealed heterozygous, which could be further used for study population genetic of *C. sinensis* (Nguyen et al. 2015).

Genetic diversity of *O. viverrini* has been intensively investigated based on a variety factors, e.g., spatial, temporal and host factors. Unlike *C. sinensis*, there is no report of genetic variation between specimens collected from different reservoir hosts. An initial report was published on different geographical isolates by Ando et al. (2001) using rDNA and mitochondrial DNA sequences but with a restricted sample size. The next study involved the establishment of 32 enzyme (allozymes) loci using multilocus enzyme electrophoresis (MEE) to comprehensively examine the genetic variation among natural populations of *O. viverrini* from 11 different geographical localities in Thailand and four localities in Lao PDR (Saijuntha et al. 2006a, b, 2007). Two major evolutionary lineages of *O. viverrini* were found, which could be additionally subdivided into at least six distinct genetic groups which correlated with five different wetland systems (Saijuntha et al. 2007). RAPD and microsatellite analyses in *O. viverrini* also showed significant differences between the isolates from Thailand and Lao PDR (Sithithaworn et al. 2007a). These comprehensive molecular systematics studies (Ando et al. 2001; Saijuntha et al. 2006a, b, 2007; Sithithaworn et al. 2007a) have transformed our perception of the systematic and taxonomic status of *O. viverrini* that *O. viverrini* is not a single species but that it is indeed a species complex "*O. viverrini* sensu lato (sl)" that contains two evolutionary lineages with many cryptic species (morphologically similar but genetically distinct species) occurring in distinct wetland systems in Thailand and Lao PDR. Interestingly, the MEE data provided evidence of potential co-evolution between *O. viverrini* and its snail host, *B. s. goniomphalos*, as there was a high concordance of lineages and specific genetic groups (Saijuntha et al. 2007; Kiatsopit et al. 2013). An additional *O. viverrini* genetic isolate from Savannakhet, Lao PDR was analyzed using 20 allozyme markers and also found to be associated with a specific wetland system (Kiatsopit et al. 2011).

Microsatellite markers and MEE have been used to explore the population genetics and systematics of *O. viverrini* from different geographical isolates (Saijuntha et al. 2007; Laoprom et al. 2010, 2012). In addition, *O. viverrini* populations collected from different years (temporal), as well as from different fish host species, was carried out by MEE (Saijuntha et al. 2007, 2009). The level of genetic differentiation between the populations from Thailand and Lao PDR was very high, whereas it was low for comparisons among populations from Thailand. The same pattern was found among different fish host species and temporal populations (Saijuntha et al. 2007, 2009). Based on the MEE and microsatellite analyses, *O. viverrini* populations almost always deviated from Hardy-Weinberg equilibrium with varying levels of heterozygote deficiencies (Saijuntha et al. 2007, 2008). In addition, microsatellite markers could be used to examine the genetic differences among *O. viverrini* populations over small-scale geographical distances within Khon Kaen Province, Thailand (Laoprom et al. 2012). MEE was also used to explore the genetic structure of *O. viverrini* populations at Vientiane Province, Lao PDR (Kiatsopit et al. 2014). The analyses based on microsatellites, together with allozyme data, revealed that the predominant mode of reproduction in *O. viverrini* is selfing (inbreeding) rather than cross-fertilization (outbreeding). The demonstration of significant genetic heterogeneity, as well as biological variation between the different geographical isolates of *O. viverrini* from Thailand and Lao PDR,

provide independent evidence that *O. viverrini* is a species complex (Saijuntha et al. 2007; Laoprom et al. 2009). The *O. viverrini*-like egg recovered from the resident of from Sanamchaikate District, Chachoengsao Province, Thailand, has been genetically characterized using mitochondrial CO1 and ND1 sequences, which was more closely related to the isolates from Lao PDR (Buathong et al. 2017). Recently, a new cryptic group of *O. viverrini* was discovered in Songkhram River Basin, Sakon Nakhon Province, Thailand, by using six independent nuclear and mitochondrial DNA markers (Pitaksakulrat et al. 2018). The polymorphic intron region of taurocyamine kinase has been characterized to explore genetic variation of *O. viverrini* and the results correspond to Pitaksakulrat et al. (2018) that a new cryptic group from Sakon Nakhon province was genetically distinct from the other *O. viverrini* (Saijuntha et al., unpublished).

Genetic variation within *O. felineus* from different geographical localities was investigated using three different polymorphic genetic markers, i.e., CO1, CO3 and ITS1 sequences (Brusentsov et al. 2013). All *O. felineus* populations were classified into three geographically isolated groups, namely from eastern Europe (the drainage basins of the Volga, the Don and the Ural rivers), northern Asia (Siberia, the Ob-Irtysh and the Yenisei river basins) and Central Asia (Kazakh, the Nura-Sarysu Basin, part of the endorheic Aral-Caspian basin). Only low genetic differentiation between these geographically distinct European and Asian *O. felineus* population was observed. This homogenization of population structure could result from potentially high levels of gene flow between populations, accompanied by active migrations of definitive hosts, including humans, during the Holocene (Brusentsov et al. 2013). More recently, ISSR and allozyme analyses were used to examine genetic variation of *O. felineus* from six rivers of Western Siberia. In addition, ISSR was also used to explore the genetic variability of metacercariae of *O. felineus* collected from different fish host species (Zhigileva et al. 2013). Again, only a low degree of genetic polymorphism and differentiation among *O. felineus* population was observed. Southern *O. felineus* samples from the Tobol and Tura rivers showed higher polymorphism levels than the samples from rivers in northern part (Zhigileva et al. 2013). However, the metacercariae of *O. felineus* collected from different fish species showed no genetic differences (Zhigileva et al. 2013). The results so far suggest that population genetic data based on other genetic markers such as microsatellite DNA are required.

6.5 Diagnosis

The most common diagnostic method for fish-borne zoonotic trematodes (FZT), which involves finding eggs in fecal samples, seems still to be far from ideal. In low egg output and a low prevalence situation, sensitivity is also low using this method is a puzzle that challenges scientific efforts. Recent advances in the diagnosis and detection of *O. viverrini* infection in human and their intermediate hosts has been reviewed (Saijuntha et al. 2018).

6.5.1 Parasitological Methods

Fecal examination is the routine method used for the diagnosis of liver fluke infection. It has the advantages of the simplicity of sample collection and of being non-invasive. Once a fecal sample is available, the modified formalin-ether (or ethyl acetate) concentration technique (FECT) (Elkins et al. 1990), the modified thick Kato smear (Hong et al. 2003b), or Stoll's dilution egg count technique can be used (Viyanant et al. 1983). Although these techniques are highly specific there are limitations because of the prepatent period of infection before eggs are produced, poor sensitivity when infection intensities are low, or intermittent egg excretion associated with bile duct obstruction. Both sensitivity and specificity vary depending on the method used but also on the experience of the examiner. The diagnostic value of these methods lies in their ability to detect relatively light infections, which occur in the majority of infected individuals, and in individuals recently treated with praziquantel. As a single examination does not

necessarily provide diagnostic certainty, repeated examinations are needed to improve diagnostic sensitivity. Thus, three consecutive Kato-Katz thick smears are more sensitive than a single examination by FECT (Lovis et al. 2009). However, even using such repeated stool examination there can be a discrepancy between egg count and worm detection so that a false negative diagnosis remains a real possibility.

In an autopsy study, adult *O. viverrini* were recovered directly from 139 livers. Examination of postmortem fecal samples from these individuals showed that only 67% were positive for *O. viverrini* infection. The detection limit using normal fecal examination was estimated to be 20 worms or approximately 1000 EPG. Individuals with low infection intensities and limited egg output are likely to be underdiagnosed by as much as ~20% (Sithithaworn et al. 1991). Although there is some evidence of density-dependent fecundity, there is in general a linear relationship between fecal egg count and worm burden.

The commercial stool concentrator kits which are designed to reduce processing time such as Parasep SF were available, however, these show a lower sensitivity than FECT although they have a higher sensitivity than the simple smear method (Sithithaworn, unpublished). Based on this preliminary study, the performance of this kit is comparable with that of the widely used Kato-Katz method.

Eggs can also be detected during treatment of bile duct obstruction either in bile from nasobiliary or percutaneous transhepatic biliary drainage (PTBD) or in the duodenal fluid. Adult worms are ejected during expulsion chemotherapy (Ramsay et al. 1989; Elkins et al. 1991; Radomyos et al. 1994; Joo and Bang 2005). Similar data to those available for *O. viverrini* are now also available for *C. sinensis*. These indicate that this species lays more eggs than *O. viverrini* (514 EPG/worm), probably due larger size of adult worms (Kim et al. 2011). Worm burdens determined by expulsion chemotherapy ranged from 1 to about 100 worms for *C. sinensis* (Shen et al. 2007; Kim et al. 2011).

In some endemic areas for liver flukes, for example in Southeast Asia, intestinal flukes coexist leading to a potential diagnostic problem if conventional fecal examination is used. The eggs of *O. viverrini*, which are identified by their characteristic rough and thick egg shells are very similar to the eggs of several species of other food-borne trematodes belonging to the families Opisthorchiidae, Heterophyidae, and Lecithodendriidae. The latter two families are commonly referred to as minute intestinal flukes (MIF) due to their small size compared to the liver flukes (Kaewkes 2003; Chai et al. 2005; De and Le 2011). These species are, like the liver flukes, fish-borne trematodes (FBT) or fish-borne zoonotic trematode (FZT) (Lan-Anh et al. 2009; Phan et al. 2010a, b). The similarity between the eggs of these other FBT species and those of *O. viverrini* and *C. sinensis* can substantially increase the likelihood of a false positive diagnosis, depending on the prevalence of these species. They thus reduce diagnostic specificity and the identification of adult worms and PCR confirmation may be necessary for correct species identification in areas where a number of species exist.

6.5.2 Immunological Methods

Several serological tests for clonorchiasis and opisthorchiasis have been developed for use in a diagnostic assay with greater sensitivity and specificity than fecal examination. These include the intradermal test (IDT), immunoelectrophoresis (IEP), indirect hemagglutination assay (IHA), indirect fluorescent antibody test (IFAT), and indirect enzyme-linked immunosorbent assay (indirect ELISA) (Wongratanacheewin et al. 2003; Kim et al. 2010; Hong and Fang 2012). Indirect ELISA is commonly preferred for the detection of antibodies although, due to the complexity of the antigen, neither sensitivity nor specificity is constant. Crude somatic extracts of adult worms of both *O. viverrini* and *C. senensis* used for ELISA provide higher sensitivities than fecal examination (Poopyruchpong et al. 1990; Wongsaroj et al. 2001; Hong and Fang 2012), while ES antigens show a superior or equivalent performance to the crude antigen (Sirisinha et al. 1990; Choi et al. 2003). Interestingly, antigen

extracted from the *Bithynia* intermediate host snails has also been used as an antibody detecting antigen for the diagnosis of human *Opisthorchis* infection (Waikagul et al. 2002; Watthanakulpanich et al. 2003) although the value of such tests has yet to be evaluated.

Recombinant antigen for serum antibody detection by ELISA has been produced from eggs and egg shells (Wongsaroj et al. 2001; Ruangsittichai et al. 2006). In addition, the propeptide of cathepsin L, glutathione S-transferases, adenylate kinase 3, phosphoglycerate kinases, phosphoglycerate mutase, lysophosphatidic acid phosphatase and cathepsin B, cathepsin F, cathepsin L-like, legumain, taurocyamine kinase have been characterized and show better diagnostic sensitivity and specificity over conventional fecal examination diagnostic methods (Hong et al. 2000, 2002; Ruangsittichai et al. 2006; Hu et al. 2007; Chen et al. 2011; Li et al. 2011, 2012). Nevertheless, the increased specificity and reduced cross reactivity of these proteins need to be tested under field conditions before they can be judged good enough to replace the commonly used native crude antigen (Hong and Fang 2012). The detection by ELISA of antibodies in non-fecal clinical samples such as urine and saliva, has been considered and saliva found to be of potential use for the serodiagnosis of opisthorchiasis (Sawangsoda et al. 2012).

In the case of clonorchiasis in China, a combination of serological and parasitological techniques could improve diagnostic accuracy and reduce the false negative diagnosis rate. ELISA used as an auxiliary diagnostic method was suggested for a large-scale screening test in monitoring the prevalence and assessing the risk factors of clonorchiasis (Han et al. 2012). A major drawback of antibody-based detection is the inability of this method to differentiate between past and present infections because of the persistence of antibodies in the patient even after a cure has been effected (Hong 1988; Ruangkunaporn et al. 1994; Johansen et al. 2010). One way of overcoming this problem is to use an antigen-based detection which indicates if current infection is present (Sirisinha et al. 1991, 1995; Chaicumpa et al. 1992). Monoclonal antibody (mAb)-based systems offer increased diagnostic sensitivity, as they are able to detect secretory products from only a few adult worms. This is effective in low-scale infections when eggs are not detectable in fecal samples (Sirisinha et al. 1995). This has been corroborated in an autopsy study (Sithithaworn et al. 1991). Studies in animal models for *C. sinensis* (Mazidur Rahman et al. 2012) and *O. viverrini* (Duanngai, unpublished) showed promising results. Recently it was suggested that copro-antigen detection is useful for detecting positive cases, again especially when fecal examination negative although the antigen level is also correlated with the intensity of infection (Watwiengkam et al. 2013).

In 2015, Worasith et al. (2015) reported a novel antigen detection method using urine samples for the diagnosis of opisthorchiasis. Recently, a comparison of urine and copro-antigen detection yielded similarly high diagnostic performances compared with standard fecal examination (Worasith, unpublished). Because of the ease and acceptance of urine specimen collection and handling, urine antigen detection has a high potential for the diagnosis and mass screening of opisthorchiasis in control and elimination programs. In particular, the antigen detection is useful approach for the detection of mild infections and for the evaluation of the effectiveness of pharmaceutical cure.

6.5.3 Molecular Biological Methods

A number of target genes from both *C. sinensis* and *O. viverrini* have been tested for their diagnostic suitability including satellite DNA, ITS1, ITS2 and mitochondrial DNA. These were used for both conventional PCR and real-time PCR diagnosis showing high specificity but variable sensitivity (Wongratanacheewin et al. 2003; Hong and Fang 2012; Qiao et al. 2012). The detection of *O. viverrini* egg DNA in human stools using PCR and based on primers complementary to the repeat DNA element showed a specificity of 98% and a sensitivity of 100% for moderate to heavy infections with more than 1000 EPG. In light infections with less than 200

EPG the sensitivity was reduced to only 68% (Wongratanacheewin et al. 2001, 2002). More recently, the retrotransposon of *O. viverrini* (*OVRTE-1*) has been found to be the new alternative genetic marker of high sensitivity and specificity for the PCR diagnosis of opisthorchiasis (Phung et al. 2014). Another PCR-based study using the same target DNA showed low sensitivity (50%) at high egg counts of more than 1000 EPG in stool samples from Lao PDR (Stensvold et al. 2006). However, if the quality of the DNA was improved by using cetyltrimethylammonium bromide (CTAB) during its preparation to remove PCR inhibitors the sensitivity was increased to 79% (Duenngai et al. 2008). PCR-positive tests occurred in a 29% of cases which were parasite negative in this study using the conventional fecal examination method indicating its potential diagnostic value for light infections. Another *O. viverrini*-specific primer pair was established which was able to detect adult worms with 1–12 ng of DNA, and metacercariae when 3 or more occurred in a fish sample (Parvathi et al. 2008). Loop-mediated isothermal amplification (LAMP) has been established for the detection of both *O. viverrini* and *C. sinensis* with a higher sensitivity than conventional PCR (Cai et al. 2010; Arimatsu et al. 2012; Le et al. 2012).

Species-specific PCRs are now also available to distinguish between the three species of liver fluke: *O. viverrini* (Ando et al. 2001; Wongratanacheewin et al. 2001), *O. felineus* (Pauly et al. 2003), *C. sinensis* (Le et al. 2006). In addition, several genetic markers/approaches involving conventional PCR, PCR-RFLP, multiplex PCR, real-time PCR and multiplex ligation-depended probe amplification (MLPA) pyrosequencing can be used to differentiate between species involved (Le et al. 2006; Sato et al. 2009; Sun et al. 2011; Sanpool et al. 2012).

The molecular methods discussed above will contribute significantly towards a more effective and accurate diagnosis of trematode infections although further simplification of the tests and an understanding of cost effectiveness under various socioeconomic scenarios is needed. In addition, the validation of DNA positive test results is required although evidence from animal models is accumulating and supported human studies. (Rahman et al. 2011; Duenngai et al. 2013).

Real-time PCR can also now be used to quantify the intensity of infection with *C. sinensis* (Kim et al. 2009a). In addition, molecular identification techniques, can be used in cases of multiparasite infections in a single host (Sato et al. 2009; Thaenkham et al. 2011). Such approaches can also be used in a food security setting to test for the presence of liver flukes in aquaculture or native fisheries products, particularly for export (Parvathi et al. 2007, 2008; Cai et al. 2010).

Due to their high specificity, such molecular diagnostic tests are likely to play an increasingly significant role in anthelminthic drug efficacy evaluations, the rigorous monitoring of reinfection patterns, and to investigate changes in the endemic range of the liver flukes (Touch et al. 2009; Traub et al. 2009).

6.6 Consequence of Infection

6.6.1 Pathogenesis, Pathology and Morbidity

Liver fluke infection causes significant pathological changes to the bile ducts which the worms inhabit. The pathology can also extend to affect both the liver and gall bladder (Rim 2005; Sithithaworn et al. 2007b). Syrian golden hamsters provide a suitable animal model to examine these changes (Bhamarapravati et al. 1978; Lee et al. 1993). During the early phase of infection with *O. viverrini* there is an acute inflammatory reaction in the large intrahepatic bile ducts as well as portal connective tissue. Once the infection has become chronic (at about 30 days post infection) hyperplasia and adenomatous formations of the bile duct epithelium occur (Sripa 2003). Granulomatous responses to both the adult flukes as well as to the eggs which they produce lead to periductal fibrosis and scarring. This is the most prominent feature during the chronic stage of infection (Bhamarapravati et al. 1978). The extensive fibrosis is associated with a significant increase in the synthesis and the hepatic

content of collagen (Hutadilok and Ruenwongsa 1983; Chotigeat and Ruenwongsa 1986). With the onset of the chronic phase of infection the inflammatory responses become less severe suggesting that immune modulation may occur. Fibrotic tissue accumulates due to repair dysfunction and an imbalance in synthesis and degradation of the fibrotic tissue. These factors may lead to cell proliferation which, in the presence of cofactors, significantly contributes to cancer development (Kenny and Bissell 2003). In humans, periductal fibrosis is a significant cause of hepatobiliary disease and leads to an increased risk of CCA development (Mairiang et al. 1992, 2012). In *O. viverrini* patients with advanced periductal fibrosis there was an 8 times higher level of IL-6 responses to *O. viverrini*-excretory/secretory products than in patients without fibrosis, indicating the role of IL-6 in the pathogenesis of advanced periductal fibrosis in opisthorchiasis (Sripa et al. 2009).

Chronic infection by the liver flukes corroborated by a marked humoral immune response indicated by the presence of parasite-specific IgG, IgA, and IgE in the serum and bile of humans infected with *O. viverrini* (Itoh et al. 1994; Akai et al. 1995). Although the IgG level against crude somatic antigen correlated with hepatobiliary abnormalities diagnosed by ultrasonography, there was weak correlation with the intensity of infection (Elkins et al. 1996).

The bile ducts which harbor the adult worms show the most significant and potentially dangerous gross and microscopic pathological changes in both *O. viverrini* and *C. sinensis* infections but development is long term taking up to seven to 15 years for *O. viverrini* (Riganti et al. 1989). Immunomodulation during both the acute and chronic phases of infection is responsible for the pathological changes observed (Rim 2005).

Light infections may be inapparent with no significant symptoms. Pathology depends on both the duration and the intensity of infection as well as to the susceptibility of the host (Behr et al. 1998; Sithithaworn et al. 2007b; Hong and Fang 2012; Armignacco et al. 2013). For heavy infections the peripheral bile ducts may become thickened beneath the fibrotic capsule of the liver. A recent outbreak of opisthorchiasis in Italy caused by *O. felineus* infection presented as a febrile syndrome with eosinophilia and cholestasis (Traverso et al. 2012). This outbreak is interesting as 37 (82%) of the 45 infected individuals showed symptoms of the disease and 8 (17.7%) were admitted to hospital for treatment.

As indicated above, inapparent infections are common with only about 5% morbidity occurs among infected individuals (Upatham et al. 1984). Once the symptoms become apparent they are usually non-specific, involving general abdominal discomfort. In such cases hepatobiliary abnormalities and/or CCA can usually be detected by ultrasonography (Choi et al. 2005; Mairiang et al. 2012).

6.6.2 Liver Flukes and Cholangiocarcinoma

Cholangiocarcinoma (CCA) is a cancer of the epithelial cells in the bile ducts arising along either the intrahepatic or extra-hepatic biliary tree (Nakeeb et al. 1996; Blechacz and Gores 2008) although studies on molecular pathogenesis are currently confined to the intrahepatic CCA type. CCA is responsible for as much as 15% of liver cancers worldwide, most of which are associated with trematode infection (Parkin et al. 1993; Parkin 2006). Large-scale epidemiological studies of CCA indicate an increase in both the incidence and mortality rates. Currently CCA is the second most frequent primary liver cancer worldwide (Khan et al. 2007). The highest incidence of CCA worldwide occurs in northeast Thailand (Sithithaworn et al. 2014). In addition to CCA induced by either *C. sinensis* or *O. viverrini*, early observations indicate that around 400 cases of this disease currently occur every year in patients heavily infected with *O. felineus* (Hotez and Alibek 2011).

The induction of cancer by these liver flukes appears to be dependent on a variety of factors including host genetic background, past exposure to infection as determined by elevated *O. viverrini* antibody levels, liver cirrhosis, chronic infection

with hepatitis C virus and heavy alcohol consumption (Tyson and El-Serag 2011).

The association between *O. viverrini* and CCA was first determined in a hospital-based, case-control study conducted in Thailand in the late 1980s (Parkin et al. 1991). A total of 103 patients with CCA were compared with an equal number of age- and sex-matched controls and elevated *O. viverrini* antibody titers were positively correlated with an increased risk of CCA (Parkin et al. 1991). This was confirmed in a repeat study based on 129 cases the cancer. This study indicated that the population-attributable risk is as high as 88% in endemic areas (Honjo et al. 2005). Another a case-control study, this time on *C. sinensis* from Korea, compared 41 patients with CCA with 406 controls and found a similarly strong association between liver fluke eggs in fecal samples and CCA (Shin et al. 1996). A recent meta-analysis including 912 cases and 4909 controls confirmed this association (Shin et al. 2010). The population-attributable risk was lower than that calculated for *O. viverrini* but was nevertheless 27.9% for men and 16.2% for women.

In patients with a *C. sinensis* infection the formation of calculi in the intrahepatic biliary passages is a characteristic pathological change. This may be associated with suppurative cholangitis, cholecystitis, and biliary abscess or the so-called cholangiohepatitis. It can eventually lead to the development of primary liver cancer, especially CCA. The occurrence of calculi is probably caused by bile stagnation, which in turn causes mechanical obstruction by *C. sinensis* worms and eggs in the bile ducts. The calculi in the intra- and extra-hepatic bile ducts are made up of bilirubin and calcium salts. The formation of such pigment stones in clonorchiasis is thought to be due to bile stagnation leading to changes in the composition of bilirubin, cholesterol, phospholipid, bile acid and the activity of bacterial glucuronidase. The goblet cell metaplasia of the bile duct epithelium is responsible for the high content of mucous secretion in the bile. This mucin-rich bile in conjunction with the worms and eggs not only cause cholestasis but also provide a suitable environment for secondary bacterial infection. This is usually due to *Escherichia coli* which cause ascending cholangitis from the intestine (Rim 2005). Studies on *C. sinensis* indicate that this species also stimulates biliary epithelial hyperplasia (Hong et al. 1993), which is considered to play a significant role in carcinogenesis (Lee et al. 1993, 1994). Clonorchiasis-associated CCA involves substantial mucin secretion, usually accompanied by extensive fibrosis (Chou and Chan 1976; Choi et al. 1988). Although the larger bile ducts are only slightly enlarged and fibrotic, they are commonly blocked by adult worms or calcium bilirubinate stones (Kim et al. 1989). Clonorchiasis-associated CCA has develops in a discrete nodular or confluent mass in which smaller ducts with adenomatous hyperplasia undergo malignant transformation occur (Hou 1956). Chronic inflammation is of particular significance for the induction of CCA due to oxidative and nitrative DNA damage (Yongvanit et al. 2012).

Although most of these studies indicate that liver flukes cause tissue damage by mechanical and chemical irritation, some recent studies suggest that parasite-specific immune responses may also play a major role (Yongvanit et al. 2012). A genetic polymorphism in the detoxifying enzyme glutathione S-transferase (GSTM1) in association with seropositivity for opisthorchiasis was found to modify the cancer risk factor for CCA (Honjo et al. 2005). Thus, gene-environment interactions (current or past infection of *O. viverrini* infection) can play a significant role in individual susceptibility to CCA.

Carcinogenesis of CCA is still not clearly understood, however, it appears to be a multistage process with a variety of factors being involved of which chronic infections and persistent inflammation are predominant (Ohshima and Bartsch 1994). It is also possible that nitric oxide (NO), which can generate DNA-reactive agents and N-nitrosamines, is involved (Yongvanit et al. 2012). Excess NO production plays an important role in a number of pathological processes, including the induction of cancer (see Yongvanit et al. 2012). If a host becomes infected with a liver fluke, macrophages and other cell types (e.g., mast cells, eosinophils, and epithelial cells) are

activated by parasite-specific T cells and cytokines and synthesize NO from L-arginine via the induction of iNOS with the aim of eliminating the intruder. Nitric oxide is not only cytotoxic, it is also genotoxic by reacting with superoxide to form the highly reactive peroxynitrite which leads to oxidative and nitrative DNA damage via the formation of 8-oxodG and 8-nitroguanine (Inoue and Kawanishi 1995). These can be used to indicate DNA damage in the affected tissues. The overproduction of NO caused by *O. viverrini* infection can also lead to the endogenous nitrosation of amine precursors to form potentially carcinogenic N-nitrosamines such as N-dimethylnitrosamine (NDMA) (Satarug et al. 1998). NDMA, which is a carcinogenic product of the nitrosation reaction, has been detected in the urine of *O. viverrini*-infected subjects. It seems to be associated with lymphoproliferative responses to active liver fluke antigens which ceases after praziquantel treatment and the death of the parasites (Satarug et al. 1998). During an active *O. viverrini* infection of either hamsters (the animal model) or humans, an isoform of cytochrome P-450 (CYP) enzymes, CYP2A6 is formed (Kirby et al. 1994; Satarug et al. 1996). NDMA requires metabolic activation, mainly by CYP2E1 and CYP2A6, before becoming carcinogenic. It is hypothesized that this increase in CYP2A6-related enzyme activity in *O. viverrini*-infected individuals is an important link between inflammatory processes due to chronic liver fluke infection and a high risk for CCA.

6.6.3 Associated Pathogens

In a study in the northeast of Thailand individuals with *O. viverrini* infection had a significantly higher rate of leptospirosis than those without. In addition, *O. viverrini* metacercariae from the fish were positive for *Leptospira interrogans*, suggesting a close association between these two pathogens (Van et al. 2017). In individuals with a *C. sinensis* infection, the abundance of *Dorea* (Lachnospiraceae), a potentially pro-inflammatory microbe, was higher than in healthy individuals, while *Variovorax* (Comamonadaceae) was only detected in infected subjects (Xu et al. 2018). The frequency, structure and pathogenic significance of multiple parasite communities has been reviewed by Petney and Andrews (1998).

6.7 Epidemiology

A somewhat dated national survey carried out by the Ministry of Public Health in Thailand in 2001 showed that helminth infections are common with a country wide total prevalence of 22.5%. Of the species involved hookworms are the most common (11.4%), while *O. viverrini* ranked second with an average prevalence of 9.6% although the central and southern areas of the country showed a very limited presence of the parasite (Jongsuksuntigul 2002). In the northeast, there is substantial variation in the prevalence of opisthorchiasis among provinces, ranging from 4% to 33% (Jongsuksuntigul 2002). In Lao PDR, *O. viverrini* is common in the lowlands among people with close ethnic ties to the majority of the northeast Thai population probably due to high levels of partially enforced migration from Lao PDR into Thailand in the past (Giboda et al. 1991). The prevalence in certain areas is as high as 36–60% (Sithithaworn et al. 2012a). This is much higher than previous records indicate (Kobayashi et al. 1996, 2000). The presence of mixed infections including *O. viverrini* and heterophyid and lecithodendriid flukes found in communities along the Mekong River, potentially make conventional fecal diagnosis difficult (Chai et al. 2005).

Limited information on the incidence of infection in endemic communities in Thailand is available (Sornmani et al. 1984; Upatham et al. 1988; Saowakontha et al. 1993). In a study of 3 villages in Khon Kaen Province, the incidence was 1.7–25% over a 6-month period (Saowakontha et al. 1993). In a central Thai village containing a migrant population from the northeast of the country the incidence was 21.6% per year (Suwannahitatorn et al. 2013). Similarly, in the north of Thailand immigrants from the northeast were more likely to be infected with *O. viverrini* than local people (Pumidonming et al. 2018). The high levels of incidence in some villages cor-

respond with the high prevalences in some areas. For example, with an incidence of 40% per year, only 6 years are required for the prevalence of an originally uninfected cohort to exceed 95% (Upatham et al. 1985). In northeast Thailand information on the rate of reinfection after treatment also show a high incidence of reinfection. After a pretreatment prevalence of 55.1%, it took one year for the prevalence to return to 54.8% (Sornmani et al. 1984). Upatham et al. (1988) reported that in an area in Chonnabot, Khon Kaen Province, where 97.4% of villagers were infected, the prevalence had reached 94% one-year post-praziquantel treatment. It is significant that individuals with a high pretreatment intensity of infection tended to have a high intensity of reinfection. This may be a predisposition to heavy infection in some individuals. This hypothesis is supported by evidence from other parasites, such as *Ascaris lumbricoides* (Elkins et al. 1986), *Necator americanus* (Schad and Anderson 1985), *Trichuris trichiura* (Bundy and Golden 1987) and *Schistosoma mansoni* (Bensted-Smith et al. 1987). Rapid reinfection after treatment shows little evidence for protective immunity although this may occur in some individuals.

Clonorchis sinensis shows considerable variation in prevalences in the Republic of Korea dependent on the river system involved (Jeong et al. 2016). In this country males (11.2%) are more commonly infected than females (6.2%) as are individuals aged between 50 and 59 years (Jeong et al. 2016).

Although the rates of *O. viverrini* and *C. sinensis* infection vary considerably between villages, communities, (Kaewpitoon et al. 2015; Jeong et al. 2016; Prakobwong et al. 2017) and provinces (Miyamoto et al. 2014; Thaewnongiew et al. 2014; Lai et al. 2016), the pattern of infection is similar. Infection is age-dependent with the youngest age groups (0–5 years) having a low prevalence and intensity of infection. These increase through the pre- and early teenage years, often reaching a plateau in late teenagers (e.g., 15–19 years). This is in contrast to data published in the 1980s that showed very high prevalences in young school children, a situation that has now improved markedly (Khuntikeo et al. 2016). In some areas, the intensity of eggs released increased with age (Upatham et al. 1984), but the worm burden declined after the age of 50–60 (Haswell-Elkins et al. 1991; Sithithaworn et al. 1991). A number of possible reasons have been suggested for this decline including the late development of an immune response, lower parasite survival in more heavily fibrosed bile ducts, death of parasite in heavily infected people, or reduced exposure to infection in older age groups. Infection in infants may be due to mothers feeding them raw fish which is often finely ground (Sadun 1955; Upatham et al. 1982, 1984). However, the reported intensities of infection under the age of 4 are invariably low and there is little evidence that young children experience frequent exposure to infection.

In general, the prevalence and average intensity of *O. viverrini* infection is either not sex related or is slightly higher among males compared to females (Wykoff et al. 1965; Upatham et al. 1982, 1984; Haswell-Elkins et al. 1991) although more heavy infections may be found among males than females. This is also the case for *C. sinensis* (Joo et al. 1997). Males could therefore be more at risk of significant pathology, including cancer, as this increases in a non-linear fashion with infection (Haswell-Elkins et al. 1994; Elkins et al. 1996).

As with other helminths, *O. viverrini*, and probably all of the liver flukes dealt with here, is highly overdispersed with the majority of worms being found in only a few heavily infected individuals (Ramsay et al. 1989). The maximum worm load was 565 with a mean of 85 (S.D. = 154). Haswell-Elkins et al. (1991) observed that 81% of 11,000 worms recovered after treatment of 246 village residents were expelled by just 25 individuals (10% of the sample population). The highest burdens were over 100 worms. Interestingly, a number of individuals who did not expel worms were nevertheless positive for eggs. In an autopsy study in Khon Kaen, northeast Thailand, in which the worm burden was accurately measured, Sithithaworn et al. (1991) found that 30 out of 181 cadavers contained 66% of all the worms recovered and that only 13 people (7%) had worm burdens greater than 400.

A preliminary analysis by Khuntikeo et al. (2018) shows that deaths caused by *O. viverrini*-induced cholangiocarcinoma cause a high socio-economic burden on the families and potentially communities involved. Data are required before a quantitative estimate of this burden can be made. On a broader level, data for the year 2009 show that in Thailand medical care and loss of wages alone costs about $120 million annually (Kaewpitoon et al. 2015).

6.8 Treatment

Treatment programs vary considerably between countries. In general, infection with *O. felineus* is so limited in Europe where only local control is necessary if a particular community or group is infected (Pozio et al. 2013). In Thailand, a trial liver fluke control program was developed as early as 1967 in Sakon Nakhon Province (Jongsuksuntigul and Imsomboon 2003). This, as with other control programs in Thailand was based on the selective treatment of infected individuals as opposed to mass treatment. One of the limitations of this approach is that although the drug of choice, praziquantel, has a high efficacy (90–95%) and there is no evidence of drug resistance, the reinfection rate is high. This suggests that control by chemotherapy alone is unlikely to be completely successful, which is supported by the "residual" prevalence found in many areas after control measures were conducted (Jongsuksuntigul and Imsomboon 2003). With the advent of praziquantel in the mid-1970s, which is effective in about 90% of cases, the duration and toxicity problems were largely eliminated (Bunnag and Harinasuta 1981). The recommended daily dose for treatment of *C. sinensis* was 3 × 25 mg/kg × 1 day with cure rate of 85% and egg reduction rate of 99.7% (Rim and Yoo 1979), for *O. viverrini* the dose of 40 mg/kg with cure rate of 90% and egg reduction rate of >99.7 (Bunnag and Harinasuta 1981; Lovis et al. 2012), and for *O. felineus* the dose of 3 × 25 mg/kg × 1 day with cure rate of 90% and egg reduction rate of 100% (Wegner 1984; Zavoikin et al. 1994).

Recent data based on higher sensitivity methods such as PCR, however, suggest, that the treatment efficacy may be lower than previous estimates suggest, an area which clearly requires urgent investigation. Nevertheless, treatment with praziquantel usually leads to the elimination of symptoms. As an alternative to praziquantel, tribendimidine has been examined and it gave an efficacy comparable to praziquantel in the treatment of *C. sinensis* infection and resulted in fewer adverse events (Qian et al. 2013) but more study is required.

6.9 Prevention and Control

Methods of prevention and control aim at breaking the transmission cycle to humans. The millions of people infected and at risk of infection, as well as the direct and indirect economic losses resulting from liver fluke infection indicate the great importance of implementing effective and long-lasting prevention and control measures. There have been a number of attempts to do this both via direct treatment and also education aimed at reducing or elimination the consumption of raw or partially cooked fish. For *O. viverrini*, when fecal contamination from humans is reduced or eliminated by mass treatment and/or improved sanitation, the prevalence of infection can be substantially reduced (Sithithaworn and Haswell-Elkins 2003). A control program initiated in 1989 in certain provinces, particularly in the north and northeast of the country, resulted in a reduction in prevalence from 35.6% in 1988 to 8.7–9.4% during 2001–2009 (Jongsuksuntigul et al. 1992; Sithithaworn et al. 2012a). However, complete elimination of infection may not be possible if domestic cat and dog reservoir hosts maintain the source of parasite therefore playing a critical role in maintaining the life cycle. The difficulty involved in detecting infected cases with only a light infection (<1000 EPG) and the problem of reinfection after treatment also present serious problems to effective, long-term control. At the social level, education has proven both difficult to implement and ineffective to reduce the consumption of raw or partially

cooked fish in Thailand (Sithithaworn and Haswell-Elkins 2003).

An easy and promising method of preventing infection is to kill the infective metacercariae before fish are consumed. Unfortunately, as the consumption of raw or partially cooked fish is a deeply imbedded tradition in areas where opisthorchiasis and clonorchiasis are most common, the relevant populations have proven refractory to change (Grundy-Warr et al. 2012).

Particularly, in Thailand and Lao PDR there are 3 types of fish dishes which act as a source of infection with *O. viverrini*. "Koi pla" is prepared from fresh raw fish which are seasoned with lemon juice and spices and consumed without heating, posing a high risk of infection. "Pla som," which is a dish made out of fish which have been fermented for 1–2 days, poses a moderate risk, while "Pla ra" which undergoes less than the usual long term, although less risky, may provide a favorable environment for metacercarial survival (Grundy-Warr et al. 2012). Other Mekong countries have their traditional potential sources of infection; fermented "Pla dak" in Lao PDR, raw "Pla hoc" in Cambodia which is similar to "Pla som," "goi ca mai" (raw fish salad) and slices of raw silver carp in Vietnam, a raw fish salad in China, and sushi in Korea (Rim 2005). In Russia, *O. felineus* infection may come through eating dried or salted fish or sliced raw fish ("stroganina") which is popular among native Siberians, as well as fish pickled in vinegar.

Salted fish is generally considered ready in a day or two. It has been shown, however, that metacercariae remain viable under high salt concentrations for up to 2 weeks. The popular dishes in Russia and Eastern Europe of slightly salted fish are not safe at all. The same is true for dried fish where greater than 12 days of drying is required to kill 99% of metacercariae. Given that the weight of fish and the temperature of drying are not constant even at fish plants or after 12-day period, dried fish remain dangerous for consumption. Cold smoking has similar effects to those of drying and salting (Yossepowitch et al. 2004).

A recent investigation in fish farms in Lao PDR supported by Food and Agriculture Organization demonstrated that some carp species commonly cultured in fish ponds contained *O. viverrini* metacercariae (unpublished data). This preliminary result suggests that apart from captured fish, culture fish can provide an additional source of infection to consumers and thus urgently need attention control body to ensure food safety.

Control efforts are primarily focused on the reduction and elimination of parasite transmission by ensuring proper food preparation, promoting the development of improved diagnostic techniques, providing chemotherapy, and improving sanitation. A combination of health education, mass treatment, and governmental aid could significantly reduce liver fluke infection. Emphasis on health education should be placed on the younger generation in school as a part of the conventional education curriculum.

Interrupting the life cycle of the parasite has always been regarded as a promising way of disease control, for example, application of molluscicides (chemicals that kill snails) to control snail populations (Petney et al. 2012; Tesana et al. 2012). Low concentrations of certain molluscicides (e.g., phenasal, niclosamide) are lethal for infected snails, sublethal for uninfected ones, and, it is presumed, nontoxic for other animals (Tesana et al. 2012). The biggest case against this approach is that it involves interference of the ecosystem which can have dramatic consequences including potential toxicity to fish in rice fields (Calumpang et al. 1995; Tesana et al. 2012). Economic factors also negate its usage as the application of molluscicides is practical only for small water bodies since the costs of treating big areas are extremely high. Additionally, it has been shown that snail populations are restored in about 5 years, hence repeated treatments are necessary. Therefore, decontamination of aquatic bodies has been abandoned in Russia.

In order to achieve overall, long-term control of liver flukes, a multidisciplinary approach is necessary. This must aim at breaking the transmission cycle at the level of the first and second intermediate hosts, i.e., at the level of general hygiene as well as at the fisheries and aquaculture levels. This must also be extended to the food production and distribution industries. Of great significance, is that the population at risk

must be educated as to how infection occurs and how it can thus be avoided, and to the risks of infection, particularly the development of CCA, should it occur.

The Food and Agriculture Organization (FAO) recognizes the necessity of determining the importance of aquaculture in comparison with capture fisheries in the likelihood of human infection with food-borne trematodes. This is a primary requirement in food safety assurance from aquaculture at both the domestic and international trade levels. Hazard Analysis and Critical Control Point (HACCP) methodologies are already available to assist control approaches at the food production stage; however, more work is required taking into account the economics of the costs accruing through implementation of these approaches.

Thailand is considered the hot spot of opisthorchiasis and CCA and the public health importance is acknowledged. In order to prioritize the health problems associated with liver fluke infection, an estimation of burden of disease (BOD) in Thailand was instigated in 1999 and data are available from 2004 (Bundhamcharoen et al. 2011). Generally, BOD are based on two measurements, namely Disability Adjusted Life Year (DALY) and, when accurate mortality data are lacking, estimated number of Years of Life Loss (YLL). DALY is a summary measure of population health for setting priorities since this measure combines both fatal and non-fatal health outcomes. BOD estimates are far from complete, especially in developing countries where resources and budget are not adequate. The BOD study in Thailand has identified a short list of the top 20 diseases based on mortality, DALY, and YLL. Of these only the top 10 diseases were selected. Liver cancer ranks 5th in males and 8th in females. A total of 27,500 people die every year of liver cancer, while YLL is estimated to be 400,000. Both of these figures show the high significance of opisthorchiasis, and CCA, as a public health problem in Thailand.

Thailand was one of the first nations to initiate a program of liver fluke control. This was based initially on funding supplied by USAID as early as 1950. This was followed up by a Thai government program supported by the Deutsche Gesellschaft für Technische Zusammenarbeit (German Society for Technical Cooperation, GTZ). These programs have been successful in reducing the prevalence of *O. viverrini* infection from approximately 63.6% in 1984–1987 to 9.6% in the year 2001 (Jongsuksuntigul and Imsomboon 2003). In spite of this success, a nationwide survey showed that there was still a residual prevalence of 8.7% in 2009. The northeast of Thailand currently still has the highest prevalence with 16.6% with northern Thailand at 10%. Both central (1.3%) and southern Thailand (0.1%) have low infection prevalences (Sithithaworn et al. 2012a). These data indicate that although the control programs have been successful in reducing the prevalence, they have not eliminated the infection.

The control programs to date have relied on the use of praziquantel (40 mg/kg) curing (>95% cure rate) infected individuals. Most infected individuals involved in the control programs are now estimated to have only light infections (EPG <1000). Treatment with praziquantel, although it is successful in killing adult worms, does not prevent reinfection. Indeed, evidence is accumulating to suggest that it may compromise the immune system or cause liver complications when used repeatedly for reinfections (Pinlaor et al. 2008). This leads us to the conclusion that the current control programs require urgent modification.

Both short-term and a long-term program components should be reassessed. The short-term program requires a modification in the current selective treatment strategy by including traditional methods applied with increased accuracy, as well as molecular diagnostics. Multiple stool samples taken from an individual can be analyzed using the Kato-Katz method or the more sensitive formalin-ethyl acetate method. In addition, the cure rate based on the administration of praziquantel must be regularly monitored. This is particularly relevant given the different genetic groups of parasites present in different areas (Andrews et al. 2008). Control approaches must also include zoonotic cycles in carnivore reservoir hosts as they will increase in significance as human prevalences decrease.

One of the most important aspects in the long-term strategy involves an education program based on food safely. Current programs have shown that long-term, continuous education is required as raw or partially cooked fish consumption is a deeply rooted, raw attitude in the areas where it occurs (Grundy-Warr et al. 2012). Ziegler et al. (2011) recommend school-based health education for young children in order to imprint the importance of food preparation and hygiene in relation to public health. Such educational programs can be promoted through participatory activities in schools. It is anticipated that the information will not only become part of the child's background knowledge as they grow to adulthood, but that it will also be discussed at home during the period of schooling. Such a program should have a major impact at the family, extended family, and village levels. An approach of this intensity and magnitude will have the potential for a long-term impact that is not present with national and international selective treatment strategies.

Regarding the control options, Bürli et al. (2018b) utilized a mathematical model and suggested that education and improved sanitation would have to have a very high coverage to lead to *O. viverrini* elimination, whereas annual drug distribution at medium coverage is sufficient. Work by Laithavewat et al. (2018) and Khuntikeo et al. (2016) indicate that the education programs carried out to date in Thailand have had a major influence both on the knowledge of school children regarding the association between *O. viverrini* infection and cholangiocarcinoma and most importantly on the prevalence of infection in these children. This is not the case in Lao PDR where a recent study found 83% of children aged 5–15 years were infected and that the likelihood of infection was positively correlated with maternal infection (Araki et al. 2018). Based on their model, Bürli et al. (2018a) suggest that best solution is a combination of drug distribution at a medium level of coverage and as high as possible coverage of education and improved sanitation.

Moreover, the One Health approach recommended by the World Health Organization is a worldwide strategy for expanding interdisciplinary collaborations and communication in all aspects of health care for humans, animals, and the environment that is applicable for liver fluke control. This helps to improve our understanding of the social, economic, and ecological dimensions of liver fluke transmission including opisthorchiasis in Thailand and other Southeast Asian countries.

An integrated control program against *C. sinensis* infection based on education, improved sanitation, and use of praziquantel in Lou Village, Guangdong Province, China, proved to be remarkably successful with reductions in parasite prevalence and intensity of infections in human and fish hosts (Huang et al. 2017).

Long-term strategies, and indeed a good deal more research, are needed to overcome the dynamic situation caused by land-use and climatic changes either taking place or predicted for the Thailand. Such changes are often coupled with dynamic changes in parasite transmission (Patz et al. 2003, 2004). Given the public health significance of *O. viverrini* infection, the Thai Ministry of Public Health recently initiated the "Esan agenda: eradicate the liver fluke to reduce CCA" which is primarily aimed at screening for CCA patients. If the cancer is recognized sufficiently early, curative surgery may lead to an effective cure. Such a strategy, however, does not attack the problem at its roots as relatively very few people are involved compared with the population at risk based on infection with *O. viverrini*. Currently, at least 26 million people are at risk of infection in the north and northeast of Thailand. Any control program aimed at reducing the long-term burden of *Opisthorchis* infection must address this population group as the initiation point for the effective control of opisthorchiasis and its associated CCA.

References

Adam R, Arnold H, Pipitgool V, Sithithaworn P, Hinz E, Storch V (1993) Studies on lophocercous cercariae from *Bithynia siamensis goniomphalos* (Prosobranchia: Bithyniidae). Southeast Asian J Trop Med Public Health 24:697–700

Akai PS, Pungpak S, Chaicumpa W, Kitikoon V, Ruangkunaporn Y, Bunnag D, Befus AD (1995) Serum antibody responses in opisthorchiasis. Int J Parasitol 25:971–973

Ando K, Sithithaworn P, Nuchjungreed C, Tesana S, Srisawangwong T, Limviroj W, Chinzei Y (2001) Nucleotide sequence of mitochondrial CO I and ribosomal ITS II genes of *Opisthorchis viverrini* in northeast Thailand. Southeast Asian J Trop Med Public Health 32(Suppl 2):17–22

Andrews RH, Sithithaworn P, Petney TN (2008) *Opisthorchis viverrini*: an underestimated parasite in world health. Trends Parasitol 24:497–501

Araki H, Ong KIC, Lorphachan L, Soundala P, Iwagami M, Shibanuma A, Hongvanthong B, Brey PT, Kano S, Jimba M (2018) Mothers' *Opisthorchis viverrini* infection status and raw fish dish consumption in Lao People's Democratic Republic: determinants of child infection status. Trop Med Health 46:29

Arimatsu Y, Kaewkes S, Laha T, Hong SJ, Sripa B (2012) Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). Parasitol Int 61:178–182

Armignacco O, Caterini L, Marucci G, Ferri F, Bernardini G, Natalini Raponi G, Ludovisi A, Bossu T, Gomez Morales MA, Pozio E (2008) Human illnesses caused by *Opisthorchis felineus* flukes, Italy. Emerg Infect Dis 14:1902–1905

Armignacco O, Ferri F, Gomez-Morales MA, Caterini L, Pozio E (2013) Cryptic and asymptomatic *Opisthorchis felineus* infections. Am J Trop Med Hyg 88:364–366

Attwood HD, Chou ST (1978) The longevity of *Clonorchis sinensis*. Pathology 10:153–156

Aung WPP, Htoon TT, Tina HH, Thinn KK, Sanpool O, Jongthawin J, Sadaow L, Phosuk I, Rodpai R, Intapan PM, Maleewong W (2017) First report and molecular identification of *Opisthorchis viverrini* infection in human communities from Lower Myanmar. PLoS One 12:e0177130

Aunpromma S, Tangkawattana P, Papirom P, Kanjampa P, Tesana S, Sripa B, Tangkawattana S (2012) High prevalence of *Opisthorchis viverrini* infection in reservoir hosts in four districts of Khon Kaen Province, an opisthorchiasis endemic area of Thailand. Parasitol Int 61:60–64

Aunpromma S, Kanjampa P, Papirom P, Tangkawattana S, Tangkawattana P, Tesana S, Boonmars T, Suwannatrai A, Uopsai S, Sukon P, Sripa B (2016) Prevalence and risk factors for *Opisthorchis viverrini* infection among cats and dogs in six districts surrounding the Ubolratana Dam, an endemic area for human opisthorchiasis in Northeastern Thailand. Southeast Asian J Trop Med Public Health 47:1153–1159

Awiruttapanich M (2004) Epidemiology of liver fluke (*Opisthorchis viverrini*) in pla kao na (*Cyclocheilichthys armatus*). Dissertation, Khon Kaen University

Bae YA, Kong Y (2003) Evolutionary course of *CsRn1* long-terminal-repeat retrotransposon and its heterogeneous integrations into the genome of the liver fluke, *Clonorchis sinensis*. Korean J Parasitol 41:209–219

Bae YA, Moon SY, Kong Y, Cho SY, Rhyu MG (2001) *CsRn1*, a novel active retrotransposon in a parasitic trematode, *Clonorchis sinensis*, discloses a new phylogenetic clade of Ty3/gypsy-like LTR retrotransposons. Mol Biol Evol 18:1474–1483

Bedier E, Chesneau P (1929) Distomatose hepatique a *Opisthorchis* au Laos (a Vientiane et Thakhek). Bull Soc Pathol Exot 22:331–334

Behr MA, Gyorkos TW, Kokoskin E, Ward BJ, MacLean JD (1998) North American liver fluke (*Metorchis conjunctus*) in a Canadian aboriginal population: a submerging human pathogen? Can J Public Health 89:258–259

Bensted-Smith R, Anderson RM, Butterworth AE, Dalton PR, Kariuki CH, Koech D, Mugambi M, Ouma JH, Arap Siongok TK, Sturrock RF (1987) Evidence for predisposition of individual patients to reinfections with *Schistosoma mansoni* after treatment. Trans R Soc Trop Med Hyg 81:651–656

Bhamarapravati N, Thammavit W, Vajrasthira S (1978) Liver changes in hamsters infected with a liver fluke of man, *Opisthorchis viverrini*. Am J Trop Med Hyg 27:787–794

Blechacz BR, Gores GJ (2008) Cholangiocarcinoma. Clin Liver Dis 12:131–150

Boonmee S, Imtawil K, Wongkham C, Wongkham S (2003) Comparative proteomic analysis of juvenile and adult liver fluke, *Opisthorchis viverrini*. Acta Trop 88:233–238

Brockelman WY, Upatham ES, Viyanant V, Ardsungnoen S, Chantanawat R (1986) Field studies on the transmission of the human liver fluke, *Opisthorchis viverrini*, in northeast Thailand: population changes of the snail intermediate host. Int J Parasitol 16:545–552

Brusentsov II, Katokhin AV, Brusentsova IV, Shekhovtsov SV, Borovikov SN, Goncharenko GG, Lider LA, Romashov BV, Rusinek OT, Shibitov SK, Suleymanov MM, Yevtushenko AV, Mordvinov VA (2013) Low genetic diversity in wide-spread Eurasian liver fluke *Opisthorchis felineus* suggests special demographic history of this trematode species. PLoS One 8:e62453

Buathong S, Leelayoova S, Mungthin M, Ruang-areerate T, Naaglor T, Suwannahitatorn P, Piyaraj P, Taamasri P, Tan-ariya P (2017) Molecular discrimination of *Opisthorchis*-like eggs from residents in a rural community of central Thailand. PLoS Negl Trop Dis 11:e0006030

Bui TN, Pham TT, Nguyen NT, Nguyen HV, Murrell D, Phan VT (2016) The importance of wild fish in the epidemiology of *Clonorchis sinensis* in Vietnam. Parasitol Res 115:3401–3408

Bundhamcharoen K, Odton P, Phulkerd S, Tangcharoensathien V (2011) Burden of disease in Thailand: changes in health gap between 1999 and 2004. BMC Public Health 11:53

Bundy DA, Golden MH (1987) The impact of host nutrition on gastrointestinal helminth populations. Parasitology 95(Pt 3):623–635

Bunnag D, Harinasuta T (1981) Studies on the chemotherapy of human opisthorchiasis: III. Minimum effective dose of praziquantel. Southeast Asian J Trop Med Public Health 12:413–417

Bürli C, Harbrecht H, Odermatt P, Sayasone S, Chitnis N (2018a) Analysis of interventions against the liver fluke, *Opisthorchis viverrini*. Math Biosci 303:115–125

Bürli C, Harbrecht H, Odermatt P, Sayasone S, Chitnis N (2018b) Mathematical analysis of the transmission dynamics of the liver fluke, *Opisthorchis viverrini*. J Theor Biol 439:181–194

Cai XQ, Xu MJ, Wang YH, Qiu DY, Liu GX, Lin A, Tang JD, Zhang RL, Zhu XQ (2010) Sensitive and rapid detection of *Clonorchis sinensis* infection in fish by loop-mediated isothermal amplification (LAMP). Parasitol Res 106:1379–1383

Cai XQ, Liu GH, Song HQ, Wu CY, Zou FC, Yan HK, Yuan ZG, Lin RQ, Zhu XQ (2012) Sequences and gene organization of the mitochondrial genomes of the liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis* (Trematoda). Parasitol Res 110:235–243

Calumpang SM, Medina MJ, Tejada AW, Medina JR (1995) Environmental impact of two molluscicides: niclosamide and metaldehyde in a rice paddy ecosystem. Bull Environ Contam Toxicol 55:494–501

Chai JY, Park JH, Han ET, Guk SM, Shin EH, Lin A, Kim JL, Sohn WM, Yong TS, Eom KS, Min DY, Hwang EH, Phommmasack B, Insisiengmay B, Rim HJ (2005) Mixed infections with *Opisthorchis viverrini* and intestinal flukes in residents of Vientiane Municipality and Saravane Province in Laos. J Helminthol 79:283–289

Chaicumpa W, Ybanez L, Kitikoon V, Pungpak S, Ruangkunaporn Y, Chongsa-nguan M, Sornmani S (1992) Detection of *Opisthorchis viverrini* antigens in stools using specific monoclonal antibody. Int J Parasitol 22:527–531

Chen D, Chen J, Huang J, Chen X, Feng D, Liang B, Che Y, Liu X, Zhu C, Li X, Shen H (2010) Epidemiological investigation of *Clonorchis sinensis* infection in freshwater fishes in the Pearl River Delta. Parasitol Res 107:835–839

Chen J, Xu H, Zhang Z, Zeng S, Gan W, Yu X, Hu X (2011) Cloning and expression of 21.1-kDa tegumental protein of *Clonorchis sinensis* and human antibody response to it as a trematode-nematode pan-specific serodiagnosis antigen. Parasitol Res 108:161–168

Choi BI, Park JH, Kim YI, Yu ES, Kim SH, Kim WH, Kim CY, Han MC (1988) Peripheral cholangiocarcinoma and clonorchiasis: CT findings. Radiology 169:149–153

Choi MH, Ryu JS, Lee M, Li S, Chung BS, Chai JY, Sithithaworn P, Tesana S, Hong ST (2003) Specific and common antigens of *Clonorchis sinensis* and *Opisthorchis viverrini* (Opisthorchidae, Trematoda). Korean J Parasitol 41:155–163

Choi CD, Savage J, Stephens DN, O'Donnell M (2005) An integrated semicompliant balloon ultrasound catheter for quantitative feedback and image guidance during stent deployment. IEEE Trans Ultrason Ferroelectr Freq Control 52:1498–1503

Chotigeat W, Ruenwongsa P (1986) Types of collagen in *Opisthorchis viverrini* infected hamster liver. Mol Biochem Parasitol 18:377–387

Chou ST, Chan CW (1976) Mucin-producing cholangiocarcinoma: an autopsy study in Hong Kong. Pathology 8:321–328

Dao TT, Abatih EN, Nguyen TT, Tran HT, Gabriel S, Smit S, Le PN, Dorny P (2016) Prevalence of *Opisthorchis viverrini*-like fluke infection in ducks in Binh Dinh Province, central Vietnam. Korean J Parasitol 54:357–361

Dao TTH, Nguyen TTG, Gabriel S, Bui KL, Dorny P, Le TH (2017) Updated molecular phylogenetic data for *Opisthorchis* spp. (Trematoda: Opisthorchioidea) from ducks in Vietnam. Parasit Vectors 10:575

De NV, Le TH (2011) Human infections of fish-borne trematodes in Vietnam: prevalence and molecular specific identification at an endemic commune in Nam Dinh province. Exp Parasitol 129:355–361

Ditrich O, Scholz T, Giboda M (1990) Occurrence of some medically important flukes (Trematoda: Opisthorchiidae and Heterophyidae) in Nam Ngum water reservoir, Laos. Southeast Asian J Trop Med Public Health 21:482–488

Doanh PN, Nawa Y (2016) *Clonorchis sinensis* and *Opisthorchis* spp. in Vietnam: current status and prospects. Trans R Soc Trop Med Hyg 110:13–20

Donthaisong C, Arunsan P, Suwannatrai K, Prasopdee S, Kulsantiwong J, Wongmaneeprateep S, Suwannatrai A, Tesana S (2014) Experimental infection of *Opisthorchis viverrini* cercariae to the cyprinid fish, *Barbonymus gonionotus*. Acta Trop 136:118–122

Duenngai K, Sithithaworn P, Rudrappa UK, Iddya K, Laha T, Stensvold CR, Strandgaard H, Johansen MV (2008) Improvement of PCR for detection of *Opisthorchis viverrini* DNA in human stool samples. J Clin Microbiol 46:366–368

Duenngai K, Boonmars T, Sithithaworn J, Sithithaworn P (2013) Diagnosis of early infection and post chemotherapeutic treatment by copro-DNA detection in experimental opisthorchiasis. Parasitol Res 112:271–278

Dung VT, Waikagul J, Thanh BN, Vo DT, Nguyen DN, Murrell KD (2014) Endemicity of *Opisthorchis viverrini* liver flukes, Vietnam, 2011-2012. Emerg Infect Dis 20:152–154

Elkins DB, Haswell-Elkins M, Anderson RM (1986) The epidemiology and control of intestinal helminths in the Pulicat Lake region of Southern India. I. Study design and pre- and post-treatment observations on *Ascaris lumbricoides* infection. Trans R Soc Trop Med Hyg 80:774–792

Elkins DB, Haswell-Elkins MR, Mairiang E, Mairiang P, Sithithaworn P, Kaewkes S, Bhudhisawasdi V, Uttaravichien T (1990) A high frequency of hepatobiliary disease and suspected cholangiocarcinoma associated with heavy *Opisthorchis viverrini* infection in a small community in north-east Thailand. Trans R Soc Trop Med Hyg 84:715–719

Elkins DB, Sithithaworn P, Haswell-Elkins M, Kaewkes S, Awacharagan P, Wongratanacheewin S (1991)

Opisthorchis viverrini: relationships between egg counts, worms recovered and antibody levels within an endemic community in northeast Thailand. Parasitology 102(Pt 2):283–288

Elkins DB, Mairiang E, Sithithaworn P, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Haswell-Elkins MR (1996) Cross-sectional patterns of hepatobiliary abnormalities and possible precursor conditions of cholangiocarcinoma associated with *Opisthorchis viverrini* infection in humans. Am J Trop Med Hyg 55:295–301

Erhardt A, Germer WD, Hörning B (1962) Die Opisthorchiasis hervorgerufen durch den Katzenleberegel *Opisthorchis felineus* (Riv.). Parasitol Schriftenr 15:1–171

Fang YY, Chen YD, Li XM, Wu J, Zhang QM, Ruan CW (2008) Current prevalence of *Clonorchis sinensis* infection in endemic areas of China. Chin J Parasitol Parasit Dis 26:99–103

Fedorova OS, Kovshirina YV, Kovshirina AE, Fedotova MM, Deev IA, Petrovskiy FI, Filimonov AV, Dmitrieva AI, Kudyakov LA, Saltykova IV, Odermatt P, Ogorodova LM (2017) *Opisthorchis felineus* infection and cholangiocarcinoma in the Russian Federation: a review of medical statistics. Parasitol Int 66:365–371

Feldmeier H, Hazay M, Sato M, Tiengkham P, Nishimoto F, Jiang H, Sopraseuth V, Moji K (2016) Morbidity assessment of *Opisthorchis viverrini* infection in rural Laos: I. Parasitological, clinical, ultrasonographical and biochemical findings. Trop Med Health 44:12

Flavell DJ, Flavell SU, Field GF (1983) *Opisthorchis viverrini*: the relationship between egg production, worm size and intensity of infection in the hamster. Trans R Soc Trop Med Hyg 77:538–545

Frazer IH, Leggatt GR, Mattarollo SR (2011) Prevention and treatment of papillomavirus-related cancers through immunization. Annu Rev Immunol 29:111–138

Fried B, Abruzzi A (2010) Food-borne trematode infections of humans in the United States of America. Parasitol Res 106:1263–1280

Giboda M, Ditrich O, Scholz T, Viengsay T, Bouaphanh S (1991) Current status of food-borne parasitic zoonoses in Laos. Southeast Asian J Trop Med Public Health 22(Suppl):56–61

Gozlan RE, Britton JR, Cowx I, Copp GH (2010) Current knowledge on non-native freshwater fish introductions. J Fish Biol 76:751–786

Grundy-Warr C, Andrews RH, Sithithaworn P, Petney TN, Sripa B, Laithavewat L, Ziegler AD (2012) Raw attitudes, wetland cultures, life-cycles: socio-cultural dynamics relating to *Opisthorchis viverrini* in the Mekong Basin. Parasitol Int 61:65–70

Haas W, Granzer M, Brockelman CR (1990) *Opisthorchis viverrini*: finding and recognition of the fish host by the cercariae. Exp Parasitol 71:422–431

Han S, Zhang X, Wen J, Li Y, Shu J, Ling H, Zhang F (2012) A combination of the Kato-Katz methods and ELISA to improve the diagnosis of clonorchiasis in an endemic area, China. PLoS One 7:e46977

Haswell-Elkins MR, Elkins DB, Sithithaworn P, Treesarawat P, Kaewkes S (1991) Distribution patterns of *Opisthorchis viverrini* within a human community. Parasitology 103(Pt 1):97–101

Haswell-Elkins MR, Mairiang E, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Sithithaworn P, Elkins DB (1994) Cross-sectional study of *Opisthorchis viverrini* infection and cholangiocarcinoma in communities within a high-risk area in northeast Thailand. Int J Cancer 59:505–509

Hering-Hagenbeck S, Schuster R (1996) A focus of opisthorchiidosis in Germany. Appl Parasitol 37:260–265

Hira PR, Al-Enizi AA, Al-Kandari S, Behbehani K (1987) Opisthorchiasis in Kuwait: first report of infections in Thai migrant workers in the Arabian Gulf. Ann Soc Bel Med Trop 67:363–368

Hong ST (1988) Changes of anti-*Clonorchis sinensis* IgG antibody in serum after praziquantel treatment in human clonorchiasis. Korean J Parasitol 26:1–8

Hong ST, Fang Y (2012) *Clonorchis sinensis* and clonorchiasis, an update. Parasitol Int 61:17–24

Hong ST, Kho WG, Kim WH, Chai JY, Lee SH (1993) Turnover of biliary epithelial cells in *Clonorchis sinensis* infected rats. Korean J Parasitol 31:83–89

Hong SJ, Seong KY, Sohn WM, Song KY (2000) Molecular cloning and immunological characterization of phosphoglycerate kinase from *Clonorchis sinensis*. Mol Biochem Parasitol 108:207–216

Hong SJ, Yun Kim T, Gan XX, Shen LY, Sukontason K, Sukontason K, Kang SY (2002) *Clonorchis sinensis*: glutathione S-transferase as a serodiagnostic antigen for detecting IgG and IgE antibodies. Exp Parasitol 101:231–233

Hong SJ, Shin JK, Kang SY, Ryu JR (2003a) Ultrastructural localization of phosphoglycerate kinase in adult *Clonorchis sinensis*. Parasitol Res 90:369–371

Hong ST, Choi MH, Kim CH, Chung BS, Ji Z (2003b) The Kato-Katz method is reliable for diagnosis of *Clonorchis sinensis* infection. Diagn Microbiol Infect Dis 47:345–347

Honjo S, Srivatanakul P, Sriplung H, Kikukawa H, Hanai S, Uchida K, Todoroki T, Jedpiyawongse A, Kittiwatanachot P, Sripa B, Deerasamee S, Miwa M (2005) Genetic and environmental determinants of risk for cholangiocarcinoma via *Opisthorchis viverrini* in a densely infested area in Nakhon Phanom, northeast Thailand. Int J Cancer 117:854–860

Hotez PJ, Alibek K (2011) Central Asia's hidden burden of neglected tropical diseases. PLoS Negl Trop Dis 5:e1224

Hou PC (1956) The relationship between primary carcinoma of the liver and infestation with *Clonorchis sinensis*. J Pathol Bacteriol 72:239–246

Hu F, Yu X, Ma C, Zhou H, Zhou Z, Li Y, Lu F, Xu J, Wu Z, Hu X (2007) *Clonorchis sinensis*: expression, characterization, immunolocalization and serological reactivity of one excretory/secretory antigen-LPAP homologue. Exp Parasitol 117:157–164

Huang Y, Zhou Z, Hu X, Wei Q, Xu J, Wu Z, Yu X (2007) A novel tegumental protein 31.8 kDa of *Clonorchis*

sinensis: sequence analysis, expression, and immunolocalization. Parasitol Res 102:77–81

Huang SY, Zhao GH, Fu BQ, Xu MJ, Wang CR, Wu SM, Zou FC, Zhu XQ (2012) Genomics and molecular genetics of *Clonorchis sinensis*: current status and perspectives. Parasitol Int 61:71–76

Huang Y, Huang D, Geng Y, Fang S, Yang F, Wu C, Zhang H, Wang M, Zhang R, Wang X, Wu S, Cao J, Zhang R (2017) An integrated control strategy takes *Clonorchis sinensis* under control in an endemic area in south China. Vector Borne Zoonotic Dis 17:791–798

Hutadilok N, Ruenwongsa P (1983) Liver collagen turnover in hamsters during infection by the human liver fluke, *Opisthorchis viverrini*. Mol Biochem Parasitol 8:71–77

IARC (2012) Biological agents: a review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum 100B:341–370

Inoue S, Kawanishi S (1995) Oxidative DNA damage induced by simultaneous generation of nitric oxide and superoxide. FEBS Lett 371:86–88

Itoh M, Pairojkul C, Thamawit W, Sithithaworn P, Tiwawech D, Uttaravicien T, Shirai T, Ito N (1994) Association of antibodies to *Opisthorchis viverrini* with hepatobiliary disease in northeastern Thailand. Am J Trop Med Hyg 51:424–429

Jeong YI, Shin HE, Lee SE, Cheun HI, Ju JW, Kim JY, Park MY, Cho SH (2016) Prevalence of *Clonorchis sinensis* infection among residents along 5 major rivers in the Republic of Korea. Korean J Parasitol 54:215–219

Jittimanee J, Sermswan RW, Kaewraemruaen C, Barta JR, Macinnes JI, Maleewong W, Wongratanacheewin S (2012) Protective immunization of hamsters against *Opisthorchis viverrini* infection is associated with the reduction of TGF-beta expression. *Acta Trop* 122:189–195

Johansen MV, Sithithaworn P, Bergquist R, Utzinger J (2010) Towards improved diagnosis of zoonotic trematode infections in Southeast Asia. Adv Parasitol 73:171–195

Jones MK, Gobert GN, Zhang L, Sunderland P, McManus DP (2004) The cytoskeleton and motor proteins of human schistosomes and their roles in surface maintenance and host-parasite interactions. Bioessays 26:752–765

Jongsuksuntigul P (2002) Parasitic diseases in Northeast Thailand. In: Seminar in parasitic diseases in Northeast Thailand. Klang Nana Tham, Khon Kaen, pp 3–18

Jongsuksuntigul P, Imsomboon T (2003) Opisthorchiasis control in Thailand. Acta Trop 88:229–232

Jongsuksuntigul P, Chaychumsri W, Techamontrikul P, Cheeradit P, Suratawanich P (1992) Studies on prevalence and intensity of intestinal helminthiasis and liver fluke in Thailand in 1991. J Med Assoc Thai 2:80–95

Joo KR, Bang SJ (2005) A bile based study of *Clonorchis sinensis* infections in patients with biliary tract diseases in Ulsan, Korea. Yonsei Med J 46:794–798

Joo CY, Chung MS, Kim SJ, Kang CM (1997) Changing patterns of *Clonorchis sinensis* infections in Kyongbuk, Korea. Korean J Parasitol 35:155–164

Ju JW, Joo HN, Lee MR, Cho SH, Cheun HI, Kim JY, Lee YH, Lee KJ, Sohn WM, Kim DM, Kim IC, Park BC, Kim TS (2009) Identification of a serodiagnostic antigen, legumain, by immunoproteomic analysis of excretory-secretory products of *Clonorchis sinensis* adult worms. Proteomics 9:3066–3078

Kaewkes S (2003) Taxonomy and biology of liver flukes. Acta Trop 88:177–186

Kaewkes S, Kaewkes W, Boonmars T, Sripa B (2012a) Effect of light intensity on *Opisthorchis viverrini* cercarial shedding levels from *Bithynia* snails—a preliminary study. Parasitol Int 61:46–48

Kaewkes W, Kaewkes S, Tesana S, Laha T, Sripa B (2012b) Fecal bacterial contamination in natural water reservoirs as an indicator of seasonal infection by *Opisthorchis viverrini* in snail intermediate hosts. Parasitol Int 61:49–51

Kaewkong W, Choochote W, Kanla P, Maleewong W, Intapan PM, Wongkham S, Wongkham C (2012a) Chromosomes and karyotype analysis of a liver fluke, *Opisthorchis viverrini*, by scanning electron microscopy. Parasitol Int 61:504–507

Kaewkong W, Imtawil K, Maleewong W, Intapan PM, Sri-Aroon P, Wongkham S, Wongkham C (2012b) Genome size estimation of liver fluke *Opisthorchis viverrini* by real-time polymerase chain reaction based method. Parasitol Int 61:77–80

Kaewpitoon N, Kaewpitoon SJ, Ueng-arporn N, Rujirakul R, Churproong S, Matrakool L, Auiwatanagul S, Sripa B (2012) Carcinogenic human liver fluke: current status of *Opisthorchis viverrini* metacercariae in Nakhon Ratchasima, Thailand. Asian Pac J Cancer Prev 13:1235–1240

Kaewpitoon N, Kootanavanichpong N, Kompor P, Chavenkun W, Kujapun J, Norkaew J, Ponphimai S, Matrakool L, Tongtawee T, Panpimanmas S, Rujirakul R, Padchasuwan N, Pholsripradit P, Eksanti T, Phatisena T, Loyd RA, Kaewpitoon SJ (2015) Review and current status of *Opisthorchis viverrini* infection at the community level in Thailand. Asian Pac J Cancer Prev 16:6825–6830

Kang TH, Yun DH, Lee EH, Chung YB, Bae YA, Chung JY, Kang I, Kim J, Cho SY, Kong Y (2004) A cathepsin F of adult *Clonorchis sinensis* and its phylogenetic conservation in trematodes. Parasitology 128:195–207

Kang S, Sultana T, Loktev VB, Wongratanacheewin S, Sohn WM, Eom KS, Park JK (2008) Molecular identification and phylogenetic analysis of nuclear rDNA sequences among three opisthorchid liver fluke species (Opisthorchiidae: Trematoda). Parasitol Int 57:191–197

Katokhin AV, Shekhovtsov SV, Konkow S, Yurlova NI, Serbina EA, Vodianitskai SN, Fedorov KP, Loktev VB, Muratov IV, Ohyama F, Makhnev TV, Pel'tek SE, Mordvinov VA (2008) Assessment of the genetic distinctions of *Opisthorchis felineus* from *O. viverrini* and *Clonorchis sinensis* by ITS2 and CO1 sequences. Dokl Biochem Biophys 421:214–217

Keiser J, Utzinger J (2005) Emerging foodborne trematodiasis. Emerg Infect Dis 11:1507–1514

Kenny PA, Bissell MJ (2003) Tumor reversion: correction of malignant behavior by microenvironmental cues. Int J Cancer 107:688–695

Khampoosa P, Jones MK, Lovas EM, Piratae S, Kulsuntiwong J, Prasopdee S, Srisawangwong T, Laha T, Sripanidkulchai B, Thitapakorn V, Tesana S (2018) Egg-hatching mechanism of human liver fluke, *Opisthorchis viverrini*: a role for leucine aminopeptidases from the snail host, *Bithynia siamensis goniomphalos*. J Parasitol 104:388–397

Khan SA, Miras A, Pelling M, Taylor-Robinson SD (2007) Cholangiocarcinoma and its management. Gut 56:1755–1756

Khuntikeo N, Sithithaworn P, Loilom W, Namwat N, Yongvanit P, Thinkhamrop B, Kiatsopit N, Andrews RH, Petney TN (2016) Changing patterns of prevalence in *Opisthorchis viverrini* sensu lato infection in children and adolescents in northeast Thailand. Acta Trop 164:469–472

Khuntikeo N, Thinkhamrop B, Bundhamcharoen K, Andrews RH, Grundy-Warr C, Yongvanit P, Loilome W, Chamadol N, Kosuwan W, Sithithaworn P, Petney TN (2018) The socioeconomic burden of cholangiocarcinoma associated with *Opisthorchis viverrini* sensu lato infection in northeast Thailand: a preliminary analysis. Adv Parasitol 102:141–163

Kiatsopit N, Sithithaworn P, Sithithaworn J, Boonmars T, Tesana S, Pitaksakulrat O, Saijuntha W, Petney TN, Andrews RH (2011) Genetic relationships within the *Opisthorchis viverrini* species complex with specific analysis of *O. viverrini* from Savannakhet, Lao PDR by multilocus enzyme electrophoresis. Parasitol Res 108:211–217

Kiatsopit N, Sithithaworn P, Saijuntha W, Boonmars T, Tesana S, Sithithaworn J, Petney TN, Andrews RH (2012) Exceptionally high prevalence of infection of *Bithynia siamensis goniomphalos* with *Opisthorchis viverrini* cercariae in different wetlands in Thailand and Lao PDR. Am J Trop Med Hyg 86:464–469

Kiatsopit N, Sithithaworn P, Saijuntha W, Petney TN, Andrews RH (2013) *Opisthorchis viverrini*: implications of the systematics of first intermediate hosts, *Bithynia* snail species in Thailand and Lao PDR. Infect Genet Evol 14:313–319

Kiatsopit N, Sithithaworn P, Saijuntha W, Pitaksakulrat O, Petney TN, Webster JP, Andrews RH (2014) Analysis of the population genetics of *Opisthorchis viverrini* sensu lato in the Nam Ngum River wetland, Lao PDR, by multilocus enzyme electrophoresis. Parasitol Res 113:2973–2981

Kim YI, Yu ES, Kim ST (1989) Intraductal variant of peripheral cholangiocarcinoma of the liver with *Clonorchis sinensis* infection. Cancer 63:1562–1566

Kim TI, Na BK, Hong SJ (2009a) Functional genes and proteins of *Clonorchis sinensis*. Korean J Parasitol 47(Suppl):S59–S68

Kim TS, Cho SH, Huh S, Kong Y, Sohn WM, Hwang SS, Chai JY, Lee SH, Park YK, Oh DK, Lee JK, Working Groups in National Institute of Health, Korea Association of Health, Promotion (2009b) A nationwide survey on the prevalence of intestinal parasitic infections in the Republic of Korea, 2004. Korean J Parasitol 47:37–47

Kim TY, Lee YS, Yun JH, Kim JJ, Choi WH, Oh IH, Song HO, Chu JP (2010) A case of probable mixed-infection with *Clonorchis sinensis* and *Fasciola* sp.: CT and parasitological findings. Korean J Parasitol 48:157–160

Kim JH, Choi MH, Bae YM, Oh JK, Lim MK, Hong ST (2011) Correlation between discharged worms and fecal egg counts in human clonorchiasis. PLoS Negl Trop Dis 5:e1339

King S, Scholz T (2001) Trematodes of the family Opisthorchiidae: a minireview. Korean J Parasitol 39:209–221

Kirby GM, Pelkonen P, Vatanasapt V, Camus AM, Wild CP, Lang MA (1994) Association of liver fluke (*Opisthorchis viverrini*) infestation with increased expression of cytochrome P450 and carcinogen metabolism in male hamster liver. Mol Carcinog 11:81–89

Kobayashi J, Vannachone B, Xeutvongsa A, Manivang K, Ogawa S, Sato Y, Pholsena K (1996) Prevalence of intestinal parasitic infection among children in two villages in Lao PDR. Southeast Asian J Trop Med Public Health 27:562–565

Kobayashi J, Vannachone B, Sato Y, Manivong K, Nambanya S, Inthakone S (2000) An epidemiological study on *Opisthorchis viverrini* infection in Lao villages. Southeast Asian J Trop Med Public Health 31:128–132

Komalamisra C (1999) Chromosomes and C-banding of *Opisthorchis viverrini*. Southeast Asian J Trop Med Public Health 30:576–579

Kwon YD, Cho PY, Hong SJ (2005) *Clonorchis sinensis*: molecular cloning and localization of myosin regulatory light chain. Parasitol Res 97:21–26

Laha T, Pinlaor P, Mulvenna J, Sripa B, Sripa M, Smout MJ, Gasser RB, Brindley PJ, Loukas A (2007) Gene discovery for the carcinogenic human liver fluke, *Opisthorchis viverrini*. BMC Genomics 8:189

Lai DH, Wang QP, Chen W, Cai LS, Wu ZD, Zhu XQ, Lun ZR (2008) Molecular genetic profiles among individual *Clonorchis sinensis* adults collected from cats in two geographic regions of China revealed by RAPD and MGE-PCR methods. Acta Trop 107:213–216

Lai DH, Hong XK, Su BX, Liang C, Hide G, Zhang X, Yu X, Lun ZR (2016) Current status of *Clonorchis sinensis* and clonorchiasis in China. Trans R Soc Trop Med Hyg 110:21–27

Laithavewat L, Grundy-Warr C, Khuntikeo N, Andrews RH, Petney TN, Yongvanit P, Banchonhattakit P, Sithithaworn P (2018) Analysis of a school-based health education model to prevent opisthorchiasis and cholangiocarcinoma in primary school children in northeast Thailand. Glob Health Promot. https://doi.org/10.1177/1757975918767622

Lan-Anh NT, Phuong NT, Murrell KD, Johansen MV, Dalsgaard A, Thu LT, Kim-Chi TT, Thamsborg SM (2009) Animal reservoir hosts and fish-borne zoonotic

trematode infections on fish farms, Vietnam. Emerg Infect Dis 15:540–546

Laoprom N, Saijuntha W, Sithithaworn P, Wongkham S, Laha T, Ando K, Andrews RH, Petney TN (2009) Biological variation within *Opisthorchis viverrini* sensu lato in Thailand and Lao PDR. J Parasitol 95:1307–1313

Laoprom N, Sithithaworn P, Ando K, Sithithaworn J, Wongkham S, Laha T, Klinbunga S, Webster JP, Andrews RH (2010) Microsatellite loci in the carcinogenic liver fluke, *Opisthorchis viverrini* and their application as population genetic markers. Infect Genet Evol 10:146–153

Laoprom N, Sithithaworn P, Andrews RH, Ando K, Laha T, Klinbunga S, Webster JP, Petney TN (2012) Population genetic structuring in *Opisthorchis viverrini* over various spatial scales in Thailand and Lao PDR. PLoS Negl Trop Dis 6:e1906

Laoprom N, Kiatsopit N, Sithithaworn P, Kopolrat K, Namsanor J, Andrews RH, Petney TN (2016) Cercarial emergence patterns for *Opisthorchis viverrini* sensu lato infecting *Bithynia siamensis goniomphalos* from Sakon Nakhon Province, Thailand. Parasitol Res 115:3313–3321

Lazutkina EA, Andreyev NI, Andreyeva SI, Gloer P, Vinarski MV (2009) On the taxonomic state of *Bithynia troschelii* var. *sibirica* Westerlund, 1886, a Siberian endemic bithyniid snail (Gastropoda: Bithyniidae). Mollusca 27:113–122

Le TH, Van De N, Blair D, Sithithaworn P, McManus DP (2006) *Clonorchis sinensis* and *Opisthorchis viverrini*: development of a mitochondrial-based multiplex PCR for their identification and discrimination. Exp Parasitol 112:109–114

Le TH, Nguyen NT, Truong NH, De NV (2012) Development of mitochondrial loop-mediated isothermal amplification for detection of the small liver fluke *Opisthorchis viverrini* (Opisthorchiidae; Trematoda; Platyhelminthes). J Clin Microbiol 50:1178–1184

Lee SU, Huh S (2004) Variation of nuclear and mitochondrial DNAs in Korean and Chinese isolates of *Clonorchis sinensis*. Korean J Parasitol 42:145–148

Lee JH, Rim HJ, Bak UB (1993) Effect of *Clonorchis sinensis* infection and dimethylnitrosamine administration on the induction of cholangiocarcinoma in Syrian golden hamsters. Korean J Parasitol 31:21–30

Lee JH, Yang HM, Bak UB, Rim HJ (1994) Promoting role of *Clonorchis sinensis* infection on induction of cholangiocarcinoma during two-step carcinogenesis. Korean J Parasitol 32:13–18

Lee JS, Kim IS, Sohn WM, Lee J, Yong TS (2006) Vaccination with DNA encoding cysteine proteinase confers protective immune response to rats infected with *Clonorchis sinensis*. Vaccine 24:2358–2366

Leiper RT (1915) Notes of the occurrence of parasites presumably rare in man. J R Army Med Corps 24:569–575

Li S, Shin JG, Cho PY, Kim TI, Hong ST, Hong SJ (2011) Multiple recombinant antigens of *Clonorchis sinensis* for serodiagnosis of human clonorchiasis. Parasitol Res 108:1295–1302

Li Y, Hu X, Liu X, Huang Y, Xu J, Zhao J, Wu Z, Yu X (2012) Serological diagnosis of clonorchiasis: using a recombinant propeptide of cathepsin L proteinase from *Clonorchis sinensis* as a candidate antigen. Parasitol Res 110:2197–2203

Lin R, Li X, Lan C, Yu S, Kawanaka M (2005) Investigation on the epidemiological factors of *Clonorchis sinensis* infection in an area of south China. Southeast Asian J Trop Med Public Health 36:1114–1117

Liu WQ, Liu J, Zhang JH, Long XC, Lei JH, Li YL (2007) Comparison of ancient and modern *Clonorchis sinensis* based on ITS1 and ITS2 sequences. Acta Trop 101:91–94

Liu GH, Li B, Li JY, Song HQ, Lin RQ, Cai XQ, Zou FC, Yan HK, Yuan ZG, Zhou DH, Zhu XQ (2012) Genetic variation among *Clonorchis sinensis* isolates from different geographic regions in China revealed by sequence analyses of four mitochondrial genes. J Helminthol 86:479–484

Lohachit C (2004-2005) Ecological studies of *Bithynia siamensis goniomphalos*, a snail intermediate host of *Opisthorchis viverrini*, in Khon Kaen Province, Northeast Thailand. Malacol Rev 37(38):1–26

Looss A (1907) On some parasites in the museum of the School of Tropical Medicine, Liverpool. Ann Trop Med Parasitol 1:123–152

Lovis L, Mak TK, Phongluxa K, Soukhathammavong P, Sayasone S, Akkhavong K, Odermatt P, Keiser J, Felger I (2009) PCR diagnosis of *Opisthorchis viverrini* and *Haplorchis taichui* infections in a Lao community in an area of endemicity and comparison of diagnostic methods for parasitological field surveys. J Clin Microbiol 47:1517–1523

Lovis L, Mak TK, Phongluxa K, Aye Soukhathammavong P, Vonghachack Y, Keiser J, Vounatsou P, Tanner M, Hatz C, Utzinger J, Odermatt P, Akkhavong K (2012) Efficacy of praziquantel against *Schistosoma mekongi* and *Opisthorchis viverrini*: a randomized, single-blinded dose-comparison trial. PLoS Negl Trop Dis 6:e1726

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database. In: The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN). Hollands Printing Ltd, Auckland

Lun ZR, Gasser RB, Lai DH, Li AX, Zhu XQ, Yu XB, Fang YY (2005) Clonorchiasis: a key foodborne zoonosis in China. Lancet Infect Dis 5:31–41

Mairiang E, Elkins DB, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, Posri S, Sithithaworn P, Haswell-Elkins M (1992) Relationship between intensity of *Opisthorchis viverrini* infection and hepatobiliary disease detected by ultrasonography. J Gastroenterol Hepatol 7:17–21

Mairiang E, Laha T, Bethony JM, Thinkhamrop B, Kaewkes S, Sithithaworn P, Tesana S, Loukas A,

Brindley PJ, Sripa B (2012) Ultrasonography assessment of hepatobiliary abnormalities in 3359 subjects with *Opisthorchis viverrini* infection in endemic areas of Thailand. Parasitol Int 61:208–211

Manivong K, Komalamisra C, Waikagul J, Radomyos P (2009) *Opisthorchis viverrini* metacercariae in cyprinoid fish from three rivers in Khammouane Province, Lao PDR. J Trop Med Parasitol 32:23–29

Mas-Coma S, Bargues MD (1997) Human liver flukes: a review. Res Rev Parasitol 57:144–225

Mazidur Rahman SM, Choi MH, Bae YM, Hong ST (2012) Coproantigen capture ELISA for detection of *Clonorchis sinensis* infection in experimentally infected rats. Parasitol Int 61:203–207

Miyamoto K, Kirinoki M, Matsuda H, Hayashi N, Chigusa Y, Sinuon M, Chuor CM, Kitikoon V (2014) Field survey focused on *Opisthorchis viverrini* infection in five provinces of Cambodia. Parasitol Int 63:366–373

Molina CD, Molina MM, Molina JM (1988) Intestinal parasites in southeast Asian refugees two years after immigration. West J Med 149:422–425

Mordvinov VA, Yurlova NI, Ogorodova LM, Katokhin AV (2012) *Opisthorchis felineus* and *Metorchis bilis* are the main agents of liver fluke infection of humans in Russia. Parasitol Int 61:25–31

Mulvenna J, Sripa B, Brindley PJ, Gorman J, Jones MK, Colgrave ML, Jones A, Nawaratna S, Laha T, Suttiprapa S, Smout MJ, Loukas A (2010) The secreted and surface proteomes of the adult stage of the carcinogenic human liver fluke *Opisthorchis viverrini*. Proteomics 10:1063–1078

Nagano I, Pei F, Wu Z, Wu J, Cui H, Boonmars T, Takahashi Y (2004) Molecular expression of a cysteine proteinase of *Clonorchis sinensis* and its application to an enzyme-linked immunosorbent assay for immunodiagnosis of clonorchiasis. Clin Diagn Lab Immunol 11:411–416

Nakeeb A, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S, Hruban RH, Lillemoe KD, Yeo CJ, Cameron JL (1996) Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. Ann Surg 224:463–473

Namsanor J, Sithithaworn P, Kopolrat K, Kiatsopit N, Pitaksakulrat O, Tesana S, Andrews RH, Petney TN (2015) Seasonal transmission of *Opisthorchis viverrini* sensu lato and a Lecithodendriid trematode species in *Bithynia siamensis goniomphalos* snails in Northeast Thailand. Am J Trop Med Hyg 93:87–93

Nawa Y, Doanh PN, Thaenkham U (2015) Is *Opisthorchis viverrini* an avian liver fluke? J Helminthol 89:255–256

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MC, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. *Nature* 405:1017–1024

Ngern-klun R, Sukontason KL, Tesana S, Sripakdee D, Irvine KN, Sukontason K (2006) Field investigation of *Bithynia funiculata*, intermediate host of *Opisthorchis viverrini* in northern Thailand. Southeast Asian J Trop Med Publ Health 37:662–672

Ngoen-Klan R, Piangjai S, Somwang P, Moophayak K, Sukontason K, Sukontason KL, Sampson M, Irvine K (2010) Emerging helminths infection in snails and cyprinoid fish in sewage treatment wetlands waters in Cambodia. Asian J Water Environ Pollut 7:13–21

Nguyen TT, Arimatsu Y, Hong SJ, Brindley PJ, Blair D, Laha T, Sripa B (2015) Genome-wide characterization of microsatellites and marker development in the carcinogenic liver fluke *Clonorchis sinensis*. Parasitol Res 114:2263–2272

Ogorodova LM, Freidin MB, Sazonov AE, Fedorova OS, Gerbek IE, Cherevko NA, Lebedeva NY (2007) A pilot screening of prevalence of atopic states and opisthorchosis and their relationship in people of Tomsk Oblast. Parasitol Res 101:1165–1168

Ohshima H, Bartsch H (1994) Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat Res 305:253–264

Onsurathum S, Pinlaor P, Charoensuk L, Haonon O, Chaidee A, Intuyod K, Laummaunwai P, Boonmars T, Kaewkes W, Pinlaor S (2016a) Contamination of *Opisthorchis viverrini* and *Haplorchis taichui* metacercariae in fermented fish products in northeastern Thailand markets. Food Control 59:493–498

Onsurathum S, Pinlaor P, Haonon O, Chaidee A, Charoensuk L, Intuyod K, Boonmars T, Laummaunwai P, Pinlaor S (2016b) Effects of fermentation time and low temperature during the production process of Thai pickled fish (pla-som) on the viability and infectivity of *Opisthorchis viverrini* metacercariae. Int J Food Microbiol 218:1–5

Park GM (2007) Genetic comparison of liver flukes, *Clonorchis sinensis* and *Opisthorchis viverrini*, based on rDNA and mtDNA gene sequences. Parasitol Res 100:351–357

Park GM, Yong TS (2001) Geographical variation of the liver fluke, *Clonorchis sinensis*, from Korea and China based on the karyotypes, zymodeme and DNA sequences. Southeast Asian J Trop Med Publ Health 32(Suppl 2):12–16

Park GM, Im K, Huh S, Yong TS (2000a) Chromosomes of the liver fluke, *Clonorchis sinensis*. Korean J Parasitol 38:201–206

Park GM, Yong TS, Im K, Lee KJ (2000b) Isozyme electrophoresis patterns of the liver fluke, *Clonorchis sinensis* from Kimhae, Korea and from Shenyang, China. Korean J Parasitol 38:45–48

Parkin DM (2006) The global health burden of infection-associated cancers in the year 2002. Int J Cancer 118:3030–3044

Parkin DM, Srivatanakul P, Khlat M, Chenvidhya D, Chotiwan P, Insiripong S, L'Abbe KA, Wild CP (1991) Liver cancer in Thailand. I. A case-control study of cholangiocarcinoma. Int J Cancer 48:323–328

Parkin DM, Ohshima H, Srivatanakul P, Vatanasapt V (1993) Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. Cancer Epidemiol Biomarkers Prev 2:537–544

Parvathi A, Sanath Kumar H, Kenchanna Prakasha B, Lu J, Xu X, Hu W, Feng Z, Karunasagar I, Karunasagar I (2007) *Clonorchis sinensis*: development and evaluation of a nested polymerase chain reaction (PCR) assay. Exp Parasitol 115:291–295

Parvathi A, Umesha KR, Kumar S, Sithithaworn P, Karunasagar I, Karunasagar I (2008) Development and evaluation of a polymerase chain reaction (PCR) assay for the detection of *Opisthorchis viverrini* in fish. Acta Trop 107:13–16

Patz JA, Githeko AK, McCarty JP, Hussein S, Confalonieri U, de Wet N (2003) Climate change and infectious diseases. In: McMichael AJ, Campbell-Lendrum DH, Corvalán CF, Ebi KL, Githeko A, Scheraga JD, Woodward A (eds) Climate change and human health: risks and responses. World Health Organization, Malta, pp 103–132

Patz JA, Daszak P, Tabor GM, Aguirre AA, Pearl M, Epstein J, Wolfe ND, Kilpatrick AM, Foufopoulos J, Molyneux D, Bradley DJ, Members of the Working Group on Land Use Change and Disease Emergence (2004) Unhealthy landscapes: Policy recommendations on land use change and infectious disease emergence. Environ Health Perspect 112:1092–1098

Pauly A, Schuster R, Steuber S (2003) Molecular characterization and differentiation of opisthorchiid trematodes of the species *Opisthorchis felineus* (Rivolta, 1884) and *Metorchis bilis* (Braun, 1790) using polymerase chain reaction. Parasitol Res 90:409–414

Petney TN, Andrews RH (1998) Multiparasite communities in animals and humans: frequency, structure and pathogenic significance. Int J Parasitol 28:377–393

Petney T, Sithithaworn P, Andrews R, Kiatsopit N, Tesana S, Grundy-Warr C, Ziegler A (2012) The ecology of the *Bithynia* first intermediate hosts of *Opisthorchis viverrini*. Parasitol Int 61:38–45

Petney TN, Andrews RH, Saijuntha W, Wenz-Mucke A, Sithithaworn P (2013) The zoonotic, fish-borne liver flukes *Clonorchis sinensis*, *Opisthorchis felineus* and *Opisthorchis viverrini*. Int J Parasitol 43:1031–1046

Petney TN, Andrews RH, Saijuntha W, Tesana S, Prasopdee S, Kiatsopit N, Sithithaworn P (2018) Taxonomy, ecology and population genetics of *Opisthorchis viverrini* and its intermediate hosts. Adv Parasitol 101:1–39

Phalee A, Wongsawad C, Chuboon S (2008) Occurrence of Metacercariae in Cyprinoid Fish from Khumtakla District, Sakonnakorn Province. In: 34th Congress on Science and Technology of Thailand. Queen Sirikit National Convention Center, Thailand

Phan VT, Ersboll AK, Nguyen KV, Madsen H, Dalsgaard A (2010a) Farm-level risk factors for fish-borne zoonotic trematode infection in integrated small-scale fish farms in northern Vietnam. PLoS Negl Trop Dis 4:e742

Phan VT, Ersboll AK, Nguyen TT, Nguyen KV, Nguyen HT, Murrell D, Dalsgaard A (2010b) Freshwater aquaculture nurseries and infection of fish with zoonotic trematodes, Vietnam. Emerg Infect Dis 16:1905–1909

Phung LT, Loukas A, Brindley PJ, Sripa B, Laha T (2014) Retrotransposon OV-RTE-1 from the carcinogenic liver flukes *Opisthorchis viverrini*: Potential target for DNA-based diagnosis. Infect Genet Evol 21:443–451

Pinlaor S, Prakobwong S, Hiraku Y, Kaewsamut B, Dechakhamphu S, Boonmars T, Sithithaworn P, Pinlaor P, Ma N, Yongvanit P, Kawanishi S (2008) Oxidative and nitrative stress in *Opisthorchis viverrini*-infected hamsters: an indirect effect after praziquantel treatment. Am J Trop Med Hyg 78:564–573

Pinlaor S, Onsurathum S, Boonmars T, Pinlaor P, Hongsrichan N, Chaidee A, Haonon O, Limviroj W, Tesana S, Kaewkes S, Sithithaworn P (2013) Distribution and abundance of *Opisthorchis viverrini* metacercariae in cyprinid fish in Northeastern Thailand. Korean J Parasitol 51:703–710

Pitaksakulrat O, Sithithaworn P, Laoprom N, Laha T, Petney TN, Andrews RH (2013) A cross-sectional study on the potential transmission of the carcinogenic liver fluke *Opisthorchis viverrini* and other fishborne zoonotic trematodes by aquaculture fish. Foodborne Pathog Dis 10:35–41

Pitaksakulrat O, Webster BL, Webster JP, Laha T, Saijuntha W, Lamberton PHL, Kiatsopit N, Andrews RH, Petney TN, Sithithaworn P (2018) Phylogenetic relationships within the *Opisthorchis viverrini* species complex with specific analysis of *O. viverrini* sensu lato from Sakon Nakhon, Thailand by mitochondrial and nuclear DNA sequencing. Infect Genet Evol 62:86–94

Poirier MJ (1886) Trematodes nouveaux ou peu connus. Bull Soc Philom 7:20–40

Polyakov AV, Katokhin AV, Bocharova TA, Romanov KV, L'vova MN, Bonina OM, Yurlova NI, Mordvinov VA (2010) Comparative analysis of karyotypes of *Opisthorchis felineus* from West Siberia. Contemp Probl Ecol 3:1–3

Poopyruchpong N, Viyanant V, Upatham ES, Srivatanakul P (1990) Diagnosis of opisthorchiasis by enzyme-linked immunosorbent assay using partially purified antigens. Asian Pac J Allergy Immunol 8:27–31

Pozio E, Armignacco O, Ferri F, Gomez Morales MA (2013) *Opisthorchis felineus*, an emerging infection in Italy and its implication for the European Union. Acta Trop 126:54–62

Prakobwong S, Suwannatrai A, Sancomerang A, Chaipibool S, Siriwechtumrong N (2017) A large scale study of the epidemiology and risk factors for the carcinogenic liver fluke *Opisthorchis viverrini* in Udon Thani Province, Thailand. Asian Pac J Cancer Prev 18:2853–2860

Prasopdee S, Kulsantiwong J, Piratae S, Khampoosa P, Thammasiri C, Suwannatrai A, Laha T, Grams R, Loukas A, Tesana S (2015) Temperature dependence of *Opisthorchis viverrini* infection in first intermediate host snail, *Bithynia siamensis goniomphalos*. Acta Trop 141:112–117

Prommas C (1927) Report of a case of *Opisthorchis felineus* in siam. Ann Trop Med Parasitol 21:9–10

Pumidonming W, Katahira H, Igarashi M, Salman D, Abdelbaset AE, Sangkaeo K (2018) Potential risk of a liver fluke *Opisthorchis viverrini* infection brought by immigrants from prevalent areas: a case study in the lower Northern Thailand. Acta Trop 178:213–218

Qian MB, Chen YD, Yan F (2013) Time to tackle clonorchiasis in China. Infect Dis Poverty 2:4

Qiao T, Zheng PM, Ma RH, Luo XB, Luo ZL (2012) Development of a real-time PCR assay for the detection of *Clonorchis sinensis* DNA in gallbladder bile and stone samples from patients with cholecystolithiasis. Parasitol Res 111:1497–1503

Radomyos P, Radomyos B, Tungtrongchitr A (1994) Multi-infection with helminths in adults from northeast Thailand as determined by post-treatment fecal examination of adult worms. Trop Med Parasitol 45:133–135

Rahman SM, Bae YM, Hong ST, Choi MH (2011) Early detection and estimation of infection burden by real-time PCR in rats experimentally infected with *Clonorchis sinensis*. Parasitol Res 109:297–303

Ramsay RJ, Sithithaworn P, Prociv P, Moorhouse DE, Methaphat C (1989) Density-dependent fecundity of *Opisthorchis viverrini* in humans, based on faecal recovery of flukes. Trans R Soc Trop Med Hyg 83:241–242

Riganti M, Pungpak S, Punpoowong B, Bunnag D, Harinasuta T (1989) Human pathology of *Opisthorchis viverrini* infection: a comparison of adults and children. Southeast Asian J Trop Med Publ Health 20:95–100

Rim HJ (2005) Clonorchiasis: an update. J Helminthol 79:269–281

Rim HJ, Yoo KS (1979) Chemotherapeutic effect of praziquantel (Embay 8440) in the treatment of *Clonorchiasis sinensis*. Korea Univ Med J 16:459–470

Rim HJ, Sohn WM, Yong TS, Eom KS, Chai JY, Min DY, Lee SH, Hoang EH, Phommasack B, Insisengmay S (2008) Fishborne trematode metacercariae detected in freshwater fish from Vientiane Municipality and Savannakhet Province, Lao PDR. Korean J Parasitol 46:253–260

Rim HJ, Sohn WM, Yong TS, Eom KS, Chai JY, Min DY, Lee SH, Hoang EH, Phommasack B, Insisiengmay S (2013) Fishborne trematode metacercariae in Luang Prabang, Khammouane, and Saravane Province, Lao PDR. Korean J Parasitol 51:107–114

Rivolta S (1884) Sopra una specie di Distoma nel gatto e nel cane. Giorn Anat Fisiol Patol Anim 16:20–28

Ruangkunaporn Y, Akai PS, Chongsa-nguan M, Sri-Yeythong S, Kitikoon V, Chaicumpa W (1994) Changes in serum antibodies to *Opisthorchis viverrini* in humans and hamsters following treatment of opisthorchiasis. Asian Pac J Allergy Immunol 12:83–84

Ruangsittichai J, Viyanant V, Vichasri-Grams S, Sobhon P, Tesana S, Upatham ES, Hofmann A, Korge G, Grams R (2006) *Opisthorchis viverrini*: identification of a glycine-tyrosine rich eggshell protein and its potential as a diagnostic tool for human opisthorchiasis. Int J Parasitol 36:1329–1339

Sadun EH (1955) Studies on *Opisthorchis viverrini* in Thailand. Am J Hyg 62:81–115

Saijuntha W, Sithithaworn P, Wongkham S, Laha T, Pipitgool V, Petney TN, Andrews RH (2006a) Genetic markers for the identification and characterization of *Opisthorchis viverrini*, a medically important food borne trematode in Southeast Asia. Acta Trop 100:246–251

Saijuntha W, Sithithaworn P, Wongkham S, Laha T, Pipitgool V, Petney TN, Chilton NB, Andrews RH (2006b) Enzyme markers to identify and characterize *Opisthorchis viverrini* in Thailand and Lao PDR. Southeast Asian J Trop Med Publ Health 37(Suppl 3):43–47

Saijuntha W, Sithithaworn P, Wongkham S, Laha T, Pipitgool V, Tesana S, Chilton NB, Petney TN, Andrews RH (2007) Evidence of a species complex within the food-borne trematode *Opisthorchis viverrini* and possible co-evolution with their first intermediate hosts. Int J Parasitol 37:695–703

Saijuntha W, Sithithaworn P, Wongkham S, Laha T, Satrawaha R, Chilton NB, Petney TN, Andrews RH (2008) Genetic variation at three enzyme loci within a Thailand population of *Opisthorchis viverrini*. Parasitol Res 103:1283–1287

Saijuntha W, Sithithaworn P, Chilton NB, Petney TN, Klinbunga S, Satrawaha R, Webster JP, Andrews RH (2009) Impact of temporal changes and host factors on the genetic structure of a population of *Opisthorchis viverrini* sensu lato in Khon Kaen Province (Thailand). Parasitology 136:1057–1063

Saijuntha W, Duenngai K, Tangkawattana S, Petney TN, Andrews RH, Sithithaworn P (2018) Recent advances in the diagnosis and detection of *Opisthorchis viverrini* sensu lato in human and intermediate hosts for use in control and elimination programs. Adv Parasitol 101:177–214

Saksirisampant W, Wiwanitkit V, Akrabovorn P, Nuchprayoon S (2002) Parasitic infections in Thai workers that pursue overseas employment: the need for a screening program. Southeast Asian J Trop Med Publ Health 33(Suppl 3):110–112

Sanpool O, Intapan PM, Thanchomnang T, Janwan P, Lulitanond V, Doanh PN, Van Hien H, Dung do T, Maleewong W, Nawa Y (2012) Rapid detection and differentiation of *Clonorchis sinensis* and *Opisthorchis viverrini* eggs in human fecal samples using a duplex real-time fluorescence resonance energy transfer PCR and melting curve analysis. Parasitol Res 111:89–96

Sanpool O, Aung WPP, Rodpai R, Maleewong W, Intapan PM (2018) Human liver fluke *Opisthorchis viverrini* (Trematoda, Opisthorchiidae) in Central Myanmar: new records of adults and metacercariae identified by morphology and molecular analysis. Acta Trop 185:149–155

Saowakontha S, Pipitgool V, Pariyanonda S, Tesana S, Rojsathaporn K, Intarakhao C (1993) Field trials in the control of *Opisthorchis viverrini* with an integrated programme in endemic areas of northeast Thailand. Parasitology 106(Pt 3):283–288

Satarug S, Lang MA, Yongvanit P, Sithithaworn P, Mairiang E, Mairiang P, Pelkonen P, Bartsch H, Haswell-Elkins MR (1996) Induction of cytochrome P450 2A6 expression in humans by the carcinogenic parasite infection, *Opisthorchiasis viverrini*. Cancer Epidemiol Biomarkers Prev 5:795–800

Satarug S, Haswell-Elkins MR, Sithithaworn P, Bartsch H, Ohshima H, Tsuda M, Mairiang P, Mairiang E, Yongvanit P, Esumi H, Elkins DB (1998) Relationships between the synthesis of N-nitrosodimethylamine and immune responses to chronic infection with the carcinogenic parasite, *Opisthorchis viverrini*, in men. Carcinogenesis 19:485–491

Sato M, Thaenkham U, Dekumyoy P, Waikagul J (2009) Discrimination of *O. viverrini*, *C. sinensis*, *H. pumilio* and *H. taichui* using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. Acta Trop 109:81–83

Sawangsoda P, Sithithaworn J, Tesana S, Pinlaor S, Boonmars T, Mairiang E, Yongvanit P, Duenngai K, Sithithaworn P (2012) Diagnostic values of parasite-specific antibody detections in saliva and urine in comparison with serum in opisthorchiasis. Parasitol Int 61:196–202

Sayasone S, Odermatt P, Phoumindr N, Vongsaravane X, Sensombath V, Phetsouvanh R, Choulamany X, Strobel M (2007) Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. Trans R Soc Trop Med Hyg 101:40–47

Schad GA, Anderson RM (1985) Predisposition to hookworm infection in humans. Science 228:1537–1540

Scholz T (2008) Family Opistorchiidae Looss, 1899. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda. CAB International and Natural History Museum, London, pp 9–49

Scholz T, Ditrich O, Giboda M (1991) Differential diagnosis of opisthorchiid and heterophyid metacercariae (Trematoda) infecting flesh of cyprinid fish from Nam Ngum Dam Lake in Laos. Southeast Asian J Trop Med Publ Health 22(Suppl):171–173

Schuster R (2002) Leberegelbefall. Denisia 184:291–315

Shekhovtsov SV, Katokhin AV, Romanov KV, Besprozvannykh VV, Fedorov KP, Yurlova NI, Serbina EA, Sithithaworn P, Kolchanov NA, Mordvinov VA (2009) A novel nuclear marker, Pm-int9, for phylogenetic studies of *Opisthorchis felineus*, *Opisthorchis viverrini*, and *Clonorchis sinensis* (Opisthorchiidae, Trematoda). Parasitol Res 106:293–297

Shekhovtsov SV, Katokhin AV, Kolchanov NA, Mordvinov VA (2010) The complete mitochondrial genomes of the liver flukes *Opisthorchis felineus* and *Clonorchis sinensis* (Trematoda). Parasitol Int 59:100–103

Shen C, Kim J, Lee JK, Bae YM, Choi MH, Oh JK, Lim MK, Shin HR, Hong ST (2007) Collection of *Clonorchis sinensis* adult worms from infected humans after praziquantel treatment. Korean J Parasitol 45: 149–152

Shimalov VV, Shimalov VT (2003) Helminth fauna of the red fox (*Vulpes vulpes* Linnaeus, 1758) in southern Belarus. Parasitol Res 89:77–78

Shin HR, Lee CU, Park HJ, Seol SY, Chung JM, Choi HC, Ahn YO, Shigemastu T (1996) Hepatitis B and C virus, *Clonorchis sinensis* for the risk of liver cancer: a case-control study in Pusan, Korea. Int J Epidemiol 25:933–940

Shin HR, Oh JK, Lim MK, Shin A, Kong HJ, Jung KW, Won YJ, Park S, Park SJ, Hong ST (2010) Descriptive epidemiology of cholangiocarcinoma and clonorchiasis in Korea. J Korean Med Sci 25:1011–1016

Sirisinha S, Sahassananda D, Bunnag D, Rim HJ (1990) Immunological analysis of *Opisthorchis* and *Clonorchis* antigens. J Helminthol 64:133–138

Sirisinha S, Chawengkirttikul R, Sermswan R, Amornpant S, Mongkolsuk S, Panyim S (1991) Detection of *Opisthorchis viverrini* by monoclonal antibody-based ELISA and DNA hybridization. Am J Trop Med Hyg 44:140–145

Sirisinha S, Chawengkirttikul R, Haswell-Elkins MR, Elkins DB, Kaewkes S, Sithithaworn P (1995) Evaluation of a monoclonal antibody-based enzyme linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. Am J Trop Med Hyg 52:521–524

Sithithaworn P, Haswell-Elkins M (2003) Epidemiology of *Opisthorchis viverrini*. Acta Trop 88:187–194

Sithithaworn P, Tesana S, Pipitgool V, Kaewkes S, Pairojkul C, Sripa B, Paupairoj A, Thaiklar K (1991) Relationship between faecal egg count and worm burden of *Opisthorchis viverrini* in human autopsy cases. Parasitology 102(Pt 2):277–281

Sithithaworn P, Pipitgool V, Srisawangwong T, Elkins DB, Haswell-Elkins MR (1997) Seasonal variation of *Opisthorchis viverrini* infection in cyprinoid fish in north-east Thailand: implications for parasite control and food safety. Bull World Health Organ 75:125–131

Sithithaworn P, Sukavat K, Vannachone B, Sophonphong K, Ben-Embarek P, Petney T, Andrews R (2006) Epidemiology of food-borne trematodes and other parasite infections in a fishing community on the Nam Ngum reservoir, Lao PDR. Southeast Asian J Trop Med Publ Health 37:1083–1090

Sithithaworn P, Nuchjungreed C, Srisawangwong T, Ando K, Petney TN, Chilton NB, Andrews RH (2007a) Genetic variation in *Opisthorchis viverrini* (Trematoda: Opisthorchiidae) from northeast Thailand and Laos PDR based on random amplified polymorphic DNA analyses. Parasitol Res 100:613–617

Sithithaworn P, Yongvanit P, Tesana S, Pairojkul C (2007b) Liver flukes. In: Murrell KD, Bernard F (eds) Foodborne parasitic zoonoses. Springer, Berlin, pp 3–52

Sithithaworn P, Andrews RH, Nguyen VD, Wongsaroj T, Sinuon M, Odermatt P, Nawa Y, Liang S, Brindley PJ, Sripa B (2012a) The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. Parasitol Int 61:10–16

Sithithaworn P, Andrews RH, Petney TN, Saijuntha W, Laoprom N (2012b) The systematics and population genetics of *Opisthorchis viverrini* sensu lato: implications in parasite epidemiology and bile duct cancer. Parasitol Int 61:32–37

Sithithaworn P, Yongvanit P, Duenngai K, Kiatsopit N, Pairojkul C (2014) Roles of liver fluke infection as risk factor for cholangiocarcinoma. J Hepatobiliary Pancreat Sci 21:301–308

Smout MJ, Laha T, Mulvenna J, Sripa B, Suttiprapa S, Jones A, Brindley PJ, Loukas A (2009) A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, promotes proliferation of host cells. PLoS Pathog 5:e1000611

Sohn WM, Yong TS, Eom KS, Pyo KH, Lee MY, Lim H, Choe S, Jeong HG, Sinuon M, Socheat D, Chai JY (2012) Prevalence of *Opisthorchis viverrini* infection in humans and fish in Kratie Province, Cambodia. Acta Trop 124:215–220

Song L, Xu Z, Yu X (2007) Molecular cloning and characterization of a phosphoglycerate mutase gene from *Clonorchis sinensis*. Parasitol Res 101:709–714

Sornmani S, Schelp FP, Vivatanasesth P, Patihatakorn W, Impand P, Sitabutra P, Worasan P, Preuksaraj S (1984) A pilot project for controlling *O. viverrini* infection in Nong Wai, Northeast Thailand, by applying praziquantel and other measures. Arzneimittelforschung 34:1231–1234

Sri-Aroon P, Butraporn P, Limsomboon J, Kerdpuech Y, Kaewpoolsri M, Kiatsiri S (2005) Freshwater mollusks of medical importance in Kalasin Province, northeast Thailand. Southeast Asian J Trop Med Publ Health 36:653–657

Sri-Aroon P, Intapan PM, Lohachit C, Phongsasakulchoti P, Thanchomnang T, Lulitanond V, Hiscox A, Phompida S, Sananikhom P, Maleewong W, Brey PT (2011) Molecular evidence of *Opisthorchis viverrini* in infected bithyniid snails in the Lao People's Democratic Republic by specific hybridization probe-based real-time fluorescence resonance energy transfer PCR method. Parasitol Res 108:973–978

Sripa B (2003) Pathobiology of opisthorchiasis: an update. Acta Trop 88:209–220

Sripa B, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, Pairojkul C, Bhudhisawasdi V, Tesana S, Thinkamrop B, Bethony JM, Loukas A, Brindley PJ (2007) Liver fluke induces cholangiocarcinoma. PLoS Med 4:e201

Sripa B, Mairiang E, Thinkhamrop B, Laha T, Kaewkes S, Sithithaworn P, Tessana S, Loukas A, Brindley PJ, Bethony JM (2009) Advanced periductal fibrosis from infection with the carcinogenic human liver fluke *Opisthorchis viverrini* correlates with elevated levels of interleukin-6. Hepatology 50:1273–1281

Sripa B, Brindley PJ, Mulvenna J, Laha T, Smout MJ, Mairiang E, Bethony JM, Loukas A (2012) The tumorigenic liver fluke *Opisthorchis viverrini*-multiple pathways to cancer. Trends Parasitol 28:395–407

Stensvold CR, Saijuntha W, Sithithaworn P, Wongratanacheewin S, Strandgaard H, Ornbjerg N, Johansen MV (2006) Evaluation of PCR based coprodiagnosis of human opisthorchiasis. Acta Trop 97:26–30

Sukontason K, Piangjai S, Muangyimpong Y, Sukontason K, Methanitikorn R, Chaithong U (1999) Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao district, Chiang Mai Province, northern Thailand. Southeast Asian J Trop Med Publ Health 30:365–370

Sun J, Xu J, Liang P, Mao Q, Huang Y, Lv X, Deng C, Liang C, de Hoog GS, Yu X (2011) Molecular identification of *Clonorchis sinensis* and discrimination with other opisthorchid liver fluke species using multiple Ligation-depended Probe Amplification (MLPA). Parasit Vectors 4:98

Suttiprapa S, Sotillo J, Smout M, Suyapoh W, Chaiyadet S, Tripathi T, Laha T, Loukas A (2018) *Opisthorchis viverrini* proteome and host-parasite interactions. Adv Parasitol 102:45–72

Suwannahitatorn P, Klomjit S, Naaglor T, Taamasri P, Rangsin R, Leelayoova S, Mungthin M (2013) A follow-up study of *Opisthorchis viverrini* infection after the implementation of control program in a rural community, central Thailand. Parasit Vectors 6:188

Suwannatrai A, Suwannatrai K, Haruay S, Piratae S, Thammasiri C, Khampoosa P, Kulsantiwong J, Prasopdee S, Tarbsripair P, Suwanwerakamtorn R, Sukchan S, Boonmars T, Malone JB, Kearney MT, Tesana S (2011) Effect of soil surface salt on the density and distribution of the snail *Bithynia siamensis goniomphalos* in northeast Thailand. Geospat Health 5:183–190

Suwannatrai A, Pratumchart K, Suwannatrai K, Thinkhamrop K, Chaiyos J, Kim CS, Suwanweerakamtorn R, Boonmars T, Wongsaroj T, Sripa B (2017) Modeling impacts of climate change on the potential distribution of the carcinogenic liver fluke, *Opisthorchis viverrini*, in Thailand. Parasitol Res 116:243–250

Tang Y, Cho PY, Kim BS, Hong SJ (2005) Molecular cloning and characterization of vitelline precursor protein B1 from *Clonorchis sinensis*. J Parasitol 91:1374–1378

Tatonova YV, Chelomina GN, Besprosvannykh VV (2012) Genetic diversity of nuclear ITS1-5.8S-ITS2 rDNA sequence in *Clonorchis sinensis* Cobbold, 1875 (Trematoda: Opisthorchidae) from the Russian Far East. Parasitol Int 61:664–674

Tatonova YV, Chelomina GN, Besprozvannykh VV (2013) Genetic diversity of *Clonorchis sinensis* (Trematoda: Opisthorchiidae) in the Russian southern Far East based on mtDNA cox1 sequence variation. Folia Parasitol 60:155–162

Tesana S, Srisawangwonk T, Kaewkes S, Sithithaworn P, Kanla P, Arunyanart C (1991) Eggshell morphology of the small eggs of human trematodes in Thailand. Southeast Asian J Trop Med Publ Health 22:631–636

Tesana S, Thapsripair P, Thammasiri C, Prasopdee S, Suwannatrai A, Harauy S, Piratae S, Khampoosa P, Kulsantiwong J, Donthaisong C, Chalokepanrat P, Viyanant V, Malone JB (2012) Effects of Bayluscide on *Bithynia siamensis goniomphalos*, the first intermediate host of the human liver fluke, *Opisthorchis viverrini*, in laboratory and field trials. Parasitol Int 61:52–55

Thaenkham U, Nuamtanong S, Vonghachack Y, Yoonuan T, Sanguankiat S, Dekumyoy P, Prommasack B, Kobayashi J, Waikagul J (2011) Discovery of *Opisthorchis lobatus* (Trematoda: Opisthorchiidae): a new record of small liver flukes in the Greater Mekong Sub-region. J Parasitol 97:1152–1158

Thaewnongiew K, Singthong S, Kutchamart S, Tangsawad S, Promthet S, Sailugkum S, Wongba N (2014) Prevalence and risk factors for *Opisthorchis viverrini* infections in upper Northeast Thailand. Asian Pac J Cancer Prev 15:6609–6612

Toledo R, Bernal MD, Marcilla A (2011) Proteomics of foodborne trematodes. J Proteomics 74:1485–1503

Touch S, Komalamisra C, Radomyos P, Waikagul J (2009) Discovery of *Opisthorchis viverrini* metacercariae in freshwater fish in southern Cambodia. Acta Trop 111:108–113

Touch S, Yoonuan T, Nuamtanong S, Homsuwan N, Phuphisut O, Thaenkham U, Waikagul J (2013) Seasonal variation of *Opisthorchis viverrini* metacercarial infection in cyprinid fish from Southern Cambodia. J Trop Med Parasitol 36:1–7

Traub RJ, Macaranas J, Mungthin M, Leelayoova S, Cribb T, Murrell KD, Thompson RC (2009) A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to Central Thailand. PLoS Negl Trop Dis 3:e367

Traverso A, Repetto E, Magnani S, Meloni T, Natrella M, Marchisio P, Giacomazzi C, Bernardi P, Gatti S, Gomez Morales MA, Pozio E (2012) A large outbreak of *Opisthorchis felineus* in Italy suggests that opisthorchiasis develops as a febrile eosinophilic syndrome with cholestasis rather than a hepatitis-like syndrome. Eur J Clin Microbiol Infect Dis 31:1089–1093

Tyson GL, El-Serag HB (2011) Risk factors for cholangiocarcinoma. Hepatology 54:173–184

Upatham ES, Sukhapanth N (1980) Field studies on the bionomics of *Bithynia siamensis siamensis* and the transmission of *Opisthorchis viverrini* in Bangna, Bangkok, Thailand. Southeast Asian J Trop Med Publ Health 11:355–358

Upatham ES, Viyanant V, Kurathong S, Brockelman WY, Menaruchi A, Saowakontha S, Intarakhao C, Vajrasthira S, Warren KS (1982) Morbidity in relation to intensity of infection in *Opisthorchiasis viverrini*: study of a community in Khon Kaen, Thailand. Am J Trop Med Hyg 31:1156–1163

Upatham ES, Viyanant V, Kurathong S, Rojborwonwitaya J, Brockelman WY, Ardsungnoen S, Lee P, Vajrasthira S (1984) Relationship between prevalence and intensity of *Opisthorchis viverrini* infection, and clinical symptoms and signs in a rural community in northeast Thailand. Bull World Health Organ 62:451–461

Upatham ES, Brockelman WY, Viyanant V, Lee P, Kaengraeng R, Prayoonwiwat B (1985) Incidence of endemic *Opisthorchis viverrini* infection in a village in northeast Thailand. Am J Trop Med Hyg 34:903–906

Upatham ES, Viyanant V, Brockelman WY, Kurathong S, Lee P, Kraengraeng R (1988) Rate of re-infection by *Opisthorchis viverrini* in an endemic northeast Thai community after chemotherapy. Int J Parasitol 18:643–649

Van Hellemond JJ, Retra K, Brouwers JF, van Balkom BW, Yazdanbakhsh M, Shoemaker CB, Tielens AG (2006) Functions of the tegument of schistosomes: clues from the proteome and lipidome. Int J Parasitol 36:691–699

Van CD, Doungchawee G, Suttiprapa S, Arimatsu Y, Kaewkes S, Sripa B (2017) Association between *Opisthorchis viverrini* and *Leptospira* spp. infection in endemic Northeast Thailand. Parasitol Int 66:503–509

Vichasri S, Viyanant V, Upatham ES (1982) *Opisthorchis viverrini*: intensity and rates of infection in cyprinoid fish from an endemic focus in Northeast Thailand. Southeast Asian J Trop Med Publ Health 13:138–141

Vinogradoff K (1892) On a new species of distomum (*Distomum sibericum*) in the human liver (in Russian). Zentralblatt für allgemeine Pathologie u pathologische Anatomie 3:910–911

Viyanant V, Brockelman WY, Lee P, Ardsungnoen S, Upatham ES (1983) A comparison of a modified quick-Kato technique and the Stoll dilution method for field examination for *Opisthorchis viverrini* eggs. J Helminthol 57:191–195

Waikagul J (1998) *Opisthorchis viverrini* metacercaria in Thai freshwater fish. Southeast Asian J Trop Med Publ Health 29:324–326

Waikagul J, Dekumyoy P, Chaichana K, Thairungroje Anantapruti M, Komalamisra C, Kitikoon V (2002) Serodiagnosis of human opisthorchiasis using cocktail and electroeluted *Bithynia* snail antigens. Parasitol Int 51:237–247

Wang X, Chen W, Huang Y, Sun J, Men J, Liu H, Luo F, Guo L, Lv X, Deng C, Zhou C, Fan Y, Li X, Huang L, Hu Y, Liang C, Hu X, Xu J, Yu X (2011) The draft genome of the carcinogenic human liver fluke *Clonorchis sinensis*. Genome Biol 12:R107

Wang YC, Feng CC, Sithithaworn P (2013) Environmental determinants of *Opisthorchis viverrini* prevalence in northeast Thailand. Geospat Health 8:111–123

Wang D, Young ND, Korhonen PK, Gasser RB (2018) *Clonorchis sinensis* and Clonorchiasis: the relevance of exploring genetic variation. Adv Parasitol 100:155–208

Watthanakulpanich D, Waikagul J, Dekumyoy P, Anantaphruti MT (2003) Studies on concomitant antigens of *Bithynia funiculata* for detection of antibody to *Opisthorchis viverrini*: effect of different centrifugal speeds on antigen preparation. Southeast Asian J Trop Med Publ Health 34(Suppl 2):114–120

Watwiengkam N, Sithithaworn J, Duenngai K, Sripa B, Laha T, Johansen MV, Sithithaworn P (2013) Improved performance and quantitative detection of copro-antigens by a monoclonal antibody based ELISA to diagnose human opisthorchiasis. Acta Trop 128:659–665

Wegner DH (1984) The profile of the trematodicidal compound praziquantel. Arzneimittelforschung 34: 1132–1136

WHO (1995) Control of foodborne trematode infections. World Health Organization, Geneva

Wongratanacheewin S, Pumidonming W, Sermswan RW, Maleewong W (2001) Development of a PCR-based method for the detection of *Opisthorchis viverrini* in experimentally infected hamsters. Parasitology 122:175–180

Wongratanacheewin S, Pumidonming W, Sermswan RW, Pipitgool V, Maleewong W (2002) Detection of *Opisthorchis viverrini* in human stool specimens by PCR. J Clin Microbiol 40:3879–3880

Wongratanacheewin S, Sermswan RW, Sirisinha S (2003) Immunology and molecular biology of *Opisthorchis viverrini* infection. Acta Trop 88:195–207

Wongsaroj T, Sakolvaree Y, Chaicumpa W, Maleewong W, Kitikoon V, Tapchaisri P, Chongsa-nguan M, Cross JH (2001) Affinity purified oval antigen for diagnosis of *Opisthorchiasis viverrini*. Asian Pac J Allergy Immunol 19:245–258

Wongsawad C, Wongsawad P, Anuntalabhochai S, Chai JY, Sukontason K (2013) Occurrence and molecular identification of liver and minute intestinal flukes metacercariae in freshwater fish from Fang-Mae Ai Agricultural Basin, Chiang Mai province, Thailand. Asian Biomed 7:97–104

Worasith C, Kamamia C, Yakovleva A, Duenngai K, Wangboon C, Sithithaworn J, Watwiengkam N, Namwat N, Techasen A, Loilome W, Yongvanit P, Loukas A, Sithithaworn P, Bethony JM (2015) Advances in the diagnosis of human opisthorchiasis: development of *Opisthorchis viverrini* antigen detection in urine. PLoS Negl Trop Dis 9:e0004157

Wykoff DE, Harinasuta C, Juttijudata P, Winn MM (1965) *Opisthorchis viverrini* in Thailand-the life cycle and comparison with *O. felineus*. J Parasitol 51:207–214

Xiao JY, Gao JF, Cai LS, Dai Y, Yang CJ, Luo L, Agatsuma T, Wang CR (2013) Genetic variation among *Clonorchis sinensis* isolates from different hosts and geographical locations revealed by sequence analysis of mitochondrial and ribosomal DNA regions. Mitochondrial DNA 24:559–564

Xu M, Jiang Z, Huang W, Yin J, Ou S, Jiang Y, Meng L, Cao S, Yu A, Cao J, Shen Y (2018) Altered gut microbiota composition in subjects infected with *Clonorchis sinensis*. Front Microbiol 9:2292

Yang G, Jing C, Zhu P, Hu X, Xu J, Wu Z, Yu X (2006) Molecular cloning and characterization of a novel lactate dehydrogenase gene from *Clonorchis sinensis*. Parasitol Res 99:55–64

Yongvanit P, Pinlaor S, Bartsch H (2012) Oxidative and nitrative DNA damage: key events in opisthorchiasis-induced carcinogenesis. Parasitol Int 61:130–135

Yossepowitch O, Gotesman T, Assous M, Marva E, Zimlichman R, Dan M (2004) Opisthorchiasis from imported raw fish. Emerg Infect Dis 10:2122–2126

Young ND, Campbell BE, Hall RS, Jex AR, Cantacessi C, Laha T, Sohn WM, Sripa B, Loukas A, Brindley PJ, Gasser RB (2010) Unlocking the transcriptomes of two carcinogenic parasites, *Clonorchis sinensis* and *Opisthorchis viverrini*. PLoS Negl Trop Dis 4:e719

Zadesenets KS, Karamysheva TV, Katokhin AV, Mordvinov VA, Rubtsov NB (2012a) Distribution of repetitive DNA sequences in chromosomes of five opisthorchid species (Trematoda, Opisthorchiidae). Parasitol Int 61:84–86

Zadesenets KS, Katokhin AV, Mordvinov VA, Rubtsov NB (2012b) Comparative cytogenetics of opisthorchid species (Trematoda, Opisthorchiidae). Parasitol Int 61:87–89

Zadesenets KS, Katokhin AV, Mordvinov VA, Rubtsov NB (2012c) Telomeric DNA in chromosomes of five opisthorchid species. Parasitol Int 61:81–83

Zavoikin VD, Zelia OP, Bronshtein AM, Sokerina OA, Iarotskii LS, Firsova RA, Mikhailov MM, Gerasimov IV (1994) The procedure for the wide use of praziquantel in a complex of measures to control opisthorchiasis. 1. The tolerance and efficacy of different doses of biltricide during outpatient use in foci. Med Parazitol 24–27

Zhang HM, Jiang H, Xu B, Tan CP (2007) Preliminary survey on the infection status of freshwater fish with metacercariae of *Metorchis orientalis* in Guangxi province. In: The 1st International symposium on geospatial health, Lijiang, Yunnan, China, pp 39–41

Zheng N, Xu J, Wu Z, Chen J, Hu X, Song L, Yang G, Ji C, Chen S, Gu S, Ying K, Yu X (2005) *Clonorchis sinensis*: molecular cloning and functional expression of novel cytosolic malate dehydrogenase. Exp Parasitol 109:220–227

Zheng N, Huang B, Xu J, Huang S, Chen J, Hu X, Ying K, Yu X (2006) Enzymatic and physico-chemical characteristics of recombinant cMDH and mMDH of *Clonorchis sinensis*. Parasitol Res 99:174–180

Zheng N, Huang B, Xu J, Huang S, Chen J, Hu X, Ji C, Ying K, Yu X (2008) Cloning and expression of mitochondrial malate dehydrogenase of *Clonorchis sinensis*. Parasitol Res 102:989–995

Zheng M, Hu K, Liu W, Hu X, Hu F, Huang L, Wang P, Hu Y, Huang Y, Li W, Liang C, Yin X, He Q, Yu X (2011) Proteomic analysis of excretory secretory products from *Clonorchis sinensis* adult worms: molecular characterization and serological reactivity of a excretory-secretory antigen-fructose-1,6-bisphosphatase. Parasitol Res 109:737–744

Zhigileva ON, Zenovkina DV, Zamyatina TA (2013) Genetic variability and population differentiation of *Opisthorchis felineus* (Trematoda) in Western Siberian rivers. Russ J Genet Appl Res 3:371–377

Zhou Z, Hu X, Huang Y, Hu H, Ma C, Chen X, Hu F, Xu J, Lu F, Wu Z, Yu X (2007) Molecular cloning and identification of a novel *Clonorchis sinensis* gene encoding a tegumental protein. Parasitol Res 101:737–742

Zhou Z, Xia H, Hu X, Huang Y, Li Y, Li L, Ma C, Chen X, Hu F, Xu J, Lu F, Wu Z, Yu X (2008) Oral administration of a *Bacillus subtilis* spore-based vaccine expressing *Clonorchis sinensis* tegumental protein 22.3 kDa confers protection against *Clonorchis sinensis*. Vaccine 26:1817–1825

Ziegler AD, Andrews RH, Grundy-Warr C, Sithithaworn P, Petney TN (2011) Fighting liverflukes with food safety education. Science 331:282–283

Intestinal Trematode Infections

7

Rafael Toledo, Maria Alvárez-Izquierdo,
Carla Muñoz-Antoli, and J. Guillermo Esteban

7.1 Introduction

It is estimated that more than one billion people are at risk of infection with food-borne trematodes and about 56 million people were infected in 2005 (Fürst et al. 2012a). According to the target organ in the definitive host, those trematodes are classified as liver, lung, and intestinal flukes. Intestinal trematodes are the largest group and about seven million people are infected worldwide (Fürst et al. 2012a). About 76 of species belonging to 14 families have been recorded infecting humans. Infection commonly occurs when humans eat raw or undercooked foods that contain the infective metacercariae. A variety of ailments are involved in the transmission of intestinal flukes (Table 7.1) and the eating habits are essential to determine the distribution of these parasitic diseases. High incidence of intestinal trematodiasis is strongly associated with populations living near freshwater bodies and the practice of eating raw or undercooked aquatic products. Thus, intestinal trematode infections are commonly considered as tropical diseases with severe endemic foci in Asia, where it is estimated that more than six million people are infected (Fürst et al. 2012a). However, the geographical limits and the population at risk are currently expanding and changing in relation to factors such as growing international markets, improved transportation systems, changes in eating habits in Western countries and demographic changes.

Despite the considerable public health impact and the emerging nature of intestinal trematodiases, these diseases are among the most neglected of the so-called neglected tropical diseases, and they are found predominantly in the world's poorest populations in low-income countries and, where these diseases are common, they exacerbate poverty. This makes necessary additional efforts to gain a better knowledge of these diseases to facilitate their control. In this chapter, we describe the biology, medical and epidemiological features, and current treatment and diagnostic tools of the main groups of intestinal flukes and the corresponding diseases.

R. Toledo (✉) · M. Alvárez-Izquierdo
C. Muñoz-Antoli · J. G. Esteban
Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain
e-mail: Rafael.Toledo@uv.es; alizma@alumni.uv.es; Carla.Munoz@uv.es; Jguillermo.Esteban@uv.es

7.2 Family Brachylaimidae

7.2.1 Background

The family Brachylaimidae contains numerous species of terrestrial trematodes that infect mammals, birds, and reptiles (Gibson and Bray 1994).

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_7

Table 7.1 Families of trematodes involved in intestinal human infections with data on the number of species involved, sources of infection, and geographical distribution

Family	Number of species cited in humans	Source of infection	Geographical distribution of human cases[a]
Brachylaimidae	1	Terrestrial snails	Oceania
Cathaemaciidae	1	Not known	Asia
Diplostomidae	2[b]	Snakes, frogs, tadpoles	Asia
Echinostomatidae	20	Freshwater fish, frogs, mussels, snails, tadpoles	Africa, Asia, Europe
Fasciolidae	1	Aquatic vegetables, contaminated water	Asia
Gastrodiscidae	1	Aquatic vegetables, crustaceans, molluscs, amphibians	Africa, Asia, Europe
Gymnophallidae	1	Oysters	Asia
Heterophyidae	26	Freshwater fishes	Africa, America, Asia, Europe
Lecithodendriidae	3	Dragonflies	Asia
Microphallidae	2	Shrimps, crabs	Asia
Nanophyetidae	1	Salmonid fishes	America, Europe
Paramphistomidae	2	Aquatic plants	Africa, Asia
Plagiorchiidae	4	Insect larvae	Asia
Strigeidae	1	Not known	Asia

[a]Imported cases are not considered
[b]One of them (*Fibricola cratera*) only in experimental infections

Brachylaima is the most representative genus within this family. This is, however, a very problematic genus that includes many poorly known species, for which the description of the adult stage is the only information available. This problem is compounded by the morphological similarity of many of the adult worms. Species of *Brachylaima* follow a three-host terrestrial life cycle (Yamaguti 1975). The first and second intermediate hosts of brachylaimids are either the same or two different species of terrestrial snail species. The definitive host can be either a mammal or a bird.

7.2.2 Species Reported in Humans and Geographical Distribution

Humans only have been reported in Australia as an incidental definitive host (three cases) for one species in the family, *Brachylaima cribbi* (Fig. 7.1). The first human infections with this trematode were described in two young children (21 months old) in rural South Australia in whom brachylaimid eggs were seen repeatedly in stools, but no adult worms were recovered. Both infants had been seen eating raw snails (Butcher et al. 1996). Thereafter, a 78-year-old woman presented with an 18-month history of intermittent diarrhea was found to be infected with a brachylaimid infection. Examination of her stools revealed the presence of brachylaimid eggs. She lived in a rural area of South Australia and ate raw vegetables which had been contaminated with helicid snails. After treatment with praziquantel a degenerate adult *Brachylaima* species was recovered in her stools (Butcher et al. 1998). The parasite was identified as *B. cribbi* (Butcher and Grove 2001).

Infections in humans usually become chronic and can persist as long as 18 months (Butcher et al. 1996, 1998). Clinical symptoms depend on the parasite load and heavy infections are associated with diarrhea with offensive stools several times a day, abdominal pain, low-grade fever, and fatigue (Butcher et al. 1996, 1998). There are no further studies on the pathology of infections with brachylaimids.

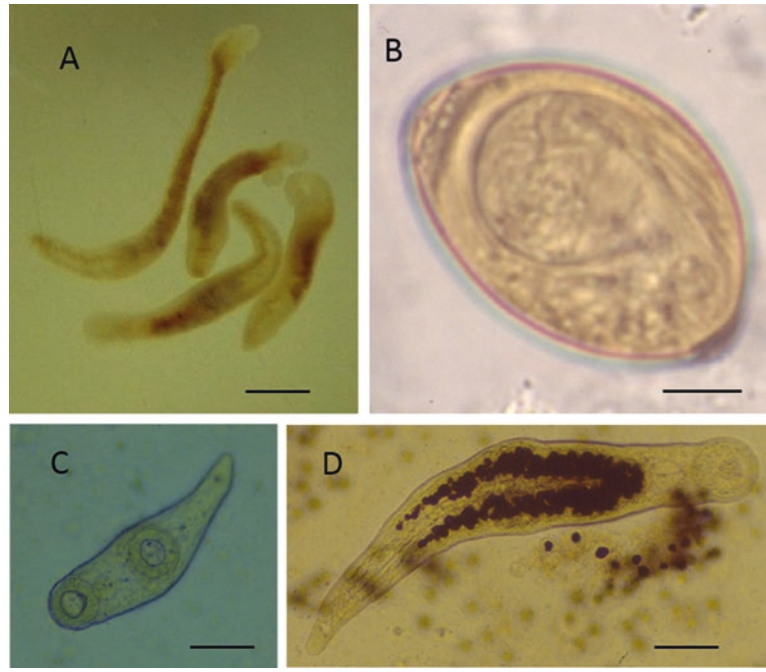

Fig. 7.1 Unstained specimens of *Brachylaima cribbi*: (**a**) adults (Scale bar: 1 mm); (**b**) fertile egg (Scale bar: 5 µm); (**c**) cercaria (Scale bar: 25 µm); and (**d**) metacercaria (Scale bar: 500 µm). Photomicrographs courtesy of Andrew R. Butcher

7.2.3 Host–Parasite Relationships and Immunology

The complexity of the life cycles of *Brachylaima* spp. may explain the scarcity of the data on host–parasite relationships in infections with brachylaimids. However, the use of different strains of mice as experimental hosts has allowed for studies on several aspects of the development of these flukes and the immunology of the infections. These studies have been focused on *B. cribbi*. However, there was variability in susceptibility in relation to gender and maturity of mice (Butcher et al. 2002). Mature female CB57BL/6J mice were significantly more resistant to *B. cribbi* infection that older mature females and adolescent females. These differences in susceptibility were attributed to physiological factors. Butcher et al. (2002) suggested that sex hormones provided a significant level of protection to *B. cribbi*.

B. cribbi evokes significant antibody responses as determined by indirect ELISA. Butcher et al. (2003) showed that humoral and/or cellular mediated immunity are important in mediating resistance and influencing the fertility of adult worms. The course of the infection in immunocompetent CB57BL/6J and immunodeficient NOD SCID mice re-infected with *B. cribbi* was assessed. In the case of CB57BL/6J mice, there were significant differences in the mean fecal eggs per gram of feces and worm fecundity, having lower egg counts and reduced fecundity with the challenge infections. In contrast, no significant differences were observed in NOD SCID mice between primary and challenge infections. The immunology of the infection in humans has not been studied.

7.3 Family Diplostomidae

7.3.1 Background

The family Diplostomidae Poirier, 1886 comprises digenean parasites of numerous orders of birds and mammals. Recently, Niewiadomska (2002) accepted a total of 41 genera within this

family grouped into subfamilies according to host specificity. A total of the 11 genera reported in mammals were included in the subfamily Alariinae Hall and Wigdor, 1960.

In general, species of the Diplostomidae have a three-host life cycle. Fork-tailed cercariae are produced in sporocysts in the gastropod first intermediate host. The cercariae emerge from the snails and penetrate and form metacercariae in fishes, amphibians, molluscs, and annelids (Hong et al. 1982). Definitive hosts become infected by the ingestion of the second intermediate host harboring metacercariae. Eggs typically hatch and penetrate the first intermediate host (Cribb et al. 2003). In some Diplostomidae, the life cycle is expanded to incorporate four hosts by inclusion of an unencysted larval stage known as mesocercariae (a form between the cercariae and the metacercariae). In this case, the definitive host becomes infected after ingestion of the second intermediate host or the paratenic host harbouring mesocercariae.

7.3.2 Species Reported in Humans and Geographical Distribution

At least members of three genera of Diplostomidae (*Alaria, Neodiplostomum,* and *Fibricola*) are known to parasitize man. However, in the case of *Alaria* spp., humans serve as a paratenic host harboring metacercariae in different tissues. Humans become infected after eating tainted frog meat (Fried et al. 2004). At the intestinal level, only *Fibricola cratera* and *Neodiplostomum seoulense* serve as parasites of humans.

Human infections with *F. cratera* can be considered as anecdotal since the only one report is an experimental infection of a human volunteer (Shoop 1989). This species is a parasite of wild mammals in North America. Frogs are the second intermediate host, and snakes act as paratenic hosts (Shoop 1989). A total of 100 metacercariae were inoculated in a human volunteer producing a patent infection that lasted 40 months. Symptoms of epigastric discomfort, loose stools, and flatulence occurred over the first year of infection but ameliorated thereafter (Shoop 1989). No further studies have been conducted in relation to the human studies with this species.

N. seoulense, formerly named *F. seoulensis* (Chai and Lee 1991), is a relatively common parasite of humans and animals in Korea (Chai and Lee 2002). Morphologically, it is characterized by a bisegmented body, a tribocytic organ, butterfly-shaped testes and a wide distribution of the vitellaria in the anterior body to the level of the ventral sucker (Fig. 7.2a) (Seo et al. 1964, 1988; Chai et al. 2009a). The freshwater snails *Hippeutis (Helicorbis) cantori, Segmentina (Polipylis) hemiaespherula,* and *Austropeplea ollula* serve as the first intermediate host (Seo

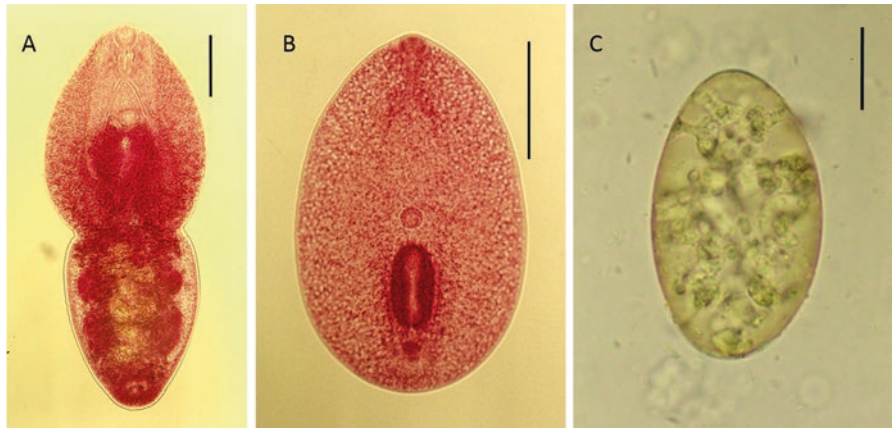

Fig. 7.2 *Neodisplostomum seoulense*: (**a**) adult specimen (Scale bar: 250 μm); (**b**) mesocercaria collected from a snake (Scale bar: 250 μm); and (**c**) egg (Scale bar: 25 μm). Photomicrographs courtesy of Woon-Mok Sohn

et al. 1988; Chung et al. 1996, 2002). Second intermediate hosts are tadpoles and frogs harboring metacercariae, and snakes may act as a paratenic host harboring the mesocercariae (Fig. 7.2b) (Hong et al. 1982; Seo et al. 1988). The site of infection in the definitive host is the duodenum but parasites may extend to the jejunum and ileum in heavy infections (Hong et al. 1983) (Fig. 7.2).

Human infections with *N. seoulense* only have been recorded in Korea (Chai et al. 2009a). This species was first implicated when a 25-year-old male suddenly suffered severe gastrointestinal symptoms (Seo et al. 1982). Another 26 cases were also reported among Korean soldiers, probably infected during their survival training. In all the cases, the infections were related with a history of consuming improperly cooked snakes and/or frogs (Seo et al. 1982; Hong et al. 1984a, b). Chai and Lee (2002) estimated the total number of human cases as 1000 in the Republic of Korea.

7.3.3 Clinical Manifestations and Pathology

The symptomatology of *N. seoulense* infections in humans has been scarcely studied and most of the current knowledge proceeds from studies on experimentally infected rodents (Toledo et al. 2006a).

The clinical manifestation and the pathology induced by *N. seoulense* is markedly related with the worm burden. It has been shown that the severity of the clinical symptoms and the mortality in experimentally infected mice is proportional to the cyst inoculum (Huh et al. 1988; Kook et al. 1998). In human infections, the symptomatology also has been shown to be dependent on the worm burden. Severe clinical manifestations only were reported in the first patient (Seo et al. 1982). This patient rapidly developed epigastric discomfort, fullness, and pain and anorexia. Thereafter, diarrhea, fever, and tenderness also appeared. After treatment with bithionol and magnesium purgation, a total of 79 adult worms were collected (Seo et al. 1982). In contrast, the remaining human cases had no clinical symptoms. The absence of clinical signs was attributed to the chronic and repeated infection with small amounts of metacercariae and a relatively low numbers of adults in the intestine (Hong et al. 1984a).

The parasite may cause mechanical and chemical damage. Each worm embraces a villous with the forebody which cause injury in the intestinal mucosa (Lee et al. 1985). Moreover, the tribocytic organ may pierce the host villi and secretes alkaline phosphatase which can lyse the villi (Huh et al. 1990; Huh and Hong 1993). The changes induced by *N. seoulense* were studied by Lee et al. (1985) in mice and rats. Shortening, widening, and fusion of the villi were observed. There was also a reduction in the number of goblet cells in the areas surrounding the worms, capillary congestion, lymphatic dilation, and inflammatory cell infiltration including lymphocytes, plasma cells, eosinophils, and occasional giant cells. In heavy infections, the changes were extended to the jejunum and gross bleeding was also observed (Lee et al. 1985). Pyo et al. (2012) showed that infection with *N. seoulense* downregulates the expression of the neuronal growth associated protein (GAP)-43 in mice suggesting that neuronal damage is induced by the parasite. Recently, Shin et al. (2016) demonstrated that *N. seoulense* reduced the fecundity in mice due to destruction of gonad tissues in a process that was infection intensity-dependent and progressive.

7.3.4 Host–Parasite Relationships and Immunology

There are some evidences supporting the existence of mechanisms of host protection against *N. seoulense*. For example, a significant reduction in worm recovery has been demonstrated in secondary infections in rats (Yu et al. 1995). In mice, it has been shown that worm survival is markedly dependent upon the genetic background of the host. The worm survival was markedly higher in BALB/c mice than in the C3H strain (Chai et al. 1998). However, little is known about the effector mechanisms of the host immune response in *N. seoulense* infections.

Several studies have shown that histamine and macrophages may have an important role on worm rejection. Histamine released by mast cells could facilitate worm expulsion by the increasing of intestinal motility and macrophages have been shown to kill worms in vitro (Shin et al. 2003, 2007). *N. seoulense* induces a mixed Th1/Th2 phenotype with overexpression of IFN-γ and IL-4 in mice (Shin et al. 2007). Antibody responses in mice are characterized by elevated levels of IgG, IgG2a, and IgA (Shin et al. 2007; Han et al. 2008). Major worm antigens appear to be located in the trybocytic organ, seminal vesicle, caeca, and vitelline follicles (Lee et al. 1997a). Han et al. (2008) detected by immunoblotting that the major antigens in the crude extract of adult worms had a molecular weight ranging from 26 to 94 kDa. Kim et al. (2008) identified two cystatin-binding cysteine proteinases, weighing 50 and 60 kDa, were recognized by the sera of humans infected with *N. seoulense*.

7.3.5 Diagnosis

Diagnosis of *N. seoulense* is usually done by detection of eggs in feces using traditional methods. The eggs are ellipsoid, thin shelled, with an inconspicuous operculum and measuring 86–99 × 55–63 μm (Seo et al. 1982) (Fig. 7.2c). They can be differentiated from other similar eggs, such as those of *Echinostoma* spp. by their clean shell surface and the absence of abopercular wrinkles at the posterior end (Chai et al. 2009a). Although immunological or DNA-based methods for diagnosis have not been developed, Kim et al. (2008) postulated that cystatin-binding cysteine proteinases could be putative antigens for serodiagnosis of *N. seoulense* infections in humans.

7.4 Family Echinostomatidae

7.4.1 Background

The family Echinostomatidae includes digeneans characterized by the presence of a prominent cephalic collar of spines (Fig. 7.3a). Adult echinostomatids are also characterized by the presence of spine-like structures, two post-ovarian testes in tandem located in the posterior part of the body and oral and ventral suckers that are close to each other.

Echinostomatids constitute a heterogeneous group of hermaphrodite trematodes that parasitize, as adult worms, numerous vertebrate hosts of all classes. The typical location is the intestine though species that parasitize other sites also exist (Toledo et al. 2009). Echinostomatids follow a three-host life cycle. The first intermediate hosts are aquatic snails in which a sporocyst, two generations of rediae and cercaria develop. Emerged cercariae freely swim and infect the second intermediate hosts, which may be several species of aquatic organisms such as snails, frogs, clams, and fishes. The definitive host, including humans, becomes infected after ingestion of the

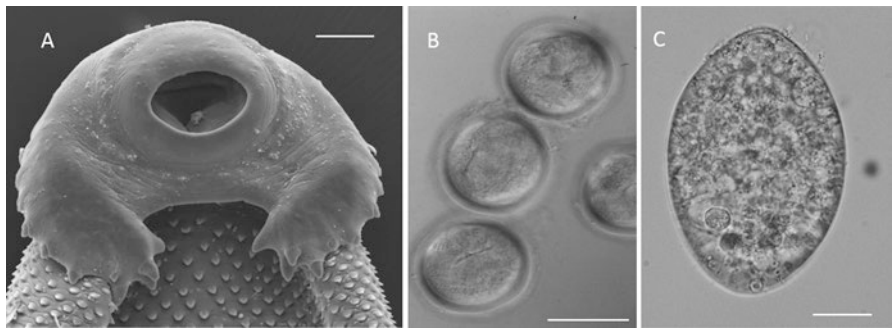

Fig. 7.3 (**a**) SEM microphotograph of the cephalic collar of spines of *Echinostoma* sp. (Scale bar: 100 μm); (**b**) metacercarial cysts of *Echinostoma* sp. (Scale bar: 100 μm); and (**c**) egg of *Echinostoma hortense* (Photomicrograph courtesy of In Sik Kim) (Scale bar: 25 μm)

second intermediate host harboring the encysted metacercariae (Fig. 7.3b). Finally, adults produce eggs that are released with the host's feces (Fig. 7.3c) (Toledo et al. 2009).

There is considerable confusion in relation to the taxonomy within the Echinostomatidae. This family has been viewed as a monophyletic taxon though the morphological similarity between its members and the diversity of the criteria adopted by the different authors for the classification have led to its division into an impressive number of taxa (Kostadinova and Gibson 2000; Fried and Toledo 2004; Kostadinova 2005; Esteban and Muñoz-Antoli 2009).

7.4.2 Species Reported in Humans and Geographical Distribution

Echinostomes commonly parasitize waterfowls and mammals associated with freshwater habitats. However, the specificity toward the definitive hosts is low determining that humans may become infected. Human echinostomiasis may occur worldwide though the distribution is usually focal though occasional cases are cited. Most of the cases are reported from East and Southeast Asia in relation to the eating habits in these areas (Graczyk and Fried 1998). Metacercariae, the infective stage, are ingested by humans in raw or undercooked freshwater fresh or brackish water molluscs, fishes, crustaceans, and amphibians (tadpoles or frogs). As a consequence, the infections are more prevalent in areas where traditional eating practices encourage the consumption of these types of foods. For example, in Philippines, human echinostome infections are related to eating raw fish or snails dipped in a salt and vinegar mixture, known as *kinilaw* and other methods of local fish preparations. In Cambodia, similar types of local food are commonly eaten which determine the prevalence observed in these areas (Belizario et al. 2007; Sohn et al. 2011a, b). Moreover, it has been suggested that drinking untreated water containing echinostome cercariae can be a source of human infection (Xiao et al. 1995).

The number and identity of the species causing human echinostomiasis is uncertain due to the absence of systematic surveys which determine that most of the available information is based on occasional case reports. Furthermore, the problematical taxonomy of the group complicates the situation since misidentifications are common. For example, *Artyfechinostomum malayanum* (Fig. 7.4a) was originally described in Malaysia as *Echinostoma malayanum*. Thereafter, *Artyfechinostomum sufrartyfex* has been considered by several authors as a synonym of *A. malayanum*. However, a recent study has suggested that the synonymy of these species is not valid (Tantrawatpan et al. 2013). Herein both species will be considered as separate taxa. Haseeb and Eveland (2000) listed a total of 21 species of echinostomes infecting humans, while Chai (2007, 2009) compiled 20 species and their identity differed with those reported by Haseeb and Eveland (2000) and Toledo and Esteban (2016) listed a total of 23 species. Table 7.2 compiled the main features of the echinostome species involved in human infections. Further details on the morphology and biology of these species can be found in the works by Chai and co-workers (Chai 2009; Chai et al. 2009a).

The highest incidence of echinostomiasis occurs in Asia, mainly in Southeast Asia (Toledo and Esteban 2016). In fact, all the species reported by Chai (2009) as causative of human echinostomiasis, with the exception of *Himasthla muehlensi*, have been reported in this area (Chai et al. 2009a).

A total of nine species have been reported infecting humans in China. Most of the cases occurred in the provinces of Fujian, Guangdong, Yunnan, Anhui, and Hubei. The mode of transmission could be the use of human excrements collected from latrines for fertilization of fish pond in these areas. Among the five members of the genus *Echinochasmus* reported in China (*E. fujianensis, E. perfoliatus, E. jiufoensis, E. liliputanus*, and *E. japonicus*), *E. fujianensis* is the most common. In several areas, the prevalence among residents reached the 3.2%, with the highest rates in children from 3 to 15 years

Fig. 7.4 Adult worms of (**a**) *Artyfechinostomum malayanum* (syn. *Echinostoma malayanum*) (Photomicrograph courtesy of Weerachai Saijuntha); (**b**) *Echinostoma hortense* (Photomicrograph courtesy of Woon-Mok Sohn); (**c**) *Echinoparyphium recurvatum*; and (**d**) *Hypoderaeum conoideum*. (Scale bars: 1 mm)

Table 7.2 Species of Echinostomatidae involved in human infections

Acanthoparyphium tyonense	Korea
Artyfechinostomum malayanum[a]	India, Indonesia, Lao PDR, Malaysia, Philippines, Singapore, Thailand
Artyfechinostomum oraoni	India
Echinoschasmus fujianensis	China
Echinoschasmus japonicus	China, Korea, Japanb, Lao PDR
Echinoschasmus jiufoensis	China
Echinoschasmus liliputanus	China
Echinochasmus perfoliatus	China, Japan
Echinoparyphium recurvatum	Egypt, Indonesia, Taiwan
Echinostoma angustitestis	China
Echinostoma cinetorchis	Japan, Korea, Taiwan
Echinostoma echinatum	Indonesia
Echinostoma hortense	China, Japan, Korea
Echinostoma ilocanum	Cambodia, India, Indonesia, Malaysia, Philippines, Thailand
Echinostoma macrorchis	Japan
Echinostoma revolutum	Cambodia, China, Egypt, Indonesia, Lao PDR, Russia, Thailand, Europe
Episthmium caninum	Thailand
Himasthla muehlensi	USA[c]
Hypoderaeum conoideum	Thailand
Isthmiophora melis	China, Rumania, Taiwan

[a]Syn. *Echinostoma malayanum*
[b]Experimental infection
[c]Imported infection

(Cheng et al. 1992; Yu and Mott 1994). The prevalence of *E. japonicus* in some counties of Guangdong and Fujian was 4.9%, whereas the prevalence of *E. perfoliatus* in these areas was lower (1.8%) (Yu and Mott 1994). Interestingly, prevalence of 13.4% of *E. liliputanus* was reported in Anhui (Xiao et al. 1992). A single case of human infection of *E. jiufoensis* has been reported during an autopsy of a 6-month-old girl in Guangzhou (Liang and Ke 1988). A total of three members of *Echinostoma* (*E. revolutum, E. hortense,* and *E. angustitestis*) have also been detected in China. *E. revolutum* has been reported in Yunnan and Guangdong provinces and two cases of *E. japonicus* infection were recorded in Fujian (Cheng et al. 1992; Chai 2009). In Liaoning Province, six patients with hepatitis were found to be infected with *E. hortense* (Fig. 7.4b) (Chen et al. 1993). More anecdotal is the infection with *Isthmiophora melis* (Chai 2009).

A total of five species of echinostomes have been reported in Indonesia infecting humans (*A. malayanum, Echinoparyphium recurvatum, Echinostoma echinatum, E. ilocanum,* and *E. revolutum*), mainly focused in the major Islands (Sumatra, Java, and Sulawesi) (Graczyk and Fried 1998). A high prevalence of *E. echinatum* was detected from 1937 to 1956 in the Lake Lindu Valley (Sulawesi). The average prevalence was 43%, but it reached 96% in some areas (Carney et al. 1980). Human infections appeared to be due to the habit of eating raw or insufficiently cooked lake molluscs, particularly bivalves (*Corbicula* spp.). Elimination of *Corbicula* clams from the diet significantly reduced the prevalences and a survey in the 1970s only revealed occasional presence of eggs (Clarke et al. 1974). In other areas, an increasing number of cases were reported in relation to the growing popularity of exotic food available in Korean and Japanese restaurants in Indonesia (Kusharyono and Sukartinah 1991; Graczyk and Fried 1998). Moreover, in the Indonesian area of Borneo (West Kalimantan), echinostome eggs are frequently observed in faces of the residents (Cross et al. 1976).

A total of four species have been recorded in Japan (*Echinochasmus perfoliatus*, *Echinostoma cinetorcis, E. hortense,* and *E. macrorchis*), Korea (*A. tyosenense, E. hortense, E. cinetorchis,* and *E. japonicus*), Thailand (*E. revolutum, E. ilocanum, Episthmium caninum,* and *Hypoderaeum conoideum*), Lao PDR (*A. malayanum, E. japonicus, E. revolutum,* and *Euparyphium* sp.), and India (*A. malayanum, A. oraoni, A sufrartyfex,* and *E. ilocanum*). However, some of these species only have been found occasionally. For example, in Japan only one case has been detected for *E. perfoliatus* and *Echinostoma macrorchis* in Japan (Majima 1927; Hirazawa 1928), while three cases were reported for *Episthmium caninum* in Thailand (Radomyos et al. 1985, 1991). In the case of *A. tyosenense* and *A. oraoni*, a total of 10 and 20 cases were reported in Korea and India, respectively (Bandyopadhyay and Nandy 1986; Bandyopadhyay et al. 1989; Chai et al. 2009a). Human infections with *E. cinetorchis* were first reported in Japan and in the Republic of Korea (Takahashi et al. 1930; Kawahara and Yamamoto 1933; Seo et al. 1980; Ryang et al. 1986; Lee et al. 1988; Jung et al. 2014). Human infections with *E. hortense* have been reported both in Japan and Korea (Chai 2009). An endemicity of this echinostome species has been reported among residents of Cheongsong-gun (Republic of Korea) with 22% of prevalence (Lee et al. 1988). *Hypoderaeum conoideum* (Fig. 7.4c) in humans only has been recorded in the northeast area of Thailand with a prevalence of 55% among the residents (Yokogawa et al. 1965).

A recent study in Laos PDR among 2074 residents in riparian villages along the Mekong River in the Khammouane Province demonstrated the presence of echinostome eggs in the feces of 1.1% of residents. A total of 55 specimens belonging to *E. revolutum, A. malayanum, E. japonicus,* and *Echinoparyphium* sp. were recovered after treatment and purgation (Chai et al. 2012). Sayasone et al. (2009a) detected three human cases of *E. japonicus*. Recently, human infections with *E. ilocanum* were recorded by the first time in Laos PDR in two riparian people from Savannakhet Province, confirming that echinostomiasis is prevalent in the Meking River (Chai et al. 2018). A prevalence of 6% was found in southern Lao PDR (Sayasone et al. 2011).

In Taiwan, three species (*E. revolutum, E. recurvatum,* and *I. melis*) were reported in humans (Lu 1982) and the prevalence of infection varied from 11 to 65% in some areas (Carney 1991). In Cambodia, *E. revolutum,* and *E. ilocanum* have been recently reported. Sohn et al. (2011a) demonstrated a prevalence of 1.0% of *E. ilocanum in* the Oddar Meanchey Province. Sohn et al. (2011b) determined a prevalence of 7.5–22.4% of *E. revolutum* in children from the Pursat Province. In the Philippines, *E. ilocanum* and *A. malayanum* infect humans with an overall prevalence of 3% though it reached 44% in some areas (Eduardo 1991). In Malaysia and Singapore, only human infections with *A. malayanum* have been recorded (Chai 2009). Recently, human echinostomiasis in Nepal was detected by the first time by Sah et al. (2018) who detected a single adult worm by endoscopy in a Hindu patient with gastrointestinal disorders. Coprological examination showed the presence of eggs of an echinostomatid.

The number of known reports outside of Asia is very limited. Only four species have been occasionally recorded. A human infection with *I. melis* was detected in 1916 in a diarrheic patient in Rumania (Beaver et al. 1984) and *E. recurvatum* (Fig. 7.4d) and *E. revolutum* have been sporadically reported in Egypt and Russia, respectively (Chai 2009). Imported cases in the USA also have been reported. *H. muehlensi* was originally described on the basis of five adult specimens from a German patient who lived in Colombia and traveled to New York, where he had eaten raw clams (Chai 2007). DeGirolami and Kimber (1983) recorded *Echinostoma* sp. from Asian refugees in the USA. Poland et al. (1985) reported 18 cases of imported echinostomiasis among a total of 20 American tourists to Kenya. A total of ten of the patients showed moderately severe abdominal cramps and loose or watery stools. A traveler group of seven Caucasians became infected with *Echinostoma* sp. In Tanzania, after ingesting raw fish from Lake Tanganika. The infection was diagnosed by finding eggs in stool samples at their return to Kenya. This constituted the first report of human echinostomiasis in East Africa (Chunge and Chunge 2017).

7.4.3 Clinical Manifestations and Pathology

The clinical symptoms of human echinostomiasis may be more severe than those produced by other intestinal trematodes though the clinical features greatly depend on the parasite load (Graczyk and Fried 1998; Chai 2007). Human morbidity and mortality due to echinostomiasis depend on a number of factors such as a prolonged latent phase, limited acute phase, and similarity with the symptomatology of other intestinal pathologies and, even, the existence of asymptomatic presentations (Graczyk and Fried 1998).

Epigastric and abdominal pain, easy fatigue, diarrhea, and weight loss are the most common symptoms in human echinostomiasis (Graczyk and Fried 1998; Chai and Lee 2002; Toledo et al. 2006a). Although this fact, other symptoms also can be detected. Several studies have shown that patients infected with *E. hortense* additionally suffered acid belching, anorexia, headache, nausea and vomiting, and urinary incontinence (Lee et al. 1986; Chai et al. 1994; Chang et al. 2005; Park and Kim 2006). Peripheral blood eosinophilia has been commonly reported (Poland et al. 1985; Lee et al. 1988). However, the levels of eosinophilia appear to be markedly dependent upon the worm load and ranged from 2 to 24% as demonstrated in *E. hortense* infections (Lee et al. 1988).

The intestinal pathology induced by echinostomes in humans has been poorly studied and most of the known data were obtained by gastroduodenal endoscopies in *E. hortense* infections. In general, the patients showed mucosal erosion and ulceration, bleeding in the stomach and the duodenum, signs of chronic gastritis, and infiltration of inflammatory cells including neutrophils (Chai et al. 1994; Chang et al. 2005; Cho et al. 2003). Interestingly, stage IIc or stage III early gastric cancer was determined by gastroduodenal fiberscopy (Chai et al. 1994). Cortés et al. (2015a) showed that echinostome infection in mice induces significant changes in the intestine affecting to the restoration of the intestinal epithelium and the control of homeostatic dysregulation, concomitantly with mitochondrial and cytoskeletal

proteins among others, which may conduct to malignant transformation.

Further details on the pathology of echinostome infections were obtained using laboratory rodents. The pathological effects of echinostomiasis are dependent on a wide variety of factors including the echinostome species, the host species, and the intensity of the infection (Toledo et al. 2006a). Echinostomes provoke inflammatory responses in the attachment sites. The surrounding areas showed marked dilation, erosion of the villi and lymphocytic infiltration (Huffman et al. 1988; Mabus et al. 1988; Toledo et al. 2006b, 2009; Muñoz-Antoli et al. 2007). Moreover, goblet cell hyperplasia, neutrophilia and infiltration of inflammatory cells, changes in the mucin expression and glycosylation, alterations of the intestine epithelial cell turnover and crypt hyperplasia with increased mitotic rates also occur (Fujino and Fried 1996; Toledo et al. 2006b; Cortés et al. 2015b, c). Cellular infiltration of lymphocytes, eosinophils, and plasma cells also were observed in the lamina propria and submucosa (Weinstein and Fried 1991; Toledo et al. 2006b).

7.4.4 Host–Parasite Relationships and Immunology

Immunology of echinostomiasis has been extensively studied in laboratory rodents. It has been shown that the rodent hosts are able to express various types of resistance to echinostome infections which suggest that the parasites can be spontaneously expulsed or, in contrast, develop a chronic infection depending on the host–parasite combination (Toledo and Fried 2005; Toledo et al. 2006a, 2009).

Although it has been shown that echinostomes alter several immunological parameters the role of these alterations in the course of the infections remains unclear (Chai 2009; Toledo 2009; Toledo et al. 2009). *Echinostoma* spp. induces changes at the cellular level and in the expression of certain glycoconjugates in the intestinal mucosa (Toledo et al. 2006a). Mastocytosis, eosinophilic infiltration, and increase in the goblet cells and mast cell populations have been commonly observed, though there are conflicting data in relation to their effect on the echinostome infections (Fujino and Fried 1993, 1996; Fujino et al. 1993, 1996a, b; Kim et al. 2000; Park et al. 2005; Toledo et al. 2006b; Muñoz-Antoli et al. 2007; Ryang et al. 2007). Furthermore, several studies have suggested that the alterations of the terminal sugar of the mucins produced by goblet cells may regulate the worm expulsion (Fujino and Fried 1993, 1996; Park et al. 2005).

Apart from the changes in cell populations, echinostomes also may induce energic antibody responses (Toledo 2009). However, they do not appear to alter the course of the infections. For example, *Echinostoma caproni* induces elevated responses of IgM, IgG, IgG1, and IgG3 in the serum of mice in which the parasite survives for more than 25 weeks (Graczyk and Fried 1994; Toledo et al. 2004; Sotillo et al. 2007). In contrast, low levels of antibodies were detected in the serum of *E. caproni*-infected rats concomitantly with an early expulsion of the worms (Sotillo et al. 2007). At the intestinal level, increases in the IgM, IgA, IgG1, and IgG2a levels were detected in mice (Sotillo et al. 2007). The response against *E. hortense* is characterized by an elevation of the serum levels of IgG1, IgE, and IgA (Cho et al. 2007). This can be explained, at least in part, by the fact that echinostomes are able to trap and degrade the surface-bound antibodies as a mechanism of immune evasion (Cortés et al. 2017a). The target antigens of these responses in *E. caproni* infections were studied by Sotillo et al. (2008). A total of four proteins (enolase, actin, HSP-70, and aldolase) appeared to be the major antigens in the *E. caproni* adult worms.

The cytokine profile in echinostome infections has been poorly studied. However, new information about this topic has been obtained in recent years. The production of cytokines in the splenocytes of mice infected with *E. hortense* has been studied (Cho et al. 2007; Ryang et al. 2007; Lee et al. 2009). These studies detected a predominance of the Th2 responses with elevated expression of IL-4 and IL-5. In *E. caproni* infections, Brunet et al. (2000) observed an elevated production of IFN-γ in the spleen cells of

experimentally infected mice. Comparative studies using hosts of different compatibility with *E. caproni* have provided further insight in the responses determining the course of the infection. The development of chronic infections appears to be related with the development of local Th1 responses with elevated levels of IFN-γ, whereas the early worm rejection is mediated by the development of a biased Th2/Th17 local phenotype (Sotillo et al. 2011; Trelis et al. 2011). Although, IFN-γ production has been associated with chronicity and elevated mucosal damage, this cytokine seems to play a dichotomous role in the infection facilitating the parasite establishment, but it may also benefit mice since it protects the mice from morbidity and mortality induced by the parasite (Cortés et al. 2014).

Recently, it has been shown that a primary infection with *E. caproni* in ICR mice induces partial resistance against subsequent homologous infections. This resistance was expressed as a reduced rate of infection, worm recovery, and worm size, indicating that primary infection induces changes in the host, making a hostile environment for the development of the parasite (Muñoz-Antoli et al. 2016a). Further studies on this topic indicated that susceptibility is determined by the lack of IL-25 expression in response to primary infection. In contrast, infection in an environment with elevated levels of IL-25, as occurs in challenge infection, results in a Th2 phenotype impairing parasite survival (Muñoz-Antoli et al. 2016b).

Interestingly, it has been found that the protein production of echinostome adult worms is markedly affected by the host milieu. Upregulation of proteins with stress or detoxification process has been observed in highly susceptible hosts which may serve to withstand the hostile Th1 environment generated in primary infections in these hosts (Cortés et al. 2016, 2018).

7.4.5 Diagnosis

Clinical diagnosis of human echinostomiasis is difficult since the infection may remain unapparent for a while or the symptoms, if present, are often unspecific. Laboratory diagnosis is based on the demonstration of eggs in feces. The eggs are oval, yellowish, thin shelled, and with an operculum at the anterior end which may be difficult to see. The size of the most human infecting echinostomes is in the range 66–145 × 43–90 mm though the eggs of several species may fall outside this range (Chai 2009). However, the difficulty entailed in the specific characterization of the eggs strongly recommends recovering the adult worms.

Although immunological methods for the diagnosis of human echinostomiasis have not been developed, several studies using laboratory rodents have shown that conventional ELISA and capture ELISA may be promising methods for the detection of human infections (Agger et al. 1993; Graczyk and Fried 1995; Toledo et al. 2003, 2004, 2005; Cho et al. 2007; Sotillo et al. 2007).

7.5 Family Fasciolidae

7.5.1 Background

The family Fasciolidae comprises large trematodes that inhabit the liver and bile ducts but members of two genera (*Fasciolopsis* and *Parafasciolopsis*) are intestinal parasites. The life cycle of the members of this family includes a metacercarial stage that encysts on pasture and other vegetation.

7.5.2 Species Reported in Humans and Geographical Distribution

Fasciolopsis buski is the only fasciolid species reported infecting the intestine of humans. This is the largest trematode parasitizing humans (8–10 × 1–3 cm) (Fig. 7.5a) and a common intestinal parasite of human and pigs in Asia (Fried et al. 2004).

In humans, *F. buski* inhabits the duodenum and the jejunum though it can extend to almost the complete intestine and, even, the stomach in heavy infections. Adult worms produce over

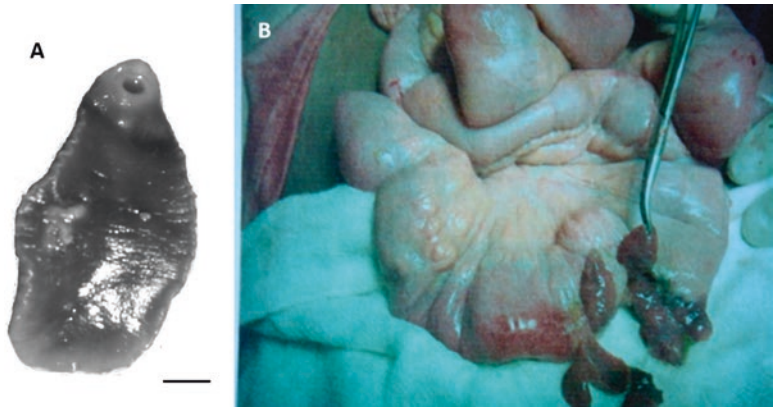

Fig. 7.5 (**a**) Unstained specimen of *Fasciolopsis buski* (Scale bar: 0.5 mm) (Photograph courtesy of Deepak Bagga); (**b**) *Fasciolopsis buski* emerging from perforation site of the ileum (Scale bar: 5 μm) (Photograph courtesy of Umesh Chandra Singh)

25,000 eggs every day. Unembryonated eggs are discharged into the intestine and stool. Eggs become embryonated in water and release miracidia, which invade a suitable snail intermediate host. Several species of genera *Segmentina* and *Hippeutis* serve as intermediate hosts. In the snail, the parasite undergoes several developmental stages (sporocysts, rediae, and cercariae). The cercariae are released from the snail and encyst as metacercariae on aquatic plants such as water chestnut, water caltrop, lotus, bamboo, and other edible plants. The mammalian final host becomes infected by ingesting metacercariae on the aquatic plants. After ingestion, the metacercariae excyst in the duodenum in about 3 months and attach to the intestinal wall. There they develop into adult flukes in approximately 3 months, attached to the intestinal wall of the mammalian hosts (humans and pigs). The adults have a life span of about 1 year.

Fasciolopsiasis is confined and endemic in Far East and Southeast Asia (Fried et al. 2004). The disease occurs focally and is linked to freshwater habitats and is associated with common social and agricultural practices and promiscuous defecation (Mas-Coma et al. 2005). Humans commonly become infected by eating raw or undercooked aquatic plants, but infection can also be contracted by the drinking or use of contaminated water or processing of the water-derived plants, e.g., using teeth to peel plants (Gilman et al. 1982; Weng et al. 1989; Fried et al. 2004). Fasciolopsiasis can be aggravated by social and economic factors such as poverty, malnutrition, and uninspected and poorly sanitized food markets (Mas-Coma et al. 2005). In fact, differences in the incidence within the same area have been found in relation to the economic status, educational background, or the standard of health and/or way of life (Jaroonvesama et al. 1986). The infection predominantly occurs in children and the worm burden may exceed 800 flukes/child (Gilman et al. 1982; Weng et al. 1989). In foci of transmission, the prevalence of infection in children ranged from 10 to 60% in countries such as China, Taiwan, India, Bangladesh, or Thailand (Muttalib and Islam 1975; Rahman et al. 1981; Bunnag et al. 1983; Shyu et al. 1984; Weng et al. 1989). Pigs may play an important role in the transmission of the parasite. The pig is the main reservoir and different infection rates have been reported, ranging from 10% in China to 52% in Taiwan (Mas-Coma et al. 2005). Fresh aquatic green fodder and untreated water used to raise pigs appear to be the source of infection in farm animals (Weng et al. 1989; D'Souza et al. 2001).

In China, infections have been reported in ten provinces reaching 85% of prevalence in some of them (Weng et al. 1989). In Bangladesh, the prevalence in schoolchildren in an endemic focus reached 50% (Gilman et al. 1982). In India, 60% of people were found to be infected and harboring 1–57 worms in Assam (Buckley 1939). In Thailand, the central area is the main endemic area with an estimation of 20% of infected people (Manning et al. 1971). Infection has also been reported from Lao PDR, Vietnam, Cambodia,

Indonesia, the Philippines, Kampuchea, Burma, and Malaysia (Mas-Coma et al. 2005). Moreover, imported cases from the Far East have been recorded in Korea, Japan, the USA, Venezuela, Australia, Guatemala, Israel, and Cuba (Mas-Coma et al. 2005).

Although several studies showed that human fasciolopsiasis decreased in the 1980s, this tendency was not maintained since people continue eating raw vegetables. Recently, Quang et al. (2008) suggested the possibility of an emergence of human fasciolopsiasis in Lao PDR.

7.5.3 Clinical Manifestations and Pathology

Clinical symptoms in *F. buski* infections in humans are related to parasite load and can be fatal in heavy infections (Gilman et al. 1982; Weng et al. 1989). In light infections, symptomatology may include anemia, eosinophilia, dizziness, and gastrointestinal symptoms. In moderate and heavy infections, there may appear severe epigastric and abdominal pain, diarrhea or bowel obstruction, nausea, acute ileus, anasarca, and eosinophilia and leucocytosis (Gilman et al. 1982; Fried et al. 2004). Eventually, it may cause intestinal perforation or obstruction and may produce apendicitis (Fig. 7.5b) (Bhattacharjee et al. 2009; Cao et al. 2015).

Moreover, adult flukes damage the intestinal mucosa and cause extensive duodenal erosions, ulceration, hemorrhage, abscesses, and catarrhal inflammation. Absorption of toxic and allergic worm metabolites causes ascitis, general edema, and facial edema (Jaroonvesama et al. 1986; Fried et al. 2004).

7.5.4 Diagnosis

Laboratory diagnosis is based on the demonstration of eggs in feces. The eggs are ellipsoidal, operculated, non-embryonated, and measuring 130–140 × 80–85 mm (Gilman et al. 1982). Immunological or molecular methods have not been developed.

7.6 Family Gastrodiscidae

7.6.1 Background

The family Gastrodiscidae contains trematodes relatively large, i.e., approximately 8–14 mm in length. They are intestinal parasites of terrestrial mammals, including man, and have been distinguished by a dorsoventrally flattened body, which has the appearance of being divided into two parts.

7.6.2 Species Reported in Humans and Geographical Distribution

Only one species of Gastrodiscidae, *Gastrodiscoides hominis*, has been found infecting humans. Adult of *G. hominis* are large flukes (8–14 × 5.5–7.5 mm). Moreover, this species is characterized by a short and cylindrical anterior part, large and discoidal posterior part, subterminal pharynx, testes lobed and in tandem, a post-testicular ovary, an ascending uterus, and a ventral genital pore.

The life cycle is not completely understood. Adult worms inhabit the caecum and colon of humans, pigs, small, monkeys, and other mammals. Unembryonated eggs are laid and, in a freshwater environment, the miracidium hatches and infects the first intermediate host. Only the snail *Helicorbis coenosus* is known to act as the first intermediate host. After the development of mother and daughter rediae, the cercaria emerges and encysts in aquatic plants, snails, tadpoles, frogs, or crayfish. Definitive host becomes infected after swallowing metacercariae with tainted vegetables or raw or undercooked crustaceans, molluscs, or amphibians (Mas-Coma et al. 2006).

G. hominis has been detected infecting humans in India, Burma, Pakistan, Myanmar, Vietnam, the Philippines, Thailand, China, Kazakhstan, Indian immigrants in Guyana, Zambia, Nigeria, and the Volga Delta in Russia (Mas-Coma et al. 2005). Although *G. homins* is mainly a parasite of pigs, high prevalence in humans has been detected in some areas. For

example, Buckley (1939) detected a prevalence of 41% in children from Assam (India).

Pathology and symptomatology of humans *G. hominis* infections are not well known. Heavy infections may induce headache, epigastric pain, and diarrhea that may be a reaction to metabolites released by the parasite (Marty and Andersen 2000). Acetabulum of the adult worm is found to drag the mucosa like a plug causing inflammation (Mas-Coma et al. 2005, 2006). In human infection, a picture similar to that detected in pigs might be expected. Surface desquamation, infiltration with eosinophils, lymphocytes, and plasma cells appear in sections of the lesions caused by the fixation of the parasites to the mucosa. Hypersecretion of mucus and necrosis of the mucous glands are also observed. The lamina propria shows infiltration of eosinophils, lymphocytes, macrophages, and plasma cells (Mas-Coma et al. 2005, 2006).

Diagnosis of human gastrodiscoidiasis is feasible by detection of eggs in feces. Egg size is about 4–6 × 5–10 mm and it is deep yellow, operculated, non-embryonated, and measuring about 150 × 70 mm (Mas-Coma et al. 2006).

7.7 Family Gymnophallidae

7.7.1 Background

Gymnophalloidiasis is the intestinal infection caused by *Gymnophalloides seoi*, belonging to the family members of the family Gymnophallidae. This family consists of a small group of marine digeneans. Most members use molluscs as the first intermediate host; the metacercariae never encysts and are usually parasitic in bivalves. With rare exceptions, charadriiform and anseriform birds are the definitive host which becomes infected after ingestion of the second intermediate host harboring the metacercariae (Cremonte et al. 2013). Although a total of five genera are accepted in the family, only *G. seoi* has been found infecting humans.

7.7.2 Species Infecting Humans and Geographical Distribution

Human infections with *G. seoi* only have been recorded in the Republic of Korea (Fried et al. 2004; Chai et al. 2009a; Toledo et al. 2012). This fluke was first discovered in 1988 in a Korean woman suffering pancreatitis and gastric discomfort (Lee et al. 1993a; Chai et al. 2003). This woman lived in the southwestern coastal island of Aphae in the Shinan County, which was subsequently found to be an endemic area. Then, more than 25 villages in western and southwestern coastal islands and 3 mainland coastal villages have been reported as endemic foci with prevalences ranging from 9.3 to 57.9% (Lee et al. 1996; Chai et al. 1997, 2009a, b; Fried et al. 2004; Guk et al. 2006; Chai 2007; Cho et al. 2010).

The adult parasite is small (0.4–0.5 × 0.2–0.3 mm) and characterized by a large oral sucker and small ventral sucker, short caeca, and a unique ventral pit (Fig. 7.6a) (Chai et al. 2003). The first intermediate host remains unknown but the second intermediate host was found to be the oyster *Crassostrea gigas* (Lee et al. 1995; Sohn et al. 1998). Humans become infected by eating raw or undercooked oysters harboring the metacercariae (Fig. 7.6b). The parasite inhabits the small intestine though a case of mucosal tissue invasion has been reported. An adult worm was incidentally found in a surgical pathology specimen of the lymph node around the colon of a 65-year-old Korean patient (Seo et al. 2006).

The symptomatology induced by *G. seoi* may vary greatly between individual patients. Gastrointestinal discomfort and indigestion are the most common symptoms. Moreover, fever, anorexia, weight loss, easy fatigue, and weakness can also appear (Chai et al. 2003). It has also been suggested that gymnophalloidiasis increases the levels of amylase in the serum and urine. In two patients, the gymnophalloidiasis was accompanied by diabetes mellitus suggesting a relationship between both diseases (Lee et al. 1995).

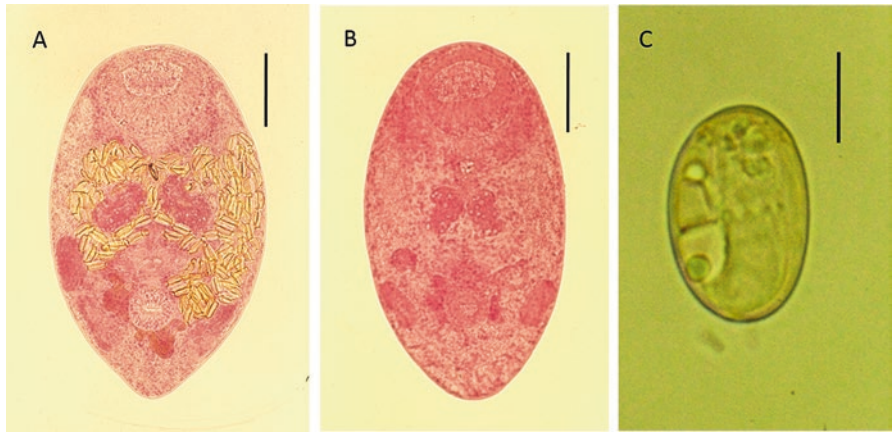

Fig. 7.6 *Gymnophalloides seoi*: (**a**) adult worm (Scale bar: 100 μm); (**b**) metacercaria collected from a naturally infected oyster (Scale bar: 100 μm); and (**c**) egg (Scale bar: 10 μm). Photomicrographs courtesy of Woon-Mok Sohn

7.7.3 Host–Parasite Relationships and Immunology

Apparently, *G. seoi* induces less pathological damage than other intestinal trematodes. Adult worms inhabit the duodenum and jejunum where they are attached by grasping or pinching the epithelial layer with the oral sucker. This induces villous atrophy and crypt hyperplasia with inflammation of the villous stroma and the crypt (Chai et al. 2001; Toledo et al. 2006a). A marked goblet cell hyperplasia dependent on CD4+ T-helper cells along the villous epithelia was observed, mainly in the jejunum (Chai et al. 2001; Guk et al. 2009). These changes were resolved at 2–3 weeks post-infection. However, it is suspected that *G. seoi* invades the pancreatic duct of humans which may cause further complications which may require medical attention. Acute pancreatitis was diagnosed in one patient of gymnophallodiasis and diabetes mellitus was accompanied in other two patients (Lee et al. 1993a, 1995).

In mice, *G. seoi* infections are spontaneously expelled within 3 weeks post-infection but the course of the infection differs depending on the strain of mice (Lee et al. 1997b; Chai et al. 1999) indicating a genetic background in the resistance to infection. Moreover, comparative studies on the development of *G. seoi* in immunocompetent and immunosuppressed mice indicated that the differential susceptibility is dependent on the host immune response (Lee et al. 1997b; Chai et al. 1999; Seo et al. 2003). Little is known about the immune effector mechanisms responsible for the short-term parasitism of *G. seoi* observed in mice. However, the observation of a strong proliferation of goblet cells previous to the worm expulsion in *G. seoi*-infected mice suggested that this can be one of the effector mechanisms involved in worm rejection (Chai et al. 2001). Lee et al. (2013) showed that *G. seoi i*nfection in mice induces overexpression of STAT6 and IL-13 enhancing goblet cell hyperplasia responsible for the worm expulsion. Increased intestinal epithelial cell turnover, and increased intestinal motility should be important mucosal defense mechanisms in *G. seoi*-infected mice (Lee et al. 2014). However, recent studies have shown that T-cell-independent mechanisms also mediate in the expulsion of *G. seoi*. In this context, alteration of the mucin quality with changes in the terminal sugar chain and elevated levels of IL-4 and IL-5 expression appears to be of importance (Guk et al. 2009). Furthermore, it has been demonstrated that *G. seoi* antigens upregulate Toll-like receptors and mucin-related genes (MUC2) in human intestinal epithelial cells via IFN-γ (Lee et al. 2010).

7.7.4 Diagnosis

Diagnosis can be done by detection of eggs in the feces. However, the detection and identification may be difficult because of: (1) low-laying capacity of the parasite and (2) identification of *G. seoi* eggs. The daily egg output/worm is estimated in 2–84 in the human host (Chai et al. 2003) which makes difficult the isolation of the eggs unless heavy infections occur. Another difficulty entailed with the diagnosis of the gymnophalloidiasis is the identification of the eggs. They are very small (approximately 23 × 13 μm) and thin and transparent shelled (Fig. 7.6c) (Lee et al. 2012). Due to their small size, the eggs can be overlooked by an inexpert analyst or misdiagnosed as a bubble or other artifacts. Moreover, differential diagnosis with other digeneans may be difficult (Lee et al. 2012).

7.8 Family Heterophyidae

7.8.1 Background

Heterophyiasis is caused by the members of the family Heterophyidae. This family contains small egg-shaped trematodes with infective metacercariae that are usually encysted in fish second intermediate hosts. The heterophyids are characterized by the possession of a gonotyle or genital sucker (Yamaguti 1971). This author accepted 13 subfamilies within the family which may be differentiated on the basis of the body morphology, the presence of a circumoral crown of spines, extension of the vitellaria and uterus, morphology of the testes, and location of the genital pore.

The definitive host becomes infected by eating raw or poorly cooked fish harboring metacercariae. The adult worms live between the villi of the anterior region of the small intestine and release fully embryonated eggs into water. The eggs are then ingested often by littorine snails (species of *Melanoides, Semisulcospira,* and others), and hatch within the snail's intestine. Intramolluscan development comprises sporocyst and redial stages and cercariae are released into the water where they typically penetrate shrimps or shore-fish, such as cunners, gudgeon, and charr, and encyst on the surface or muscles of the fish. Metacercariae may remain viable for years (Fried et al. 2004; Toledo et al. 2006a; Sohn 2009).

7.8.2 Species Infecting Humans and Geographical Distribution

A total of 26 species of heterophyids have been reported infecting humans (Table 7.3). Humans become infected by eating fish or shrimps harboring viable metacercariae, which mature into adults in 5–10 days. Adult heterophyids attach and live embedded the intestinal mucosa producing embryonated eggs that reach water reservoir due to the lack or non-use of sanitary latrines which allow for the maintenance of the life cycle.

Probably, *Heterophyes heterophyes* and *Metagonimus yokogawai* (Fig. 7.7a, b) are the most relevant species from the standpoint of human disease. *H. heterophyes* was first discovered in an Egyptian and is a common parasite in the Nile Delta around the lakes Manzala, Borollos, and Edco (Yu and Mott 1994). In the period 1984–1991, the prevalence in the five governorates of the Delta ranged from 0.001 to 1% and the population at risk was estimated as 933,000. People became infected by consuming recently salted or insufficiently baked fish (Sheir and Aboul-Enein 1970). In the villages of Khuzestan (Iran), the prevalence in humans ranged from 2 to 24% (Yu and Mott 1994). In Asia, several foci have been identified but this parasite could be confused with *Heterophyes nocens* (Chai et al. 2009a). Imported cases in Japan were reported from people who returned from Egypt, Saudi Arabia, and Sudan (Chai et al. 2009a). In Western Europe, human infections with *H. heterophyes* have been recorded sporadically. For example, Martínez-Alonso et al. (1999) described the infection of a woman after eating raw fish in a Chinese restaurant in Spain. *M. yokogawai* is probably the most common intestinal fluke infecting humans in the Far East. It has been recorded in China, Japan, Korea, Taiwan,

Table 7.3 Species of Heterophyidae involved in human infections

Genus	Species	Geographical distribution of human cases
Apophallus	*A. donicus*	USA
Ascocotyle	*A. longa*[a]	Brazil[a]
Centrocestus	*C. armatus*	Japan, Korea
	C. caninus	Thailand
	C. cuspidatus	Egypt
	C. formosanus	Japan, Taiwan, Vietnam, Lao PDR
	C. kurokawai	Japan
Cryptocotyle	*C. lingua*	Greenland
Haplorchis	*H. pleurolophocerca*	Egypt
	H. pumilio	China, Egypt, Korea, Lao PDR, Philippines, Taiwan, Thailand, Vietnam
	H. taichui	China, Lao PDR, Philippines, Thailand, Vietnam
	H. vanissimus	Philippines
	H. yokogawai	China, Egypt, India, Indonesia, Lao PDR, Malaysia, Philippines, Taiwan, Thailand
Heterophyes	*H. dispar*	Korea[b]
	H. heterophyes	Egypt, Iran, Korea[b], Sudan
	H. nocens	China, Japan, Korea
Heterophyopsis	*H. continua*	Japan, Korea
Metagonimus	*M. miyatai*	Japan, Korea
	M. takahashii	Korea
	M. yokogawai	All Far East, India, Balkan states, Israel, Siberia, Spain
Procerovum	*P. calderoni*	Africa, China, Philippines
Pygidiopsis	*P. summa*	Japan, Korea
Stellanchasmus	*S. falcatus*	Hawaii, Japan, Korea, Palestine, Philippines, Thailand, Vietnam
	S. pseudocirratus	Hawaii, Philippines
Stictodora	*S. fuscata*	Korea
	S. lari	Korea

[a]Referred to as *Phagicola* sp. by Chieffi et al. (1992) in Brazil
[b]Imported cases

and Indonesia (Yu and Mott 1994). In Korea, the prevalence reached 4.8% (Seo et al. 1981), with special incidence in riverside communities where raw sweetfish is commonly eaten (Youn 2009). In particular, the eastern parts of Gyeongbuk Province, Gangjingun (Tamjin River), Boseong (Boseong River), Hadonggun (Seomjin River), and Samcheok (Osip Stream) were the most important endemic areas with a prevalence ranging from 20 to 70% (Chai and Lee 2002; Lee et al. 2008). In China, *M. yokogawai* is distributed in Guangdong, Anhui, Hubei and Zhejiang (Chai et al. 2009a). In India, only three cases have been reported, the first two cases occurred with a Muslim family in upper Assam (Mahanta et al. 1995) and more recently an isolated case was found in a 6-year-old female in New Delhi (Uppal and Wadhwa 2005).

Another ten species of Heterophyidae have been recorded in the Republic of Korea (Table 7.3). *H. nocens* commonly infects humans though the identity of this species is confused and may be a synonym of *H. heterophyes*. Prevalence ranging from 17 to 70% was detected among residents in the southwestern coastal areas (Chai et al. 2004). Human infections with *Metagonimus miyatai* (Fig. 7.7c) have been detected in people from Gum, Namhan, and Hantaan rivers (Chai et al. 2009a). In the upper reaches of Namhan River have also recorded human infections with *Metagonimus takahashi* (Chai et al. 1993). *Pygidiopsis summa* is widely distributed in the western and southwestern coastal areas of Korea (Eom et al. 1985). Recently, a prevalence of 4.9% of heterophyid infections was described in South Jeolla Province

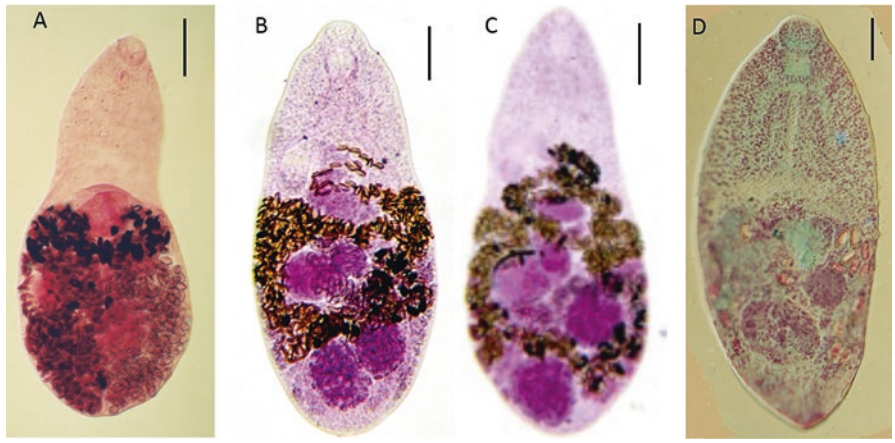

Fig. 7.7 Adult worms of (**a**) *Heterophyes heterophyes* (Scale bar: 50 μm); (**b**) *Metagonimus yokogawai* (Scale bar: 150 μm) (Photomicrograph courtesy of Hideto Kino); (**c**) *Metagonimus miyatai* (Scale bar: 75 μm) (Photomicrograph courtesy of Hideto Kino); and (**d**) *Haplorchis taichui*. (Scale bar: 100 μm)

(Cho et al. 2010). Sporadic cases of *Centrocestus armatus* (1 case), *Haplorchis pumilio* (1) *Heterophiopsis continua* (10), *Stellantchasmus falcatus* (4), *Stictodora fuscata* (14), and *S. lari* (6) have also been recorded (Chai and Lee 2002; Chai et al. 2009a; Chung et al. 2011). Furthermore, two imported cases of *Heterophyes dispar* infections were reported in men returning from Saudi Arabia (Chai et al. 1986). A recent study demonstrated that intestinal trematodiasis constituted an unrecognized food safety risk in Vietnam and heterophyids were the most prevalent intestinal trematodes. Elevated prevalence was detected for *H. pumilio, Haplorchis taichui* (Fig. 7.3d), *Haplorchis yokogawai,* and *S. falcatus* in several areas (Trung Dung et al. 2007).

In Japan, seven additional species of heterophyids have been recorded infecting humans (Table 7.3) (Chai et al. 2009a; Yu and Mott 1994). Of particular interest are the endemic foci of *H. nocens* detected in Shizuoka Prefecture (Kino et al. 2006). In Lao PDR and Vietnam, several studies have shown the *Haplorchis* spp. (*H. pumilio, H. taichui,* and *H. yokogawai*) and *Centrocestus formosanus* have a significant impact among residents (Trung Dung et al. 2007; Chai et al. 2009b, 2013; Sayasone et al. 2009b; De and Le 2011).

7.8.3 Clinical Manifestations and Pathology

Commonly, low-grade infections with heterophyids are of no clinical consequences. Heavy infections are associated with diarrhea, mucus-rich feces, abdominal pain, dyspepsia anorexia, nausea, and vomiting (Toledo et al. 2006a). Symptoms frequently subside spontaneously after 1 month although the flukes may remain for more than 1 year, and only episodic diarrhea may appear (Sripa et al. 2010). Several complications are associated with heterophyiasis. Anaphylaxis was developed by a woman after infection with *H. heterophyes* after eating raw fish in Spain (Martínez-Alonso et al. 1999). Occasionally worm eggs may enter the circulatory system via the crypts of Lieberkhün which may be fatal (Marty and Andersen 2000; Sripa et al. 2010). Eggs can be transported to other organs and they have been found in cardiac muscles in autopsies, spinal cord or brain, where they become trapped and elicit granulomatous lesions and fibrosis. Signs of heterophyid myocarditis may include subepicardial edema, epicardium hemorrhage, fragmentation of muscle fibers, cardiac enlargement, embolism of the capillaries, cough, dyspnea, cyanosis, fatigue, edema and ascites,

palpitation, loss of reflexes, and abnormal heart sounds. Eggs or worms in the spinal cord or brain may cause neurological disease, transverse myelitis, and loss of sensory and motor function (Sripa et al. 2010). Recently, *H. taichui* has been identified as a possible etiologic causative agent of irritable Bowel syndrome-like symptoms (Watthanakulpanich et al. 2010).

The adult worms parasitize mainly the duodenum though they may extend more distally in heavy infections (Lee et al. 1981). The pathology induced by heterophyids has been mainly studied in laboratory animals. Adult worms live embedded in the intestinal mucosa causing villous atrophy, hypertrophy of crypts of Lieberkhün, enlargement of mesenteric lymph nodes, and inflammatory responses with cell infiltration (Toledo et al. 2006a). Ashour et al. (2014) showed that atrophic villi covered by poorly differentiated epithelial cells were observed in the second week post-infection in mice with *H. heterophyes*. Moreover, marked hyperplasia of the intestinal crypts with abundant inflammatory cellular infiltrate in the lamina propria, as well as apoptosis of cells lining the intestinal villi was noted. Both caspase-3 and NF-jB showed positive staining in the intestinal epithelial cells with varying grades of intensity over the length of infection. In humans, Chi et al. (1988) studied the pathology of a human case of metagonimiasis. The parasitism was incidentally detected in an intestinal segment that was removed surgically for treating intestinal perforation related to a malignant histiocytosis. The adult worms of *M. yokogawai* were found free in the jejunal lumen as well as impacted in the intervillous spaces. The pathological findings in this human case were similar to those in the recovering phase of infection in experimental animals. The main histological lesions were massive lymphoplasmacytic and eosinophilic infiltration in the stroma, erosion of the enterocytes in the areas surrounding the worms, goblet cell depletion, and occasional villous edema. Sukontason et al. (2005) studied the pathology induced by *H. taichui* on three human cases revealing mucosal ulceration, mucosal and submucosal hemorrhages, fusion and shortening villi, chronic inflammation, and fibrosis of the submucosa.

7.8.4 Host–Parasite Relationships and Immunology

The fact that pathology induced by heterophyids is spontaneously restored indicated the development of host protective immunity. Several effector mechanisms have been suggested to act against heterophyids (Toledo et al. 2006a). Intraepithelial lymphocytes and the lamina propria lymphocytes acting as CD8+ cytotoxic and IgA producing cells, respectively, may be of importance. Moreover, mucosal mast cells and goblet cells appear to be also involved in the worm expulsion in rodents (Chai et al. 2009a). Regarding antibody responses, it is known that both systemic and local antibody responses occur in these infections. The specific IgG levels in the serum of *M. yokogawai*-infected cats rise from 7 dpi and the maximum levels were observed at 2–4 wpi with both antigens (Cho et al. 1987). Elevated levels of IgG, IgM, and IgE have been detected in the serum of humans infected with *H. heterophyes* (El-Ganayni et al. 1989; Martínez-Alonso et al. 1999; Pica et al. 2003). In the intestine, the levels of IgG, IgM, and IgA were increased (El-Ganayni et al. 1989). Systemic IgG response has also been detected in humans infected with *H. taichui* and *M. yokogawai*, respectively (Ditrich et al. 1991; Lee et al. 1993b). There is some controversy on the potential role on the antibody response in the course of heterophyid infections. An inverse relationship between the antibody levels and the load of the infection has been often observed, suggesting that humoral response may be involved in worm expulsion (El-Ganayni et al. 1989; Ditrich et al. 1991). In contrast to these findings, Cho et al. (1987) detected that specific IgG levels were directly related to the number of *M. yokogawai* adult worms in experimentally infected cats.

7.8.5 Diagnosis

Diagnosis can be done by detection of eggs in the feces. However, the detection and identification may be difficult because of: (1) similarity of the eggs of the heterophyids; (2) low-laying capacity of the parasite and (3) existence of extraintestinal

cases. Due to the similarity of the eggs of heterophyids, when eggs are detected it is usually referred to as "heterophyid infection." The specific diagnosis may require the recovery of adult worms after treatment and purgation (Chai et al. 2009a). Because of this fact, close examination of the egg sizes may be of help (Lee et al. 2012). Moreover, light infections can be easily missed since the egg laying capacity of the heterophyids is low (Sripa et al. 2010). For example, the daily egg output of *H. taichui* is estimated as 82 eggs/worm (Sato et al. 2010). More problematic is the diagnosis of the extraintestinal cases which only can be detected by surgery or autopsy.

Few studies have been done concerning the immunodiagnosis of heterophyid infections. ELISA and western-blot methods showed a good level of sensitivity but cross-reactivity with other trematodes occurred (Ditrich et al. 1991; Lee et al. 1993b). Similarly, several molecular-based methods for the diagnosis have been developed recently (Van Van et al. 2009; Wongsawad and Wongsawad 2009; Wongsawad et al. 2009a, b; Sato et al. 2010; Thaenkham et al. 2011).

7.9 Other Families of Intestinal Trematodes Infecting Humans

7.9.1 Family Cathaemaciidae

Only one species belonging to this family, *Cathaemacia cabrerai*, has been recorded infecting humans. A single case was reported in the Philippines (Jueco and Monzon 1984). No further information about this parasite is available.

7.9.2 Family Lecithodendriidae

The lecithodendriids are characterized by possessing an oval body and a spinose tegument. The testes are opposite and a cirrus sac is present. The position of the genital pore is variable, the vitellaria are in lateral clusters, and the eggs are operculate and embryonated when laid. Adults of these digeneans are parasites of the digestive tract of amphibians, birds, or mammals (typically bats). Three species of this family have been found infecting humans: *Prosthodendrium molenkampi, Phaneropsolus bonnei,* and *P. spinicirrus* (Fried et al. 2004).

P. molenkampi was first discovered infecting humans in Indonesia but thereafter, high prevalence was detected in Thailand reaching 19% (Radomyos et al. 1998). Moreover, human infections have been reported in Lao PDR (Chai et al. 2009b; Sayasone et al. 2009b). *P. bonnei* was first discovered concurrently with *P. molenkampi* in humans in Indonesia. Later, it was recorded in Malaysia, India, Thailand and, more recently, in Lao PDR (Chai et al. 2009a; Sayasone et al. 2009b). Metacercariae of both species have been detected in dragonflies and it is thought that people become infected after eating this odonata which is common in these areas (Chai et al. 2009a). This may explain that concurrent infections have been commonly reported (Sayasone et al. 2009b). Only one human infection by *P. spinicirrus* has been recorded in Thailand (Kaewkes et al. 1991). In general, lecithodendriids do not appear to produce clinical disease in humans (Fried et al. 2004).

7.9.3 Family Microphallidae

This family includes trematode parasites of vertebrates and is characterized by possession of a pyriform body, spinose tegument, short intestinal caeca, testes opposite in the hindbody, vitellaria posterior to the testes and the uterus confined to the hindbody. Eggs are operculated and non-embryonated. The life cycle includes three hosts: a mollusc, an arthropod, and a vertebrate. In some species, the cycle is abbreviated by eliminating the second intermediate (arthropod) host. In these species, the cercaria encysts within the sporocyst and the infected mollusc is eaten by the definitive host (Fried et al. 2004).

Two species of this family have been recorded in humans: *Spelotrema brevicaeca* and *Gynaecotyla squatarolae*. In *S. brevicaeca*, cercariae encyst in the abdomen and cephalothorax of shrimps or crabs, which appears to be the source of human infections. This species was reported as the cause of fatal involvement in the

heart and spinal cord of humans in the Philippines. Fluke eggs were found in the heart, brain, and spinal cord of persons who died of acute cardiac dilatation (Fried et al. 2004; Chai et al. 2009a).

The first human infection of *G. squatarolae* was recently reported in Korea (Chung et al. 2011). A 50-year-old female was found to be infected with this trematode concomitantly with other trematodes such as *G. seoi* and *H. pumilio*. It is known that *G. squatarolae* metacercariae encyst in crabs (*Macrophthalmus* spp.) and the woman had usually eaten brackish crabs in soy sauce.

7.9.4 Family Nanophyetidae

Only one species belonging to this family, *Nanophyetus salmincola*, has been recorded in humans. This parasite is characterized by a pyriform body and the presence of two large testes in the posterior half of the body (Fig. 7.8a). The life cycle of *N. salmincola* involves the release of non-embryonated eggs in the feces of the mammalian host. The miracidium hatches and penetrates the freshwater snail, *Oxytrema silicula*. Cercariae emerge from snails, penetrate the skin and gills of salmonid fishes, and encyst in the muscles and connective tissue. The adults develop in the intestine of mammals following ingestion of the infected fish (Fried et al. 2004).

Human nanophyetiasis is endemic in the far-eastern part of Russia with an average prevalence of 5% in some areas (Yu and Mott 1994). In local ethnic minorities, the prevalence is higher and reaches 60% in some localities (Chai et al. 2004). In the northwest USA, Eastburn et al. (1987) reported ten human infections. Patients became infected after eating improperly cooked salmon or trout. The main symptoms were gastrointestinal complaint of abdominal discomfort, diarrhea, nausea, and vomiting. Peripheral blood eosinophilia was also observed.

It should be noted that *Nanophyetus schikhobalowi* was described from natives of far-eastern Siberia though this fluke is currently considered as a subspecies of *N. salmincola* (Chai 2007).

7.9.5 Family Paramphistomidae

The family Paramphistomidae is restricted to paramphistomoid digeneans, parasitic in mammals, which lack pharyngeal sacs, a cirrus sac and a ventral pouch. However, this family has often been used as a repository with a varying number of subfamilies and there is confusion on its taxonomy.

Members of Paramphistomidae rarely infect humans. Only two species, *Fischoederius elongatus* and *Watsonius watsoni*, have been recorded. Information about *F. elongatus* is scarce. This is a parasite of humans in China (Yu and Mott 1994).

W. watsoni was first discovered in a patient from West Africa who died of severe diarrhea (Beaver et al. 1984). In both species, the source of infection it is suspected to be the ingestion of aquatic plants.

7.9.6 Family Plagiorchiidae

The members of this family are characterized by having a spinose tegument, the ventral sucker in the anterior half of the body, long intestinal caeca, testes opposite, tandem or oblique and located in the hindbody. A cirrus sac is present and the genital pore is anterior to the acetabulum. The ovary is pretesticular and the vitelline follicles are adjacent to the intestinal ceca and are variable in length (Fig. 7.8b). The eggs are operculate and embryonated (Schell 1985). The life cycle typically involves embryonated eggs being eaten by pulmonate snails, in which sporocysts develop and produce xiphidiocercariae. Emerged cercariae encyst in insect larvae, but encystment in freshwater fishes also occurs. Definitive hosts become infected after eating the second intermediate host harboring the cysts.

Occasional human infections with *Plagiorchis harinasutai* (in Thailand), *P. javensis* (in Indonesia), *P. muris* (Fig. 7.8b) (in Japan and Korea), and *P. philippinensis* (in the Philippines) have been recorded (Asada et al. 1962; Radomyos et al. 1989; Yu and Mott 1994; Hong et al. 1996).

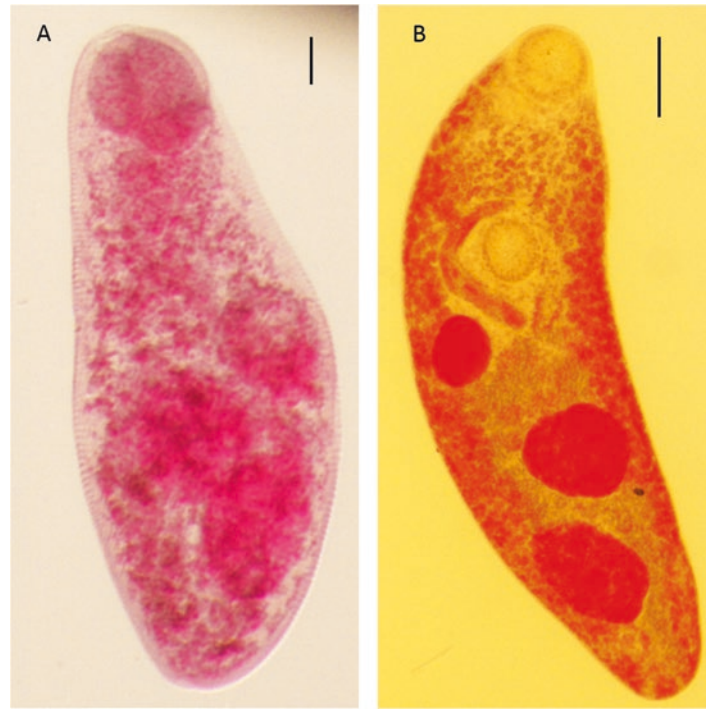

Fig. 7.8 Adult worms of (**a**) *Nanophyetus salmincola* (Scale bar: 100 μm); and (**b**) *Plagiorchis muris* (Scale bar: 200 μm)

7.9.7 Family Strigeidae

The family Strigeidae includes trematodes with a cup-shaped forebody and a tribocytic organ (an accessory sucker). The hindbody is cylindrical or ovoid and contains the reproductive organs. The short uterus contains operculate, non-embryonated eggs. The adult flukes are parasites of the intestine of birds and mammals (Fried et al. 2004).

Within this family only *Cotylurus japonicus* has been recorded parasitizing the intestine of humans. The first infection was reported from a 13-year-old girl in China (Chen and Cai 1985). Further details about the human infections are scarce and the source of the infection is unknown (Marty and Andersen 2000).

7.10 Treatment of Intestinal Trematode Infections

At present, the drug of choice for the treatment of intestinal trematode infections is praziquantel. Although the exact mechanism of action of praziquantel has not been elucidated, it has been postulated that a disruption of Ca2+ homestasis occurs as praziquantel induces a rapid contraction of the trematodes (Greenberg 2005). Praziquantel exhibits a broad spectrum against trematodes and has an excellent safety profile (Keiser and Utzinger 2004). All treatment schedules with praziquantel are well tolerated, with only few adverse events including abdominal pain, dizziness, headache, vomiting, nausea, and urticaria and less commonly may also appear rash, hypotension, and sudden expulsion of the worms which may produce obstruction (Keiser and Utzinger 2004; Fürst et al. 2012b). Furthermore, there are no reports of food-borne trematodes resistant to praziquantel. All the used treatments consist of a single dose of 25 mg/kg for echinostomiasis and heterophyiasis and 10 mg/kg for gymnophalloidiasis (Keiser and Utzinger 2004, 2010; Toledo et al. 2011, 2012). Other drugs such as mebendazole, thymol, carbon tetrachloride, and tetrachloroethylene also have been used.

The limited number of available drugs for food-borne trematodiases, together with the concerns in

relation to the development of resistance against these drugs, has encouraged the search for alternative drugs. In this context, preliminary studies have shown that a number of compounds might be further developed for the treatment of intestinal trematodiases using *Echinostoma caproni* as an experimental model (Keiser and Utzinger 2010). Artemisinin, the active constituent of the herb *Artemisia annua*, is a sesquiterpene lactone that contains an unusual peroxide bridge. In recent years, the activity of artemisinin and its derivatives including artemether, artesunate, and dihydroartemisinin against food-borne trematodes has been investigated in vitro and in vivo. In trematodes, it has been shown that artemether disrupts the tegument (Keiser and Utzinger 2009). Complete healing of *E. caproni* infections was achieved in mice using a single oral dose of 200 mg/kg (Keiser et al. 2006a). Recently, artesunate has successfully studied against heterophyids in experimentally infected mice (Fathy 2011). Due to the problems that artemisinins have in relation to their bioavailability, preparation, and pharmacokinetics, many synthetic peroxide analogues have been prepared and investigated. One of them, ozonide OZ78, has shown to be an effective flukicide in the rodent model. Complete *E. caproni* worm burden reduction was achieved in acute and chronic infections in mice using a dose of 1000 mg/kg (Keiser et al. 2006b). The increasing interest in medicinal plants as new sources of antiparasitic drugs has led to study several extracts as flukicides. Recently, Ferreira et al. (2011) showed that ethanolic extracts of *Artemisia annua, A. absinthium, and Asimina triloba* kill *E. caproni,* probably in relation to their elevated content in artemisinin and acetogenins. Garlic (*Allium sativum*) has been shown to have certain degree of efficacy against echinostomatids (Cortés et al. 2017b).

7.11 Control of the Intestinal Trematode Infections

Eating raw or improperly cooked freshwater fish and fresh or brackish water snails, snakes, bivalves, or aquatic vegetables should be avoided to prevent intestinal trematode infections. There is evidence that various types of marinades and food preparations commonly used may not affect the viability of the metacercariae. Various physical and chemical factors have been studied to determine their effects on the viability of encysted metacercariae of *E. caproni*. Viability was equated with chemical excystation in an alkaline trypsin-bile salts medium. Of numerous marinades tested, the one that was most harmful to isolated and in situ cysts was vinegar. Concentrated solutions of NaCl and sucrose had no effect on the viability of isolated and in situ cysts, suggesting that their use in food preparations for molluscs would not be effective in killing echinostomatid cysts in tainted snail tissues (Saxton and Fried 2009). Similarly, Wongsawad et al. (2005) studied the effect of several factors on the viability of the metacercaria of *S. falcatus*. The authors concluded that the worms were killed in NaCl at 20, 30, and 40% within 12, 6, and 2 h, respectively. Acetic acid at 5 and 10% killed the metacercaria within 12 and 6 h while at 20 and 30%, within 2 h. The killing effect of 3% vinegar was found within 18 h and of 5% vinegar within 12 h. Lemon juice showed no killing effect.

The nature of intestinal trematode infections does not justify the establishment of a separate control program because it can be controlled along with other food-borne diseases for which there are sustained WHO control programs. The control of human intestinal trematodiasis via blocking or interruption of the life cycle can be achieved through proper diagnosis, followed by pharmacologic treatment and prevention of reinfection. The control should be focused predominantly on a reduction or elimination of the transmission of the disease. In theory, the means of control in endemic regions can include: a reduction of the sources of infection, particularly human beings, through effective treatment; the protection of fish ponds and aquaculture systems from contamination with feces from people and other definitive hosts; the treatment or sterilization of feces; the control of snail host populations; and the implementation of education campaigns. Therefore, in principle, the prevention and control is relatively simple. Since infec-

tion of the definitive host is only contracted through the ingestion of metacercariae, the most practical measure for preventing and controlling human infection is to eliminate the consumption of raw, undercooked aliments susceptible to harbor the metacercariae. However, this strategy may be difficult to implement in some endemic regions because of the ancient eating traditions. Thus, only adequate cooking will render safe for human consumption. Nonetheless, together with education awareness programs focused particularly on teaching young children about the parasites, its life cycle, and the disease it causes, prevention and control could be successful. Targeting children may have the advantage that they are less entrenched than adults in their customs and eating habits. Significant changes for the control of intestinal trematodiasis may include: (1) the development of effective broad-spectrum anthelmintic, (2) an understanding by WHO of the differences between intestinal helminths and arthropod-borne infectious agents, and (3) the implementation of control programs in school-age children, with strong community therapy programs delivering multiple treatments against concurrent helminthic infections. A decreasing pattern in some food-borne trematode diseases along with industrialization, health education, and alteration of environment has been observed in certain areas of Southeast Asia. This is particularly true for Taiwan and mainland China, where industrial developments and wastewater discharges pollute streams and rivers, practically destroying those aquatic animals involved in trematode life cycles. The WHO control programs operating through the essential components of diagnosis, treatment, and prevention for the control of human zoonotic trematodiases have not been successful against several intestinal trematodiases although they have been for other trematode diseases.

7.12 Concluding Remarks

Despite the significant public health impact of the intestinal trematodiases, these diseases are among the most neglected tropical diseases. There was a general lack of knowledge about these infections and their causative species. Traditionally, intestinal trematode infections were considered as minor diseases confined to low-income countries, mainly in Asia, and the presence in the press media and funding research were practically inexistent. In recent years, this picture is changing by a number of factors. The number of infected is counted in the millions and an elevated percentage of the world's population is considered to live at risk for infection. Currently, the geographical limits and the population at risk are currently expanding and changing in relation to factors such as growing international markets, improved transportation systems, new eating habits in developed countries and demographic changes. Moreover, the number of species causing human intestinal trematode infections is high which makes difficult their correct knowledge, several severe endemic foci have been described and some species can cause serious health problems. This is aggravated since as occurs with other neglected tropical diseases, there are several gaps in our knowledge on the intestinal trematode infections.

In this context, new approaches to these diseases are needed. Current control strategies are reasonable and logical though approaches based on new technologies for training of health workers, treatment, diagnosis, and control of these diseases should be implemented. At present, a limited number of drugs are available, which is aggravated by the little incentive to invest in the discovery and development of new trematodicidal drugs. Moreover, the identification of parasite-specific proteins could clearly facilitate the design of new tools for rapid and cheap diagnosis, which may help to control the transmission of the parasite. In this context, the identification of potential targets for vaccination seems to be one of the best ways to control these parasite infections. The emergence of the intestinal trematodiases associated with the new risks of transmission introduced by the "globalized world" makes necessary the development of updated maps of distributions and risk of transmission and all these measures should also be accompanied by programs of training of health personnel and the population in general to change

risk eating habits. These approaches can provide the tools necessary for a proper understanding and control of the intestinal trematode infections.

Acknowledgements This work was supported supported by Ministerio de Economía y Competitividad (Madrid, Spain) (grant number: BFU2016-75639-P) and by Ministerio de Sanidad y Consumo (Madrid, Spain) (No. RD12/0018/0013, Red de Investigación Cooperativa en Enfermedades Tropicales—RICET, IV National Program of I+D+I 2008-2011, ISCIII—Subdirección General de Redes y Centros de Investigación Cooperativa and FEDER).

References

Agger MK, Simonsen PE, Vennervald BJ (1993) The antibody response in serum, intestinal wall and intestinal lumen of NMRI mice infected with *Echinostoma caproni*. J Helminthol 67:169–178

Asada JI, Otagaki H, Morita M, Takeuchi T, Sakai Y, Kojishi T, Okahashi K (1962) A case report on the human infection with *Plagiorchis muris* Tanabe, 1922 in Japan. Jpn J Parasitol 11:512–516

Ashour DS, Othman AA, Radi DA (2014) Insights into regulatory molecules of intestinal epithelial cell turnover during experimental infection by *Heterophyes heterophyes*. Exp Parasitol 143:48–54

Bandyopadhyay AK, Nandy A (1986) A preliminary observation on the prevalence of echinostomes in a tribal community near Calcutta. Ann Trop Med Parasitol 80:373–375

Bandyopadhyay AK, Manna B, Nandy A (1989) Human infection of *Artyfechinostomum oraoni* n. sp. (Paryphostominae: Echinostomatidae) in a tribal community, "Oraons" in West Bengal, India. Indian J Parasitol 13:191–196

Beaver PC, Jung RC, Cupp EW (1984) Clinical parasitology, 9th edn. Lea and Febiger, Philadelphia

Belizario VY, Geronilla GG, Anastacio MBM, de Leon WU, Suba-an AU, Sebastian AC, Bangs MJ (2007) *Echinostoma malayanum* infection, in the Philippines. Emerg Infect Dis 13:1130–1131

Bhattacharjee HK, Yadav D, Bagga D (2009) Fasciolopsis presenting as intestinal perforation: a case report. Trop Gastroenterol 30:40–41

Brunet LR, Joseph S, Dunne DW et al (2000) Immune responses during the acute stages of infection with the intestinal trematode *Echinostoma caproni*. Parasitology 120:565–571

Buckley JJC (1939) Observations on *Gastrodiscoides hominis* and *Fasciolopsis buski* in Assam. J Helminthol 17:1–12

Bunnag D, Radomyos P, Harinasuta T (1983) Field trial on the treatment of fasciolopsiasis with praziquantel. Southeast Asian J Trop Med Public Health 14:216–219

Butcher AR, Grove DI (2001) Description of the life-cycle stages of *Brachylaima cribbi* n. sp. (Digenea: Brachylaimidae) derived from eggs recovered from human faeces in Australia. Syst Parasitol 49:211–221

Butcher AR, Talbot GA, Norton RE, Kirk MD, Cribb TH, Forsyth JR, Knight B, Cameron AS (1996) Locally acquired *Brachylaima* sp. (Digenea: Brachylaimidae) intestinal fluke infection in two South Australian infants. Med J Aust 164:475–478

Butcher AR, Parasuramar PS, Thompson CS, Grove DI (1998) First report of the isolation of an adult worm of the genus *Brachylaima* (Digenea: Brachylaimidae), from the gastrointestinal tract of a human. Int J Parasitol 28:607–610

Butcher AR, Palethorpe HM, Grove DI (2002) Effects of sex and age on the susceptibility of C57BL/6J mice to infection with *Brachylaima cribbi* and the course of the infection in NOD SCID mice. Parasitol Res 88:668–674

Butcher AR, Palethorpe HM, Grove DI (2003) Response to re-infection with *Brachylaima cribbi* in immunocompetent and immunodeficient mice. Parasitol Int 52:219–228

Cao YH, Ma YM, Qiu F, Zhang XQ (2015) Rare cause of appendicitis: mechanical obstruction due to *Fasciolopsis buski i*nfestation. World J Gastroenterol 21:3146–3149

Carney WP (1991) Echinostomiasis—a snail-borne intestinal disease. Southeast Asian J Trop Med Public Health 22:206–211

Carney WP, Sudomo M, Purnomo A (1980) Echinostomiasis: a disease that disappeared. Trop Geogr Med 32:101–105

Chai JY (2007) Intestinal flukes. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites, vol 1. Springer, New York, pp 53–115

Chai JY (2009) Echinostomes in humans. In: Fried B, Toledo R (eds) The biology of Echinostomes. From the molecule to the community. Springer, New York, pp 147–183

Chai JY, Lee SH (1991) Intestinal trematodes infecting humans in Korea. Southeast Asian J Trop Med Public Health 22:163–170

Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 51:129–154

Chai JY, Seo BS, Lee SH, Hong SJ, Sohn WM (1986) Human infections by *Heterophyes heterophyes* and *H. dispar* imported from Saudi Arabia. Korean J Parasitol 24:82–88

Chai JY, Huh S, Yu JR (1993) An epidemiological study of metagonimiasis along the upper reaches of the Namhau River. Korean J Parasitol 31:99–108

Chai JY, Hong ST, Lee SH, Lee GC, Min YI (1994) A case of echinostomiasis with ulcerative lesions in the duodenum. Korean J Parasitol 32:201–204

Chai JY, Kim IM, Seo M, Guk SM, Kim JL, Sohn WM, Lee SH (1997) A new focus of *Heterophyes nocens*, *Pygidiopsis summa*, and other intestinal flukes in a

coastal area of Muan-gun, Chollanam-do. Korean J Parasitol 35:233–238

Chai JY, Kim TK, Cho WH, Seo M, Kook J, Guk SM, Lee SH (1998) Intestinal mastocytosis and goblet cell hyperplasia in BALB/c and C3H mice infected with *Neodiplostomum seoulense*. Korean J Parasitol 36:109–119

Chai JY, Chung WJ, Kook J, Seo M, Park YK, Guk SM, Choi MH, Lee SH (1999) Growth and development of *Gymnophalloides seoi* in immunocompetent and immunosupressed C3H/HeN mice. Korean J Parasitol 37:21–26

Chai JY, Lee HS, Hong SJ, Yoo JH, Guk SM, Seo M, Choi MH, Lee SH (2001) Intestinal histopathology and in situ postures of *Gymnophalloides seoi* in experimentally infected mice. Korean J Parasitol 39:31–41

Chai JY, Choi MH, Yu JR, Lee SH (2003) *Gymnophalloides seoi*, a new human intestinal trematode. Trends Parasitol 19:109–112

Chai JY, Park JH, Han ET, Shin EH, Kim JL, Guk SM, Hong KS, Lee SH, Rim HJ (2004) Prevalence of *Heterophyes nocens* and *Pygydiopsis summa* infections among residents in Western and Southern coastal islands of the Republic of Korea. Am J Trop Med Hyg 71:617–622

Chai JY, Shin EH, Lee SH, Rim HJ (2009a) Foodborne intestinal flukes in Southeast Asia. Korean J Parasitol 47:S69–S102

Chai JY, Han ET, Shin EH, Sohn WM, Yong TS, Eom KS, Min DY, Um JY, Park MS, Hoang EH, Phommasack B, Insisiengmay B, Lee SH, Rim HJ (2009b) High prevalence of *Haplorchis taichui, Phaneropsolus molenkampi,* and other helminth infections among people in Khammouane province, Lao PDR. Korean J Parasitol 47:243–247

Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Hoang EH, Phammasack B, Insisiengmay B, Rim HJ (2012) Echinostome flukes recovered from humans in Khammouane Province, Lao PDR. Korean J Parasitol 50:269–272

Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Lee MY, Lim H, Insisiengmay B, Phommasack B, Rim HJ (2013) Centrocestus formosanus (Heterophyidae): human infections and the infection source in Lao PDR. J Parasitol 99:531–536

Chai JY, Sohn WM, Cho J, Eom KS, Yong TS, Min DY, Hoang EH, Phommasack B, Insisiengmay B, Rim HJ (2018) *Echinostoma ilocanum* infection in two residents of Savannakhet Province, Lao PDR. Korean J Parasitol 56:75–79

Chang YD, Sohn WM, Ryu JH, Kang SY, Hong SJ (2005) A human infection of *Echinostoma hortense* in duodenal bulb diagnosed by endoscopy. Korean J Parasitol 43:57–60

Chen CE, Cai GD (1985) Morphological testimony: demonstration of a case of human infestation with *Cotylurus japonicus* Ishii, 1932 (Trematoda, Strigeidae). Bull Hum Med 10:31–34

Chen XQ, Feng GX, Qian ZF (1993) Survey on infection due to *Echinostoma hortense* in Liaoning Province. Chin J Parasitol Parasit Dis 11:226

Cheng YZ, Lin JX, Fang YY, Lin AQ, Chen BJ, Zhunag HJ (1992) Discovery of human infection with *Echinostoma angustitestis*. Chin J Zoonoses 8:7–8

Chi JG, Kim CW, Kim JR, Hong ST, Lee SH (1988) Intestinal pathology in human metagonimiasis with ultrastructural observations of parasites. J Korean Med Sci 3:171–177

Chieffi PP, Gorla MC, Torres DM, Dias RM, Mangini AC, Monteiro AV, Woiciechovski E (1992) Human infection by *Phagicola* sp. (Trematoda, Heterophyidae) in the *municipality* of Registro, São Paulo State, Brazil. J Trop Med Hyg 95:346–348

Cho SY, Kim SI, Kang SY (1987) Specific IgG responses in experimental cat metagonimiasis. Korean J Parasitol 25:149–153

Cho CM, Tak WY, Kweon YO, Kim SK, Choi YH, Kong HH, Chung DI (2003) A human case of *Echinostoma hortense* (Trematoda: Echinostomatidae) infection diagnosed by gastroduodenal endoscopy in Korea. Korean J Parasitol 41:117–120

Cho YK, Ryang YS, Kim IS, Park SK, Im JA, Lee KJ (2007) Differential immune profiles following experimental *Echinostoma hortense infection* in BALB/c and C3H/HeN mice. Parasitol Res 100:1053–1061

Cho SH, Cho PY, Lee DM, Kim TS, Kim IS, Hwang EJ, Na BK, Sohn WM (2010) Epidemiological survey on the infection of intestinal flukes in residents of Muan-gun, Jeollanam-do, the Republic of Korea. Korean J Parasitol 48:133–138

Chung PR, Jung Y, Kim DS (1996) *Segmentina (Polypylis) hemisphaerula* (Gastropoda: Planorbidae): a new molluscan intermediate host of a human intestinal fluke *Neodiplostomum seoulensis* (Trematoda: Diplostomatidae) in Korea. J Parasitol 82:336–338

Chung PR, Jung Y, Park YK, Hwang MG (2002) Host specificity of *Austropeplea ollula* (Gastropoda: Lymnaeidae) to miracidial infection with a human intestinal fluke *Neodiplostomum seoulense* (Trematoda: Diplostomatidae) in Korea. J Parasitol 88:630–631

Chung OS, Lee HJ, Kim YM, Sohn WM, Kwak SJ, Seo M (2011) First report of human infection with *Gynaecotyla squatarolae* and first Korean record of *Haplorchis pumilio* in a patient. Parasitol Int 60:227–229

Chunge RN, Chunge CN (2017) Infection with *Echinostoma* sp. in a group of travellers to Lake Tanganyika, Tanzania, in January 2017. J Travel Med 24(5): 1–3

Clarke MD, Carney WP, Cross JH, Hadidjaja P, Oemijati S, Joesoef A (1974) Schistosomiasis and other human parasitoses of Lake Lindu in Central Suwalesi (Celebes), Indonesia. Am J Trop Med Hyg 23:385–392

Cortés A, Sotillo J, Muñoz-Antoli C, Fried B, Esteban JG, Toledo R (2014) Intestinal IFN-γ production is associated with protection from clinical signs, but not with elimination of worms, in *Echinostoma caproni* infected-mice. Parasitol Res 113:2037–2045

Cortés A, Sotillo J, Muñoz-Antoli C, Fried B, Esteban JG, Toledo R (2015a) Altered protein expression in the ileum of mice associated with the development of chronic infections with *Echinostoma caproni* (Trematoda). PLoS Negl Trop Dis 9:e0004082

Cortés A, Muñoz-Antoli C, Martín-Grau C, Esteban JG, Grencis RK, Toledo R (2015b) Differential alterations in the small intestine epithelial cell turnover during acute and chronic infection with *Echinostoma caproni* (Trematoda). Parasit Vectors 8:334

Cortés A, Muñoz-Antoli C, Sotillo J, Fried B, Esteban JG, Toledo R (2015c) *Echinostoma caproni* (Trematoda): differential in vivo mucin expression and glycosylation in high- and low-compatible hosts. Parasite Immunol 37:32–42

Cortés A, Sotillo J, Muñoz-Antolí C, Trelis M, Esteban JG, Toledo R (2016) Definitive host influences the proteomic profile of excretory/secretory products of the trematode *Echinostoma caproni*. Parasit Vectors 9:185

Cortés A, Sotillo J, Muñoz-Antolí C, Molina-Durán J, Esteban JG, Toledo R (2017a) Antibody trapping: a novel mechanism of parasite immune evasion by the trematode *Echinostoma caproni*. PLoS Negl Trop Dis 11:e0005773

Cortés A, García-Ferrús M, Sotillo J, Esteban JG, Toledo R, Muñoz-Antolí C (2017b) Effects of dietary intake of garlic on intestinal trematodes. Parasitol Res 116:2119–2129

Cortés A, Muñoz-Antolí C, Álvarez-Izquierdo M, Sotillo J, Esteban JG, Toledo R (2018) Adaptation of the secretome of *Echinostoma caproni* may contribute to parasite survival in a Th1 milieu. Parasitol Res 117:947–957

Cremonte F, Pina S, Gilardoni C, Rodrigues P, Chai JY, Ituarte C (2013) A new species of gymnophallid (Digenea) and an amended diagnosis of the genus *Gymnophalloides* Fujita, 1925. J Parasitol 99:85–92

Cribb TH, Bray RA, Olson PD, Littlewood DT (2003) Life cycle evolution in the digenea: a new perspective from phylogeny. Adv Parasitol 54:197–254

Cross JH, Clarke MD, Cole WC (1976) Parasitic infection in humans in West Kalimantan (Borneo) Indonesia. Trop Geogr Med 28:121–130

De NV, Le TH (2011) Human infections of fish-borne trematodes in Vietnam: prevalence and molecular specific identification at an endemic commune in Nam Dinh province. Exp Parasitol 129:355–361

DeGirolami PC, Kimber J (1983) Intestinal parasites among Southeast Asian refugees in Massachusetts. Am J Clin Pathol 79:502–504

Ditrich O, Kopacek P, Giboda M, Gutvirth J, Scholz T (1991) Serological differentiation of human small fluke infections using *Opisthorchis viverrini* and *Haplorchis taichui* antigens. Southeast Asian J Trop Med Public Health 22:174–178

D'Souza PE, Jagannath MS, Prasanna KM (2001) A note on the occurrence of *Fasciolopsis buski* in pigs. Cheiron 30:178–179

Eastburn RL, Fritsche TR, Terhune CA (1987) Human intestinal infection with *Nanophyetus salminocola* from salmonoid fishes. Am J Trop Med Hyg 36:586–591

Eduardo SL (1991) Food-borne zoonoses in the Philippines. Southeast Asian J Trop Med Public Health 22:16–22

El-Ganayni GA, Youssef ME, Handousa AE, Bou-Zakham AA, Hegazi MM (1989) Serum and intestinal immunoglobulins in heterophyiasis. J Egypt Soc Parasitol 19:219–223

Eom KS, Son SY, Lee JS, Rim HJ (1985) Heterophyid trematodes (*Heterophyopsis continua, Pygidiopsis summa* and *Heterophyes heterophyes nocens*) from domestic cats in Korea. Korean J Parasitol 23:197–202

Esteban JG, Muñoz-Antoli C (2009) Echinostomes: systematics and life cycles. In: Fried B, Toledo R (eds) The biology of Echinostomes. From the molecule to the community. Springer, New York, pp 1–34

Fathy FM (2011) Anthelmintic effect of artesunate in experimental heterophyiid infection. J Egypt Soc Parasitol 41:469–483

Ferreira JFS, Peaden P, Keiser J (2011) In vitro trematocidal effects of crude alcoholic extracts of *Artemisia annua, A. absinthium, Asimina triloba* and *Fumaria officinalis*. Parasitol Res 109:1585–1582

Fried B, Toledo R (2004) Criteria for species determination in the "*revolutum*" group of *Echinostoma*. J Parasitol 90:917

Fried B, Graczyk TK, Tamang L (2004) Food-borne intestinal trematodiases in humans. Parasitol Res 93:159–170

Fujino T, Fried B (1993) Expulsion of *Echinostoma trivolvis* (Cort, 1914) Kanev, 1985 and retention of *E. caproni* Richard, 1964 (Trematoda, Echinostomatidae) in C3H mice: pathological, ultrastructural, and cytochemical effects on the host intestine. Parasitol Res 79:286–292

Fujino T, Fried B (1996) The expulsion of *Echinostoma trivolvis* from C3H mice: differences in glycoconjugates in mouse versus hamster small intestinal mucosa during infection. J Helminthol 70:115–121

Fujino T, Fried B, Tada I (1993) The expulsion of *Echinostoma trivolvis*: worm kinetics and intestinal cytopathology in conventional and congenitally athymic BALB/c mice. Parasitology 106:297–304

Fujino T, Fried B, Ichikawa H, Tada I (1996a) Rapid expulsion of the intestinal trematodes *Echinostoma trivolvis* and *E. caproni* from C3H mice by trapping with increased goblet cell mucins. Int J Parasitol 26:319–324

Fujino T, Ichikawa H, Fried B, Fukuda K (1996b) The expulsion of *Echinostoma trivolvis*: suppressive effects of dexamethasone on goblet cell hyperplasia and worm rejection in C3H/HeN mice. Parasite 3:283–289

Fürst T, Keiser J, Utzinger J (2012a) Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect Dis 12:210–221

Fürst T, Sayasone S, Odermatt P, Keiser J, Utzinger J (2012b) Manifestations, diagnosis, and management of foodborne trematodiasis. Br Med J 26:e4093

Gibson DI, Bray RA (1994) The evolutionary expansion and host-parasite relationship of the Digenea. Int J Parasitol 24:1213–1226

Gilman RH, Mondal G, Maskud M, Alam K, Rutherford E, Gilman JB, Khan MU (1982) Endemic focus of *Fasciolopsis buski* in Bangladesh. Am J Trop Med Hyg 31:796–802

Graczyk TK, Fried B (1994) ELISA method for detecting anti-*Echinostoma caproni* (Trematoda: Echinostomatidae) immunoglobulins in experimentally infected ICR mice. J Parasitol 80:544–549

Graczyk TK, Fried B (1995) An enzyme-linked immunosorbent assay for detecting anti-*Echinostoma trivolvis* (Trematoda) IgG in experimentally infected ICR mice. Cross-reactivity with *E. caproni*. Parasitol Res 81:710–712

Graczyk TK, Fried B (1998) Echinostomiasis: a common but forgotten food-borne disease. Am J Trop Med Hyg 58:501–504

Greenberg RM (2005) Are Ca2+ channels targets of praziquantel action? Int J Parasitol 35:1–9

Guk SM, Park JH, Shin EH, Kim JL, Lin A, Chai JY (2006) Prevalence of *Gymnophalloides seoi* infection in coastal villages of Haenamgun and Yeongam-gun, Republic of Korea. Korean J Parasitol 44:1–5

Guk SM, Lee JH, Kim HJ, Kim WH, Shin EH, Chai JY (2009) CD4+ T-cell-dependent goblet cell proliferation and expulsion of *Gymnophalloides seoi* from the intestine of C57BL/6 mice. J Parasitol 95:581–590

Han ET, Chen JH, Chai JY (2008) Antibody responses in sera of different mouse strains experimentally infected with *Neodiplostomum seoulense*. Korean J Parasitol 46:279–283

Haseeb MA, Eveland LK (2000) Human echinostomiasis: mechanisms of pathogenesis and host resistance. In: Fried B, Graczyk TK (eds) Echinostomes as experimental models for biological research. Kluwer Academic Publishers, Dordrecht, pp 83–98

Hirazawa I (1928) On *Echinochasmus perfoliatus* (Ratz) found in man. Tokyo Iji Shinshi 2577:1328–1334

Hong ST, Hong SJ, Lee SH, Seo BS, Chi JG (1982) Studies on intestinal trematodes in Korea: VI. On the metacercaria and the second intermediate host of *Fibricola seoulensis*. Korean J Parasitol 20:101–111

Hong SJ, Lee SH, Seo BS, Hong ST, Chai JY (1983) Studies on intestinal trematodes in Korea: IX. Recovery rate and development of *Fibricola seoulensis* in experimental animals. Korean J Parasitol 21:224–233

Hong ST, Chai JY, Lee SH (1984a) Ten human cases of *Fibricola seoulensis* infection and mixed one with *Stellantchasmus* and *Metagonimus*. Korean J Parasitol 24:95–97

Hong ST, Cho TK, Hong SJ, Chai JY, Lee SH, Seo BS (1984b) Fifteen human cases of *Fibricola seoulensis* infection in Korea. Korean J Parasitol 22:61–65

Hong SJ, Woo HC, Chai JY (1996) A human case of *Plagiorchis muris* (Tanabe, 1922: Digenea) infection in the Republic of Korea: freshwater fish as a possible source of infection. J Parasitol 82:647–649

Huffman JE, Iglesias D, Fried B (1988) *Echinostoma revolutum*: pathology of extraintestinal infection in the golden hamster. Int J Parasitol 18:873–874

Huh S, Hong ST (1993) Transmission electron microscopic findings of the tribocytic organ of *Fibricola seoulensis*. Korean J Parasitol 31:315–320

Huh S, Chai JY, Hong ST, Lee SH (1988) Clinical and histopathological findings in mice heavily infected with *Fibricola seoulensis*. Korean J Parasitol 26:45–53

Huh S, Lee SH, Seo BS (1990) Histochemical findings of the tribocytic organ and tegument of *Fibricola seoulensis*. Korean J Parasitol 28:155–160

Jaroonvesama N, Charoenlarp K, Areekul S (1986) Intestinal absorption studies in *Fasciolopsis buski* infection. Southeast Asian J Trop Med Public Health 17:582–586

Jueco NL, Monzon RB (1984) *Cathaemacia cabrerai* sp. n. (Trematoda: Cathaemaciidae) a new oarqasite of man in the Philippines. Southeast Asian J Trop Med Public Health 15:427–429

Jung WT, Lee KJ, Kim HJ, Kim TH, Na BK, Sohn WM (2014) A case of *Echinostoma cinetorchis* (Trematoda: Echinostomatidae) infection diagnosed by colonoscopy. Korean J Parasitol 52:287–290

Kaewkes S, Elkins DB, Haswell-Elkins MR, Sithithaworn P (1991) *Phaneropsolus spinicirrus* n. sp. (Digenea: Lecithodendriidae), a human parasite in Thailand. J Parasitol 77:514–516

Kawahara S, Yamamoto E (1933) Human cases of *Echinostoma cinetorchis*. Tokyo Iji Shinshi 2840:1794–1796

Keiser J, Utzinger J (2004) Chemotherapy for major food-borne trematodes: a review. Expert Opin Pharmacother 5:1711–1726

Keiser J, Utzinger J (2009) Food-borne trematodiases. Clin Microbiol Rev 22:466–483

Keiser J, Utzinger J (2010) The drugs we have and the drugs we need against major helminth infections. Adv Parasitol 73:197–230

Keiser J, Brun R, Fried B, Utzinger J (2006a) Trematocidal activity of praziquantel and artemisinin derivatives: in vitro and in vivo investigations on adult *Echinostoma caproni*. Antimicrob Agents Chemother 50:803–805

Keiser J, Utzinger J, Tanner M, Dong Y, Vennerstrom JL (2006b) The synthetic peroxide OZ78 is effective against *Echinostoma caproni* and *Fasciola hepatica*. J Antimicrob Chemother 58:1193–1197

Kim I, Im JA, Lee KJ, Ryang YS (2000) Mucosal mast cell responses in the small intestine of rats infected with *Echinostoma hortense*. Korean J Parasitol 38:139–143

Kim TY, Han KY, Shin EH, Chai JY (2008) Antigenic properties of cystatin-binding cysteine proteinases from *Neodiplostomum seoulense*. J Parasitol 94:654–658

Kino H, Suzuki Y, Oishi H, Suzuki S, Yamagiwa S, Ishiguro M (2006) Geographical distribution of *Metagonimus yokogawai,* and *M. miyatai* in Shizuoka Prefecture, Japan, and their site preferences in the sweetfish, *Plecoglossus altivelis*, and hamsters. Parasitol Int 55:201–206

Kook J, Nawa Y, Le SH, Chai JY (1998) Pathogenicity and lethality of a minute intestinal fluke, *Neodiplostomum seoulense*, to various strains of mice. Korean J Parasitol 84:1178–1183

Kostadinova A (2005) Family Echinostomatidae Looss, 1899. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 2. CABI Publishing, Wallingford, pp 9–64

Kostadinova A, Gibson DI (2000) The systematics of the Echinostomes. In: Fried B, Graczyk TK (eds) Echinostomes as experimental models for biological research. Kluwer Academic Publishers, Dordrecht, pp 31–57

Kusharyono C, Sukartinah S (1991) The current status of foodborne parasitic zoonoses in Indonesia. Southeast Asian J Trop Med Public Health 22:8–10

Lee JB, Chi JG, Lee SK, Cho SY (1981) Study on the pathology of metagonimiasis in experimentally infected cat intestine. Korean J Parasitol 19:109–129

Lee SH, Yoo BH, Hong ST, Chai JY, Seo BS, Chi JG (1985) A histopathological study on the intestine of mice and rats experimentally infected by *Fibricola seoulensis*. Korean J Parasitol 23:58–72

Lee SK, Chung NS, Ko IH, Ko HI, Chai JY (1986) Two cases of natural human infection by *Echinostoma hortense*. Kisaengchunghak Chapchi 24:77–81

Lee SK, Chung NS, Ko IH (1988) An epidemiological survey of *Echinostoma hortense* infection in Chongsong-gun, Kyongbuk Province. Korean J Parasitol 26:199–206

Lee SH, Chai JY, Hong ST (1993a) *Gymnophalloides seoi* n. sp. (Digenea: Gymnophallidae), the first report of human infection by a gymnophallid. Korean J Parasitol 79:677–680

Lee SC, Chung YB, Kong Y, Kang SY, Cho SY (1993b) Antigenic protein fractions of *Metagonimus yokogawai* reacting with patient sera. Korean J Parasitol 31:43–48

Lee SH, Chai JY, Seo M, Choi MH, Kim DC, Lee SK (1995) Two cases of *Gymnophalloides seoi* infection accompanied by diabetes mellitus. Korean J Parasitol 33:61–64

Lee SH, Sohn WM, Hong SJ, Huh S, Seo M, Choi MH, Chai JY (1996) A nationwide survey of naturally produced oysters for infection with *Gymnophalloides seoi* metacercariae. Korean J Parasitol 34:107–112

Lee JC, Kong Y, Lee SU, Huh S (1997a) Localization of worm antigen in *Neodiplostomum seoulense* by immuno-electronmicroscopy. Korean J Parasitol 35:95–104

Lee SH, Park SK, Seo M, Guk SM, Choi MH, Chai JY (1997b) Susceptibility of various species of animals and strains of mice to *Gymnophalloides seoi* infection and the effects of immunosuppression in C3H/HeN mice. J Parasitol 83:883–886

Lee JJ, Kim HJ, Kim MJ, Yi Lee JW, Jung BK, Lee JY, Shin EH, Kim JL, Chai JY (2008) Decrease of *Metagonimus yokogawai* endemicity along the Tamjin River basin. Korean J Parasitol 46:289–291

Lee KJ, Jin D, Chang BS, Teng YC, Kim DH (2009) The immunological effects of electrolyzed reduced water on the *Echinostoma hortense* infection in C57BL/6 mice. Biol Pharm Bull 32:456–462

Lee KD, Guk SM, Chai JY (2010) Toll-like receptor 2 and Muc2 expression on human intestinal epithelial cells by *Gymnophalloides seoi* adult antigen. J Parasitol 96:58–66

Lee JJ, Jung BK, Lim H, Lee MY, Choi SY, Shin EH, Chai JY (2012) Comparative morphology of minute intestinal fluke eggs that can occur in human stools in the Republic of Korea. Korean J Parasitol 50:207–213

Lee JJ, Kim D, Pyo KH, Kim MK, Kim HJ, Chai JY, Shin EH (2013) STAT6 expression and IL-13 production in association with goblet cell hyperplasia and worm expulsion of *Gymnophalloides seoi* from C57BL/6 mice. Korean J Parasitol 51:589–594

Lee SH, Jung BK, Park JH, Shin EH, Chai JY (2014) Increased intestinal epithelial cell turnover and intestinal motility in *Gymnophalloides seoi*-infected C57BL/6 mice. Korean J Parasitol 52:273–280

Liang C, Ke XL (1988) *Echinochasmus jiufoensis* sp. nov., a human parasite from Guangzhou (Trematoda: Echinostomatidae). Acta Zootaxa Sin 13:4–8

Lu SC (1982) Echinostomiasis in Taiwan. Int J Zoonoses 9:33–38

Mabus J, Huffman JE, Fried B (1988) Humoral and cellular response to infection with *Echinostoma revolutum* in the golden hamster, *Mesocricetus auratus*. J Helminthol 62:127–132

Mahanta J, Narain K, Srivastava VK (1995) Heterophyid eggs in human stool samples in Assam: first report for India. J Commun Dis 27:142–145

Majima M (1927) On *Echinostoma macrorchis* found in man. Kumamoto Igakkai Zasshi 2252:2260

Manning GS, Brockelman WY, Yiyant V (1971) An analysis of the prevalence of *Fasciolopsis buski* in central Thailand using catalyitic models. Am J Epidemiol 93:354–360

Martínez-Alonso JC, Armentia A, Vega JM, Callejo A, Gómez A (1999) Anaphylactic reaction concurrent with *Heterophyes heterophyes* infestation. Rev Esp Alergol Immunol Clín 14:37–39

Marty AM, Andersen EM (2000) Fasciolopsiasis and other intestinal trematodiasis. In: Meyers WM, Neafie RC, Marty A, Wear DJ (eds) Pathology of infectious diseases, vol I. Helminthiases. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC

Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278

Mas-Coma S, Bargues MD, Valero MA (2006) Gastrodiscoidiasis, a plant-borne zoonotic dis-

ease caused by the intestinal amphistome fluke *Gastrodiscoides hominis* (Trematoda: Gastrodiscidae). Rev Iber Parasitol 66:75–81

Muñoz-Antoli C, Sotillo J, Monteagudo C, Fried B, Marcilla A, Toledo R (2007) Development and pathology of *Echinostoma caproni* in experimentally infected mice. J Parasitol 93:854–859

Muñoz-Antoli C, Cortés A, Martín-Grau C, Fried B, Esteban JG, Toledo R (2016a) Partial resistance to homologous challenge infections of the digenean *Echinostoma caproni* in ICR mice. J Helminthol 90:428–433

Muñoz-Antoli C, Cortés A, Santano R, Sotillo J, Esteban JG, Toledo R (2016b) Interleukin-25 induces resistance against intestinal Trematodes. Sci Rep 6:34142

Muttalib MA, Islam N (1975) *Fasciolopsis buski* in Bangladesh: a pilot study. J Trop Med Hyg 78:135–137

Niewiadomska K (2002) Family Diplostomidae Poirier, 1886. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CABI Publishing, Wallingford, pp 167–198

Park CJ, Kim J (2006) A human case of *Echinostoma hortense* infection diagnosed by endoscopy in area of southwestern Korea. Korean J Med 71:229–234

Park KY, Lee KJ, Kim IS (2005) Reaction of mast cells and goblet cells in the small intestine of C57BL/6 and C3H/HeN mice infected with *Echinostoma hortense*. Korean J Biomed Lab Sci 11:259–266

Pica R, Castellano C, Cilia C, Errico FO (2003) Intestinal fluke infections: the heterophyids. Clin Ter 154:61–63

Poland GA, Navin TR, Sarosi GA (1985) Outbreak of parasitic gastroenteritis among travelers returning from Africa. Arch Intern Med 145:2220–2221

Pyo KH, Kang EY, Jung BK, Moon JH, Chai JY, Shin EH (2012) Depressed neuronal growth associated protein (GAP)-43 expression in the small intestines of mice experimentally infected with *Neodiplostomum seoulense*. Korean J Parasitol 50:89–93

Quang TD, Duong TH, Richard-Lenoble D, Odermatt P, Khammanivong K (2008) [Emergence in humans of fascioliasis (from *Fasciola gigantica*) and intestinal distomatosis (from *Fasciolopsis buski*) in Laos]. Sante 18:119–124 (in French)

Radomyos P, Bunnag D, Harinasuta T (1985) Report of *Episthmium caninum* (Verma, 1935) Yamaguti, 1958 (Digenea: Echinostomatidae) in man. Southeast Asian J Trop Med Public Health 16:508–511

Radomyos P, Bunnag D, Harinasuta C (1989) A new intestinal fluke, *Plagiorchis harinasutai*, n. sp. Southeast Asian J Trop Med Public Health 20:101–107

Radomyos P, Charoenlarp P, Harinasuta T (1991) Human *Episthmium caninum* (Digenea: Echinostomatidae) infection: report of two more cases. J Trop Med Parasitol 14:48–50

Radomyos B, Wongsaroj T, Wilrairatana P, Radomyos P, Praevanich R, Meesomboon V, Jongsuksuntikul P (1998) Opisthorchiasis and intestinal fluke infections in northern Thailand. Southeast Asian J Trop Med Public Health 29:123–127

Rahman KM, Idris M, Khan AKA (1981) A study of fasciolopsiasis in Bangladesh. J Trop Med Hyg 84:81–86

Ryang YS, Ahn YK, Kim WT, Shin KC, Lee KW, Kim TS (1986) Two cases of human infection by *Echinostoma cinetorchis*. Kisaengchunghak Chapchi 24:71–76

Ryang YS, Yang EJ, Kim JL, Lee KJ, Sung HJ, Kim JB, Kim IS (2007) Immune response and inhibitory effect of ketotifen on the BALB/c and C3H/HeN mice infected with *Echinostoma hortense*. Parasitol Res 101:1103–1110

Sah R, Khadka S, Hamal R, Poudyal S (2018) Human echinostomiasis: a case report. BMC Res Notes 11:17

Sato M, Pongvongsa T, Sanguankiat S, Yoonuan T, Dekumyoy P, Kalambaheti T, Keomoungkhoun M, Phimmayoi I, Boupha B, Moji K, Waikagul J (2010) Copro-DNA diagnosis of *Opisthorchis viverrini* and *Haplorchis taichui* infection in an endemic area of Lao PDR. Southeast Asian J Trop Med Public Health 41:28–35

Saxton T, Fried B (2009) An update on metacercarial excystment of trematodes. Parasitol Res 105:1185–1191

Sayasone S, Tesana S, Utzinger J, Hatz C, Akkhavong K, Odermatt P (2009a) Rare human infection with the trematode *Echinochasmus japonicus* in Lao PDR. Parasitol Int 58:106–109

Sayasone S, Vonghajack Y, Vanmany M, Rasphone O, Tesana S, Utzinger J, Akkhavong K, Odermatt P (2009b) Diversity of human intestinal helminthiasis in Lao PDR. Trans R Soc Trop Med Hyg 103:247–254

Sayasone S, Mak TK, Vanmany M, Rasphone O, Vounatsou P, Utzinger J, Akkhavong K, Odermatt P (2011) Helminth and intestinal protozoa infections, multiparasitism and risk factors in Champasack province, Lao People's Democratic Republic. PLoS Negl Trop Dis 5:e1037

Schell SC (1985) Trematodes of North America, north of Mexico. University Press of Idaho, Idaho

Seo BS, Rim HJ, Lee CW (1964) Studies on the parasitic helmiths of Korea: I. Trematodes of rodents. Korean J Parasitol 2:20–26

Seo ES, Cho SY, Chai JY (1980) Studies on intestinal trematodes in Korea. I. A human case of *Echinostoma cinetorchis* infection with an epidemiological investigation. Seoul J Med 21:21–29

Seo BS, Lee SH, Cho SY, Chai JY, Hong ST, Han IS, Sohn JS, Cho BH, Ahn SR, Lee SK, Chung SC, Kang KS, Shim HS, Hwang IS (1981) An epidemiologic study on clonorchiasis and metagonimiasis in riverside areas in Korea. Korean J Parasitol 19:137–150

Seo BS, Lee SH, Hong ST, Hong SJ, Kim CY, Lee HY (1982) Studies on intestinal trematodes in Korea: V. A human case infected by *Fibricola seoulensis* (Trematoda: Diplostomatidae). Korean J Parasitol 20:93–99

Seo BS, Lee SH, Chai JY, Hong SJ, Hong ST (1988) The life cycle and larval development of *Fibricola seoulensis* (Trematoda: Diplostomatidae). Korean J Parasitol 26:179–188

Seo M, Guk SM, Han ET, Chai JY (2003) Role of intestinal goblet cells in the expulsion of *Gymnophalloides seoi* from mice. J Parasitol 89:1080–1082

Seo M, Chun H, Ahn G, Jang KT, Guk SM, Chai JY (2006) A case of colonic lymphoid tissue invasion by *Gymnophalloides seoi* in a Korean man. Korean J Parasitol 44:87–89

Sheir ZM, Aboul-Enein ME (1970) Demographic, clinical and therapeutic appraisal of heterophyiasis. J Trop Med Hyg 73:148–152

Shin EH, Kim TH, Hong SJ, Park JH, Guk SM, Chai JY (2003) Effects of anti-allergic drugs on intestinal mastocytosis and worm expulsion of rats infected with *Neodiplostomum seoulense*. Korean J Parasitol 41:81–87

Shin EH, Lee SH, Kim JL, Chai JY (2007) T-helper-1 and T-helper-2 immune responses in mice infected with the intestinal fluke *Neodiplostomum seoulense*: their possible roles in worm expulsion and host fatality. J Parasitol 93:1036–1045

Shin EH, Im TK, Park YK, Cho J, Kim JL, Chai JY (2016) Fecundity reduction of BALB/c mice after survival from lethal *Neodiplostomum seoulense* infection. Parasitol Res 115:2051–2059

Shoop WL (1989) Experimental human infection with *Fibricola cratera* (Trematoda: Neodiplostomidae). Korean J Parasitol 27:249–252

Shyu LY, Lee HH, Chen ER (1984) A preliminary study on epidemiology of fasciolopsiasis in Tainan Hsien, south Taiwan. Chin J Microbiol Immunol 17:118–120

Sohn WM (2009) Fish-borne zoonotic trematode metacercariae in the Republic of Korea. Korean J Parasitol 47:S103–S113

Sohn WM, Ryang YS, Chai JY, Lee SH (1998) Discovery of *Gymnophalloides seoi* metacercariae in oysters from islands of the West Sea known as the habitats of paleartic oystercatchers. Korean J Parasitol 36:163–169

Sohn WM, Kim HJ, Yong TS, Eom KS, Jeong HG, Kim JK, Kang AR, Kim MR, Park JM, Ji SH, Sinuon M, Socheat D, Chai JY (2011a) *Echinostoma ilocanum* infection in Oddar Meanchey Province, Cambodia. Korean J Parasitol 49:187–190

Sohn WM, Chai JY, Yong TS, Eom KS, Yoon CH, Sinuon M, Socheat D, Lee SH (2011b) *Echinostoma revolutum* infection in children, Pursat Province, Cambodia. Emerg Infect Dis 17:117–119

Sotillo J, Muñoz-Antoli C, Marcilla A, Fried B, Guillermo Esteban J, Toledo R (2007) *Echinostoma caproni*: kinetics of IgM, IgA and IgG subclasses in the serum and intestine of experimentally infected rats and mice. Exp Parasitol 116:390–398

Sotillo J, Valero L, Sánchez del Pino MM, Fried B, Esteban JG, Marcilla A, Toledo R (2008) Identification of antigenic proteins from *Echinostoma caproni* (Trematoda: Echinostomatidae) recognized by mouse immunoglobulins M, A and G using an immunoproteomic approach. Parasite Immunol 30:271–279

Sotillo J, Trelis M, Cortés A, Fried B, Marcilla A, Esteban JG, Toledo R (2011) Th17 responses in *Echinostoma caproni* infections in hosts of high and low compatibility. Exp Parasitol 129:307–311

Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ (2010) Food-borne trematodiases in Southeast Asia epidemiology, pathology, clinical manifestation and control. Adv Parasitol 72:305–350

Sukontason K, Unpunyo P, Sukontason KL, Piangiai S (2005) Evidence of *Haplorchis taichui* infection as pathogenic parasite: three case reports. Scand J Infect Dis 37:388–390

Takahashi S, Ishii T, Ueno N (1930) A human case of *Echinostoma cinetorchis*. Tokyo Iji Shinshi 2757:141–144

Tantrawatpan C, Saijuntha W, Sithithaworn P, Andrews RH, Petney TN (2013) Genetic differentiation of Artyfechinostomum *malayanum* and *A. sufrartyfex* (Trematoda: Echinostomatidae) based on internal transcribed spacer sequences. Parasitol Res 112:437–441

Thaenkham U, Phuphisut O, Pakdee W, Homsuwan N, Sa-nguankiat S, Waikagul J, Nawa Y, Dung do T (2011) Rapid and simple identification of human pathogenic heterophyid intestinal fluke metacercariae by PCR-RFLP. Parasitol Int 60:503–506

Toledo R (2009) Immunology and pathology of echinostomes in the definitive host. In: Fried B, Toledo R (eds) The biology of Echinostomes. From the molecule to the community. Springer, New York, pp 185–206

Toledo R, Esteban JG (2016) An update on human echinostomiasis. Trans R Soc Trop Med Hyg 110:37–45

Toledo R, Fried B (2005) Echinostomes as experimental models for interactions between adult parasites and vertebrate hosts. Trends Parasitol 21:251–254

Toledo R, Espert A, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2003) Development of an antibody-based capture enzyme-linked immunosorbent assay for detecting *Echinostoma caproni* (Trematoda) in experimentally infected rats: kinetics of coproantigen excretion. J Parasitol 89:1227–1231

Toledo R, Espert A, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2004) Kinetics of *Echinostoma caproni* (Trematoda: Echinostomatidae) antigens in feces and serum of experimentally infected hamsters and rats. J Parasitol 90:752–758

Toledo R, Espert A, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2005) Kinetics of antibodies and antigens in serum of mice experimentally infected with *Echinostoma caproni* (Trematoda: Echinostomatidae). J Parasitol 91:978–980

Toledo R, Esteban JG, Fried B (2006a) Immunology and pathology of intestinal trematodes in their definitive hosts. Adv Parasitol 63:285–365

Toledo R, Monteagudo C, Espert A, Fried B, Esteban JG, Marcilla A (2006b) *Echinostoma caproni*: intestinal pathology in the golden hamster, a highly compatible host, and the Wistar rat, a less compatible host. Exp Parasitol 112:164–171

Toledo R, Esteban JG, Fried B (2009) Recent advances in the biology of echinostomes. Adv Parasitol 69:147–204

Toledo R, Bernal MD, Marcilla A (2011) Proteomics of foodborne trematodes. J Proteome 74:1485–1503

Toledo R, Esteban JG, Fried B (2012) Current status of food-borne trematode infections. Eur J Clin Microbiol Infect Dis 31:1705–1718

Trelis M, Sotillo J, Monteagudo C, Fried B, Marcilla A, Esteban JG, Toledo R (2011) *Echinostoma caproni* (Trematoda): differential in vivo cytokine responses in high and low compatible hosts. Exp Parasitol 127:387–397

Trung Dung D, Van De N, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, Murrell KD (2007) Fishborne zoonotic intestinal trematodes, Vietnam. Emerg Infect Dis 13:1828–1833

Uppal B, Wadhwa V (2005) Rare case of *Metagonimus yokogawai*. Indian J Med Microbiol 23:61–62

Van Van K, Dalsgaard A, Blair D, Le TH (2009) *Haplorchis pumilio* and *H. taichui* in Vietnam discriminated using ITS-2 DNA sequence data from adults and larvae. Exp Parasitol 123:146–151

Watthanakulpanich D, Waikagul J, Maipanich W, Nuamtanong S, Sanguankiat S, Pubampen S, Praevanit R, Mongkhonmu S, Nawa Y (2010) *Haplorchis taichui* as a possible etiologic agent of irritable bowel syndrome-like symptoms. Korean J Parasitol 48:225–229

Weinstein MS, Fried B (1991) The expulsion of *Echinostoma trivolvis* and retention of *Echinostoma caproni* in the ICR mouse: pathological effects. Int J Parasitol 21:255–257

Weng YL, Zhuang ZL, Jiang HP, Lin GR, Lin JJ (1989) Studies on ecology of *Fasciolopsis* buski and control strategy of fasciolopsiasis. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 7:108–111

Wongsawad P, Wongsawad C (2009) Development of PCR-based diagnosis of minute intestinal fluke, *Haplorchis taichui*. Southeast Asian J Trop Med Public Health 40:919–923

Wongsawad C, Kawin S, Wongsawad P, Paratasilpin T (2005) Some factors affecting *Stellantchasmus falcatus* metacercaria in laboratory. Southeast Asian J Trop Med Public Health 36:11711–11719

Wongsawad C, Wongsawad P, Chai JY, Anuntalabhochai S (2009a) *Haplorchis taichui*, Witenberg, 1930: development of a HAT-RAPD marker for the detection of minute intestinal fluke infection. Exp Parasitol 123:158–161

Wongsawad C, Wongsawad P, Chuboon S, Anuntalabhochai S (2009b) Copro-diagnosis of *Haplorchis taichui* infection using sedimentation and PCR-based methods. Southeast Asian J Trop Med Public Health 40:924–928

Xiao X, Wang TP, Lu DB, Gao JF, Xu LF, Wu WD, Mei JD, Xu FL (1992) The first record of human infection with *Echinochasmus liliputanus*. Chin J Parasitol Parasit Dis 10:132–135

Xiao X, Dabing L, Tianping W, Gao J, Zhu C, Zhang B, An J, Pong H, Xu M, Wu W (1995) Studies on mode of human infection with *Echinochasmus liliputanus*. Chin J Parasitol Parasit Dis 13:197–199

Yamaguti S (1971) Synopsis of digenetic trematodes of vertebrates. Keigaku Publishing Company, Japan

Yamaguti S (1975) A synoptical review of the life histories of digenetic trematodes of vertebrates: with special reference to the morphology of their larval forms. Keigaku Publishing Company, Japan

Yokogawa M, Harinasutam C, Charoenlarp P (1965) *Hypoderaeum conoideum* (Block, 1782) Diez, 1909, a common intestinal fluke of man in the north-east Thailand. Jpn J Parasitol 14:148–153

Youn H (2009) Review of zoonotic parasites in medical and veterinary fields in the Republic of Korea. Korean J Parasitol 47:S133–S141

Yu S, Mott K (1994) Epidemiology and morbidity of food-borne intestinal trematode infections. Trop Dis Bull 91:R125–R152

Yu JR, Hong ST, Chai JY, Lee SH (1995) The effect of reinfection with *Neodiplostomum seoulensis* on the histopathology and activities of brush border membrane bound enzymes in the rat small intestine. Korean J Parasitol 33:37–43

Part III

Trematodes of Interest in Veterinary and Wildlife Disease

Schistosomatoidea and Diplostomoidea

8

Petr Horák, Jana Bulantová, and Libor Mikeš

8.1 Introduction

This chapter is focused on important non-human parasites of the order Diplostomida sensu (Olson et al. 2003). Although recent mitochondrial genomic data suggested the order to be paraphyletic, phylogenies based on ultra-conserved genomic elements support its validity. This mitonuclear discordance might be related to ancient, rapid radiation in the Digenea (Locke et al. 2018). Members of the superfamilies Schistosomatoidea (Schistosomatidae, Aporocotylidae, and Spirorchiidae) and Diplostomoidea (with focus on Diplostomidae and Strigeidae) will be characterized. All these flukes have indirect life cycles with cercariae having the ability to penetrate body surfaces of invertebrate/vertebrate intermediate hosts or vertebrate definitive hosts. In some cases, invasions of accidental (non-compatible) vertebrate hosts (including humans) are also reported. Penetration of the host body, migration to the target tissues/organs, and/or egg laying/deposition frequently induce pathological changes in the tissues and, therefore, outbreaks of infections caused by these parasites in animal farming/breeding may lead to economic losses.

P. Horák (✉) · J. Bulantová · L. Mikeš
Department of Parasitology, Faculty of Science, Charles University, Prague 2, Czechia
e-mail: petr.horak@natur.cuni.cz; jana.bulantova@natur.cuni.cz; libor.mikes@natur.cuni.cz

8.2 Schistosomatidae

Members of the family Schistosomatidae are exceptional organisms among digenean trematodes: they are gonochoristic, with males and females mating in the blood vessels of definitive hosts. As for other trematodes, only some members of Didymozoidae are gonochoristic, and only adults of Aporocotylidae and Spirorchiidae inhabit blood circulation of their hosts. The family Schistosomatidae comprises 14 genera parasitizing mammalian and avian hosts. Besides the genus *Schistosoma* having medical and veterinary importance (human and mammalian parasites), three genera (*Bivitellobilharzia*, *Heterobilharzia*, *Schistosomatium*) infect mammals, and ten genera (*Allobilharzia*, *Anserobilharzia*, *Austrobilharzia*, *Bilharziella*, *Dendritobilharzia*, *Gigantobilharzia*, *Jilinobilharzia*, *Macrobilharzia*, *Ornithobilharzia*, *Trichobilharzia*) cause avian diseases (Figs. 8.1, 8.2, 8.3, 8.4, and 8.5).

8.2.1 Bird Schistosomes

8.2.1.1 Life Cycle

Bird schistosomes have a two-host life cycle. Generally, the eggs containing mature miracidia are released via feces from the infected host to the water environment where miracidia hatch in a while. In case of *Trichobilharzia regenti*, however, the miracidia hatch directly in the host

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_8

Fig. 8.1 (**a**) Adult male and (**b**) adult female of *Bilharziella polonica* as an example of avian schistosome species with dorsoventrally flattened body, which is in contrast to slender body plan of most schistosomes; note the absence of canalis gynaecophorus in the male; carmine staining; scale bars 500 μm. (**c**) The intermediate hosts of *B. polonica* are planorbid snails, here represented by *Planorbarius corneus*. (Samples (**a**) and (**b**) are deposited in the Helminthological Collection, see Acknowledgements)

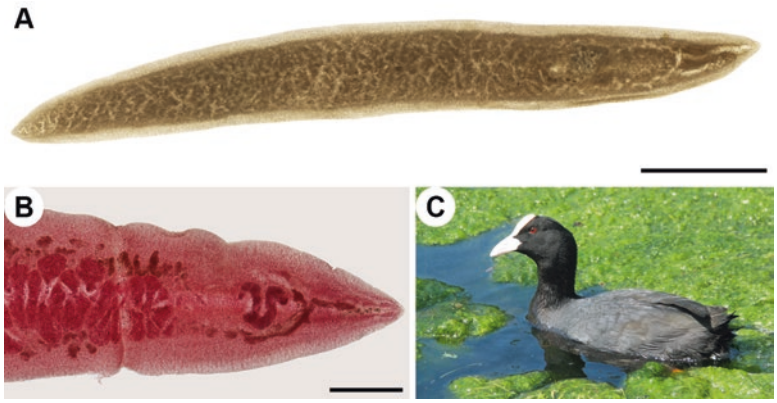

Fig. 8.2 (**a**) Adult *Dendritobilharzia pulverulenta* with dorsoventrally flattened body; scale bar 1 mm. (**b**) Detail of the anterior part of *D. pulverulenta* stained with carmine; scale bar 200 μm. (**c**) The most common definitive host of *D. pulverulenta*—the common coot (*Fulica atra*). (Sample (**a**) has been provided by Dr. J. Sitko, Moravian Ornithological Station, Comenius Museum; Sample (**b**) is deposited in the Helminthological Collection, see Acknowledgements)

nasal tissue and leave the host upon contact with water. Miracidia are active swimmers and seek after appropriate intermediate snail host. In order to find the host, miracidia possess receptors to be able to respond to physical factors (gravity, light) as well as chemical cues. Concerning the latter stimuli, carbohydrates bound in miracidia-attracting glycoproteins (MAGs, miraxons) emitted by the snail hosts seem to serve for chemoorientation and host recognition by *Trichobilharzia szidati* (syn. *Trichobilharzia ocellata*) (Kalbe et al. 1997; Haas 2003).

Subsequent larval development of bird schistosomes, characterized by an asexual multiplication in intermediate hosts, proceeds in freshwater, brackish, or saltwater snails. The details of intramolluscan development are known for a limited number of bird schistosomes. With regard to morphogenesis of intramolluscan larval stages, and also their influence on snail physiology and immunity, *T. szidati* seems to be the most studied model (Horák and Kolářová 2005). The parasite inhibits certain immune functions, such as phagocytosis, efficiency of encapsulation and

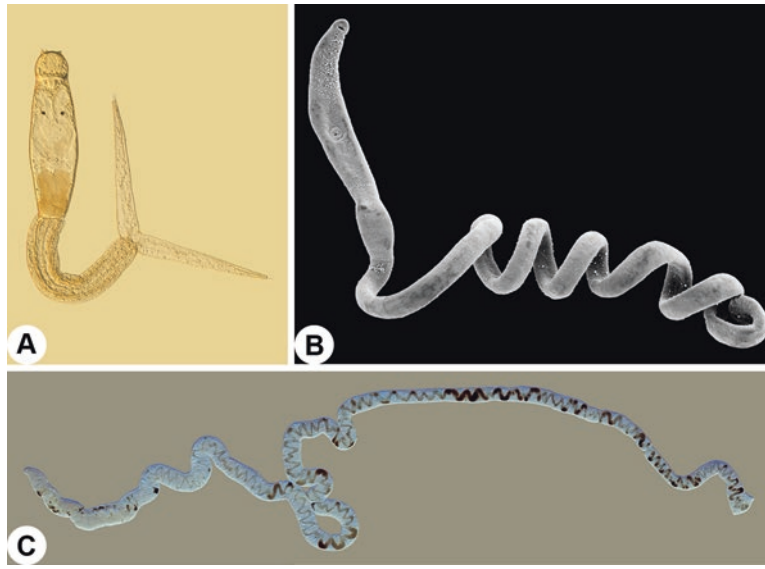

Fig. 8.3 (**a**) Ocellate furcocercaria of *Trichobilharzia regenti* (approx. length: 800 μm) released from the intermediate snail host *Radix labiata*. (**b**) Advanced male schistosomulum of *T. regenti* (approx. length: <10 mm) isolated from the spinal cord of a duck 11 days post infection and then cultivated 3 days in vitro. (**c**) Fully developed adult female removed from the definitive host 21 days post infection (approx. length: 11 mm)

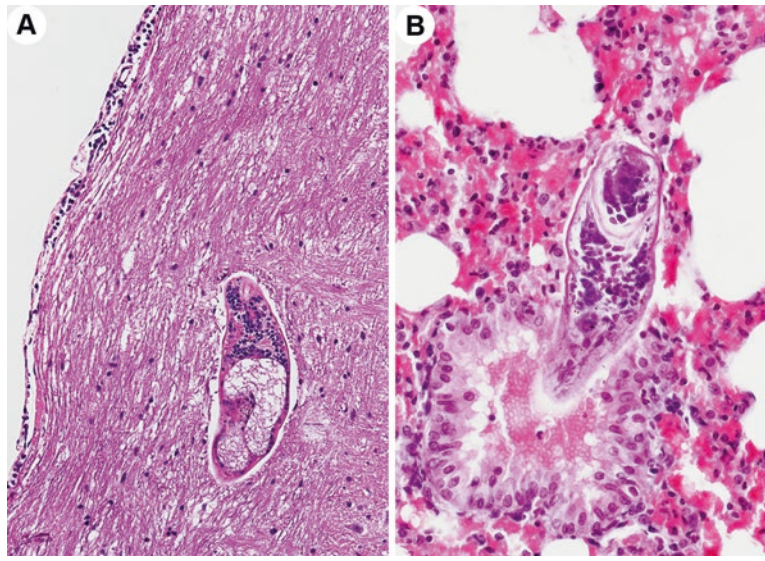

Fig. 8.4 Mouse represents a convenient model for studies of avian schistosome migration in accidental mammalian hosts. In both situations depicted, the length of migrating schistosomula at different intervals post infection varies between 300 and 400 μm. (**a**) Section of the spinal cord of experimentally infected mouse 7 days post infection with a migrating *Trichobilharzia regenti* schistosomulum. (**b**) Section of the lungs of experimentally infected mouse 3 days post infection with a *Trichobilharzia szidati* schisosomulum leaving the circulatory system and entering an alveolus. (Sample (**a**) has been provided by Dr. L. Panská, Department of Parasitology, Faculty of Science, Charles University; Micrograph (**b**) author: Mgr. T. Macháček, Department of Parasitology, Faculty of Science, Charles University)

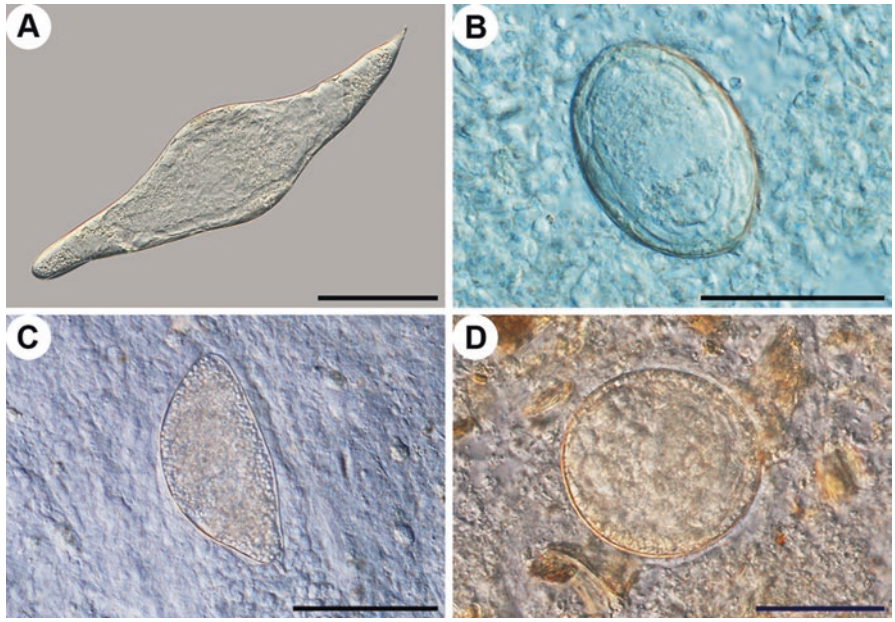

Fig. 8.5 Egg variability in different genera/species of avian schistosomes; scale bars 50 μm. (**a**) Spindle-shaped egg of *Trichobilharzia regenti* from the nasal mucosa of *Anas platyrhynchos*. (**b**) Oval egg of *Trichobilharzia filiformis* from the mucosa of small intestine of *Cygnus olor*. (**c**) Asymmetric egg of *Allobilharzia* sp. from the mucosa of small intestine of *Cygnus olor*. (**d**) Rounded egg of *Dendritobilharzia pulverulenta* from the small intestine of *Fulica atra*

bacterial killing, and stimulates production of humoral defense molecules, e.g., molluscan defense molecule (MDM) and granularin. Moreover, the developing trematode larvae induce production of schistosomin, a molecule released probably from snail hemocytes and connective tissue cells, and competing with snail hormones for receptor binding sites on the target reproductive tissues (inhibition of oviposition) (De Jong-Brink et al. 2001).

Several weeks (depending on temperature) after exposure to miracidia, the snails start to liberate cercariae (Fig. 8.3a). These freely swimming larvae respond to some physical and chemical stimuli, and exhibit specific behavior in the aquatic environment. *Trichobilharzia szidati* recognizes substrates being warmer than the ambient water and attaches to the surfaces containing ceramides and cholesterol. As the infection of a new host comprises several steps, the penetration itself is triggered by other chemical signals—unsaturated fatty acids for *T. szidati* and free sterols for *Austrobilharzia terrigalensis* (Haas 2003). Besides the action of cercarial muscles, secretions of cercarial penetration glands containing histolytic enzymes play a crucial role in skin penetration. Whereas some human schistosomes use serine peptidases (e.g., cercarial elastase) as the main penetration enzymes, in *T. szidati* and *T. regenti* (and also in *Schistosoma japonicum*) cysteine peptidases (cathepsin B-like) from the penetration glands are supposed to play a role (Kašný et al. 2011). After the attachment, the body of cercariae enters the skin, whereas the tail is removed. Once in the host, cercarial body transforms to schistosomulum. Morphologically, the muscular head organ turns into an oral sucker capable of food intake (Bulantová et al. 2011). In order to evade host immune attack, transforming schistosomula modify their body surface: (1) Thick glycocalyx on the surface is rapidly removed and a new surface double membrane of the tegument is formed. (2) The density of surface antigens drops to the level undetectable by specific antibodies and some lectin probes (Horák et al. 1998; Řimnáčová et al. 2017).

In birds, the blood-inhabiting adult schistosomes live either in visceral organs or nasal mucosa; therefore, we distinguish visceral and nasal schistosomes. These two groups also differ

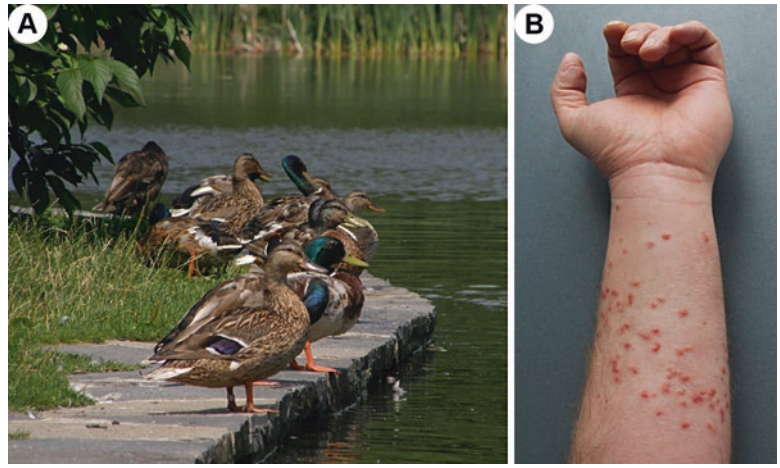

Fig. 8.6 (**a**) Water bodies with a proven occurrence of cercarial dermatitis are usually inhabited by snails and birds (mainly mallards). (**b**) Cercarial dermatitis on the forearm of a sensitized patient 3 days post exposure to the infectious agent

in migratory routes within the host body, and in some cases in the tissues/cells used as a food source for migrating schistosomula (Leontovyč et al. 2019); this statement is however based on a few experimental models. After penetration of the host skin, visceral schistosomes enter blood vessels and use the blood circulation to reach the final location; during the migration schistosomula can therefore be found in the lungs, liver, etc. On the other hand, nasal schistosomes search for peripheral nerves and use them to crawl to the spinal cord and brain. This temporary location is typical in the phase before reaching the nasal area of their avian hosts. Depending on the infection dose, some migrating schistosomula may be found in ectopic locations (e.g., nasal *T. regenti* in the lungs).

In addition to the infections of compatible definitive hosts, cercariae of some genera of bird schistosomes (e.g., *Austrobilharzia*, *Bilharziella*, *Gigantobilharzia*, *Trichobilharzia*) have been confirmed as the causative agent of human cercarial dermatitis (Fig. 8.6). It means that the human skin possesses components that may be recognized by cercariae as signals for attachment and penetration of the accidental host. It is remarkable that the content of attractive fatty acids is even higher in the human skin when compared with the bird skin (Haas and Van de Roemer 1998; Haas 2003).

8.2.1.2 Occurrence

Bird schistosomes can be found around the world (Horák and Kolářová 2011; Horák et al. 2015). Geographical latitude plays a minor role provided the intermediate snail and the definitive bird hosts are available for transmission. Therefore, bird schistosomes and cercarial dermatitis have been reported, e.g., from Iceland and Norway (Skírnisson et al. 2009; Soleng and Mehl 2011).

The prevalence rate in birds may differ with regard to local conditions, but in some cases it can be high, reaching even 90–100% (e.g., the USA and New Zealand) (Davis 2006; Brant and Loker 2009). Waterfowl (sensu lato) seems to serve as dominant definitive host; nevertheless, other groups of birds (e.g., passerines) have also been reported (Horák and Kolářová 2011; Horák et al. 2015). On the other hand, the infections of snail intermediate hosts are less frequent. In many localities, the prevalence rate does not exceed 1%. However, outbreaks with prevalences up to 50% have also been recorded (e.g., Denmark and Russia) (Larsen et al. 2004; Beer and Voronin 2007). Predominantly, pulmonate snails of the families Lymnaeidae, Physidae, and Planorbidae are used for larval development of bird schistosomes. Nevertheless, other groups of snails may also be involved in the life cycles of some genera/species (Horák and Kolářová 2011; Horák et al. 2015). The quantity of cercariae emerged to the environment from individual snails reflects mainly the size of intermediate hosts. While the great pond snail (*Lymnaea stagnalis*) can produce around 3000 cercariae per day (Soldánová et al. 2016), the small planorbids (e.g., *Gyraulus* spp.) release tens of cercariae per day.

8.2.1.3 Damage to the Definitive and Accidental Hosts

As mentioned above, the infective larvae—cercariae—penetrate the skin of bird hosts; usually feet serve as the entry site because the rest of the body covered by feathers with secretions of the uropygial (preen) glands is not attractive (Haas 2003). Penetration of the bird feet may lead to local inflammatory reactions and petechiae. Subsequent migration through the body and damage to the inner organs differ with regard to the two types of schistosomes.

Schistosomula of visceral schistosomes migrating via blood system can temporarily accumulate in the lungs, where hemorrhages, inflammation, and subsequent tissue repair can be observed (Chanová et al. 2007). Later on, the target tissues (e.g., the vessels of liver and intestine) and adjacent organs can be affected by the presence of adult worms and eggs. During severe infections, more distant organs, including the central nervous system (Prüter et al. 2017), can also be impaired. Generally, tissue lesion, inflammatory reaction, formation of granuloma and local thickening occur. Blood circulation may be affected by thrombi, hemorrhages, and perivascular cell infiltration (Horák et al. 2002; Horák and Kolářová 2011). Death has also been ascribed to schistosome infections: in birds infected experimentally or in waterfowl (Horák et al. 2002).

Our knowledge of nasal schistosomes is based on one experimental model only—*T. regenti*. Schistosomula migrate through the peripheral nerves, spinal cord, and brain in order to reach nasal mucosa (Horák et al. 1999); in the central nervous system, schistosomula (Fig. 8.4a) feed on the nervous tissue (Horák et al. 1999; Lichtenbergová et al. 2011). The mode of migration of and the uptake of nervous tissue components by *T. regenti* are unique among schistosomes. Heavy infections may be accompanied by leg paralysis, balance/orientation disorders and death. In the nasal area, adult worms (Fig. 8.3c) and eggs induce lesions, hemorrhages, and granulomas (Kolářová et al. 2001; Chanová and Horák 2007).

Cercariae of bird schistosomes may also attack accidental (incompatible) mammalian hosts, including humans. As far as human infections are concerned, an allergic skin reaction (cercarial dermatitis) develops mainly in sensitized persons after repeated contacts with the agent. Cellular and humoral immune reactions, and clinical symptoms and signs have thoroughly been characterized (Horák et al. 2013, 2015; Macháček et al. 2018). These reactions represent an unpleasant event, but they provide immediate protection against the invading worms, because the worms are subsequently killed in the skin. On the other hand, there are numerous experiments with small laboratory mammals showing that the infection of primarily affected (i.e., non-sensitized) hosts is accompanied by transformation of some cercariae to schistosomula, and their subsequent migration to different inner organs. For example, schistosomula of visceral and nasal schistosomes can be found in the lungs and the spinal cord (Fig. 8.4) of experimental mammals, respectively (Horák and Kolářová 2001; Horák et al. 2008). In the nervous tissue, immune cell infiltrates, activation of microglia and astrocytes, and damage to the nerve cells (axon injury and demyelination) are associated with mechanical damage caused by migrating worms (Lichtenbergová et al. 2011; Macháček et al. 2016). After a few days/weeks, the migrating worms die in the incompatible host due to unknown reasons (probably due to immune attacks, lack of nutrients, etc.).

Due to various climate/environmental factors linked with changes in distribution and density of snails and birds, the number of reports on the occurrence of bird schistosomes and cercarial dermatitis is growing, and cercarial dermatitis is now regarded as a re-emerging disease (Horák and Kolářová 2011; Soldánová et al. 2013; Horák et al. 2015).

8.2.1.4 Diagnosis and Control

In definitive hosts, patent infections by visceral schistosomes can be diagnosed directly, i.e., by finding schistosome eggs in feces (Fig. 8.5). Nasal schistosomes lay their eggs in the nasal mucosa, and miracidia hatch already in the host tissue. Therefore, bill lavage may be used to prove the presence of miracidia. Postmortem

diagnosis is based on a careful examination of presumptive affected organs and tissues; adult worms (often slim and threadlike) and eggs can be found in this case (Kolářová et al. 2010). Concerning indirect detection of schistosome infections, there are some data on the presence of specific antibody in sera of birds and mammals (Horák and Kolářová 2005; Turjanicová et al. 2015). Nevertheless, routine and reliable serological tests are not available for definitive/accidental hosts due to cross-reactivity at the level of schistosome species and genera, or due to a high variability of individual immune responses (Macháček et al. 2018); search for a suitable antigen is in progress. In addition, molecular detection of schistosome species has successfully been applied in the field (Hertel et al. 2002; Horák et al. 2013; Rudko et al. 2018), and development of a tool for identification of parasite DNA in body fluids of experimental animals is in progress.

Manifestation of cercarial dermatitis in humans (Fig. 8.6) consists of intensively itching maculo-papulo-vesicular eruptions and local edema. In some cases, also a generalized reaction (fever, cough, diarrhea, and local lymph node swelling) may occur (Horák et al. 2013, 2015). Clinical signs are usually more intense and rapid in sensitized persons (Macháček et al. 2018).

The intermediate snail hosts can be checked by their exposure to illumination which usually provokes release of ocellate cercariae. In addition, water samples can be filtered and subjected to, e.g., qPCR-based cercariometry (Rudko et al. 2018), or fatty acid-based traps can be used to detect cercariae in water reservoirs (Graczyk and Shiff 2000). Due to species similarity of cercariae, molecular techniques represent the most reliable way of determination (Dvořák et al. 2002; Horák et al. 2013).

Control lies in preventive measures and chemotherapy. Concerning preventive measures, access of birds to schistosome-infested water reservoirs can be obstructed (incl. the reduction of food offer and nesting places). Also, populations of snail intermediate hosts can be reduced in different ways: changes of biotopes (e.g., removal of aquatic vegetation, reduction of water levels for winter season), and mechanical, chemical (e.g., niclosamide), or biological (e.g., toxic bacterial products, introduction of snail-eating fish, e.g., tench *Tinca tinca*) intervention against the snails. Chemotherapy of birds (if applied) is based on praziquantel. Prevention of cercarial dermatitis consists in restricted access to schistosome-infested water reservoirs, protective clothing or sun creams containing niclosamide (Wulff et al. 2007); the treatment is only symptomatic (Horák et al. 2002, 2015).

8.2.2 Mammalian Schistosomes

There is no exception in the life cycles within the family Schistosomatidae in terms of the number of hosts; all life cycles include an intermediate snail host and a definitive avian/mammalian host. Also mammalian schistosomes of the genera *Bivitellobilharzia*, *Heterobilharzia*, and *Schistosomatium*, and animal-parasitizing members of the genus *Schistosoma* follow this pattern although the intermediate hosts are not always known. Some of these schistosomes belong to economically important parasites, causing pathology and sometimes death of livestock. In addition, cercariae of some representatives have been shown to penetrate the skin and inflict dermatitis on humans (e.g., *Heterobilharzia americana*, *Schistosoma turkestanicum*, *Schistosoma bovis*, *Schistosoma spindale*).

8.2.2.1 *Bivitellobilharzia*

Bivitellobilharzia spp. parasitize elephants in Africa (*B. loxodontae*), India, and Sri Lanka (*B. nairi*); the latter species has also been found in the greater one-horned rhinoceros (*Rhinoceros unicornis*) from Nepal (Devkota et al. 2014). The reported prevalence in south India is about 4% (Vimalraj et al. 2012), and in Africa (Republic of Congo and Central African Republic) about 33% (Kinsella et al. 2004). The intermediate hosts are unknown. Although the parasites may be responsible for death of the hosts (Agatsuma et al. 2004), the information on the life cycle, biology, and pathogenicity is scarce.

8.2.2.2 *Schistosomatium*

Schistosomatium sp. is a parasite of rodents and lagomorphs in North America. Larval development occurs in lymnaeid snails. With regard to the host spectrum, economic importance of the parasite is low. On the other hand, the worms may be used as a suitable laboratory model. For example, cercarial peptidases (Dvořák et al. 2008), regulatory peptides (Basch and Gupta 1988), or impact on host physiology (Raiczyk and Hall 1988; Schwanz 2006) have been studied.

8.2.2.3 *Heterobilharzia*

Heterobilharzia americana is becoming an economically important parasite of carnivores, lagomorphs, rodents, and some other mammals in the USA; lymnaeids (e.g., *Fossaria* spp. and *Pseudosuccinea* spp.) serve as the intermediate hosts. Raccoon (*Procyon lotor*) is the most important reservoir host in which the prevalence reaches up to 70%. Dogs seem to be the most important domestic definitive hosts although the number of reported infections is rather low. They suffer lethargy, weight loss, anorexia, diarrhea, vomiting, etc. Adults of *H. americana* inhabit and lay eggs in the mesenteric venules. Some eggs leave the host body via feces, whereas the others spread hematogenously to other tissues, primarily the liver where granulomatous/eosinophilic inflammation and fibrosis can be found (Corapi et al. 2011a; Stone et al. 2011). Heterobilharziosis is likely to become a more important emerging disease of domestic dogs (Flowers et al. 2002; Rodriguez et al. 2014). Recently, the species has been reported as a pathogen of horses, causing hepatic granulomas (Corapi et al. 2011b, 2012). Direct diagnosis is based on finding the eggs in feces; ELISA may also be used to detect circulating antigens. The animals can be treated with praziquantel (Flowers et al. 2002) or benzimidazoles.

8.2.2.4 *Schistosoma* spp. in Animals

Currently, there are about 25 known species of the genus *Schistosoma*; eight of them have been confirmed as parasites of humans (Aldhoun and Littlewood 2012; Standley et al. 2012). Besides humans, members of the genus *Schistosoma* parasitize different groups of mammals. Recent estimates indicate that over 165 million cattle in Africa and Asia are infected with various species of *Schistosoma* (Singh et al. 2004). In some cases, they may cause significant economic losses to the livestock industry. At least three examples of these important species can be mentioned.

8.2.2.5 *Schistosoma turkestanicum*

Schistosoma turkestanicum (formerly *Orientobilharzia turkestanicum*) (Aldhoun and Littlewood 2012) occurs in Asia (predominantly in the middle and far East) and was recently found in Europe (Hungary—see below). Adult worms (Fig. 8.7a, b) are localized in portal and intestinal veins of a range of mammals, including cattle, sheep, goats, water buffaloes, horses, donkeys, mules, and camels; infections of field rats from populations in Turkey have also been reported. Lymnaeid snails serve as intermediate hosts (Wang et al. 2009). Prevalence in definitive hosts can reach high percentages; it can be as high as 100% (e.g., in Iran) (Arfaa et al. 1965). Recently, *S. turkestanicum* appeared in southern Hungary, Europe. Here, the fluke has been found in the liver/portal veins of red deer (*Cervus elaphus*) in a prevalence of 31%. *Radix auricularia* (Fig. 8.7c) serves as the intermediate host (Majoros et al. 2010). Molecular data show that the Hungarian population of *S. turkestanicum* has been native since the Ice Age and probably established itself during the last interglacial period as red deer moved to Europe from North Africa and the Middle East (Lawton and Majoros 2013).

In the endemic countries, intensity and seriousness of the infection may frequently be associated with the timing and quantity of rainfall; the highest intensity of infection has been reported from China where a sheep harbored over 40,000 worms, and a cattle animal hosted over 58,000 worms (Wang et al. 2009). Goats seem to be more vulnerable to the infection than cattle and sheep. Wang et al. (2009) referred to an unpublished outbreak that showed a 40% fatality rate. Pathogenicity of *S. turkestanicum* towards their definitive hosts is similar to that of other *Schistosoma* species. In addition to the infections

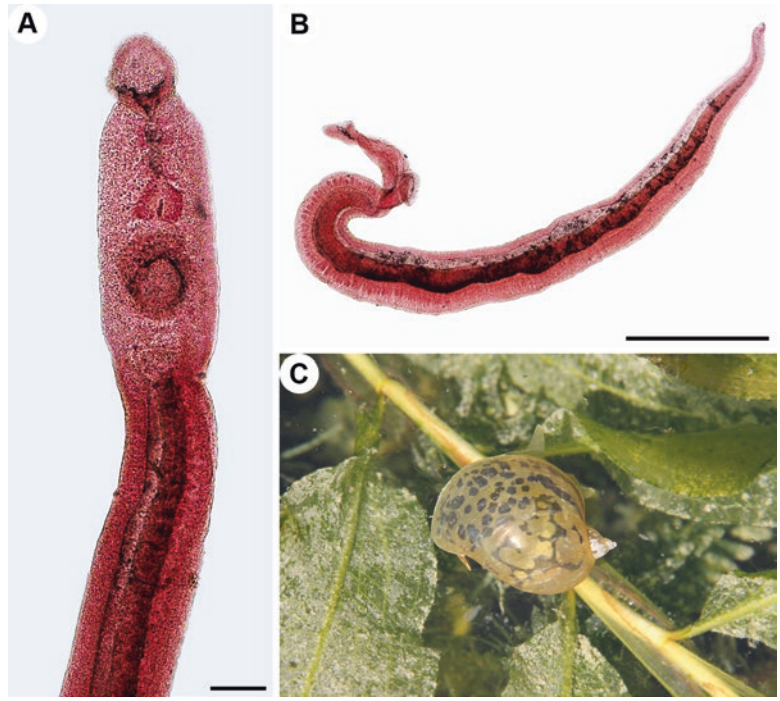

Fig. 8.7 (**a**) Frontal view at the anterior part of adult male of *Schistosoma turkestanicum*; carmine staining; scale bar 200 µm. (**b**) Body of an adult male of *S. turkestanicum* which represents also a European species occurring in red deer (cercariae are capable of causing cercarial dermatitis of humans); note the presence of a long canalis gynaecophorus typical for the genus *Schistosoma*; carmine staining; scale bar 1 mm. (**c**) The most common intermediate host of *S. turkestanicum* in Europe—*Radix auricularia*

of animals, cercariae of *S. turkestanicum* may cause human cercarial dermatitis as reported from Iran (Sahba and Malek 1979), China (Liu et al. 1976), and Hungary (Majoros et al. 2010; Juhász et al. 2016). This can happen because free fatty acids serve again as stimuli of cercarial penetration (Shakarbaev et al. 2001). In definitive hosts, diagnosis is based on detection of the eggs in feces, postmortem examination of slaughtered animals, and use of serological tests. Praziquantel seems to be the drug of choice. Cercarial dermatitis requires measures similar to those against avian schistosomes.

8.2.2.6 Indian Schistosomes

A variety of domesticated animals in south and southeast Asia can be infected by members of the *Schistosoma indicum* group (Agrawal and Rao 2018). *Schistosoma indicum*, *S. spindale*, and *S. nasale* represent species having veterinary importance. All three species are transmitted by the same intermediate snail host, *Indoplanorbis exustus*. *Schistosoma indicum* can be found on the Indian subcontinent in equines, sheep, camel, goats, cattle, buffalos, etc. Infections affect liver and large intestine and can be accompanied by debility, diarrhea, and death. *Schistosoma spindale* lives in vessels of the small and large intestines of buffalos, cattle, goats, sheep, equines, and rodents. It occurs on the Indian subcontinent and in southeast Asia (Thailand, Indonesia, Malaysia, Vietnam); for example, in south India, 68% of cattle from Bangalore were infected (Sumanth et al. 2004a), and a seroprevalence reaching 35% was detected in schistosome-infected dairy cattle and buffaloes in different agro-ecological zones of south India (Lakshmanan et al. 2018). Unlike *T. szidati* (see above), *S. spindale* cercariae use higher temperatures, but no chemical cues for host identification (attachment to the skin surface); penetration itself is triggered by free fatty acid fraction of bovine skin-surface lipids (Haas et al. 1990). The infection affects liver where hepatic lesions with periportal cell infiltration and periportal epithelioid cell granulomas within perilobular zones can be found. Submucosal and mucosal granulomas develop in the small and large intestines (Fransen et al. 1990). Severe infections of cattle may lead to diarrhoea, anemia, and death. The only nasal species among mammalian

schistosomes is *S. nasale* occurring in India, Sri Lanka, Myanmar, and Bangladesh. For example, in Sri Lanka the overall prevalence in cattle is about 12.6% (De Bont et al. 1989). In south India, 72.7% of cattle from Bangalore (Sumanth et al. 2004a), and 11.1% of cattle and 23.4% of buffaloes from Wayanad were positive (Ravindran and Kumar 2012). It causes snoring disease in buffalos, cattle, sheep, and goats. Histopathologically, granuloma formation around the eggs deposited in the nasal mucosa can be seen; in most severe cases cauliflower-like growths obstructing the nasal cavity can be observed (De Bont et al. 1989). It seems the animals differ in their susceptibility. In cattle, the infection leads to severe pathological lesions and pronounced symptoms, whereas in buffalos the course of infection is mild; buffalos show an innate tolerance to the parasite (Dutt 1967; De Bont et al. 1989). Although both species, *S. nasale* and *T. regenti*, mate and lay eggs in the nasal mucosa of their mammalian and avian hosts, respectively, the mode of migration from the skin to the final location seems to be different: *T. regenti* follows peripheral nerves in order to enter the spinal cord and brain, and after that it immediately moves to the nasal area (Horák et al. 1999), whereas *S. nasale* seems to use blood circulation because many immature/mature worms can be found in the lungs, liver, mesenteric veins, and heart of experimental animals (Sahay and Sahai 1978; Sapate et al. 2001). Diagnosis can routinely be performed by detection of eggs in feces (visceral schistosomes) or nasal mucous secretions (*S. nasale*). Also, serological tests for detection of specific antibody or antigens in sera and fecal samples (sandwich ELISA) have successfully been tested (Singh et al. 2004; Sumanth et al. 2004b; Sreenivasa Murthy et al. 2013; Lakshmanan et al. 2018). Chemotherapy of the infections is available, e.g., praziquantel (Rahman et al. 1988) or triclabendazole (Singh and Agrawal 2005) can be applied as drugs of choice.

8.2.2.7 *Schistosoma bovis*

Schistosoma bovis occurs in southwest Asia, Africa, and Europe (Spain and Mediterranean islands) (Moné et al. 1999; De la Torre-Escudero et al. 2012a). In some areas, it can represent a serious veterinary problem, e.g., in Sudan where prevalence in cattle can reach nearly 90% (Majid et al. 1980). In other areas, lower prevalence rates have been reported, e.g., in Tanzanian cattle 34% (Makundi et al. 1998), and in Ethiopian cattle 22% (Yihunie et al. 2019). Cattle, horses, donkeys, sheep, goats, camels, pigs, antelopes, and rodents can serve as the definitive hosts. Recent studies have reported its zoonotic potential through the formation of *S. bovis* × *Schistosoma haematobium* hybrids (Boissier et al. 2016; De la Torre-Escudero et al. 2017; Oey et al. 2019). The intermediate hosts are represented by various species of the genus *Bulinus*, and in Spain by *Planorbarius metidjensis*. The adult worms parasitize intestinal mesenteric veins, and the disease can be linked with diarrhea and weight loss although most infections in endemic countries are subclinical. Histopathological examination disclosed intestinal and liver lesions (granulomatous inflammatory reactions caused by the eggs and adults), and also other organs can be affected in heavy infections (Ferreras-Estrada et al. 1998). *Schistosoma bovis* is another suitable organism serving for molecular biology research. For example, it has been shown that *S. bovis* adult males express enolase on their tegumental surface; the protein is a receptor of plasminogen that plays a role in the activation of the host fibrinolytic system. In this way, the worms most probably avoid blood clot formation on their surface (De la Torre-Escudero et al. 2010). In addition, *S. bovis* annexin having fibrinolytic and anticoagulant properties has been demonstrated on the tegument of schistosomula and adult worms (De la Torre-Escudero et al. 2012b). These essential molecules, together with other candidates (e.g., peroxiredoxin, paramyosin, cathepsin B) obtained by proteomic approaches (De la Torre-Escudero et al. 2017), offer potential vaccine targets for the control of schistosomiasis in ruminants (De la Torre-Escudero et al. 2011). In the past, vaccination of ruminants having a protective effect has been realized by irradiated schistosome larvae, and later on by recombinant *S. bovis* glutathione S-transferase (Boulanger et al. 1999). Diagnosis lies in coprology as the most frequently used approach, but detection of specific

antibodies against the parasite (recombinant) antigens has also been introduced; the latter method is more sensitive for detecting light infections and can be applied in large-scale examinations (De la Torre-Escudero et al. 2012a). As for treatment, praziquantel has been shown to be highly effective (Johansen et al. 1996; Monrad et al. 2006).

8.3 Aporocotylidae (syn. Sanguinicolidae)

Taxonomy of the family has a complicated history. For the blood flukes of fish, the family Sanguinicolidae was erected by Von Graff (1907). This family-group name has been widely accepted throughout the twentieth century. Nevertheless, some authors maintained the family name Aporocotylidae Odhner, 1912 for marine species of fish blood flukes (Yamaguti 1971) although it has been evident that the diagnostic characters of the two families are often uncertain and inconsistent, and there is little correlation between hosts and parasites (Stunkard 1983). Therefore, Overstreet and Køie (1989) considered Aporocotylidae to be a junior synonym of Sanguinicolidae, and the latter taxon name has been accepted by Smith (2002) in *the Keys to the Trematoda*. However, a deep and critical examination of the literature from the beginning of the twenty-first century (Bullard et al. 2009) revealed that "Aporocotylidae Odhner, 1912 is the earliest available family-group name for these flukes." Currently, the family consists of 39 genera (March 2019) parasitizing the circulatory system and body cavities of marine and freshwater teleost fish and chondrichthyans. Aporocotylidae is apparently a highly radiated group of flukes, with numerous new genera and species being described recently, mainly from marine environments (Cribb and Bray 2011). Some species are of significant economic importance as pathogens of farm fish. Literature on blood flukes of cold-blooded vertebrates has extensively been reviewed by Smith (1972, 1997a, b). A review on blood fluke infections in marine cultured fish was published by Shirakashi and Ogawa (2016). Available GenBank sequences and molecular studies related to life history, taxonomy, and phylogeny of fish blood flukes were summarized and reviewed by Orélis-Ribeiro et al. (2014).

8.3.1 Life Cycle

Relatively few complete life cycles of aporocotylids have been described, especially in marine species (Cribb et al. 2011); all of them are dixenous. There are still many associations between intermediate hosts and aporocotylid larval stages with unresolved species determination. Molecular approaches recently help to solve these problems.

Contrary to schistosomes, aporocotylids are hermaphrodites. Eggs are mostly reported non-operculate, egg shell is thin and elastic, reported by Smith (1972) to contain elastin; however, McMichael-Phillips et al. (1992) did not detect elastin in egg shells of *Sanguinicola inermis* by histochemical tests. Miracidia hatch directly in the gill tissue and leave the host into the water environment. Mechanism of hatching was not studied in detail; the larvae leave the egg shell upon rupture, actively liberate themselves from gill filaments and seek for and invade an intermediate host, mostly by active penetration (Smith 1972; Kirk and Lewis 1993). Pigmented eye-spot occurs in, e.g., *S. inermis*, suggesting the possibility of orientation based on phototaxis. Miracidia may be armed with a stylet used for mechanical disruption of host tissues; moreover, histolysis based on action of proteolytic enzymes contained in the apical gland or paired lateral glands is highly probable (McMichael-Phillips et al. 1992).

The association of aporocotylids with a broad range of clades of marine/freshwater gastropods, marine bivalves, and also marine polychaete annelids is intriguing and unique among trematodes; not long ago they were the only known group of digeneans that may use a non-mollusc as the first intermediate host (De Buron et al. 2018). However, it seems that the host spectrum of particular species is somewhat restricted,

e.g., to a single family of intermediate hosts (Cribb et al. 2001). The development inside the intermediate host is not known for all genera. Most aporocotylids produce a few generations of daughter sporocysts, usually one or two, subsequently giving rise to cercariae (Smith 1972; Kirk and Lewis 1993; Sugihara et al. 2017). A clear evidence of rediae was surprisingly found in *Aporocotyle simplex* from terebellid polychaetes (Køie 1982; Køie and Petersen 1988), while various *Cardicola* spp. reported from members of the same family of polychaetes (and *C. parvus* from polycirrids) produce sporocysts (Cribb et al. 2011; Sugihara et al. 2014; Ogawa et al. 2017; Siegel et al. 2018). As many as 1800 sporocysts per host and 75 cercariae per sporocyst were found in a study from Japan, suggesting that $>10^5$ cercariae can be released from one infected polychaete (Sugihara et al. 2015). Seasonality in infection rate and specific spatial distribution of infected polychaetes may occur since both abiotic and biotic factors likely influence infection in the intermediate terebellid hosts (Fukuda et al. 2017; Shirakashi et al. 2017). Parasitic castration of both polychaete and mollusc intermediate hosts was observed (Køie 1982; Vazquez et al. 2013). In molluscs, the development usually takes place in the digestive gland. The records of localization in the gill hemocoel or gonads have been published for unidentified aporocotylid species in marine venerid, mactrid, or pharid clams (Gilardoni et al. 2011; Vazquez et al. 2013; Carvalho et al. 2015). A donacid marine bivalve was recently found to be the host of a chondrichthyan aporocotylid developing in gonads of the mollusc; the authors hypothesized that the lineage of blood flukes from cartilaginous fishes may be specialized on bivalves as intermediate hosts (Cribb et al. 2017a).

Cercariae are usually apharyngeate, non-ocellate, brevifurcate, without acetabulum. Typically, a dorsal fin occurs on the body (lophocercaria type, Fig. 8.8a). However, exceptions occur in, e.g., *Cardicola* spp. which possess a simple tail and lack the dorsal fin (Cribb et al. 2011; Sugihara et al. 2014). The apical part of cercaria is armed with a muscular conus called "head or cephalic organ," through which the ducts of penetration glands run to the openings at the apex. Four pairs of penetration glands have been reported for *S. inermis* (Kirk and Lewis 1993) and five pairs for *Aporocotyle simplex* (Køie 1982). No studies have been done on the content of penetration glands, though proteolytic enzymes can be expected to play a role in histolysis. Periodicity of cercarial shedding from the infected snails was studied by, e.g., Martin and Vazquez (1984); light and temperature may affect the process (Erickson and Wallace 1959). Neither phototaxis nor response to shadow stimuli was observed in cercariae of *Sanguinicola lophophora* (Erickson and Wallace 1959), whereas Meade (1967) found phototropic response in *Sanguinicola klamathensis*. Response to cues, either chemical or physical, produced by hosts has not been studied. Cercariae penetrate their hosts mostly via gills and skin of the body or fins. Less scaled parts seem to be preferred (Meade 1967; Køie 1982; Sommerville and Iqbal 1991; Kirk and Lewis 1993). During penetration, transformation of cercariae takes place, which is characterized by tail loss, dissolving of dorsal fin and formation of a double outer surface membrane common to all three families of blood flukes (McLaren and Hockley 1977). Qualitative and quantitative changes in surface glycocalyx composition of transforming larvae have been proved by altered reactivity with fluorescent lectin probes (Mikeš and Stiegeler, unpublished). Infection process, migration, and development in fish have been studied in greater detail in *S. inermis*, a parasite of cyprinid fishes. Maximum cercariae entered body of carp fingerlings within 30 min. The success rate of penetration was between 1 and 3%. A sharp decline in the number of worms occurring in the skin was observed to start 60 days p.i.; 43% of them were present in the skin even 90 days p.i., the onset of egg production by adults in the heart/gills was 73 d.p.i. (Sommerville and Iqbal 1991). This is in contrast with the data of, e.g., Kirk and Lewis (1993, 1996), who showed that around 1/3 of cercariae penetrated the fish, and faster migration occurred through the connective tissue and muscles towards the ventral and dorsal blood systems. The worms that penetrated the anterior part of

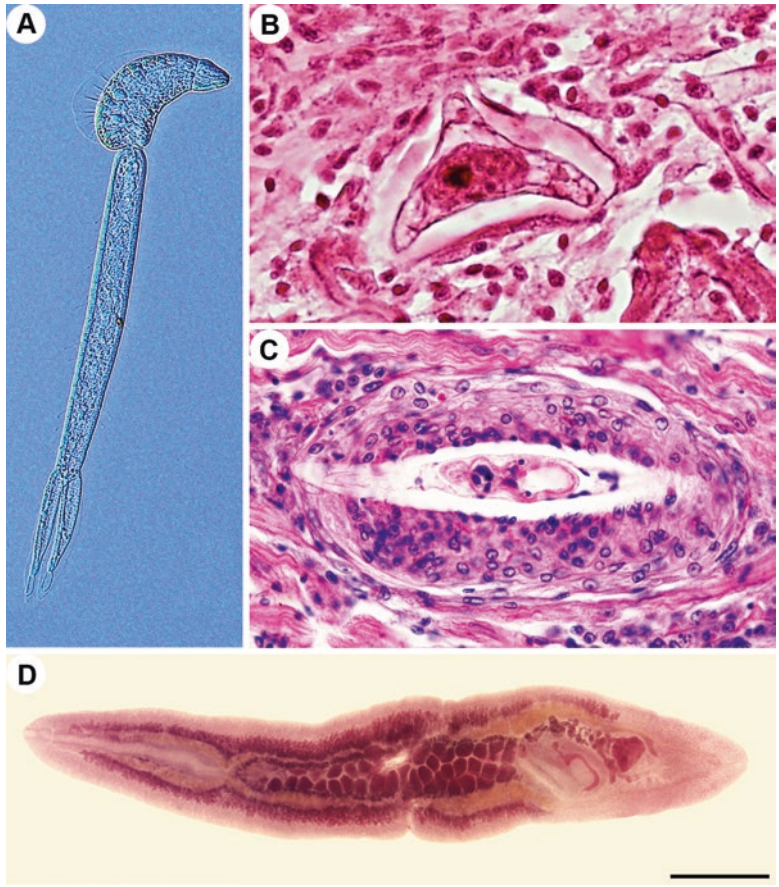

Fig. 8.8 (**a**) Lophocercaria of *Sanguinicola inermis* (approx. length: 350 μm) with a typical dorsal fin and forked tail, released from the intermediate host, *Radix auricularia*. (**b**) Section showing the triangular egg of *Sanguinicola inermis* (size approx. 30 μm) trapped in the gill tissue of young carp; miracidium with pigmented eyespot is located inside. (**c**) Spindle-shaped egg of *Cardicola laruei* (approx. length: 100 μm) in the cardiac muscle of the spotted seatrout, *Cynoscion nebulosus*. Granulomatous reaction around the egg is visible. (**d**) *Aporocotyle margolisi* adult worm from the blood system of the North Pacific hake *Merlucius productus*; scale bar 1 mm. (Micrograph (**a**) author: Dr. M. Soldánová, Institute of Parasitology, Biology Center of the Czech Academy of Sciences; Micrograph (**c**) author: Prof. I. Dyková, Institute of Botany and Zoology, Faculty of Science, Masaryk University; Sample (**d**) is deposited in the Helminthological Collection, see Acknowledgements)

fish were more successful in reaching the heart, localization in the gills and cardiac regions has been reported by the 21st to 28th days post infection. Maximum life span of adults was 70 days, and the eggs contained fully developed miracidia within 7 days from being laid. The rate of migration seems to be strongly affected by temperature.

Adult aporocotylid flukes are usually localized in the heart, bulbus arteriosus, branchial vessels or other large arteries. Few exceptions are represented by the occurrence in veins of the body cavity, e.g., in case of *Psettarium nolani* (formerly *Sasala nolani*, see Yong et al. 2016) that produces eggs conspicuously accumulating in the host gut wall (Bray et al. 2012). Occasionally reported cases of extravascular localization of adult flukes may probably arise from coincidental or aberrant migration, or rupture of blood vessels. However, there are indications that localization of some aporocotylids within body cavity of South American pimelodid catfish species may be natural (Orélis-Ribeiro and Bullard 2015, 2016). The range of fish species

serving as hosts for aporocotylids is extremely broad. Both specialists and generalists have been recognized within the family. As summarized by Cribb et al. (2001), members of the family "are reported from at least 45 families of fishes and from a total of 18 orders of fishes including elasmobranchs, holocephalans and teleosts; they are one of only a handful of families to exploit all three of these taxa."

8.3.2 Occurrence

Fish blood flukes are cosmopolitan. Our knowledge of general distribution is incomplete as some areas of the world have not been explored by fish parasitologists. Research in the new regions is usually followed by descriptions of new species or genera. Particularly, marine environments are still gray areas and promise numerous discoveries in terms of diversity of Aporocotylidae, their biology, host associations, and ecology. Also, the information on distribution of particular species is often incomplete; better data used to be available on freshwater species, particularly those parasitizing fish of economic importance. This situation has recently been changed with the massive onset of marine farming of tuna (and some other) fishes around the world and economic problems caused by blood fluke infections. In marine species, intermediate hosts are mostly unknown; since the life cycles of digeneans are obligatory dependent on appropriate intermediate hosts, the knowledge of their distribution could help to assess the expansion of parasites in fish. Nevertheless, the situation can be complicated by regular migration of some fish species, as depicted, e.g., for *Cardicola forsteri* and *Cardicola orientalis* in tuna fishes (*Thunnus* spp.) by molecular approaches (Aiken et al. 2007; Forte-Gil et al. 2016). Moreover, the international trade with marine fish has resulted in the spread of hitherto unknown parasites into indigenous farmed and wild fish (Ogawa 2015). Prevalence of the flukes in fish hosts varies greatly among various reports and, apparently, there is no common pattern. It can be near 100%, especially in sites with a high concentration of fish. Therefore, the prevalence and intensity of infections are often higher in extensively farmed fish (Padrós et al. 2001; Aiken et al. 2006). On the other hand, prevalence in wild populations of fish is commonly lower (McVay et al. 2011) as evidenced for *Cardicola laruei* in sea trouts *Cynoscion* spp. from the Gulf of Mexico and Atlantic Ocean (4–10%). Some parasites migrate to a specific site within the host and start to lay eggs during a distinctive season, causing peaks of intense infections and related fish mortality (Ogawa et al. 1989).

The prevalence of larval stages in intermediate hosts used to be rather low, usually below 10%. Faltýnková et al. (2007) found 3.2% prevalence of *Sanguinicola* sp. in *Valvata* snails in Lake Konnevesi, Finland. Køie (1982) referred to about 7% of *Artacama proboscidea* polychaetes from Oresund, Denmark, being infected by *Aporocotyle simplex*, but up to 18.4% of *Terebella* sp. were found to be infected by sporocysts of *Cardicola opisthorchis* at a tuna farm in Japan (Sugihara et al. 2015).

8.3.3 Damage to the Definitive Hosts

Some species of fish blood flukes act as serious pathogens causing mass mortalities and significant economic loss in fish industry. Pathological effects of aporocotylids on marine and freshwater fishes were thoroughly reviewed by Bullard and Overstreet (2002) and Kirk (2012), respectively. Similar to other blood flukes (schistosomes and spirorchiids), the main pathology arises from the presence of eggs in blood vessels and various organs/tissues. The eggs are laid by worms or passed by blood circulation from different sites of infection into the gill filaments of the host (Fig. 8.8b), from which miracidia can escape into the water. Additionally, a significant amount of eggs is sequestered in blood vessels or tissues of other organs including heart (Fig. 8.8c), kidney, liver, pancreas, spleen, etc. This may lead to embolism, hypoxia, infarction, and necrosis. Granulomatous inflammation is formed in the areas surrounding the eggs and may finally result

in irreversible tissue damage. Adult worms of some species may invade endocardium and myocardium and cause fibro-granulomatous endocarditis with accompanying endocardial thrombi blocking normal blood flow and emboli causing infarctions (Warren et al. 2017). The level of harm is obviously dose-dependent; heavy infections may be fatal, while in cases of non-lethal parasitemia various degree of debilitation occurs and decreased weight gain may be achieved.

Acute mortality in fish can be produced by a large number of miracidia escaping simultaneously from a heavily infected host, which is accompanied by severe hemorrhaging in the gills. Similarly, a high number of penetrating cercariae (and migrating successive stages) may kill the host, perhaps due to excessive amount of bioactive substances (e.g. peptidases) released from penetration glands into the host tissues. Experimental exposure of individual carp fingerlings to 2500 cercariae of *S. inermis* was followed by severe edema, epidermal hemorrhage, and death within a few hours (Kirk and Lewis 1992; Mikeš, personal observation); however, such an extensive immediate exposure is not probable under natural conditions due to dilution of cercariae in the environment and mobility of the hosts, which can apparently perceive higher number of penetrating larvae (Mikeš, personal observation).

8.3.4 Diagnosis and Control

With regard to the localization of adult flukes (Fig. 8.8d), complete helminthological dissection of fish is necessary for reliable confirmation/exclusion of infection by aporocotylids. Adult flukes or tissue-migrating juvenile stages can be found almost anywhere in the body of the host. Special attention must be paid to the vascular system, namely heart and bulbus arteriosus, branchial arteries, aortas and other large arteries, lymphatic system; nevertheless, aporocotylids have been found also in mesenteric veins (Bray et al. 2012), veins surrounding brain and optic lobes (Alama-Bermejo et al. 2011; Palacios-Abella et al. 2018), body cavity (Orélis-Ribeiro and Bullard 2016), and elsewhere. Due to small dimension, the worms are often hard to find and the use of dissecting microscope is necessary. Methods of collection and preservation of trematodes from fishes for taxonomy and systematics have been published by Cribb and Bray (2010).

As for the presence of aporocotylid eggs, various organs/tissues should be checked microscopically. Preparation of compression mounts of soft tissues is the method of choice; alternatively, histological processing can be performed. An improved and time-saving method for microscopical examination of gills called T-two test had a sensitivity >96% (Palacios-Abella et al. 2017). Determination of fluke species based solely on egg morphology may not be reliable due to relative uniformity of eggs among some species, or lack of data on egg morphology in particular species.

Intravital approaches to diagnosis of infections may include biopsy of gill filaments or cardiac puncture, which require certain skills and equipment. There is always some risk of unattended bacterial or fungal infection of manipulated fish connected with these invasive methods. Theoretically, diagnosis based on capture of miracidia escaping from live fish isolated in appropriate tanks would be possible, followed by molecular species identification.

Immunodiagnosis of aporocotylidosis based on specific antibody response of the hosts is a new approach that is still in its infancy. The methods are known for a long time, but limitations are linked with poor knowledge of fish immune system, e.g., variations in immune responses of different species of fishes, specific responses against diverse blood flukes, unknown nature of fluke antigens and their cross-reactivity with those of other parasites. Thus, the application of immunodiagnostics may be useful in particular cases and conditions, and will rather be targeted to aquaculture, where the risk of infection by a specific parasite can be assessed and immune response of particular host species has been studied. First promising results in immunodiagnosis of *Cardicola forsteri* infections have been reported by Aiken et al. (2008) and Kirchhoff et al. (2012).

Molecular diagnostic tools are also available and constantly develop. The most used genetic markers include ribosomal 28S DNA, 18S DNA and ITS2 region, and mitochondrial gene coding for COX I (Cribb et al. 2001; Nolan and Cribb 2006; Aiken et al. 2007; Repulles-Albelda et al. 2008; Ogawa et al. 2011). A rapid method of trematode barcoding based on 800 bp sequence of 18S rDNA gene seems to be a promising tool for species identification (Routtu et al. 2014). Real-time qPCR was successfully employed to quantitatively detect *Cardicola orientalis*, *C. forsteri*, and *C. opisthorchis* DNA within the blood, gills, and heart of cultured southern and pacific bluefin tuna juveniles (Polinski et al. 2013; Pennacchi et al. 2016). Nevertheless, practical applicability and outputs of molecular techniques largely depend on the set of sequence data available.

Various measures can be taken to control the abundance of fish blood flukes. Indeed, regular monitoring of stocks is desirable on the site of production as well as veterinary inspection before transfer, and examination and quarantine of imported fish. Wherever possible, the chance of contact between farm and wild populations of fish should be avoided or kept to a minimum because the intermediate hosts serving for transmission may co-occur at the same time.

Several approaches can lead to interruption of the life cycle of aporocotylids. Intervention to and management of the environment in order to decrease populations of intermediate hosts is one of the possibilities. Thus, "summering" or "wintering" (i.e., keeping ponds without water over the summer or winter) is traditionally practiced in the Czech Republic and surrounding countries with carp farming in order to interrupt transmission of *Sanguinicola inermis* and various other digeneans and cestodes. It can be accompanied by application of compounds relatively harmless to the environment, like burnt lime, on the surface of mud. Application of other chemicals used in the past (e.g., copper sulfate) is arguable due to their ecotoxicity. Macrophytes serving as diet for some freshwater gastropods can be removed from littoral zones, which increases expenses and therefore it is practiced just in restricted areas, e.g., in little start-up ponds used for small fry and fingerlings. Introduction of a proportion of snail-eating fish, e.g., tench, into the stocks of fish of main economical importance in extensively managed ponds may cut down the population of snails.

In marine fish farming, careful selection of sites for location of pens or net cages is important as the excessive occurrence of intermediate hosts will promote parasite transmission; moving aquaculture further offshore may be a solution. Comparison of prevalences of *Cardicola forsteri* in caged southern bluefin tuna showed 85% of infected fish kept near shore in contrast to 0% in the offshore cohort (Kirchhoff et al. 2011). Terebellid polychaetes, the intermediate hosts of *Cardicola* spp., are often cryptic within balanoid shells. These usually adhere to accompanying ropes and floats with tuna cages in tuna farms. Thus, these matrixes may be potent infection sources for caged fish and their regular cleaning and removal of the shells from fish breeding sites may aid interrupting parasite transmission (Sugihara et al. 2014).

Possibilities of antihelminthic therapy have been checked in bluefin tunas (*Thunnus orientalis*). Among four compounds tested, praziquantel was the most effective against adult flukes and therefore being considered as the drug of choice for treatment of *Cardicola* infections (Hardy-Smith et al. 2012; Shirakashi et al. 2012a). Nowadays, this drug is regularly used for oral treatment of blood fluke infections in farmed fish; effective doses and pharmacokinetics have been well studied in bluefin tuna (Ishimaru et al. 2013). However, praziquantel apparently has no significant effect on the viability of miracidia inside the eggs, thus not guaranteeing total interruption of transmission to intermediate hosts (Shirakashi et al. 2012a).

Preliminary data on immune response in early age sea cage cultured juveniles of pacific bluefin tuna documented up-regulation of immune-related genes coding IgM, MHC-I, TCRß, and IL-1ß in gills of fish infected by *C. orientalis* and *C. opisthorchis*. Although the response was not protective, the authors believe that "immunisation

at an early age may have potential as a health strategy" (Pennacchi et al. 2016).

There are few examples of aporocotylids, both marine and freshwater, causing damage to fish industry on a larger scale. Some of them arose from recent increase of the number of pen-reared fish species in marine aquaculture (Bullard and Overstreet 2002).

8.3.5 *Cardicola*

Cardicola is currently the largest marine aporocotylid genus (Cribb et al. 2011) found in perciform and mugiliform teleost fishes representing nine families, reported mainly from the Atlantic and Pacific Oceans and South China Sea (Smith 2002). However, there are indications that future taxonomic revision of the genus may alter the situation (Nolan et al. 2014). A list of hosts, infection sites and localities for *Cardicola* spp. was provided by Bullard (2013), but several new species have been described ever since. *Cardicola forsteri* became an emerging pathogen causing massive mortalities in cage-reared southern bluefin tuna in coastal areas of Australia (Hayward et al. 2010; Dennis et al. 2011). A survey performed in nine companies in Spencer Gulf, South Australia, over a 3-year period revealed >62% average prevalence of the infection and average intensity of ca. 6 flukes per host. Both the prevalence and intensity were significantly higher in fish harvested in winter compared to autumn. No effect of intensity or abundance on the condition of the infected tuna was recorded and the variations of the epizootic parameters were dependent on company only, which may be related with different husbandry measures, different average tuna sizes in each company, or location of the operations (Aiken et al. 2015). *Cardicola forsteri* has also a big potential of further emergence in aquacultures in other parts of the world, being able to infect both pacific and northern bluefin tunas suffering from cardicolosis in, e.g., Japanese and Spanish coastal farms (Aiken et al. 2007; Shirakashi et al. 2016). Moreover, three other species of *Cardicola* have been found in wild and caged tuna in Western Mediterranean, among them *C. opisthorchis* being the most abundant one in sea-farmed fish (Palacios-Abella et al. 2015; Forte-Gil et al. 2016). In Japan, mortality of pacific blue tunas is caused mainly by *C. orientalis* and *C. opisthorchis*; in a heavily infected individual, a total number of >4.5 million eggs was estimated in the gills at one side of the fish (Ogawa et al. 2011; Shirakashi et al. 2012b). *Cardicola opisthorchis* and another cardicoline species cause trickling mortalities in cultured northern bluefin tuna and sea bream *Sparus aurata*, respectively, in Spain (Padrós et al. 2001; Ogawa et al. 2011).

8.3.6 *Sanguinicola*

Sanguinicola spp. are mostly parasites of cyprinid and some other freshwater teleosts of Europe, Asia, northeast Africa, and North America (Smith 2002), and probably also South America. There is only one species reported so far from marine teleosts (*S. maritimus*) in Australian waters (Nolan and Cribb 2005). *Sanguinicola inermis* was reported from lymnaeid snails of the genus *Radix* in Europe (*R. auricularia* and *R. peregra*, although the identity of the latter host is uncertain due to confusion in taxonomy of the genus (Huňová et al. 2012)). Its main definitive host is common carp (*Cyprinus carpio*), some other cyprinids serve as less efficient hosts (Kirk and Lewis 1994). The seasonal cycle of development in carp fisheries with peaks of fish infection in spring/early summer and late summer in temperate regions was summarized by Kirk (2012). In extensive carp breedings, massive mortality caused by *S. inermis* may occur, especially in young fish (year 0–1+). Serious outbreaks in carps have repeatedly been reported from various parts of Europe; salmonid hatcheries in North America have encountered problems with huge losses due to infections by *S. davisi*, *S. klamathensis*, and *S. fontinalis* (Kirk and Lewis 1994). Currently, as a result of integrated approaches to the control of sanguinicolosis, large outbreaks are not reported from Europe and North America.

8.4 Spirorchiidae

Spirorchiids are one of the three families of blood flukes (Fig. 8.9). Nearly 100 species have been found so far in both marine and freshwater turtles, and a single spirorchiid-like species was described from crocodiles. Although their economic importance is rather inconsiderable in comparison to blood flukes of mammals and fish, they may seriously threaten populations of endangered species of turtles in some parts of the world (Werneck et al. 2008; Flint et al. 2010a, b). Besides, the group has drawn much attention in phylogenetic and evolutionary studies. Molecular analyses have clearly shown the paraphyly within the "Spirorchiidae" assemblage, which represents

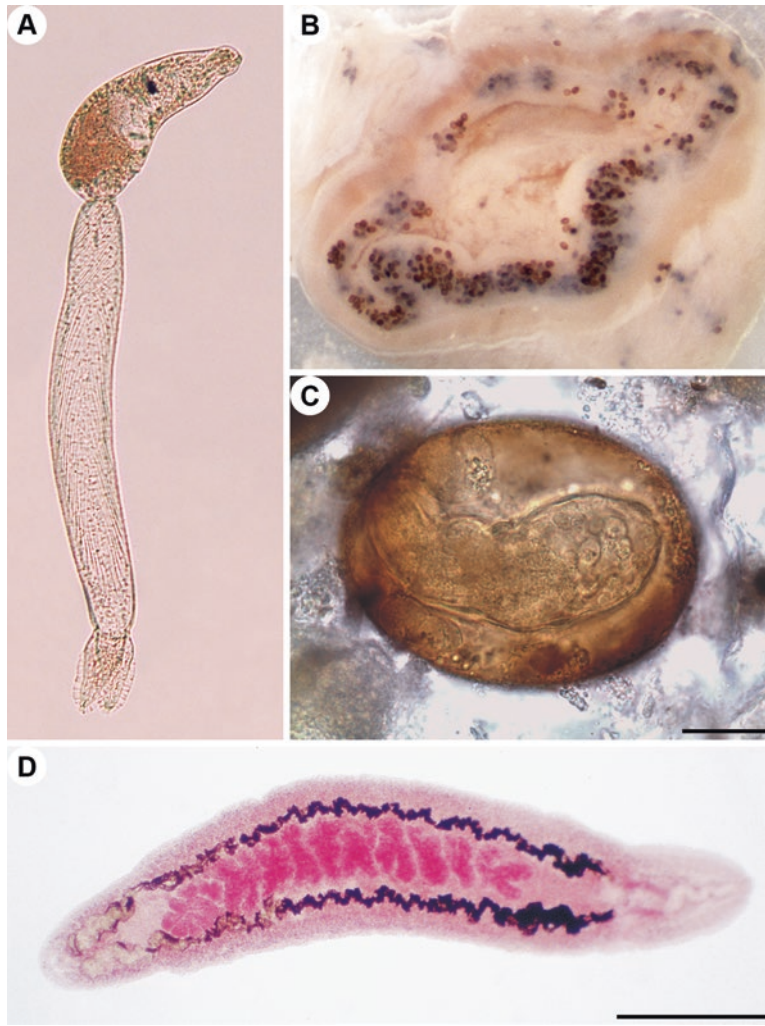

Fig. 8.9 (**a**) Lateral view of a spirorchiid brevifurcate apharyngeate ocellate cercaria from an australian planorbid snail (approx. length: 1 mm). (**b**) Gross cross-section of *Emys orbicularis* intestine with numerous dark-brown eggs of *Spirorchis elegans* deposited in the submucosal tissue refering to a fatal case of infection as described by Iglesias et al. (2015). (**c**) Fully embryonated miracidium-containing egg of *S. elegans* obtained from feces of *E. orbicularis* from the same case. Scale bar 30 µm. (**d**) Adult specimen of *S. elegans* stained with Semichon's acetocarmine (note dark deposits of hematin resulting from host blood digestion in the intestinal caeca). Scale bar 500 µm. (Micrograph (**a**): Courtesy of Dr. S. V. Brant, Division of Parasites, Museum of Southwestern Biology, University of New Mexico; Micrograph (**b**–**d**) author: Dr. R. Iglesias, Laboratory of Parasitology, Faculty of Biology, University of Vigo)

a basal lineage within the monophyletic Schistosomatoidea (Platt 1992; Platt and Brooks 1997; Snyder 2004; Orélis-Ribeiro et al. 2014; De Buron et al. 2018). The clade including *Spirorchis* spp., paraphyletic *Spirhapalum*, *Vasotrema*, *Baracktrema*, and *Unicaecum* is a likely placeholder for "Spirorchiidae Stunkard, 1921" (Roberts et al. 2016). However, the diversity of the group currently comprising 21 genera of turtle blood flukes is yet not fully understood (Cribb et al. 2017b; Stacy et al. 2017; Roberts et al. 2018), as the majority of turtle species have probably never been examined for parasites.

Biology, diversity, taxonomy, and phylogeny of spirorchiids have been reviewed by, e.g., Yamaguti (1971), Smith (1972, 1997a, b), Platt (2002), and Orélis-Ribeiro et al. (2014). Besides localization within the host, they share more common features typical for the other families of blood flukes, having dixenous life cycle, furcocercariae (conspicuously ocellate in known species except *Neospirorchis* spp.) that actively penetrate the definitive host while employing large penetration glands, well-developed head organ, double surface membrane in adults, and eggs sequestered in the host tissues that are the main source of pathologies. As in the aporocotylids, they are hermaphrodites (but note the dioecious *Griphobilharzia* at the end of this section). Eggs are released from the digestive tract of the host with feces, thus resembling most schistosomatids. Postmortem liberation of eggs from host tissues to the aquatic environment seems to be likely due to natural disintegration of carcasses or digestion in the predator alimentary tract. Larval development, where known, takes place in gastropods—planorbids and physids (Heterobran-chia) and ampullariids (Caenogastropoda) in case of freshwater species, and fissurellids (Vetigastropoda) and vermetids (Caenogastropoda) in only two known life cycles of marine species (Cribb et al. 2017b). However, recent investigations surprisingly showed the possibility that some "spirorchiids" may employ polychaete annelids as intermediate hosts, which used to be so far an attribute described only in some species of aporocotylid blood flukes (De Buron et al. 2018).

The first life cycle for a marine turtle blood fluke was definitively identified in a closed system of the Oceanografic Aquarium, Valencia, Spain. *Amphiorchis* sp. parasitizing *Thylaeodus* sp. (a vermetid gastropod) caused spirorchiidosis in captive-hatched neonate *Caretta caretta* turtles. The source of parasite eggs was an adult turtle rehabilitated in the same place (Cribb et al. 2017b). The adults can be found in various parts of the host body, including heart, pericardial cavity, arteries, veins, esophagus, and even abdominal coelom or connective tissues (Holliman and Fisher 1968), but some species can be more organ-specific. Eggs are typically disseminated in virtually all tissues. Chronic infections can result in generalized disease and may be accompanied by high morbidity and mortality rates. Spirorchiidosis can produce neurological signs in infected animals, like hemiplegia and loss of reflex activity caused by focal destruction of brain tissues due to inflammatory reactions surrounding the eggs (Holliman et al. 1971). Further pathological effects involve generalized granulomatous response, aneurysms, arteritis, endocarditis, vasculitis, and hemorrhagic lesions (Glazebrook et al. 1981; Santoro et al. 2007; Stacy et al. 2010; Chen et al. 2012). Egg burden in turtle faeces cannot generally be regarded as indicative of disease severity, since mostly weak correlations were found between fecal and tissue (splenic) egg counts (Marchiori et al. 2018). In green turtles (*Chelonia mydas*) and some other marine species, spirorchiidosis is often associated with a herpes virus-related fibropapillomatosis; this leads to general emaciation and anasarca, and neoplasy in the gastrointestinal tract, lungs, liver, kidney, muscles, dermis, etc. (Aguirre et al. 1998; Yonkers et al. 2015). However, the causal relationships require further research (Herbst et al. 1999). Alien species of spirorchiids have been introduced to various parts of the world with the invasive freshwater turtle species *Trachemys scripta elegans* (red-eared slider) (Oi et al. 2012). These may threaten indigenous turtle species—for example, an outbreak of severe spirorchiidosis in a local subpopulation of the European pond turtle (*Emys orbicularis*) due to *Spirorchis elegans* spillover was recorded in Spain (Iglesias et al. 2015).

Several reports have been published on high prevalence of infections in both freshwater and marine turtle species, often reaching more than 80% in various parts of the world (Holliman et al. 1971; Santoro et al. 2007; Stacy et al. 2010; Chen et al. 2012). For example, spirorchiidosis was the most prevalent cause of death (41.8%) in Queensland green sea turtles between 2006 and 2009 (Flint et al. 2010b). On the other hand, infections caused by two spirorchiid species did not have a causal effect on the death nor a strong impact on the general health status in 168 *Caretta caretta* individuals found stranded along the northwestern Adriatic coast between 2009 and 2015 (Marchiori et al. 2017). However, despite these findings, the impact of blood fluke infections on wild populations of most particular turtle species is largely unknown.

Methods for collection and preservation of spirorchiid trematodes from turtles are based on necropsy and helminthological dissection. However, the adults of some species are microscopic and may occur in small blood vessels, making them difficult to find and collect intact (Stacy et al. 2010). Some improvements of collection methods using citrated saline and a separatory funnel were described by Snyder and Clopton (2005). Since parasite eggs are frequently present in the absence of adult flukes (Flint et al. 2010a, b), a highly specific and sensitive method based on terminal restriction fragment length polymorphism (T-RFLP) was developed and validated as a useful tool for intravital diagnostics of mixed spirorchiid ova in turtle tissues (Chapman et al. 2016, 2017). Treatment of spirorchiidosis is possible, and praziquantel has been proved an efficient drug in several studies (e.g., Adnyana et al. 1997; Jacobson et al. 2003). The practical use is related to wild turtles caught and introduced to farms, aquaria, or rehabilitation centers.

Griphobilharzia amoena is the only species reported so far from a non-testudine host that is the crocodile *Crocodylus johnstoni* living in Australia and Irian Jaya. The life cycle is not known. Historically, this fluke has been considered as a basal schistosomatid (Platt et al. 1991; Snyder 2004), the missing link in schistosome evolution, and was presented in this way in The Keys to the Trematoda (Khalil 2002). However, molecular studies revealed that it is more closely related to spirorchiids from freshwater turtles, namely the genus *Hapalorhynchus* (Brant and Loker 2005; Roberts et al. 2018). Unlike all known spirorchiids from turtles, and much like schistosomatids, *G. amoena* is a gonochoristic fluke supporting the evidence of independent origins of the dioecy among the Schistosomatoidea. The species may be relictual in extant crocodilians, possibly being a member of once broader group reduced by periods of extinction in crocodile-line archosaur hosts (Platt et al. 2013).

8.5 Diplostomidae and Strigeidae

As for the superfamily Diplostomoidea which has recently been re-considered as monophyletic (Locke et al. 2018), our review provides a general information on Diplostomidae and Strigeidae. These two families are paraphyletic (Blasco-Costa and Locke 2017; Hernández-Mena et al. 2017) and comprise parasites with adults living in the digestive tract of birds and mammals. Members of these digenean taxa are remarkable in two aspects: (a) Body of the adult worms is composed of two parts—foliate, spatulate, calyciform, or bulbiform forebody, and cylindrical or coniform hindbody. Besides oral and ventral suckers, the forebody has a ventrally located massive holdfast (tribocytic or Brandes' organ) and, in some representatives, pseudosuckers (lappets). The holdfast probably serves also as a digestive-absorptive organ. (b) Some life cycles include the stage of an unencysted mesocercaria, an additional type of juvenile that follows the stage of furcocercous cercaria, and precedes the stage of metacercaria. Therefore, these flukes can have three- or four-host life cycles that can be extended by paratenic hosts with mesocercariae (see below). Herein, examples of economically/medically important genera of Diplostomidae will be presented.

8.5.1 *Alaria*

8.5.1.1 Life Cycle

The life cycle of *Alaria* spp. has been reviewed by Möhl et al. (2009). Various freshwater pulmonate snails serve as the first intermediate host; asexual reproduction of larval stages within these snails leads to the formation of furcocercous cercariae leaving the snail. Highly motile cercariae search for a frog (second intermediate host) in which they transform to mesocercariae. If a tadpole is infected, mesocercariae survive metamorphosis of the host. The infected frogs can be caught by a predator, and mesocercariae released in the intestine migrate through the predator body, settle in different organs/tissues, but they do not develop further. Therefore, these predators serve as paratenic (transport) hosts. Mesocercariae can survive several host transitions unharmed. Further development of mesocercariae takes place in a broad spectrum of definitive carnivore hosts (Canidae, Felidae, Mustelidae). Mesocercariae transform to diplostomulum-type metacercariae that occur in the lungs, and the adult worms (2–4 mm long) can be found in the small intestine of these hosts. Migration in the definitive host does not include a true stationary phase and is accompanied by a continuous transformation mesocercaria-metacercaria-adult worm. The eggs produced by adults leave the host via feces, and miracidia develop in the outer environment.

Some definitive hosts (e.g., felids) may serve, under certain circumstances (e.g., pregnancy), as paratenic hosts (amphiparatenesis). In this case, mesocercariae do not transform to metacercariae, and transmammary transmission of mesocercariae to the offspring may occur (Foster et al. 2009).

8.5.1.2 Occurrence

The genus *Alaria* consists of about eight species; *A. alata* (Fig. 8.10) parasitizing carnivores occurs in Europe and the former Soviet republics, whereas the remaining species (including *A. americana* and *A. marcianae*) can be found in North and South America (Moks et al. 2006). Prevalence of infection in the definitive hosts in some areas can reach 50–90% (European wolves in Estonia (Moks et al. 2006), red foxes in Poland (Kozlowska 1957; Karamon et al. 2018; Tylkowska et al. 2018), and raccoon dogs in Germany (Thiess et al. 2001)). Prevalence rates

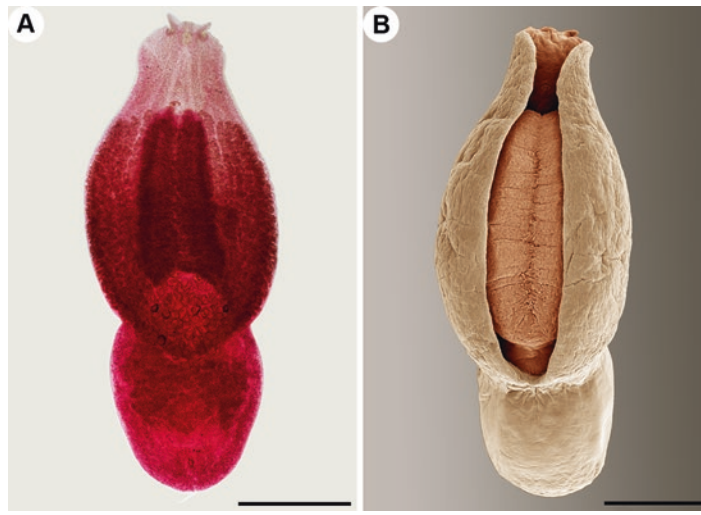

Fig. 8.10 (**a**) Strigeid trematode *Alaria alata*, carmine staining; note the dark-red Brandes' organ on the ventral part; scale bar 1 mm. (**b**) Scanning electron micrograph depicts the organization of *A. alata* forebody with the Brandes' organ (holdfast, in pink); scale bar 1 mm. (The original *Alaria* samples have been provided by Dr. R. Salamatin, Department of General Biology and Parasitology, Medical University of Warsaw)

of mesocercariae in the second intermediate hosts (frogs) (Patrelle et al. 2015) and the paratenic hosts (wild animals) can also be quite high, particularly in omnivores like wild boars.

8.5.1.3 Damage to the Intermediate or Paratenic Hosts

From the veterinary and medical viewpoints, mesocercaria seems to be the stage of enormous interest. It is particularly non-specific towards its hosts because diverse groups of vertebrates can be infected (snakes, rodents, moles, birds, felids, etc.). Mesocercaria is probably the most pathogenic stage causing tissue lesions. It can cause damage to the abdominal organs, lungs, eye, somatic muscle, and subcutaneous tissue; severe and sometimes fatal illnesses were reported also in humans (Freeman et al. 1976). As shown for traditional German meat products, a risk for the consumers is low if the respective food technological procedures are carried out properly (González-Fuentes et al. 2014).

8.5.1.4 Diagnosis and Treatment

Whereas the methods to detect *Alaria* spp. in the definitive host (proof of the eggs in stool samples, postmortem examination of the intestine for adult worms) are available, detection of mesocercariae in the meat of paratenic hosts (mainly wild boar) is under discussion. Use of compression method or pooled digestion method according to the protocols for *Trichinella* larvae seem to have limited sensitivity. Recently, a new detection method based on larval migration technique was introduced (Riehn et al. 2010). As well as, a new PCR assay for identification and characterization of *A. alata* mesocercariae has successfully been tested; it can be used for diagnostic and epidemiological purposes (Riehn et al. 2011). Praziquantel can be used for the treatment of intestinal infections with adult worms.

8.5.2 *Diplostomum*

Members of the genus are widely distributed throughout the world; taxonomy, morphology, biology, and diversity in snail and fish hosts have been reviewed by, e.g., Niewiadomska (1996, 2002), Chappell (1995), Faltýnková et al. (2016), and Scholz et al. (2016). Although species can be recognized according to the morphology of adults, a reliable determination based on morphology of larval stages is often challenging or even impossible and has led to confusion in the literature. In Europe and North America, diplostomid cercariae from lymnaeid snails or metacercariae from fish eyes have often been referred to as *D. spathaceum*. Recent investigations, commonly supported by molecular data, depicted much higher diversity and enabled accuracy in taxonomy of larval stages from snails and fish, and adult flukes from birds (Galazzo et al. 2002; Locke et al. 2010; Georgieva et al. 2013; Blasco-Costa et al. 2014; Selbach et al. 2015). Thus, for example, some studies having declared *D. spathaceum* as an experimental species in the past had, in fact, dealt with *D. pseudospathaceum* (Louhi et al. 2010; Seppälä et al. 2012a). Recently, barcoding employing cox I gene may be a useful tool for species and lineage identification, which is also important for the assessment of the distributional and host ranges of fish eye flukes and their transmission ecology (Chibwana et al. 2013; Brabec et al. 2015; Locke et al. 2015; Kudlai et al. 2017). Reliable identification is desirable, as pathology, monitoring, and control measures may vary among species (Chappell 1995) and *Diplostomum* spp. are often used in veterinary-related, ecological, and immunological studies. As Niewiadomska (1996) stressed, many early records of different *Diplostomum* spp. in fishes from Europe are based on erroneous identifications; the problem has been similar in cercariae. This fact somewhat disparages the span and accuracy of detected spectra of hosts. Moreover, mixed-species and multiple-genotype diplostomosis is common in naturally infected freshwater fishes (Rauch et al. 2005; Rellstab et al. 2011), and such co-occurrence may affect infection success of particular parasites (Seppälä et al. 2012a). On the other hand, the importance of genetic compatibility for the extent of outcrossing between different clones of *D. pseudospathaceum* was indicated experimentally (Rieger et al. 2013).

8.5.2.1 Life Cycle

The typical life cycle includes freshwater gastropods and fish as first and second intermediate hosts, respectively. Fish-eating birds from the orders Charadriiformes (e.g., gulls and terns), Anseriformes (e.g., diving ducks), Gaviiformes (e.g., loons), Pelecaniformes (e.g., herons), and some others serve as definitive hosts for adult worms (Fig. 8.11c). Furcocercariae (Fig. 8.11a) develop within sporocysts in the digestive gland of snails. Occasionally, larval progenesis has been observed to produce metacercariae in sporocysts (Lester and Lee 1976). Intramolluscan stages of *D. spathaceum* s. lat. suppress the defense system of *Lymnaea stagnalis* in terms of decreased phagocytic activity of hemocytes and reduced agglutinating activity of the hemolymph (Riley and Chappell 1992). Parasite-induced gigantism and reduced reproduction was confirmed in *L. stagnalis* infected by *D. pseudospathaceum* (Seppälä et al. 2013). This species also appeared to be dominant by outcompeting other sporocyst-producing species and preventing co-infection by redia-producing species in trematode communities parasitizing *L. stagnalis* (Soldánová et al. 2012). Huge numbers of cercariae may be released from the infected snails, almost 40,000 per individual in 24 h; production of *D. spathaceum* s. lat. cercariae is lower during night hours (Karvonen et al. 2004a). Interclonal variations in parasite transmission traits such as cercarial output, activity, survival, and infection success are not affected by current nutritional status of snail hosts (Louhi et al. 2013). After leaving the intermediate host, the cercariae respond to various cues, either environmental or host-related. In *D. pseudospathaceum* sensu Niewiadomska (1984), and Niewiadomska and Kiseliene (1994), the stimuli include direction and intensity of light radiation, dark and light stimuli, water currents, touch and gradient of CO_2; enduring contact with the host is stimulated by small hydrophilic compounds of carbohydrate character, whereas penetration is triggered by sialylated O-glycoproteins of fish mucus and fatty acids of the skin (Haas et al. 2002). Penetration is facilitated by action of muscular head organ and secretion of a cathepsin L-like cysteine peptidase released from four unicellular penetration glands (Moczoň 1994; Mikeš and Man 2003). During invasion of fish, loss of tail and major change in the carbohydrate composition of the surface glycocalyx characterize

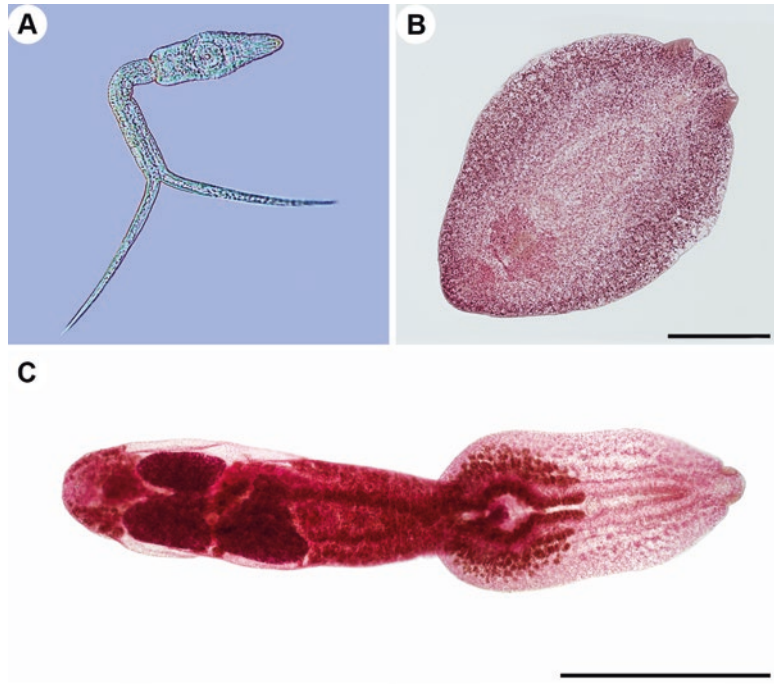

Fig. 8.11 (**a**) Typical resting position of *Diplostomum parviventosum* furcocercaria (approx. length: 600 µm) released from the intermediate host, *Radix auricularia*. (**b**) Carmine-stained diplostomulum extracted from the eye lens of common barbel, *Barbus barbus*; scale bar 200 µm. (**c**) Adult carmine-stained *Diplostomum* sp.; small intestine of the gull *Larus argentatus*; scale bar 1 mm. (Micrograph (**a**) author: Dr. M. Soldánová, Institute of Parasitology, Biology Center of the Czech Academy of Sciences)

transformation of cercaria to early diplostomulum (Mikeš and Stiegeler, unpublished). In an experiment, fish were able to avoid habitats rich in *Diplostomum* cercariae and grouping behavior seemed to enhance the effectivity of parasite avoidance (Mikheev et al. 2013). Low oxygen levels in the water correlate positively with elevated infection intensity in fish, most likely due to higher exposure of gills to cercariae, caused by increased ventilation (Mikheev et al. 2014). Mortalities may occur especially in fry or small fish due to excessive amount of invading parasites.

Rapid migration towards the anterior part of the host is accomplished mostly through the subcutaneous connective tissue and muscles of the trunk (Ratanarat-Brockelman 1974); however, studies on migration routes revealed inconsistent results and can involve also circulatory system (Haas et al. 2007). Within the host, the orientation of *D. pseudospathaceum* is mediated by Cl^- ions, glucose, arginine residues, and melatonin (Haas et al. 2007). Depending on parasite species, the typical localizations of unencysted metacercariae of diplostomulum type are eye lens, retina, aqueous humor, and/or brain or spinal cord (Fig. 8.11b). The number of diplostomula in the fish eye lenses seems to correlate positively and linearly both with infection dose and time of exposure in experimental infection (Voutilainen 2013). Fish length and age, but not sex, may significantly affect the structure of *Diplostomum* spp. infracommunities in the lenses (Désilets et al. 2013). Temperature-dependent maturation of long-living metacercariae of *D. spathaceum* s. str., until becoming infective for a final host, takes about 8 weeks in the eye (Whyte et al. 1991). Metacercariae of *Diplostomum* spp. in the eye may be mistaken for some specimens of *Tylodelphys* spp. (e.g., *T. clavata*), which in contrast apparently prefer localization in the vitreous body.

8.5.2.2 Damage to the Intermediate Fish Host

Pathobiology of fish eye flukes of the genus *Diplostomum* has been reviewed repeatedly (Chappell et al. 1994; Karvonen 2012). The main effect lies in the formation of cataracts, exophthalmia or even destruction of eye lenses and complete blindness, especially in chronic and heavy infections. Mechanical disengagement between the retinal pigmentary epithelium and the neurosensory retina may occur, with damaged cones and rods in the outer segment and epithelium reduced to a single layer of pigmentary cells (Padrós et al. 2018). Direct mortality has only rarely been reported, but restraint of vision may alter feeding and anti-predatory behavior that may lead to debilitation of fish and effortless catch by predators. Behavioral traits in fish infected by *D. pseudospathaceum* are manipulated specifically, leading to predisposition of fish to bird predators, but vulnerability to non-host fish predators remains unaffected (Seppälä et al. 2012b). Differences in the infection intensity may occur between male and female fish hosts (Karvonen and Lindström 2018). Some diplostomid species invading the cranial cavity and brain alter swimming behavior or personality traits in infected fish (Kekäläinen et al. 2014; Correa et al. 2014).

An example of species with affinity to CNS can be *D. phoxini*. Its metacercariae express a specific uneven pattern of distribution in minnows *Phoxinus phoxinus*; the parasites are most abundant in brain regions known to be important in the control of antipredator responses, like cerebellum, medulla oblongata, and optic lobes, suggesting manipulation of host behavior by the parasite (Barber and Crompton 1997). Yet the effect can be a consequence of pathology since significant granular necrotic reactions frequently occur around metacercariae (Dezfuli et al. 2007). Some members of a few other genera of diplostomids develop in the CNS. Metacercariae of *Austrodiplostomum mordax* and *Tylodelphys destructor* reported from Americas were mainly observed in the interlobular infoldings, meninges, ventricles, and cerebellum of the brain, causing hemorrhaging, cell necrosis, inflammation, nerve fiber disruption and fibrosis, namely in case of higher parasite load (Heckmann 1992; Siegmund et al. 1997). In *Ornithodiplostomum ptychocheilus*, the post-penetration larval stages readily associate with cranial nerves to access

directly the brain or migrate along/via peripheral nerves to the spinal cord/neural canal first, and then through to the brain (Hendrickson 1979; Matisz et al. 2010).

Unlike fish blood flukes, the eye flukes do not represent an extreme risk to the fish industry in terms of direct mortality (perhaps except for hatcheries). Nevertheless, the impaired vision/blindness and associated decrease of food uptake mean lower weight gain which, together with the risk of higher predation in open freshwater fisheries, may result in significant economic loss that has still not been well elucidated. There is only an experimental evidence of increased parasite-induced overwinter mortality in juvenile European bitterling (which is not a species of economic importance) (Michálková and Ondračková 2014). Actually, the prevalence and abundance of some eye fluke species may be quite high both in snail and fish hosts, like in *D. spathaceum* and *D. pseudospathaceum* (Buchmann and Bresciani 1997; Karvonen et al. 2006; Soldánová et al. 2012). Rapid transmission occurs in places where all three hosts meet extensively due to high population densities and specific conditions (as can be seen in eutrophicated, crowded carp ponds rich in vegetation and nesting colonies of gulls in South Bohemia, Czech Republic).

Human infections by fish eye flukes have not been recognized. Cercariae of *D. spathaceum* s. lat. are able to enter the cornea of some mammals and humans under experimental conditions. Although being mostly immobilized within 24 h in experimental animals, they possibly might represent a potential risk to bathers due to superficial pathological changes in eyes and temporary conjunctival inflammation (Lester and Freeman 1976).

8.5.2.3 Diagnosis and Control

Diagnosis of diplostomosis in fish is primarily based on inspection of predilection sites in dissected fish (eye, brain). Alternatively, intravital diagnosis of the disease can be done by ophthalmological microscope (Karvonen et al. 2004b), indeed, with some limitations in the field. Species diagnosis should be supported by common genetic markers (ITS regions and cytochrome c oxidase I gene), especially in case of larval and juvenile stages showing high degree of morphological similarity. Sophisticated analyses of trematode parasite communities in fish eye lenses can currently be performed by methods of next generation sequencing (Rellstab et al. 2011).

Prevention of diplostomosis can rely on removal of snail hosts and management of the environment or water supply to aquaculture (Field and Irwin 1994; also see the previous parts of the chapter). Freshwater mussels may decrease the abundance of cercariae in water reservoirs thanks to their filtration capacity (Gopko et al. 2017). Immunoprophylaxis is not available, though preliminary attempts have been made to immunize trout by cercarial homogenates or low doses of cercariae, all of them only partly successful (Speed and Pauley 1985; Whyte et al. 1990; Karvonen et al. 2010). Significant cross-reactivity in terms of fish adaptive immune response was found among various *D. pseudospathaceum* genotypes (Rellstab et al. 2013). On the other hand, increased resistance to the infection by this species in repeatedly infected sticklebacks could not be explained by a prominent adaptive immune response—rather cells of the innate immunity acted in the defense against the parasite (Scharsack and Kalbe 2014). Effectiveness of potential antihelminthic chemotherapy by praziquantel was tested experimentally in barbel (*Barbus barbus*). The exposure of infected fish to 10 and 20 mg/L of the drug in a 4-day treatment bath resulted in complete extermination of *D. spathaceum* s. lat. metacercariae. The estimated 96-h LD50 for the barbel was 28.6 mg/L (Zusková et al. 2018). Despite the latter result, antihelminthic treatment of diplostomosis is not used in common practice.

8.5.3 Black Spot Disease

Members of another group of diplostomid flukes form neascus-type metacercariae in the superficial layers of their second intermediate fish hosts (Lane and Morris 2000). Again, fish-eating birds serve as definitive hosts.

Fish can be infected by furcocercous cercariae that emerge from freshwater snails and penetrate the skin of fish. Once in the skin or the underlying tissues, the larvae release secretions to form a hyaline metacercarial cyst wall. Usually, the fish host reacts by formation of the outer fibrous capsule, and the space between the cyst and the capsule is filled with a viscous material (Wittrock et al. 1991). These processes attract fish melanocytes and, as a consequence, melanin is deposited around the parasite (Fig. 8.12a); black spots in the surface layers of fish are then visible by naked eye (Fig. 8.12b). Parasite-induced black spots can be differentiated from other patterns formed by melanin-containing cells by their size, intensity, and random distribution (Tobler and Schlupp 2008).

As for pathogenic effect, the penetrating cercariae may cause mechanical damage, hemorrhage, and secondary infections. Loss of fish weight and body lipids, together with increased oxygen requirements, may occur. As a consequence, fish ability to overwinter (survive) may be diminished (Lane and Morris 2000). In addition, poor condition and parasite-induced changes in fish behavior may increase susceptibility to predation. For example, the Prussian carp *Carassius auratus* infected by *Posthodiplostomum cuticola* can be more often consumed by its predator, the perch *Perca fluviatilis* (Ondračková et al. 2006).

8.5.3.1 *Uvulifer*

Uvulifer spp. are one of the causative agents of black spot disease. For example, *U. ambloplitis* parasitizes the intestine of the belted kingfisher (*Megaceryle alcyon*) in the USA (Lane and Morris 2000). The unembryonated eggs are voided to the outer environment with feces. After several weeks, miracidia leave the eggs and penetrate the body surface of intermediate snail host (*Planorbella* spp.). Cercariae produced by sporocysts after several weeks of the asexual reproduction leave the snail host and enter the skin of fish (e.g., sunfish *Lepomis* spp. and yellow perch *Perca flavescens*). Black spots of 1–3 mm can be

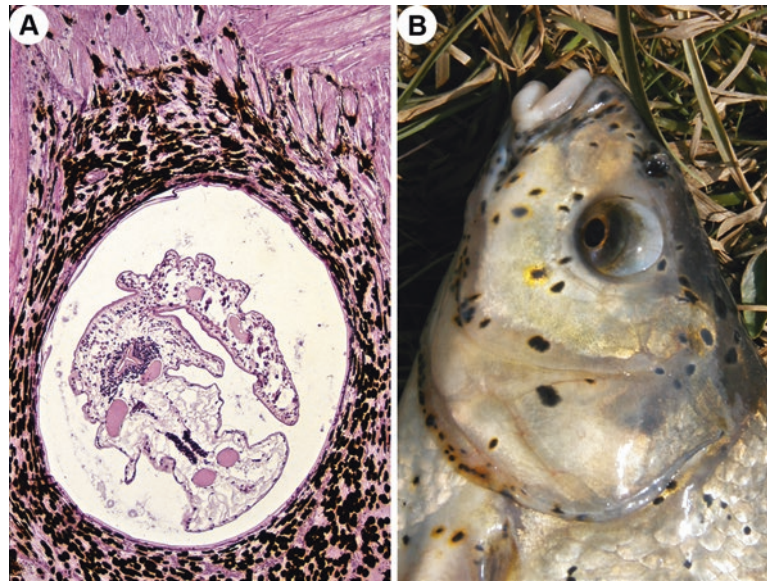

Fig. 8.12 (**a**) Histological section showing a neascus-type metacercaria of *Posthodiplostomum cuticola* from the European bitterling, *Rhodeus sericeus*. Dark-brown deposits of melanin around the parasite cyst are visible; the size of the resulting black spot is variable. (**b**) Black spot disease caused by metacercariae of *Posthodiplostomum* sp. in the common bream, *Abramis brama*. (Micrograph (**a**) author: Prof. I. Dyková, Institute of Botany and Zoology, Faculty of Science, Masaryk University; Micrograph (**b**): Courtesy of Dr. M. Ondračková, Institute of Vertebrate Biology of the Czech Academy of Sciences)

found in the skin, tail base, fins, and musculature. When kingfisher catches the infected fish, the life cycle is completed.

8.5.3.2 *Posthodiplostomum*

Posthodiplostomum cuticola is another fluke causing black spot disease (black spots of about 1 mm). It is a common parasite of freshwater fishes; it has been found in over 70 fish species in Palearctic region. Within the life cycle, planorbid snails (*Planorbis planorbis*, *Planorbarius corneus*) serve as the first intermediate host (Ondračková et al. 2004). There is a broad range of second intermediate hosts, but the members of Cyprinidae are the most frequently attacked fishes; in some cases (e.g. in Croatia) 100% prevalence of infection has been recorded (Zrnčić et al. 2009). Fish-eating birds, usually a heron or other members of Ardeidae, complete the life cycle as definitive hosts.

Besides poor conditions and potential mortality of fish, black spots often render the infected fish undesirable to consumers. Concerning preventive measures in fish farming, contact of fish with cercariae should be avoided because the infected fish cannot be treated. Therefore, a control of fish-eating birds and snails at the locality should be used.

It should be noted that species of the same genus have also a different location in the body of their second intermediate hosts. For example, *Posthodiplostomum minimum*/*Posthodiplostomum centrarchi* encyst within the body cavity (Schleppe and Goater 2004; Stoyanov et al. 2017); the parasites are frequently found in North America—in lakes in north-central Alberta, Canada, and the prevalence of infection in adult minnows is typically 100% (Sandland et al. 2001). The latter species is also present in Europe (Kvach et al. 2017, 2018; Stoyanov et al. 2017).

Acknowledgements The recent research of the authors was supported by the project "Centre for Research of Pathogenicity and Virulence of Parasites" (No. CZ.02.1.01/0.0/0.0/16_019/0000759) funded by European Regional Development Fund and Ministry of Education, Youth and Sports of the Czech Republic, Czech Science Foundation grant (No. 18-11140S), and Charles University institutional support (PROGRES Q43, UNCE/SCI/012—204072/2018, and SVV 260432/2018). We appreciate helpfulness of our colleagues at different institutions (Dr. S. V. Brant, Prof. I. Dyková, Dr. R. Iglesias, Mgr. T. Macháček, Dr. M. Ondračková, Dr. L. Panská, Dr. R. Salamatin, Dr. J. Sitko, Dr. M. Soldánová) who provided samples and/or photos of helminths mentioned in our chapter (see their affiliations in the figure legends), and colleagues at the Institute of Parasitology, Biology Center of the Czech Academy of Sciences (Prof. T. Scholz, Doc. R. Kuchta, Dr. B. Škoríková) who enabled us to take photos of helminths in the Helminthological Collection.

References

Adnyana W, Ladds PW, Blair D (1997) Efficacy of praziquantel in the treatment of green sea turtles with spontaneous infection of cardiovascular flukes. Aust Vet J 75:405–407

Agatsuma T, Rajapakse RPVJ, Kuruwita VY (2004) Molecular taxonomic position of the elephant schistosome, *Bivitellobilharzia nairi*, newly discovered in Sri Lanka. Parasitol Int 53:69–75

Agrawal MC, Rao VG (2018) Some facts on south asian schistosomiasis and need for international collaboration. Acta Trop 180:76–80

Aguirre AA, Spraker TR, Balazs GH, Zimmerman B (1998) Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian islands. J Wildl Dis 34:91–98

Aiken HM, Hayward CJ, Nowak BF (2006) An epizootic and its decline of a blood fluke, *Cardicola forsteri*, in farmed southern bluefin tuna, *Thunnus maccoyii*. Aquaculture 254:40–45

Aiken HM, Bott NJ, Mladineo I, Montero FE, Nowak BF, Hayward CJ (2007) Molecular evidence for cosmopolitan distribution of platyhelminth parasites of tunas (*Thunnus* spp.). Fish Fish 8:167–180

Aiken HM, Hayward CJ, Crosbie P, Watts M, Nowak BF (2008) Serological evidence of an antibody response in farmed southern bluefin tuna naturally infected with the blood fluke *Cardicola forsteri*. Fish Shellfish Immunol 25:66–75

Aiken HM, Hayward CJ, Nowak BF (2015) Factors affecting abundance and prevalence of blood fluke, *Cardicola forsteri*, infection in commercially ranched southern bluefin tuna, *Thunnus maccoyii*, in Australia. Vet Parasitol 210:106–113

Alama-Bermejo G, Montero FE, Raga JA, Holzer AS (2011) *Skoulekia meningialis* n. gen., n. sp. (Digenea: Aporocotylidae Odhner, 1912), a parasite surrounding the brain of the Mediterranean common two-banded seabream *Diplodus vulgaris* (Geoffroy Sain-Hilaire, 1817) (Teleostei: Sparidae): description, molecular phylogeny, habitat and pathology. Parasitol Int 60:34–44

Aldhoun JA, Littlewood DTJ (2012) *Orientobilharzia* Dutt & Srivastava, 1955 (Trematoda: Schistosomatidae), a

junior synonym of *Schistosoma* Weinland, 1858. Syst Parasitol 82:81–88

Arfaa F, Sabaghian H, Ale-Dawood H (1965) Studies on *Ornithobilharzia turkestanicum* (Skrjabin, 1913) Price, 1929 in Iran. Ann Parasitol Hum Comp 40:45–50

Barber I, Crompton DWT (1997) The distribution of the metacercariae of *Diplostomum phoxini* in the brain of minnows, *Phoxinus phoxinus*. Folia Parasitol 44:19–25

Basch PF, Gupta BC (1988) Immunocytochemical localization of regulatory peptides in six species of trematode parasites. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 91:565–570

Beer SA, Voronin MV (2007) Cercariases in urban ecosystems. Nauka, Moscow

Blasco-Costa I, Locke SA (2017) Life history, systematics and evolution of the Diplostomoidea Poirier, 1886: progress, promises and challenges emerging from molecular studies. Adv Parasitol 98:167–225

Blasco-Costa I, Faltýnková A, Georgieva S, Skírnisson K, Scholz T, Kostadinova A (2014) Fish pathogens near the Arctic Circle: molecular, morphological and ecological evidence for unexpected diversity of *Diplostomum* (Digenea: Diplostomidae) in Iceland. Int J Parasitol 44:703–715

Boissier J, Grech-Angelini S, Webster BL, Allienne J-F, Huyse T, Mas-Coma S, Toulza E, Barré-Cardi H, Rollinson D, Kincaid-Smith J, Oleaga A, Galinier R, Foata J, Rognon A, Berry A, Mouahid G, Henneron R, Moné H, Noel H, Mitta G (2016) Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study. Lancet Infect Dis 16:971–979

Boulanger D, Schneider D, Chippaux J-P, Sellin B, Capron A (1999) *Schistosoma bovis*: vaccine effects of a recombinant homologous glutathione S-transferase in sheep. Int J Parasitol 29:415–418

Brabec J, Kostadinova A, Scholz T, Littlewood DTJ (2015) Complete mitochondrial genomes and nuclear ribosomal RNA operons of two species of *Diplostomum* (Platyhelminthes: Trematoda): a molecular resource for taxonomy and molecular epidemiology of important fish pathogens. Parasit Vectors 8:336

Brant SV, Loker ES (2005) Can specialized pathogens colonize distantly related hosts? Schistosome evolution as a case study. PLoS Pathog 1:e38

Brant SV, Loker ES (2009) Molecular systematics of the avian schistosome genus *Trichobilharzia* (Trematoda: Schistosomatidae) in North America. J Parasitol 95:941–963

Bray RA, Cribb TH, Littlewood DTJ (2012) *Sasala nolani* gen. n., sp n. (Digenea: Aporocotylidae) from the body-cavity of the guineafowl puffer fish *Arothron meleagris* (Lacepede) (Tetraodontiformes: Tetraodontidae) from off Moorea, French Polynesia. Zootaxa 3334:29–41

Buchmann K, Bresciani J (1997) Parasitic infections in pond-reared rainbow trout *Oncorhynchus mykiss* in Denmark. Dis Aquat Org 28:125–138

Bulantová J, Chanová M, Houžvičková L, Horák P (2011) *Trichobilharzia regenti* (Digenea: Schistosomatidae): changes of body wall musculature during the development from miracidium to adult worm. Micron 42:47–54

Bullard SA (2013) *Cardicola langeli* sp. n. (Digenea: Aporocotylidae) from heart of sheepshead, *Archosargus probatocephalus* (Actinopterygii: Sparidae) in the Gulf of Mexico, with an updated list of hosts, infection sites and localities for *Cardicola* spp. Folia Parasitol 60:17–27

Bullard SA, Overstreet RM (2002) Potential pathological effects of blood flukes (Digenea: Sanguinicolidae) on pen-reared marine fishes. Proc Gulf Caribb Fish Inst 53:10–25

Bullard SA, Jensen K, Overstreet RM (2009) Historical account of the two family-group names in use for the single accepted family comprising the "fish blood flukes". Acta Parasitol 54:78–84

Carvalho YBM, Poersch LHD, Romano LA (2015) Parasites and pathological condition of two edible clams from the Cassino beach, southern Brazil. Bull Eur Assoc Fish Pathol 35:41–49

Chanová M, Horák P (2007) Terminal phase of bird schistosomiasis caused by *Trichobilharzia regenti* (Schistosomatidae) in ducks (*Anas platyrhynchos* f. domestica). Folia Parasitol 54:105–107

Chanová M, Vuong S, Horák P (2007) *Trichobilharzia szidati*: the lung phase of migration within avian and mammalian hosts. Parasitol Res 100:1243–1247

Chapman PA, Traub RJ, Kyaw-Tanner MT, Owen H, Flint M, Cribb TH, Mills PC (2016) Terminal restriction fragment length polymorphism for the identification of spirorchiid ova in tissues from the green sea turtle, *Chelonia mydas*. PLoS One 11:e0162114

Chapman PA, Owen H, Flint M, Soares Magalhães RJ, Traub RJ, Cribb TH, Kyaw-Tanner MT, Mills PC (2017) Molecular epidemiology and pathology of spirorchiid infection in green sea turtles (*Chelonia mydas*). Int J Parasitol Parasites Wildl 6:39–47

Chappell LH (1995) The biology of diplostomatid eyeflukes of fishes. J Helminthol 69:97–101

Chappell LH, Hardie LJ, Secombes CJ (1994) Diplostomiasis: the disease and host-parasite interactions. In: Pike AW, Lewis JW (eds) Parasitic diseases of fish. Samara Publishing, Wales

Chen HC, Kuo RJ, Chang TC, Hus CK, Bray RA, Cheng IJ (2012) Fluke (Spirorchiidae) infections in sea turtles stranded on Taiwan: prevalence and pathology. J Parasitol 98:437–439

Chibwana FD, Blasco-Costa I, Georgieva S, Hosea KM, Nkwengulila G, Scholz T, Kostadinova A (2013) A first insight into the barcodes for African diplostomids (Digenea: Diplostomidae): brain parasites in *Clarias gariepinus* (Siluriformes: Clariidae). Infect Genet Evol 17:62–70

Corapi WV, Ajithdoss DK, Snowden KF, Spaulding KA (2011a) Multi-organ involvement of *Heterobilharzia americana* infection in a dog presented for systemic mineralization. J Vet Diagn Investig 23:826–831

Corapi WV, Birch SM, Carlson KL, Chaffin MK, Snowden KF (2011b) *Heterobilharzia americana* infection as a

cause of hepatic parasitic granulomas in a horse. J Am Vet Med Assoc 239:1117–1122

Corapi WV, Snowden KF, Rodrigues A, Porter BF, Buote MS, Birch SM, Jackson ND, Eden KB, Whitley DB, Mansell J, Edwards JF, Hardy J, Chaffin MK (2012) Natural *Heterobilharzia americana* infection in horses in Texas. Vet Pathol 49:552–556

Correa LL, Souza GTR, Takemoto RM, Ceccarelli PS, Adriano EA (2014) Behavioral changes caused by *Austrodiplostomum* spp. in *Hoplias malabaricus* from the São Francisco River, Brazil. Parasitol Res 113:499–503

Cribb TH, Bray RA (2010) Gut wash, body soak, blender and heat-fixation: approaches to the effective collection, fixation and preservation of trematodes of fishes. Syst Parasitol 76:1–7

Cribb TH, Bray RA (2011) Trematode families and genera: have we found them all? Trends Parasitol 27:149–154

Cribb TH, Bray RA, Littlewood DTJ (2001) The nature and evolution of the association among digeneans, molluscs and fishes. Int J Parasitol 31:997–1011

Cribb TH, Adlard RD, Hayward CJ, Bott NJ, Ellis D, Evans D, Nowak BF (2011) The life cycle of *Cardicola forsteri* (Trematoda: Aporocotylidae), a pathogen of ranched southern bluefin tuna, *Thunnus maccoyii*. Int J Parasitol 41:861–870

Cribb TH, Chick RC, O'Connor W, O'Connor S, Johnson D, Sewell KB, Cutmore SC (2017a) Evidence that blood flukes (Trematoda: Aporocotylidae) of chondrichthyans infect bivalves as intermediate hosts: indications of an ancient diversification of the Schistosomatoidea. Int J Parasitol 47:885–891

Cribb TH, Crespo-Picazo JL, Cutmore SC, Stacy BA, Chapman PA, García-Párraga D (2017b) Elucidation of the first definitively identified life cycle for a marine turtle blood fluke (Trematoda: Spirorchiidae) enables informed control. Int J Parasitol 47:61–67

Davis NE (2006) A survey of waterfowl for echinostomes and schistosomes from Lake Wanaka and the Waitaki River watershed, New Zealand. J Helminthol 80:33–40

De Buron I, Colon BL, Siegel SV, Oberstaller J, Rivero A, Kyle DE (2018) First evidence of polychaete intermediate hosts for *Neospirorchis* spp. marine turtle blood flukes (Trematoda: Spirorchiidae). Int J Parasitol 48(14):1097–1106

De Bont J, Van Aken D, Vercruysse J, Fransen J, Southgate VR, Rollinson D (1989) The prevalence and pathology of *Schistosoma nasale* Rao, 1933 in cattle in Sri Lanka. Parasitology 98:197–202

De Jong-Brink M, Bergamin-Sassen M, Solis Soto M (2001) Multiple strategies of schistosomes to meet their requirements in the intermediate snail host. Parasitology 123:S129–S141

De la Torre-Escudero E, Manzano-Román R, Pérez-Sánchez R, Siles-Lucas M, Oleaga A (2010) Cloning and characterization of a plasminogen-binding surface-associated enolase from *Schistosoma bovis*. Vet Parasitol 173:76–84

De la Torre-Escudero E, Manzano-Román R, Valero L, Oleaga A, Pérez-Sánchez R, Hernándes-González A, Siles-Lucas M (2011) Comparative proteomic analysis of *Fasciola hepatica* juveniles and *Schistosoma bovis* schistosomula. J Proteome 74:1534–1544

De la Torre-Escudero E, Manzano-Román R, Pérez-Sánchez R (2012a) Molecular cloning, characterization and diagnostic performance of the *Schistosoma bovis* 22.6 antigen. Vet Parasitol 190:530–540

De la Torre-Escudero E, Manzano-Román R, Siles-Lucas M, Pérez-Sánchez R, Moyano JC, Barrera I, Oleaga A (2012b) Molecular and functional characterization of a *Schistosoma bovis* annexin: fibrinolytic and anticoagulant activity. Vet Parasitol 184:2–36

De la Torre-Escudero E, Pérez-Sánchez R, Manzano-Román R, Oleaga A (2017) *Schistosoma bovis*-host interplay: proteomics for knowing and acting. Mol Biochem Parasitol 215:30–39

Dennis MM, Landos M, D'Antignana T (2011) Case-control study of epidemic mortality and *Cardicola forsteri*-associated disease in farmed southern bluefin tuna (*Thunnus maccoyii*) of South Australia. Vet Pathol 48:846–855

Désilets HD, Locke SA, McLaughlin JD, Marcogliese DJ (2013) Community structure of *Diplostomum* spp. (Digenea: Diplostomidae) in eyes of fish: main determinants and potential interspecific interactions. Int J Parasitol 43:929–939

Devkota R, Brant SV, Thapa A, Loker ES (2014) Sharing schistosomes: the elephant schistosome *Bivitellobilharzia nairi* also infects the greater one-horned rhinoceros (*Rhinoceros unicornis*) in Chitwan National Park, Nepal. J Helminthol 88:32–40

Dezfuli BS, Capuano S, Simoni E, Giari L, Shinn AP (2007) Histopathological and ultrastructural observations of metacercarial infections of *Diplostomum phoxini* (Digenea) in the brain of minnows *Phoxinus phoxinus*. Dis Aquat Org 75:51–59

Dutt SC (1967) Studies on *Schistosoma nasale* Rao, 1933. I. Morphology of the adults, egg and larval stages. Indian J Vet Sci 37:249–262

Dvořák J, Vaňáčová Š, Hampl V, Flegr J, Horák P (2002) Comparison of European *Trichobilharzia* species based on ITS1 and ITS2 sequences. Parasitology 124:307–313

Dvořák J, Mashiyama ST, Braschi S, Sajid M, Knudsen GM, Hansell E, Lim KC, Hsieh I, Bahgat M, Mackenzie B, Medzihradszky KF, Babbitt PC, Caffrey CR, McKerrow JH (2008) Differential use of protease families for invasion by schistosome cercariae. Biochimie 90:345–358

Erickson DG, Wallace FG (1959) Studies on blood flukes of the genus *Sanguinicola*. J Parasitol 45:310–322

Faltýnková A, Niewiadomska K, Santos MJ, Valtonen ET (2007) Furcocercous cercariae (Trematoda) from freshwater snails in central Finland. Acta Parasitol 52:310–317

Faltýnková A, Sures B, Kostadinova A (2016) Biodiversity of trematodes in their intermediate mollusc and fish hosts in the freshwater ecosystems of Europe. Syst Parasitol 93:283–293

Ferreras-Estrada MC, García-Iglesias MJ, Pérez-Martínez C, Manga-González MY, Ramajo-Martín V, Escudero-Diez A, García-Marín JF (1998) A pathological study of experimental long-standing *Schistosoma bovis* infection in sheep. J Comp Pathol 119:479–484

Field JS, Irwin SWB (1994) The epidemiology, treatment and control of diplostomiasis on a fish farm in Northern Ireland. In: Pike AW, Lewis JW (eds) Parasitic diseases of fish. Samara Publishing, Wales

Flint M, Blair D, Patterson-Kane J, Mills PC (2010a) Blood flukes (Spirorchiidae) as a major cause of marine turtle mortality in Queensland. In: Proceedings of the XII international congress of parasitology (ICOPA), Melbourne, Australia. Medimond, Italy, pp 57–61

Flint M, Patterson-Kane JC, Limpus CJ, Mills PC (2010b) Health surveillance of stranded green turtles in southern Queensland, Australia (2006–2009): an epidemiological analysis of causes of disease and mortality. EcoHealth 7:135–145

Flowers JR, Hammerberg B, Wood SL, Malarkey DE, Van Dam GJ, Levy MG, McLawhorn MD (2002) *Heterobilharzia americana* infection in a dog. J Am Vet Med Assoc 220:193–196

Forte-Gil D, Holzer AS, Pecková H, Bartošová-Sojková P, Peñalver J, Dolores EM, Muñoz P (2016) Molecular and morphological identification of *Cardicola* (Trematoda: Aporocotylidae) eggs in hatchery-reared and migratory Atlantic bluefin tuna (*Thunnus thynnus* L.). Aquaculture 450:58–66

Foster GW, Kinsella JM, Sheppard BJ, Cunningham MW (2009) Transmammary infection of free-ranging Florida panther neonates by *Alaria marcianae* (Trematoda: Diplostomatidae). J Parasitol 95:238–239

Fransen J, De Bont J, Vercruysse J, Van Aken D, Southgate VR, Rollinson D (1990) Pathology of natural infections of *Schistosoma spindale* Montgomery, 1906, in cattle. J Comp Pathol 103:447–455

Freeman RS, Stuart PF, Cullen SJ, Ritchie AC, Mildon A, Fernandes BJ, Bonin R (1976) Fatal human infection with mesocercariae of the trematode *Alaria americana*. Am J Trop Med Hyg 25:803–807

Fukuda Y, Miyamura K, Hitaka E, Kimoto K, Sanada Y, Asai T, Ogawa K (2017) Blood fluke infection of Japanese amberjack *Seriola quinqueradiata* in fish farms along the western coastal area of Bungo Channel, Japan. Fish Pathol 52:191–197

Galazzo DE, Dayanandan S, Marcogliese DJ, McLaughlin JD (2002) Molecular systematics of some North American species of *Diplostomum* (Digenea) based on rDNA-sequence data and comparisons with European congeners. Can J Zool 80:2207–2217

Georgieva S, Soldánová M, Perez-Del-Olmo A, Dangel DR, Sitko J, Sures B, Kostadinova A (2013) Molecular prospecting for European *Diplostomum* (Digenea: Diplostomidae) reveals cryptic diversity. Int J Parasitol 43:57–72

Gilardoni C, Posadas G, Kroeck MA, Cremonte F (2011) Monorchiid and aporocotylid cercariae (Digenea) parasitising the purple clam *Amiantis purpurata* (Bivalvia, Veneridae) from the Southwestern Atlantic coast. Acta Parasitol 56:385–391

Glazebrook JS, Campbell RS, Blair D (1981) Pathological changes associated with cardiovascular trematodes (Digenea: Spirorchidae) in a green sea turtle *Chelonia mydas* (L). J Comp Pathol 91:361–368

González-Fuentes H, Hamedy A, Von Borell E, Luecker E, Riehn K (2014) Tenacity of *Alaria alata* mesocercariae in homemade German meat products. Int J Food Microbiol 176:9–14

Gopko M, Mironova E, Pasternak A, Mikheev V, Taskinen J (2017) Freshwater mussels (*Anodonta anatina*) reduce transmission of a common fish trematode (eye fluke, *Diplostomum pseudospathaceum*). Parasitology 114:1971–1979

Graczyk TK, Shiff CJ (2000) Recovery of avian schistosome cercariae from water using penetration stimulant matrix with an unsaturated fatty acid. Am J Trop Med Hyg 63:174–177

Haas W (2003) Parasitic worms: strategies of host finding, recognition and invasion. Zoology 106:349–364

Haas W, Van de Roemer A (1998) Invasion of the vertebrate skin by cercariae of *Trichobilharzia ocellata*: penetration processes and stimulating host signals. Parasitol Res 84:787–795

Haas W, Granzer M, Brockelman CR (1990) Finding and recognition of the bovine host by the cercariae of *Schistosoma spindale*. Parasitol Res 76:343–350

Haas W, Stiegeler P, Keating A, Kullmann B, Rabenau H, Schönamsgruber E, Haberl B (2002) *Diplostomum spathaceum* cercariae respond to a unique profile of cues during recognition of their fish host. Int J Parasitol 32:1145–1154

Haas W, Wulff C, Grabe K, Meyer V, Haeberlein S (2007) Navigation within host tissues: cues for orientation of *Diplostomum spathaceum* (Trematoda) in fish towards veins, head and eye. Parasitology 134:1013–1023

Hardy-Smith P, Ellis D, Humphrey J, Evans M, Evans D, Rough K, Valdenegro V, Nowak B (2012) *In vitro* and *in vivo* efficacy of anthelmintic compounds against blood fluke (*Cardicola forsteri*). Aquaculture 334:39–44

Hayward CJ, Ellis D, Foote D, Wilkinson RJ, Crosbie PB, Bott NJ, Nowak BF (2010) Concurrent epizootic hyperinfections of sea lice (predominantly *Caligus chiastos*) and blood flukes (*Cardicola forsteri*) in ranched southern bluefin tuna. Vet Parasitol 173:107–115

Heckmann RA (1992) Host records and tissue locations for *Diplostomum mordax* (metacercariae) inhabiting the cranial cavity of fishes from Lake Titicaca, Peru. J Parasitol 78:541–543

Hendrickson GL (1979) *Ornithodiplostomum ptychocheilus*: migration to the brain of the fish intermediate host, *Pimephales promelas*. Exp Parasitol 48:245–258

Herbst LH, Jacobson ER, Klein PA, Balazs GH, Moretti R, Brown T, Sundberg JP (1999) Comparative pathology and pathogenesis of spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). Vet Pathol 36:551–564

Hernández-Mena DI, García-Varela M, Pérez-Ponce de León G (2017) Filling the gaps in the classification of the Digenea Carus, 1863: systematic position of the Proterodiplostomidae Dubois, 1936 within the superfamily Diplostomoidea Poirier, 1886, inferred from nuclear and mitochondrial DNA sequences. Syst Parasitol 94:833–848

Hertel J, Hamburger J, Haberl B, Haas W (2002) Detection of bird schistosomes in lakes by PCR and filter-hybridization. Exp Parasitol 101:57–63

Holliman RB, Fisher JE (1968) Life cycle and pathology of *Spirorchis scripta* Stunkard, 1923 (Digenea: Spirorchiidae) in *Chrysemys picta picta*. J Parasitol 54:310–318

Holliman RB, Fisher JE, Parker JC (1971) Studies on *Spirorchis parvus* (Stunkard, 1923) and its pathological effects on *Chrysemys picta picta*. J Parasitol 57:71–77

Horák P, Kolářová L (2001) Bird schistosomes—do they die in mammalian skin? Trends Parasitol 17:66–69

Horák P, Kolářová L (2005) Molluscan and vertebrate immune responses to bird schistosomes. Parasite Immunol 27:247–255

Horák P, Kolářová L (2011) Snails, waterfowl and cercarial dermatitis. Freshw Biol 56:779–790

Horák P, Kovář L, Kolářová L, Nebesářová J (1998) Cercaria-schistosomulum surface transformation of *Trichobilharzia szidati* and its putative immunological impact. Parasitology 116:139–147

Horák P, Dvořák J, Kolářová L, Trefil L (1999) *Trichobilharzia regenti*, a pathogen of the avian and mammalian central nervous system. Parasitology 119:577–581

Horák P, Kolářová L, Adema CM (2002) Biology of the schistosome genus *Trichobilharzia*. Adv Parasitol 52:155–233

Horák P, Mikeš L, Rudolfová J, Kolářová L (2008) Penetration of *Trichobilharzia* cercariae into mammals: dangerous or negligible event? Parasite 15:299–303

Horák P, Schets FM, Kolářová L, Brant SV (2013) Trichobilharzia. In: Liu D (ed) Molecular detection of human parasitic pathogens. Taylor and Francis, CRC Press, Boca Raton, pp 455–465

Horák P, Mikeš L, Lichtenbergová L, Skála V, Soldánová M, Brant SV (2015) Avian schistosomes and outbreaks of cercarial dermatitis. Clin Microbiol Rev 28:165–190

Huňová K, Kašný M, Hampl V, Leontovyč R, Kuběna A, Mikeš L, Horák P (2012) *Radix* spp.: identification of trematode intermediate hosts in the Czech Republic. Acta Parasitol 57:273–284

Iglesias R, García-Estévez JM, Ayres C, Acuña A, Cordero-Rivera A (2015) First reported outbreak of severe spirorchiidiasis in *Emys orbicularis*, probably resulting from a parasite spillover event. Dis Aquat Org 113:75–80

Ishimaru K, Mine R, Shirakashi S (2013) Praziquantel treatment against *Cardicola* blood flukes: determination of the minimal effective dose and pharmacokinetics in juvenile Pacific bluefin tuna. Aquaculture 402–403:24–27

Jacobson ER, Harman GR, Maxwell LK, Laille EJ (2003) Plasma concentrations of praziquantel in loggerhead sea turtles, *Caretta caretta*, following oral administration of single and multiple doses. Am J Vet Res 64:304–308

Johansen MV, Monrad J, Christensen NØ (1996) Effects of praziquantel on experimental *Schistosoma bovis* infection in goats. Vet Parasitol 62:83–91

Juhász A, Dán Á, Dénes B, Kucsera I, Danka J, Majoros G (2016) A rare zoonosis in Hungary: cercarial dermatitis caused by *Schistosoma turkestanicum* blood-fluke. Orv Hetil 157:1579–1586

Kalbe M, Haberl B, Haas W (1997) Miracidial host-finding in *Fasciola hepatica* and *Trichobilharzia ocellata* is stimulated by species-specific glycoconjugates released from the host snails. Parasitol Res 83:806–812

Karamon J, Dąbrowska J, Kochanowski M, Samorek-Pieróg M, Sroka J, Różycki M, Bilska-Zając E, Zdybel J, Cencek T (2018) Prevalence of intestinal helminths of red foxes (*Vulpes vulpes*) in central Europe (Poland): a significant zoonotic threat. Parasit Vectors 11:436

Karvonen A (2012) Diplostomum spathaceum and related species. In: Woo PTK, Buchmann K (eds) Fish parasites: pathobiology and protection. CAB International, London

Karvonen A, Lindström K (2018) Spatiotemporal and gender-specific parasitism in two species of gobiid fish. Ecol Evol 8:6114–6123

Karvonen A, Kirsi S, Hudson PJ, Valtonen ET (2004a) Patterns of cercarial production from *Diplostomum spathaceum*: terminal investment or bet hedging? Parasitology 129:87–92

Karvonen A, Seppälä O, Valtonen ET (2004b) Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. Parasitology 129:473–478

Karvonen A, Savolainen M, Seppälä O, Valtonen ET (2006) Dynamics of *Diplostomum spathaceum* infection in snail hosts at a fish farm. Parasitol Res 99:341–345

Karvonen A, Halonen H, Seppälä O (2010) Priming of host resistance to protect cultured rainbow trout *Oncorhynchus mykiss* against eye flukes and parasite-induced cataracts. J Fish Biol 76:1508–1515

Kašný M, Mikeš L, Dolečková K, Hampl V, Dvořák J, Novotný M, Horák P (2011) Cathepsins B1 and B2 of *Trichobilharzia* spp., bird schistosomes causing cercarial dermatitis. In: Robinson MW, Dalton JP (eds) Cysteine proteases of pathogenic organisms. Landes Bioscience, Springer Science and Business Media, pp 136–154

Kekäläinen J, Lai Y-T, Vainikka A, Sirkka I, Kortet R (2014) Do brain parasites alter host personality?—Experimental study in minnows. Behav Ecol Sociobiol 68:197–204

Khalil LF (2002) Family Schistosomatidae Stiles & Hassall, 1898. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CAB International and The Natural History Museum, London, pp 419–432

Kinsella JM, Deem SL, Blake S (2004) Endoparasites of African forest elephants (*Loxodonta africana cyclotis*) from the Republic of Congo and Central African Republic. Comp Parasitol 71:104–110

Kirchhoff NT, Rough KM, Nowak BF (2011) Moving cages further offshore: effects on southern bluefin tuna, *T. maccoyii*, parasites, health, and performance. PLoS One 6:e23705

Kirchhoff NT, Leef MJ, Valdenegro V, Hayward CJ, Nowak BF (2012) Correlation of humoral immune response in southern bluefin tuna, *T. maccoyii*, with infection stage of the blood fluke, *Cardicola forsteri*. PLoS One 7:e45742

Kirk RS (2012) *Sanguinicola inermis* and related species. In: Woo PTK, Buchmann K (eds) Fish parasites: pathobiology and protection. CAB International, London, pp 270–281

Kirk PS, Lewis JW (1992) The laboratory maintenance of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). Parasitology 104:121–127

Kirk RS, Lewis JW (1993) The life-cycle and morphology of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). Syst Parasitol 25:125–133

Kirk RS, Lewis JW (1994) Sanguinicoliasis in cyprinid fish in the UK. In: Pike AW, Lewis JW (eds) Parasitic diseases of fish. Samara Publishing, Wales

Kirk RS, Lewis JW (1996) Migration and development of the blood fluke *Sanguinicola inermis* Plehn, 1905 (Trematoda: Sanguinicolidae) in carp *Cyprinus carpio* L. Parasitology 113:279–285

Køie M (1982) The redia, cercaria and early stages of *Aporocotyle simplex* Odhner, 1900 (Sanguinicolidae)—a digenetic trematode which has a polychaete annelid as the only intermediate host. Ophelia 21:115–145

Køie M, Petersen ME (1988) A new annelid intermediate host (*Lanassa nordenskioeldi* Malmgren, 1866) (Polychaeta: Terebellidae) for *Aporocotyle* sp. and a new final host family (Pisces: Bothidae) for *Aporocotyle simplex* Odhner, 1900 (Digenea: Sanguinicolidae). J Parasitol 74:499–502

Kolářová L, Horák P, Čada F (2001) Histopathology of CNS and nasal infections caused by *Trichobilharzia regenti* in vertebrates. Parasitol Res 87:644–650

Kolářová L, Horák P, Skírnisson K (2010) Methodical approaches in the identification of areas with a potential risk of infection by bird schistosomes causing cercarial dermatitis. J Helminthol 84:327–335

Kolářová L, Horák P, Skírnisson K, Marečková H, Doenhoff M (2013) Cercarial dermatitis, a neglected allergic disease. Clin Rev Allergy Immunol 45:63–74

Kozlowska J (1957) On the knowledge of the helminth fauna of wild and bred foxes. Acta Parasitol Pol 5:181–192

Kudlai O, Oros M, Kostadinova A, Georgieva S (2017) Exploring the diversity of *Diplostomum* (Digenea: Diplostomidae) in fishes from the River Danube using mitochondrial DNA barcodes. Parasit Vectors 10:592

Kvach Y, Jurajda P, Bryjová A, Trichkova T, Ribeiro F, Přikrylová I, Ondračková M (2017) European distribution for metacercariae of the North American digenean *Posthodiplostomum cf. minimum centrarchi* (Strigeiformes: Diplostomidae). Parasitol Int 66:635–642

Kvach Y, Matvienko N, Bryjová A, Ondračková M (2018) Aquaculture as a possible vector in the spread of Posthodiplostomum centrarchi (Hoffman, 1958) (Digenea: Diplostomidae) in Europe. BioInvasions Rec 7(4):427–432

Lakshmanan B, Devada K, Joseph S, Gleeja VL, Aravindakshan TV, Himachala K, Sankar S (2018) Seroprevalence of bovine intestinal schistosomosis in different agro- ecological zones of south India using excretory-secretory antigen based ELISA. Vet Parasitol 262:51–55

Lane RL, Morris JE (2000) Biology, prevention, and effects of common grubs (digenetic trematodes) in freshwater fish. Tech Bull Ser Iowa State Univ 115:1–6

Larsen AH, Bresciani J, Buchmann K (2004) Increasing frequency of cercarial dermatitis at higher latitudes. Acta Parasitol 49:217–221

Lawton SP, Majoros G (2013) A foreign invader or a reclusive native? DNA bar coding reveals a distinct European lineage of the zoonotic parasite *Schistosoma turkestanicum* (syn. *Orientobilharzia turkestanicum* (Dutt and Srivastava, 1955)). Infect Genet Evol 14:186–193

Leontovyč R, Young ND, Korhonen PK, Hall RS, Bulantová J, Jeřábková V, Kašný M, Gasser RB, Horák P (2019) Molecular evidence for distinct modes of nutrient acquisition between visceral and neurotropic schistosomes of birds. Sci Rep 9:1347

Lester RJG, Freeman RS (1976) Survival of 2 trematode parasites (*Diplostomum* spp) in mammalian eyes and associated pathology. Can J Ophthalmol 11:229–234

Lester RJG, Lee TDG (1976) Infectivity of the progenetic metacercaria of *Diplostomum spathaceum*. J Parasitol 62:832–833

Lichtenbergová L, Lassmann H, Jones M, Kolářová L, Horák P (2011) *Trichobilharzia regenti*: host immune response in the pathogenesis of neuroinfection in mice. Exp Parasitol 128:328–335

Liu Z, Zhao X, Niu SF (1976) A survey of the etiological agent of rice-field dermatitis with studies on the life-cycle of *Orientobilharzia turkestanica* var. *tuberculata* in Jinlin province. Acta Zool Sin 22:279–287

Locke SA, McLaughlin JD, Dayanandan S, Marcogliese DJ (2010) Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome c oxidase I and internal transcribed spacer sequences. Int J Parasitol 40:333–343

Locke SA, Al-Nasiri SF, Caffara M, Drago F, Kalbe M, Lapierre AR, McLaughlin JD, Nie P, Overstreet RM, Souza GT, Takemoto RM, Marcogliese DJ (2015) Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes

of freshwater fish, as revealed by DNA barcodes. Int J Parasitol 45:841–855

Locke SA, Van Dam A, Caffara M, Pinto HA, López-Hernández D, Blanar CA (2018) Validity of the Diplostomoidea and Diplostomida (Digenea, Platyhelminthes) upheld in phylogenomic analysis. Int J Parasitol 48(13):1043–1059

Louhi KR, Karvonen A, Rellstab C, Jokela J (2010) Is the population genetic structure of complex life cycle parasites determined by the geographic range of the most motile host? Infect Genet Evol 10:1271–1277

Louhi KR, Karvonen A, Rellstab C, Jokela J (2013) Genotypic and phenotypic variation in transmission traits of a complex life cycle parasite. Ecol Evol 3:2116–2127

Macháček T, Panská L, Dvořáková H, Horák P (2016) Nitric oxide and cytokine production by glial cells exposed *in vitro* to neuropathogenic schistosome *Trichobilharzia regenti*. Parasit Vectors 9:579

Macháček T, Turjanicová L, Bulantová J, Hrdý J, Horák P, Mikeš L (2018) Cercarial dermatitis: a systematic follow-up study of human cases with implications for diagnostics. Parasitol Res 117:3881–3895

Majid AA, Marshall TF, Hussein MF, Bushara BO, Taylor MG, Nelson GS, Dargie JD (1980) Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. I Epizootiological observations on *Schistosoma bovis* in the White Nile province. Am J Trop Med Hyg 29:435–441

Majoros G, Dán Á, Erdélyi K (2010) A natural focus of the blood fluke *Orientobilharzia turkestanica* (Skrjabin, 1913) (Trematoda: Schistosomatidae) in red deer (*Cervus elaphus*) in Hungary. Vet Parasitol 170:218–223

Makundi AE, Kassuku AA, Maselle RM, Boa ME (1998) Distribution, prevalence and intensity of *Schistosoma bovis* infection in cattle in Iringa district, Tanzania. Vet Parasitol 75:59–69

Marchiori E, Negrisolo E, Cassini R (2017) Cardiovascular flukes (Trematoda: Spirorchiidae) in *Caretta caretta* Linnaeus, 1758 from the Mediterranean Sea. Parasit Vectors 10:467

Marchiori E, Cassini R, Ricci I, Marcer F (2018) Qualitative and quantitative methods for estimating Spirorchiidiasis burden in sea turtles. Int J Parasitol Parasites Wildl 7(3):409–414

Martin S, Vazquez R (1984) Biology and behaviour of the cercariae of a *Sanguinicola* sp. in the river Cilloruelo (Salamanca, Spain). Ann Parasitol Hum Comp 59:231–236

Matisz CE, Goater CP, Bray D (2010) Migration and site selection of *Ornithodiplostomum ptychocheilus* (Trematoda: Digenea) metacercariae in the brain of fathead minnows (*Pimephales promelas*). Parasitology 137:719–731

McLaren DJ, Hockley DJ (1977) Blood flukes have a double outer membrane. Nature 269:147–149

McMichael-Phillips DF, Lewis JW, Thorndyke MC (1992) Ultrastructure of the egg of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae). J Nat Hist 26:895–904

McVay MJ, Bakenhaster MD, Bullard SA (2011) *Cardicola laruei* Short, 1953 (Digenea: Aporocotylidae) from heart of seatrouts, *Cynoscion* spp. (Perciformes: Sciaenidae) in the Gulf of Mexico and Atlantic Ocean: taxonomic redescription, first observations of egg and miracidium, and comments on geographic distribution and host specificity. Comp Parasitol 78:291–305

Meade TG (1967) Life history studies on *Cardicola klamathensis* (Wales, 1958) Meade and Pratt, 1965 (Trematoda: Sanguinicolidae). Proc Helminthol Soc Wash 34:210–212

Michálková V, Ondračková M (2014) Experimental evidence for parasite-induced over-winter mortality in juvenile *Rhodeus amarus*. J Fish Biol 84:1377–1388

Mikeš L, Man P (2003) Purification and characterization of a saccharide-binding protein from penetration glands of *Diplostomum pseudospathaceum*—a bifunctional molecule with cysteine protease activity. Parasitology 127:69–77

Mikheev VN, Pasternak AF, Taskinen J, Valtonen TE (2013) Grouping facilitates avoidance of parasites by fish. Parasit Vectors 6:301

Mikheev VN, Pasternak AF, Valtonen ET, Taskinen J (2014) Increased ventilation by fish leads to a higher risk of parasitism. Parasit Vectors 7:281

Moczoň T (1994) Histochemistry of proteinases in the cercariae of *Diplostomum pseudospathaceum* (Trematoda, Diplostomatidae). Parasitol Res 80:684–686

Möhl K, Große K, Hamedy A (2009) Biology of *Alaria* spp. and human exposition risk to *Alaria* mesocercariae—a review. Parasitol Res 105:1–15

Moks E, Jõgisalu I, Saarma U, Talvik H, Järvis T, Valdmann H (2006) Helminthologic survey of the wolf (*Canis lupus*) in Estonia, with an emphasis on *Echinococcus granulosus*. J Wildl Dis 42:359–365

Moné H, Mouahid G, Morand S (1999) The distribution of *Schistosoma bovis* Sonsino, 1876 in relation to intermediate host mollusc-parasite relationships. Adv Parasitol 44:99–138

Monrad J, Sörén K, Johansen MV, Lindberg R, Ornbjerg N (2006) Treatment efficacy and regulatory host responses in chronic experimental *Schistosoma bovis* infections in goats. Parasitology 133:151–158

Niewiadomska K (1984) Present status of *Diplostomum spathaceum* (Rudolphi, 1819) and differentiation of *Diplostomum pseudospathaceum* nom. nov. (Trematoda: Diplostomatidae). Syst Parasitol 6:81–86

Niewiadomska K (1996) The genus *Diplostomum*—taxonomy, morphology and biology. Acta Parasitol 41:55–66

Niewiadomska K (2002) Family Diplostomidae Poirier, 1886. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CAB International and The Natural History Museum, London, pp 167–196

Niewiadomska K, Kiseliene V (1994) *Diplostomum* cercariae (Digenea) in snails from Lithuania. II. Survey of species. Acta Parasitol 39:179–186

Nolan MJ, Cribb TH (2005) *Sanguinicola maritimus* n. sp. (Digenea: Sanguinicolidae) from Labridae (Teleostei: Perciformes) of southern Australian waters. Syst Parasitol 61:99–106

Nolan MJ, Cribb TH (2006) *Cardicola* Short, 1953 and *Braya* n. gen. (Digenea: Sanguinicolidae) from five families of tropical Indo-Pacific fishes. Zootaxa 1265:1–80

Nolan MJ, Miller TL, Cutmore SC, Cantacessi C, Cribb TH (2014) *Cardicola beveridgei* n. sp. (Digenea: Aporocotylidae) from the mangrove jack, *Lutjanus argentimaculatus* (Perciformes: Lutjanidae), and *C. bullardi* n. sp. from the Australian spotted mackerel, *Scomberomorus munroi* (Perciformes: Scombridae), from the northern Great Barrier Reef. Parasitol Int 63:735–745

Oey H, Zakrzewski M, Gravermann K, Young ND, Korhonen PK, Gobert GN, Nawaratna S, Hasan S, Martínez DM, You H, Lavin M, Jones MK, Ragan MA, Stoye J, Oleaga A, Emery AM, Webster BL, Rollinson D, Gasser RB, McManus DP, Krause L, Platt RN (2019) Whole-genome sequence of the bovine blood fluke *Schistosoma bovis* supports interspecific hybridization with S. haematobium. PLoS Pathog 15(1):e1007513

Ogawa K (2015) Diseases of cultured marine fishes caused by Platyhelminthes (Monogenea, Digenea, Cestoda). Parasitology 142:178–195

Ogawa K, Hattori K, Hatai K, Kubota S (1989) Histopathology of cultured marine fish, *Seriola purpurascens* (Carangidae) infected with *Paradeontacylix* spp. (Trematoda: Sanguinicolidae) in its vascular system. Fish Pathol 28:75–81

Ogawa K, Ishimaru K, Shirakashi S, Takami I, Grabner D (2011) *Cardicola opisthorchis* n. sp. (Trematoda: Aporocotylidae) from the Pacific bluefin tuna, *Thunnus orientalis* (Temminck & Schlegel, 1844), cultured in Japan. Parasitol Int 60:307–312

Ogawa K, Shirakashi S, Tani K, Shin SP, Ishimaru K, Honryo T, Sugihara Y, Uchida H (2017) Developmental stages of fish blood flukes, *Cardicola forsteri* and *Cardicola opisthorchis* (Trematoda: Aporocotylidae), in their polychaete intermediate hosts collected at Pacific bluefin tuna culture sites in Japan. Parasitol Int 66:972–977

Oi M, Araki J, Matsumoto J, Nogami S (2012) Helminth fauna of a turtle species introduced in Japan, the red-eared slider turtle (*Trachemys scripta elegans*). Res Vet Sci 93:826–830

Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int J Parasitol 33:733–755

Ondračková M, Šimková A, Gelnar M, Jurajda P (2004) *Posthodiplostomum cuticola* (Digenea: Diplostomatidae) in intermediate fish hosts: factors contributing to the parasite infection and prey selection by the definitive bird host. Parasitology 129:761–770

Ondračková M, Dávidová M, Gelnar M, Jurajda P (2006) Susceptibility of Prussian carp infected by metacercariae of *Posthodiplostomum cuticola* (v. Nordmann, 1832) to fish predation. Ecol Res 21:526–529

Orélis-Ribeiro R, Bullard S (2015) Blood flukes (Digenea: Aporocotylidae) infecting body cavity of South American catfishes (Siluriformes: Pimelodidae): two new species from rivers in Bolivia, Guyana and Peru with a re-assessment of *Plehniella* Szidat, 1951. Folia Parasitol 62:050

Orélis-Ribeiro R, Bullard S (2016) Two new genera of fish blood flukes (Digenea: Aporocotylidae) from catfishes in the Peruvian Amazon. J Parasitol 102:357–368

Orélis-Ribeiro R, Arias CR, Halanych KM, Cribb TH, Bullard SA (2014) Diversity and ancestry of flatworms infecting blood of nontetrapod craniates "fishes". Adv Parasitol 85:1–64

Overstreet RM, Køie M (1989) *Pearsonellum corventum*, gen. et sp. nov. (Digenea: Sanguinicolidae), in serranid fishes from the Capricornia Section of the Great Barrier Reef. Aust J Zool 37:71–79

Padrós F, Zarza C, Crespo S (2001) Histopathology of cultured seabream *Sparus aurata* infected with sanguinicolid trematodes. Dis Aquat Org 44:47–52

Padrós F, Knudsen R, Blasco-Costa I (2018) Histopathological characterisation of retinal lesions associated to *Diplostomum* species (Platyhelminthes: Trematoda) infection in polymorphic Arctic charr *Salvelinus alpinus*. Int J Parasitol Parasites Wildl 7:68–74

Palacios-Abella JF, Rodriguez-Llanos J, Mele S, Montero FE (2015) Morphological characterisation and identification of four species of *Cardicola* Short, 1953 (Trematoda: Aporocotylidae) infecting the Atlantic bluefin tuna *Thunnus thynnus* (L.) in the Mediterranean Sea. Syst Parasitol 91:101–117

Palacios-Abella JF, Rodriguez-Llanos J, Villora-Montero M, Mele S, Raga JA, Montero FE (2017) Diagnostic accuracy of the light microscope method to detect the eggs of *Cardicola* spp. in the gill filaments of the bluefin tuna. Vet Parasitol 247:26–32

Palacios-Abella JF, Raga JA, Mele S, Montero FE (2018) Blood fluke diseases in *Pagellus* spp. (Sparidae): pathology and description of a new species of Skoulekia in the blackspot seabream *P. bogaraveo* (Brünnich). Aquaculture 495:472–483

Patrelle C, Portier J, Jouet D, Delorme D, Ferté H (2015) Prevalence and intensity of *Alaria alata* (Goeze, 1792) in water frogs and brown frogs in natural conditions. Parasitol Res 114:4405–4412

Pennacchi Y, Shirakashi S, Nowak BF, Bridle AR (2016) Immune reactivity in early life stages of sea-cage cultured Pacific bluefin tuna naturally infected with blood flukes from genus *Cardicola* (Trematoda: Aporocotylidae). Fish Shellfish Immunol 58:490–499

Platt TR (1992) A phylogenic and biogeographic analysis of the genera of Spirorchinae (Digenea: Spirorchidae) parasitic in freshwater turtles. J Parasitol 78:616–629

Platt TR (2002) Family Spirorchiidae Stunkard, 1921. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CAB International and The Natural History Museum, London, pp 453–468

Platt TR, Brooks DR (1997) Evolution of the schistosomes (Digenea: Schistosomatoidea): the origin of dioecy and colonization of the venous system. J Parasitol 83:1035–1044

Platt TR, Blair D, Purdie J, Melville L (1991) *Griphobilhazia amoena* n. sp. (Digenea: Schistosomatidae), a parasite of the freshwater crocodile *Crocodylus johnstoni* (Reptilia: Crocodylia) from Australia, with the erection of a new subfamily, Griphobilharziinae. J Parasitol 77:65–68

Platt TR, Hoberg EP, Chisholm LA (2013) On the morphology and taxonomy of *Griphobilharzia amoena* Platt and Blair, 1991 (Schistosomatoidea), a dioecious digenetic trematode parasite of the freshwater crocodile, *Crocodylus johnstoni*, in Australia. J Parasitol 99:888–891

Polinski M, Hamilton DB, Nowak B, Bridle A (2013) SYBR, TaqMan, or both: highly sensitive, non-invasive detection of *Cardicola* blood fluke species in Southern Bluefin Tuna (*Thunnus maccoyii*). Mol Biochem Parasitol 191:7–15

Prüter H, Sitko J, Krone O (2017) Having bird schistosomes in mind—the first detection of *Bilharziella polonica* (Kowalewski 1895) in the bird neural system. Parasitol Res 116:865–870

Rahman SA, Sano M, Jagannath MS, Prabhakar KS, D'Souza PE, Prem G (1988) Efficacy of praziquantel against *Schistosoma nasale* infection in cattle. Trop Anim Health Prod 20:19–22

Raiczyk GB, Hall JC (1988) *Schistosomatium douthitti*: biochemical and morphological effects of an experimental infection in mice. Exp Parasitol 65:187–201

Ratanarat-Brockelman C (1974) Migration of *Diplostomum spathaceum* (Trematoda) in the fish intermediate host. Z Parasitenkd 43:123–134

Rauch G, Kalbe M, Reusch TBH (2005) How a complex life cycle can improve a parasite's sex life. J Evol Biol 18:1069–1075

Ravindran R, Kumar A (2012) Nasal schistosomiasis among large ruminants in Wayanad, India. Southeast Asian J Trop Med Public Health 43:586–588

Rellstab C, Louhi KR, Karvonen A, Jokela J (2011) Analysis of trematode parasite communities in fish eye lenses by pyrosequencing of naturally pooled DNA. Infect Genet Evol 11:1276–1286

Rellstab C, Karvonen A, Louhi KR, Jokela J (2013) Genotype-specific vs. cross-reactive host immunity against a macroparasite. PLoS One 8:e78427

Repulles-Albelda A, Montero FE, Holzer AS, Ogawa K, Hutson KS, Raga JA (2008) Speciation of the *Paradeontacylix* spp. (Sanguinicolidae) of *Seriola dumerili*. Two new species of the genus *Paradeontacylix* from the Mediterranean. Parasitol Int 57:405–414

Rieger JK, Haase D, Reusch TBH, Kalbe M (2013) Genetic compatibilities, outcrossing rates and fitness consequences across life stages of the trematode *Diplostomum pseudospathaceum*. Int J Parasitol 43:485–491

Riehn K, Hamedy A, Grosse K, Zeitler L, Lücker E (2010) A novel detection method for *Alaria alata* mesocercariae in meat. Parasitol Res 107:213–220

Riehn K, Hamedy A, Alter T, Lücker E (2011) Development of a PCR approach for differentiation of *Alaria* spp. mesocercariae. Parasitol Res 108:1327–1332

Riley EM, Chappell LH (1992) Effect of infection with *Diplostomum spathaceum* on the internal defense system of *Lymnaea stagnalis*. J Invertebr Pathol 59:190–196

Řimnáčová J, Mikeš L, Turjanicová L, Bulantová J, Horák P (2017) Changes in surface glycosylation and glycocalyx shedding in *Trichobilharzia regenti* (Schistosomatidae) during the transformation of cercaria to schistosomulum. PLoS One 12:e0173217

Roberts JR, Platt TR, Orélis-Ribeiro R, Bullard SA (2016) New genus of blood fluke (Digenea: Schistosomatoidea) from Malaysian freshwater turtles (Geoemydidae) and its phylogenetic position within Schistosomatoidea. J Parasitol 102:451–462

Roberts JR, Arias CR, Halanych KM, Dang BT, Bullard SA (2018) A new genus and species of turtle blood fluke (Digenea: Schistosomatoidea) from the Mekong snail-eating turtle, *Malayemys subtrijuga* (Schlegel & Müller) (Testudines: Geoemydidae) in Vietnam, with a reassessment of related Asiatic turtle blood flukes and molecular phylogeny. Syst Parasitol 95:133–145

Rodriguez JY, Lewis BC, Snowden KF (2014) Distribution and characterization of *Heterobilharzia americana* in dogs in Texas. Vet Parasitol 203:35–42

Routtu J, Grunberg D, Izhar R, Dagan Y, Guttel Y, Ucko M, Ben-Ami F (2014) Selective and universal primers for trematode barcoding in freshwater snails. Parasitol Res 113:2535–2540

Rudko SP, Reimink RL, Froelich K, Gordy MA, Blankespoor CL, Hanington PC (2018) Use of qPCR-based cercariometry to assess swimmer's itch in recreational lakes. EcoHealth 15:827–839

Sahay MN, Sahai BN (1978) Studies on the susceptibility of the laboratory animals, kids, and lambs to experimental infection with *Schistosoma nasale* Rao 1933. J Parasitol 64:1135–1136

Sahba GH, Malek EA (1979) Dermatitis caused by cercariae of *Orientobilharzia turkestanicum* in the Caspian Sea area of Iran. Am J Trop Med Hyg 28:912–913

Sandland GJ, Goater CP, Danylchuk AJ (2001) Population dynamics of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*) from four northern-Alberta Lakes. J Parasitol 87:744–748

Santoro M, Morales JA, Rodríguez-Ortíz B (2007) Spirorchiidiosis (Digenea: Spirorchiidae) and lesions associated with parasites in Caribbean green turtles (*Chelonia mydas*). Vet Rec 161:482–486

Sapate PP, Bhilegaonkar NG, Maske DK (2001) Development of *Schistosoma nasale* in hamster and mastomyes and their pathogenicity. Indian Vet J 78:14–17

Scharsack JP, Kalbe M (2014) Differences in susceptibility and immune responses of three-spined sticklebacks

(*Gasterosteus aculeatus*) from lake and river ecotypes to sequential infections with the eye fluke *Diplostomum pseudospathaceum*. Parasit Vectors 7:109

Schleppe JL, Goater CP (2004) Comparative life-histories of two diplostomid trematodes, *Ornithodiplostomum ptychocheilus* and *Posthodiplostomum minimum*. J Parasitol 90:1387–1390

Scholz T, Besprozvannykh W, Boutorina TE, Choudhury A, Cribb TH, Ermolenko AV, Faltýnková A, Shedko MB, Shimazu T, Smit NJ (2016) Trematode diversity in freshwater fishes of the Globe I: 'Old World'. Syst Parasitol 93:257–269

Schwanz LE (2006) Schistosome infection in deer mice (*Peromyscus maniculatus*): impacts on host physiology, behavior and energetics. J Exp Biol 209:5029–5037

Selbach C, Soldánová M, Georgieva S, Kostadinova A, Sures B (2015) Integrative taxonomic approach to the cryptic diversity of *Diplostomum* spp. in lymnaeid snails from Europe with a focus on the '*Diplostomum mergi*' species complex. Parasit Vectors 8:300

Seppälä O, Karvonen A, Rellstab C, Louhi K-R, Jokela J (2012a) Reciprocal interaction matrix reveals complex genetic and dose-dependent specificity among coinfecting parasites. Am Nat 180:306–315

Seppälä O, Karvonen A, Valtonen ET (2012b) Behavioural mechanisms underlying 'specific' host manipulation by a trophically transmitted parasite. Evol Ecol Res 14:73–81

Seppälä O, Karvonen A, Kuosa M, Haataja M, Jokela J (2013) Are sick individuals weak competitors? Competitive ability of snails parasitized by a gigantism-inducing trematode. PLoS One 8:e79366

Shakarbaev E, Haberl B, Loy C, Haas W (2001) Recognition of cattle skin by cercariae of *Orientobilharzia turkestanica*. Parasitol Res 87:705–707

Shirakashi S, Ogawa K (2016) Blood fluke infections in marine cultured fish. Fish Pathol 51:92–98

Shirakashi S, Andrews M, Kishimoto Y, Ishimaru K, Okada T, Sawada Y, Ogawa K (2012a) Oral treatment of praziquantel as an effective control measure against blood fluke infection in Pacific bluefin tuna (*Thunnus orientalis*). Aquaculture 326:15–19

Shirakashi S, Kishimoto Y, Kinami R, Katano H, Ishimaru K, Murata O, Itoh N, Ogawa K (2012b) Morphology and distribution of blood fluke eggs and associated pathology in the gills of cultured Pacific bluefin tuna, *Thunnus orientalis*. Parasitol Int 61:242–249

Shirakashi S, Tani K, Ishimaru K, Shin SP, Honryo T, Uchida H, Ogawa K (2016) Discovery of intermediate hosts for two species of blood flukes *Cardicola orientalis* and *Cardicola forsteri* (Trematoda: Aporocotylidae) infecting Pacific bluefin tuna in Japan. Parasitol Int 65:128–136

Shirakashi S, Tani K, Ishimuru K, Honryo T, Shin SP, Uchida H, Ogawa K (2017) Spatial and temporal changes in the distribution of blood fluke infection in *Nicolea gracilibranchis* (Polychaeta: Terebellidae), the intermediate host for *Cardicola orientalis* (Digenea: Aporocotylidae), at a tuna farming site in Japan. J Parasitol 103:541–546

Siegel SV, Rivero AV, Oberstaller J, Colon BL, De Buron I, Kyle DE (2018) Blood flukes *Cardicola parvus* and *C. laruei* (Trematoda: Aporocotylidae): life cycles and cryptic infection in spotted seatrout, *Cynoscion nebulosus* (Teleost: Sciaenidae). Parasitol Int 67:150–158

Siegmund I, Franjola R, Torres P (1997) Diplostomatid metacercariae in the brain of silversides from Lake Rinihue, Chile. J Wildl Dis 33:362–364

Singh BK, Agrawal MC (2005) Triclabendazole on bovine hepatic schistosomosis. Indian J Anim Sci 75:654–655

Singh A, Singh A, Chaudhri SS (2004) Visceral schistosomiasis of domestic animals in India: humoral immune status of infected cattle, sheep and goats against major polypeptide antigens of *Schistosoma indicum* and *S. spindale*. Parasite Immunol 26:167–175

Skírnisson K, Aldhoun JA, Kolářová L (2009) A review on swimmer's itch and the occurrence of bird schistosomes in Iceland. J Helminthol 83:165–171

Smith JW (1972) The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates and some comparison with the schistosomes. Helminthol Abstr Ser A 41:161–204

Smith JW (1997a) The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates: part 1. A review of the published literature since 1971, and bibliography. Helminthol Abstr 66:255–294

Smith JW (1997b) The blood flukes (Digenea: Sanguinicolidae and Spirorchidae) of cold-blooded vertebrates: part 2. Appendix I: comprehensive parasite-host list; Appendix II: comprehensive host-parasite list. Helminthol Abstr 66:329–344

Smith JW (2002) Family Sanguinicolidae von Graff, 1907. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 1. CAB International and The Natural History Museum, London, pp 433–452

Snyder SD (2004) Phylogeny and paraphyly among tetrapod blood flukes (Digenea: Schistosomatidae and Spirorchiidae). Int J Parasitol 34:1385–1392

Snyder SD, Clopton RE (2005) New methods for the collection and preservation of spirorchiid trematodes and polystomatid monogeneans from turtles. Comp Parasitol 72:10–107

Soldánová M, Kuris AM, Scholz T, Lafferty KD (2012) The role of spatial and temporal heterogeneity and competition in structuring trematode communities in the great pond snail, *Lymnaea stagnalis* (L.). J Parasitol 98:460–471

Soldánová M, Selbach C, Kalbe M, Kostadinova A, Sures B (2013) Swimmer's itch: etiology, impact, and risk factors in Europe. Trends Parasitol 29:65–74

Soldánová M, Selbach C, Sures B (2016) The Early Worm Catches the Bird? Productivity and patterns of *Trichobilharzia szidati* cercarial emission from *Lymnaea stagnalis*. PLoS One 11:e0149678

Soleng A, Mehl R (2011) Geographical distribution of cercarial dermatitis in Norway. J Helminthol 85:345–352

Sommerville C, Iqbal NAM (1991) The process of infection, migration, growth and development of *Sanguinicola inermis* Plehn, 1905 (Digenea: Sanguinicolidae) in carp, *Cyprinus carpio*. J Fish Dis 14:211–219

Speed P, Pauley GB (1985) Feasibility of protecting rainbow trout, *Salmo gairdneri* Richardson, by immunizing against the eye fluke, *Diplostomum spathaceum*. J Fish Biol 26:739–744

Sreenivasa Murthy GS, D'Souza PE, Shrikrishna Isloor K (2013) Evaluation of a polyclonal antibody based sandwich ELISA for the detection of faecal antigens in *Schistosoma spindale* infection in bovines. J Parasit Dis 37:47–51

Stacy BA, Foley AM, Greiner E, Herbst LH, Bolten A, Klein P, Manire CA, Jacobson ER (2010) Spirorchiidiasis in stranded loggerhead *Caretta caretta* and green turtles *Chelonia mydas* in Florida (USA): host pathology and significance. Dis Aquat Org 89:237–259

Stacy BA, Chapman PA, Foley AM, Greiner EC, Herbst LH, Bolten AB, Klein PA, Manire¶ CA, Jacobson ER (2017) Evidence of diversity, site, and host specificity of sea turtle blood flukes (Digenea: Schistosomatoidea: "Spirorchiidae"): a molecular prospecting study. J Parasitol 103(6):756–767

Standley CJ, Mugisha L, Dobson AP, Stothard JR (2012) Zoonotic schistosomiasis in non-human primates: past, present and future activities at the human-wildlife interface in Africa. J Helminthol 86:131–140

Stone RH, Frontera-Acevedo K, Saba CF, Ambrose D, Moorhead AR, Brown CA (2011) Lymphosarcoma associated with *Heterobilharzia americana* infection in a dog. J Vet Diagn Investig 23:1065–1070

Stoyanov B, Georgieva S, Pankov P, Kudlai O, Kostadinova A, Georgiev BB (2017) Morphology and molecules reveal the alien *Posthodiplostomum centrarchi* Hoffman, 1958 as the third species of *Posthodiplostomum* Dubois, 1936 (Digenea: Diplostomidae) in Europe. Syst Parasitol 94:1–20

Stunkard HW (1983) The marine cercariae of the Woods Hole, Massachusetts region, a review and revision. Biol Bull 164:14–162

Sugihara Y, Yamada T, Tamaki A, Yamanishi R, Kanai K (2014) Larval stages of the bluefin tuna blood fluke *Cardicola opisthorchis* (Trematoda: Aporocotylidae) found from *Terebella* sp. (Polychaeta: Terebellidae). Parasitol Int 63:295–299

Sugihara Y, Yamada T, Ogawa K, Yokoyama F, Matsukura K, Kanai K (2015) Occurrence of the bluefin tuna blood fluke *Cardicola opisthorchis* in the intermediate host *Terebella* sp. Fish Pathol 50:105–111

Sugihara Y, Yamada T, Iwanaga S, Kanai K (2017) Transplantation of *Cardicola opisthorchis* (Trematoda: Aporocotylidae) sporocysts into the intermediate host, *Terebella* sp. (Polychaeta: Terebellidae). Parasitol Int 66:839–842

Sumanth S, D'Souza PE, Jagannath MS (2004a) A study of nasal and visceral schistosomosis in cattle slaughtered at an abattoir in Bangalore, South India. Rev Sci Tech Int Office Epizootics 23:937–942

Sumanth S, D'Souza PE, Jagannath MS (2004b) Serodiagnosis of nasal and visceral schistosomosis in cattle by counter current immuno electrophoresis. Vet Arhiv 74:427–433

Thiess A, Schuster R, Nöckler K, Mix H (2001) Helminth findings in indigenous raccoon dogs *Nyctereutes procyonoides* (Gray, 1843). Berl Munch Tierarztl Wochenschr 114:273–276

Tobler M, Schlupp I (2008) Influence of black spot disease on shoaling behaviour in female western mosquitofish, *Gambusia affinis* (Poeciliidae, Teleostei). Environ Biol Fish 81:29–34

Turjanicová L, Mikeš L, Pecková M, Horák P (2015) Antibody response of definitive hosts against antigens of two life stages of the neuropathogenic schistosome *Trichobilharzia regenti*. Parasit Vectors 8:400

Tylkowska A, Pilarczyk B, Pilarczyk R, Zyśko M, Tomza-Marciniak A (2018) The presence of *Alaria alata* fluke in the red fox (*Vulpes vulpes*) in north-western Poland. Jpn J Vet Res 66:203–208

Vazquez N, Bruno EP, Marquez F, Van der Molen S, Gilardoni C, Cremonte F (2013) A histopathological survey of the razor clam *Ensis macha* (Pharidae) along the Patagonian Argentina coast. J Invertebr Pathol 112:253–259

Vimalraj PG, Jayathangaraj MG, Sridhar R, Tma S, Latchumikanthan A (2012) Elephant schistosome (*Bivitellobilharzia nairi*) in free-ranging Asian elephants (*Elephas maximus*) of Sathyamangalam forest division of Tamil Nadu State. J Vet Parasitol 26:80–81

Von Graff L (1907) Das Schmarotzertum im Tierreich und seine Bedeutung für die Artbildung. Verlag von Quelle and Meyer, Leipzig

Voutilainen A (2013) Experimental infection of Arctic charr *Salvelinus alpinus* (L.) with the cercariae of diplostomid eye flukes *Diplostomum* spp. Bull Eur Assoc Fish Pathol 33:199–205

Wang CR, Chen J, Zhao JP, Chen AH, Zhai YQ, Li L, Zhu XQ (2009) *Orientobilharzia* species: neglected parasitic zoonotic agents. Acta Trop 109:171–175

Warren MB, Orélis-Ribeiro R, Ruiz CF, Dang BT, Arias CR, Bullard SA (2017) Endocarditis associated with blood fluke infections (Digenea: Aporocotylidae: *Psettarium* cf. *anthicum*) among aquacultured cobia (*Rachycentron canadum*) from Nha Trang Bay, Vietnam. Aquaculture 468:549–557

Werneck MR, Gallo BMG, Silva RJ (2008) Spirorchiids (Digenea: Spirorchiidae) infecting a hawksbill sea turtle *Eretmochelys imbricata* (Linnaeus 1758) from Brazil. Arq Bras Med Vet Zootec 60:663–666

Whyte SK, Chappell LH, Secombes CJ (1990) Protection of rainbow trout, *Oncorhynchus mykiss* (Richardson), against *Diplostomum spathaceum* (Digenea): the role of specific antibody and activated macrophages. J Fish Dis 13:281–291

Whyte SK, Secombes CJ, Chappell LH (1991) Studies on the infectivity of *Diplostomum spathaceum* in

rainbow trout (*Oncorhynchus mykiss*). J Helminthol 65:169–178

Wittrock DD, Bruce CS, Johnson AD (1991) Histochemistry and ultrastructure of the metacercarial cysts of blackspot trematodes *Uvulifer ambloplitis* and *Neascus pyriformis*. J Parasitol 77:454–460

Wulff C, Haeberlein S, Haas W (2007) Cream formulation protecting against cercarial dermatitis by *Trichobilharzia*. Parasitol Res 101:91–97

Yamaguti S (1971) Synopsis of digenetic Trematodes of vertebrates. Keigaku Publishing Company Ltd, Tokyo

Yihunie A, Urga B, Alebie G (2019) Prevalence and risk factors of bovine schistosomiasis in northwestern Ethiopia. BMC Vet Res 15(1):12

Yong RQY, Cutmore SC, Bray RA, Miller TL, Semarariana IW, Palm HW, Cribb TH (2016) Three new species of blood flukes (Digenea: Aporocotylidae) infecting pufferfishes (Teleostei: Tetraodontidae) from off Bali, Indonesia. Parasitol Int 65:432–443

Yonkers SB, Schneider R, Reavill DR, Archer LL, Childress AL, Wellehan JF Jr (2015) Coinfection with a novel fibropapilloma-associated herpesvirus and a novel *Spirorchis* sp. in an eastern box turtle (*Terrapene carolina*) in Florida. J Vet Diagn Investig 27:408–413

Zrnčić S, Oraić D, Mihaljević Ž, Ćaleta M, Zanella D, Jelić D, Jelić M (2009) First observation of *Posthodiplostomum cuticola* (Nordmann, 1832) metacercariae in cypriniformes from Croatia. Helminthologia 46:112–116

Zusková E, Plačková V, Máchová J (2018) Efficacy and toxicity of praziquantel in helminth-infected barbel (*Barbus barbus* L.). J Fish Dis 41:643–649

Amphistomes

9

Veena Tandon, Bishnupada Roy,
Jollin Andrea Shylla, and Sudeep Ghatani

9.1 General Morphology

The body of the amphistome fluke is elongate, conical, cylindrical and flattened dorsoventrally. In some species, the body may be differentiated into two parts: cylindrical anteriorly and somewhat expanded posteriorly, with a well-developed posterior sucker (acetabulum). Amphistomes are variable in size, those of the domesticated ruminants measure from 3 to 20 mm in length and 1.5–7 mm in width. A live amphistome appears light pink to red, whereas some species may be slightly deeper in colour.

The acetabulum is a strong muscular organ placed at the posterior end of the body. It may be terminal, subterminal or almost ventral. It varies in size in different amphistomes, and its dorsoventral diameter in relation to body length is of some importance in the taxonomy of the group. The size of the acetabulum may be characteristic in some genera (e.g. *Explanatum*), and due to its strongly muscular nature it can be regarded as a relatively stable morphological character. The much more valuable taxonomic characteristics are, however, exhibited by the structure of the muscular series of the acetabulum. The muscles are oblique, circular, longitudinal and radial; on the dorsal and ventral sides, particularly along the exterior and interior borders, the circular muscle fibres are arranged in a definite pattern.

The digestive system of amphistomes comprises of the mouth, pharynx, oesophagus and two intestinal caeca. The mouth is a small circular aperture at the anterior end of the body. The pharynx is a globular muscular organ situated anteriorly. It may be terminal or subterminal, with a spindle-shaped pharyngeal cavity. The pharynx may be simple, with or without a lip sphincter or with two primary sacs, one on either side, or with a pharyngeal bulb with paired secondary sacs. The pharyngeal bulb is a transversely elongate, muscular structure arising from the posterior region of the pharynx and separated from it by a constriction. It occurs only in the family Gastrodiscidae and the Brumptidae. The relation of the length of the pharynx to that of the body and the structure of musculature of the pharynx are of considerable importance in the taxonomy of amphistomes. The oesophagus is a thin tube arising from the basal portion of the pharynx, which distally bifurcates into two intestinal caeca.

V. Tandon (✉)
Biotech Park, Lucknow, Uttar Pradesh, India

B. Roy
Department of Zoology, North-Eastern Hill University, Shillong, Meghalaya, India

J. A. Shylla
St. Anthony's College, Shillong, Meghalaya, India

S. Ghatani
Department of Zoology, Sikkim University, Gangtok, Sikkim, India
e-mail: sghatani@cus.ac.in

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_9

It may be a straight tube or J-shaped and, in some species, provided with an oesophageal bulb at the distal end. The intestinal caeca run laterally towards the posterior end of the body and end blindly, in the pre- or mid-acetabular zone. They may be wavy, straight, coiled or forming loops.

The excretory system consists of two main excretory canals, each passing laterally outer to the pharynx and following the course of the intestinal caecum of its side. Posteriorly dorsal or anterodorsal to acetabulum, the two canals bend inwards and meet to form the excretory vesicle. The excretory canal arises from the vesicle that opens on the dorsal side, somewhat medially close to posterior extremity of the body. The opening may be pre-vesicular, invesicular or post-vesicular. The relationship of the opening (excretory pore) to the Laurer's canal opening is of great taxonomic importance.

The reproductive system of this hermaphroditic parasite group consists of the male and female genital systems. The male genital system comprises the testes, vasa efferentia, a vas deferens and an ejaculatory duct. Testes are usually two in number, occasionally single, usually situated between the intestinal caeca but may be caecal or extracaecal. Testes may be in tandem, diagonally arranged or juxtaposed; they may be entire or lobed or deeply lobed. A vas efferens arises from each testis and the two vasa efferentia unite in front of the anterior testis to form the vas deferens. It is divided into three parts: the first part is known as vesicula seminalis, the second as the pars musculosa and the last as the pars prostatica, which continues into the ejaculatory duct. The degree of development of the pars prostatica and pars musculosa is a character of generic significance. Most paramphistomoids parasitic in mammals do not have a cirrus sac, but it is usually present in taxa from lower vertebrates. There is no cirrus and is functionally replaced by a genital papilla composed of erectile tissue and containing lymph vessels. In some species, the ejaculatory duct unites with the terminal portion of the uterus and leads into a delicate chamber—the hermaphroditic sac, which opens out through the genital papilla. The genital papilla is surrounded by a cavity or depression called the genital atrium, and the walls of the genital papilla may be studded with sphincter papillae. The wall of the genital atrium is strengthened by genital atrial-radial muscles and, in some cases, may be additionally presented with a cluster of muscle fibres forming the genital sphincter. In some amphistomes, another cavity or depression, the ventral atrium, is seen around the genital atrium. In case of the family Gastrothylacidae, the ventral atrium is enormously developed to form the ventral pouch, an internal blindly ending sac that opens to the exterior by a single aperture on the ventral surface of the body close to the anterior extremity. The genital pore may be surrounded by a distinct genital sucker in some species.

The genital papilla and various structures, which may or may not be present, such as the sphincter papillae, genital and ventral folds, genital and ventral atria, genital sphincter, genital sucker, genital pillar, muscle fibres and tegumental papillae are collectively known as the terminal genitalium, a specialized term used only in case of paramphistomoids but not in other digeneans. The term was introduced by Eduardo (1982) and has been well adopted and established in the literature. The majority of the components hold taxonomic importance at species-level identification, but possession of a true genital sucker has generic-level significance. The degree of development and the size of the terminal genitalium are also characters in themselves and can be significant at the generic level.

The female genital system consists of the ovary, oviduct, Mehlis' gland, Laurer's canal, vitellaria and their ducts, uterus and metraterm. The ovary is more or less spherical in shape and is usually situated in the post-testicular region in front of the acetabulum. The oviduct emerges from the ovary and after a short free course enters the Mehlis' gland complex, emerging from it as the uterus. The Laurer's canal arises from the oviduct at a place where the latter enters the Mehlis' gland complex, which then runs towards the dorsal side of the body to open either anterior or posterior to the excretory pore. The vitellaria are usually follicular and confined to the lateral regions of the

body. Their extent is variable at the anterior and the posterior region. The Mehlis' gland complex is a compact structure enclosing the dilated part of the oviduct, the ootype. The ootype receives a duct of the yolk reservoir which opens into two transverse vitelline ducts, one from each side. The uterus traverses its way towards the anterior side and opens into the hermaphroditic sac through a metraterm—the muscular distal end of the uterus. The hermaphroditic sac is formed from the union of the ejaculatory duct and the metraterm and opens at the tip of the genital papilla. The genital pore lies in the ventro-median line just below the bifurcation of the intestinal caeca (Sey 1991; Gupta 1993; Jones 2005a) (Fig. 9.1).

9.2 Classification

Several schemes have been proposed in the history of amphistome systematics, according to which the amphistomes are represented by various ranks—familial (Fischoeder 1903; Stunkard 1925; Fuhrmann 1928; Näsmark 1937), superfamilial (Stiles and Goldberger 1910; Maplestone 1923; Travassos 1934; Southwell and Kirshner 1937; Baer and Joyeux 1961; Yamaguti 1971), subordinal (Szidat 1936; Skrjabin 1949; La Rue 1957) or ordinal rank (Travassos et al. 1969; Odening 1974; Brooks et al. 1985).

Fischoeder (1901) coined the word 'Paramphistomidae' and proposed it as a family

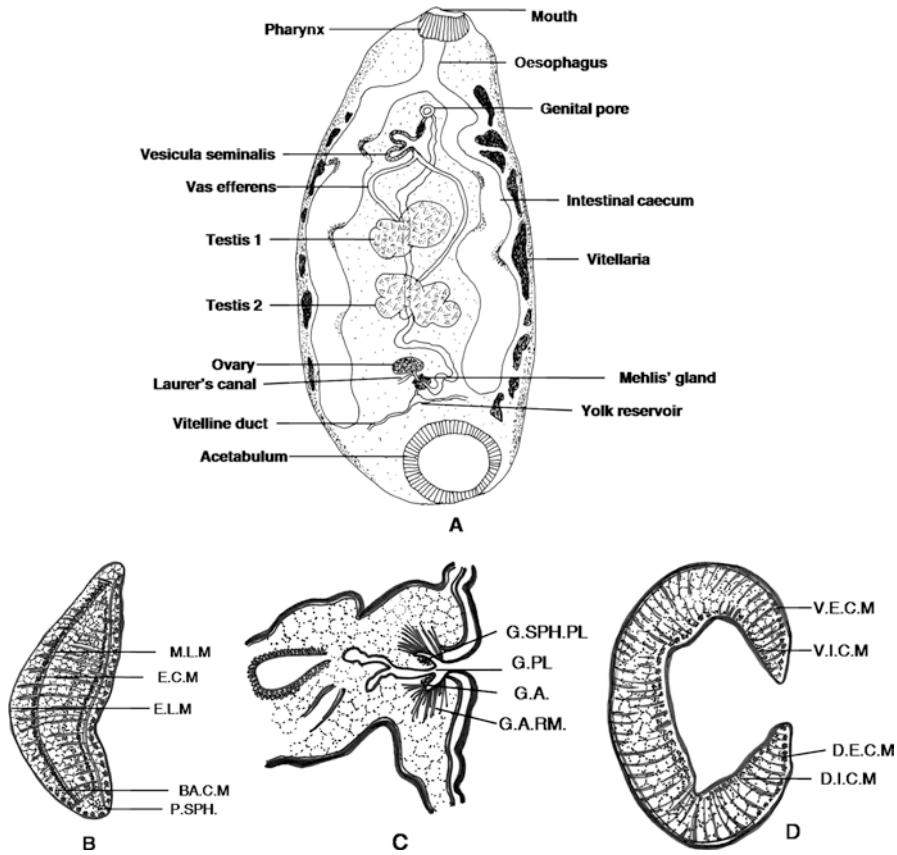

Fig. 9.1 (**a**) Generalized diagram of a typical amphistome fluke. (**b**) A median sagittal section of acetabulum. *D.E.C.M* dorsal exterior circular muscles, *D.I.C.M* dorsal interior circular muscles, *V.E.C.M* ventral exterior circular muscles, *V.I.C.M* ventral interior circular muscles. (**c**) A median sagittal section of one half of pharynx. *M.L.M* middle longitudinal muscles, *E.C.M* external circular muscles, *E.L.M* external longitudinal muscles, *BA.C.M* basal circular muscles. (**d**) A median sagittal section of one half of genital atrium. *G.SPH.PL* genital sphincter papilla, *G.PL* genital papilla, *G.A* genital atrium, *G.A.RM* genital atrial radial muscles

name for all amphistomes. The family Paramphistomidae was raised to the rank of a superfamily, Paramphistomoidea by Stiles and Goldberger (1910) who included in it three families Paramphistomidae Fischoeder, 1901 for those amphistomes in which the ventral pouch is absent; Gastrothylacidae Stiles & Goldberger, 1910 for those with a ventral pouch; and Gastrodiscidae Monticelli, 1892 in which the ventral pouch is absent and the body is divided into cephalic and caudal portions. Maplestone (1923) treated the group as a suborder under the name 'Amphistomata' and placed under it the families Paramphistomidae, Gastrothylacidae and Gastrodiscidae. Stunkard (1925), on the other hand, recognized the family Paramphistomidae and included in it the subfamilies Diplodiscinae Cohn, 1904; Schizamphistominae Looss, 1912; Paramphistominae Fischoeder, 1901; Cladorchinae Fischoeder, 1901; Gastrodiscinae Monticelli, 1892; Gastrothylacinae Stiles & Goldberger, 1910; Zygocotylinae Stunkard, 1916; Balanorchinae Stunkard, 1917; and Brumptinae Stunkard, 1925. Fukui (1929) treated the amphistomes as a single family, although he grouped the families Angiodictyidae Looss, 1902, and two new families created by him—Opistholebetidae and Opisthoporidae alongside them in the Amphistomata. Travassos (1934) recognized the superfamily Paramphistomoidea and included in it six families, namely, Paramphistomidae; Gastrodiscidae; Opistholebetidae; Gyliauchnidae Fukui, 1929; Cephaloporidae Yamaguti, 1934; and Microscaphidiidae Looss, 1900. Näsmark (1937) revised the family Paramphistomidae on the basis of histological structures of the muscular organs such as pharynx, genital atrium and acetabulum. Principles elaborated by Näsmark (1937) are important contributions to the classification of amphistomes.

Skrjabin (1949) recognized two superfamilies, the Paramphistomoidea Fischoeder, 1901, and the Cladorchoidea Fischoeder, 1901, which he was the first to elevate to superfamily rank, within the order Paramphistomata. La Rue (1957) adopted the suborder Paramphistomata and the superfamily Paramphistomoidea but not the Cladorchoidea. Yamaguti (1971) recognized the Paramphistomoidea, but reduced the Cladorchoidea to family status. Mehra (1980) accepted the suborder Paramphistomata with two superfamilies, Paramphistomoidea and Notocotyloidea La Rue, 1957.

Sey (1988, 1991), following Odening's (1974) review of higher-level classification, categorized the amphistomes into three suborders, namely, Heronimata Skrjabin & Schulz, 1937; Zonocotylata Sey, 1988; and Paramphistomata Szidat, 1936, all under the order Amphistomida (Lühe 1909) Odening, 1974. Heronimata is a monotypic taxon; its taxonomic rank is determined by monostome type, by its special morphological structure of the adults and by the ancient type of reproduction. The taxon Zonocotylata is a reduced one, including one genus and its species. It has morphological characters of its own (reproductive system, fixative apparatus, etc.), which justified its taxonomic position in the systematics of the Amphistomida. Among the three suborders, Paramphistomata has proven to be the most successful developmental line whose representatives can be found in all higher taxa of the vertebrate definitive hosts. This group includes the typical forms of amphistome species comprising the amphistomes sensu stricto. As per the classificatory scheme suggested by Sey (1988, 1991), Paramphistomata is divided into two superfamilies: Cladorchoidea Skrjabin, 1949, and Paramphistomoidea Stiles & Goldberger, 1910, based on the presence or absence of cirrus pouch. Recently, based on molecular data pertaining to complete small subunit rDNA and partial (D1–D3) large subunit rDNA sequences, Olson et al. (2003) indicated a close relationship of the superfamily Paramphistomoidea with members of the superfamilies Microscaphidioidea Looss, 1900 and Pronocephaloidea Looss, 1899. Based on this data and also considering the fact that these digenean groups lack an oral sucker, the superfamilies Paramphistomoidea and Microscaphidioidea were placed together in the new suborder Pronocephalata along with the pronocephaloid families. However, treating the superfamily as the basic unit of classification, Jones et al. (2005) proposed a different taxonomic hierarchy for the group of amphistomid digenea. As per this system

of classification, amphistomes are placed under the superfamily Paramphistomoidea, the latter being one of the seven superfamilies under the order Echinostomida (Jones 2005b), with the superfamily Cladorchoidea treated as a synonym of Paramphistomoidea. Jones (2005a) reduced the Heronimata to superfamily status treating it as a distinct superfamily, the Heronimoidea Ward, 1917, which is represented by a single genus. Similarly, Zonocotylata was reduced to family level and was placed together with other amphistome families.

9.2.1 Superfamily Paramphistomoidea Fischoeder, 1901 (Syn. Cladorchoidea Fischoeder, 1901)

Diagnosis as per Jones (2005a): Body tiny to large, may be conical or dorsoventrally flattened. Acetabulum (ventral sucker) at or close to posterior extremity, usually simple but may show various modifications; rarely, non-acetabulate, modified attachment organ present. Tegumentary papillae often present but tegument always unarmed. Ventral pouch present or absent. Oral opening terminal or subterminal. Oral sucker absent. Pharynx present, with or without pharyngeal sacs; if present, sacs may be paired intra- or extramural or, rarely, single; paired extramural sacs may be primary, arising directly from pharynx, or secondary, arising from pharyngeal bulb. Oesophagus with or without pharyngeal bulb or sphincter. Intestinal bifurcation in anterior half of body. Caeca two, end blindly; posterior extent variable. Testes usually two, occasionally single, usually intercaecal but may be caecal or extracaecal; relative positions variable. Male duct may be differentiated into tubular, coiled, seminal vesicle (pars seminalis), muscular, tubular pars musculosa, prostatic region (pars prostatica) and ejaculatory duct. Cirrus sac present or absent. Hermaphroditic sac present or absent. Genital sucker present or absent. Cirrus absent, functionally replaced by genital papilla. Genital pore opens on mid-ventral surface, usually near level of intestinal bifurcation, or into ventral pouch if present. Ovary usually posttesticular. Mehlis' gland close to ovary. Laurer's canal present, crosses excretory vesicle or duct or not. Canalicular seminal receptacle absent. Uterine seminal receptacle often present. Uterus mainly intercaecal, usually dorsal to testes and ventral to male ducts, may pass between testes. Vitellarium usually follicular; follicles usually in two lateral fields, variable in anterior and posterior extent, confluent or not in midline. Excretory vesicle usually dorsal or anterodorsal to acetabulum, rarely intertesticular, often saccate; pore usually opens close to posterior extremity, rarely at intertesticular level. Lymphatic system present. In gastrointestinal tract of all groups of vertebrates, larval stages in molluscs, cercariae encyst on vegetation; cosmopolitan. Type genus: *Paramphistomum* Fischoeder, 1901.

In a combination of the classificatory system proposed by Jones et al. (2005) and Sey (1988, 1991), the superfamily Paramphistomoidea Fischoeder, 1901, is treated as the basic unit of classification and further divided into 12 families (Jones 2005b). Of these, only six, viz. Paramphistomidae Fischoeder, 1901; Gastrodiscidae Monticelli, 1892; Gastrothylacidae Stiles & Goldberger, 1910; **Olveriidae** Yamaguti, 1958; Balanorchiidae Stunkard, 1925; and Stephanopharyngidae Stiles & Goldberger, 1910, are represented in mammalian hosts of veterinary significance (Fig. 9.2).

9.2.2 Key Characters of Amphistomid Genera of Veterinary Significance

9.2.2.1 *Gigantocotyle* Näsmark, 1937

Acetabulum enormous; genital sucker absent; pars musculosa well developed. Type species: *G. gigantocotyle* (Brandes in Otto, 1896) Näsmark, 1937.

9.2.2.2 *Explanatum* Fukui, 1929

Acetabulum enormous; genital sucker absent; pars musculosa weakly developed. Type species: *E. explanatum* (Creplin 1847) Fukui, 1929.

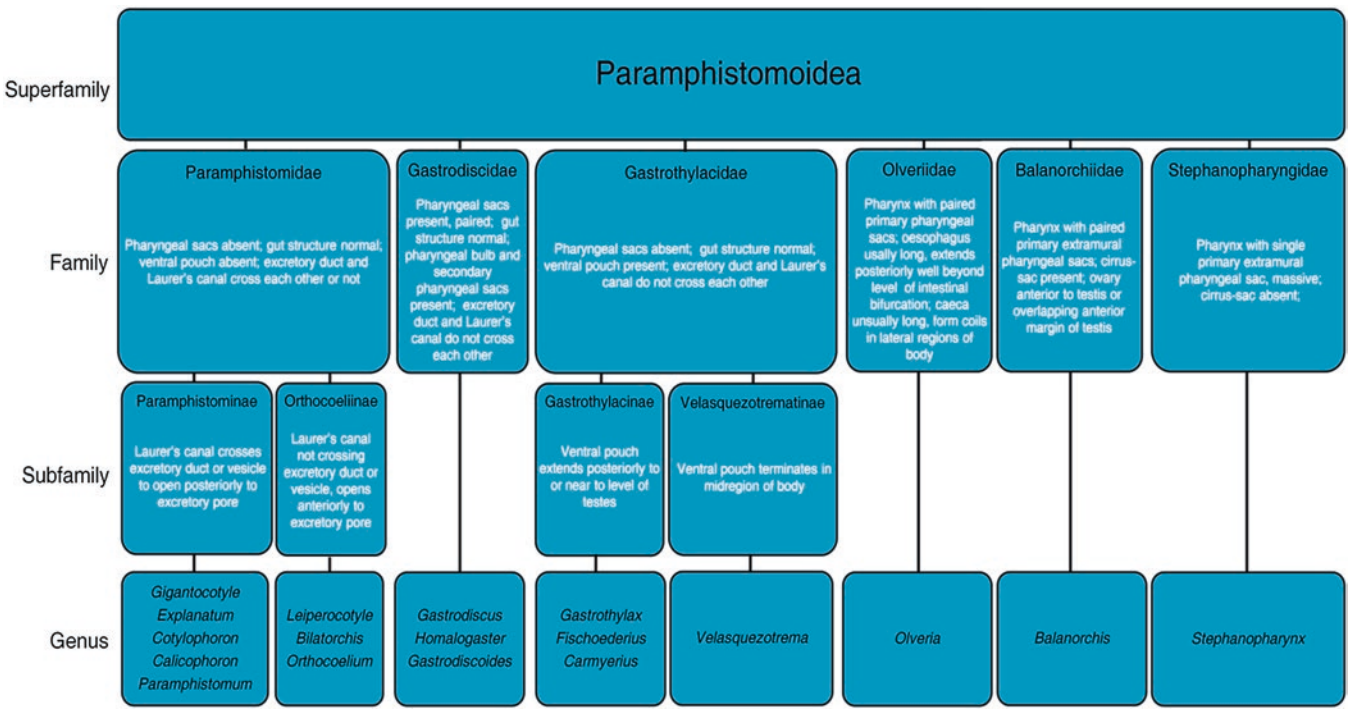

Fig. 9.2 Schematic representation of the classification of amphistomes (following Sey 1988, 1991; Jones et al. 2005) of veterinary significance up to genus level along with key characters up to subfamily level

9.2.2.3 *Cotylophoron* Stiles & Goldberger, 1910

Acetabulum small to medium; genital sucker present; pars musculosa strongly developed. Type species: *C. cotylophorum* Stiles & Goldberger, 1910.

9.2.2.4 *Calicophoron* Näsmark, 1937

Acetabulum moderate in size; genital sucker absent; pars musculosa well developed. Type species: *C. calicophorum* (Fischoeder 1901) Näsmark, 1937.

9.2.2.5 *Paramphistomum* Fischoeder, 1901

Acetabulum medium-sized; genital sucker absent; pars musculosa very weakly developed. Type species: *P. cervi* Fischoeder, 1901.

9.2.2.6 *Leiperocotyle* Eduardo, 1980

Genital sucker present; pars musculosa strongly developed; in rumen of Giraffidae and Bovidae. Type species: *L. okapi* Eduardo, 1980.

9.2.2.7 *Bilatorchis* Eduardo, 1980

Genital sucker absent; ventral atrium normal sized; testes symmetrical; acetabulum simple. Type species: *B. papillogenitalis* Eduardo, 1980.

9.2.2.8 *Orthocoelium* Stiles & Goldberger, 1910

Genital sucker absent; ventral atrium of normal size; testes tandem or diagonal; body not ringed with ridges; body conical, not flattened with inrolled margins; pars musculosa strongly developed; in rumen of Bovidae and Cervidae. Type species: *O. orthocoelium* Stiles & Goldberger, 1910.

9.2.2.9 *Gastrodiscus* Leuckart in Cobbold

Ventral surface bears large conspicuous invaginable papillae; anterior region conical; posterior region discoid with inrolled margins, covered ventrally with large invaginable papillae; testes diagonal. Type species: *G. aegyptiacus* Leuckart in Cobbold.

9.2.2.10 *Homalogaster* Poirier, 1883

Ventral surface bears large non-invaginable papillae; anterior extremity roughly triangular in ventral view; posterior region without inrolled margins, covered ventrally between triangular anterior region and acetabulum with large non-invaginable papillae; testes tandem. Type species: *H. paloniae* Poirier, 1883.

9.2.2.11 *Gastrodiscoides* Leiper, 1913

Ventral surface without conspicuous papillae, bears papillar ridges; surface of acetabulum not covered by papillae; testes tandem, lobed; genital pore prebifurcal at level of oesophageal bulb; anterior region of body conical, posterior region discoidal and dorsoventrally flattened. Type species: *G. hominis* (Lewis and McConnell 1876) Leiper, 1913.

9.2.2.12 *Gastrothylax* Poirier, 1883

Ventral pouch extends to or near to level of testes; uterus loops from one side of body to the other; testes symmetrical. Type species: *G. crumenifer* (Creplin 1847) Poirier, 1883.

9.2.2.13 *Carmyerius* Stiles & Goldberger, 1910

Ventral pouch extends to or near to level of testes; uterus in midline throughout its length; testes symmetrical. Type species: *C. gregarius* (Looss 1896) Stiles & Goldberger, 1910.

9.2.2.14 *Fischoederius* Stiles & Goldberger, 1910

Ventral pouch extends to or near to level of testes; uterus in midline throughout its length; testes tandem in midline, one anterodorsal to other. Type species: *F. elongates* (Poirier, 1883) Stiles & Goldberger, 1910.

9.2.2.15 *Velasquezotrema* Eduardo & Javellana, 1987

Key characters same as that of subfamily. Type species: *V. brevisaccus* (Eduardo, 1981) Eduardo & Javellana, 1987.

9.2.2.16 *Olveria* Thapar & Sinha, 1945

Key characters same as that of family—oesophagus long and J-shaped. Type species: *O. indica* Thapar & Sinha, 1945.

9.2.2.17 *Balanorchis* Fischoeder, 1901

Key characters same as that of family. Type and only species: *B. anastrophus* Fischoeder, 1901.

9.2.2.18 *Stephanopharynx* Fischoeder, 1901

Key characters same as that of family. Type and only species: *S. compactus* Fischoeder, 1901.

9.3 Life Cycle

Amphistomes require two hosts to complete their life cycle (Fig. 9.3); a vertebrate definitive host and a snail intermediate host. Infected animals excrete eggs in the faeces. The eggs are large in size measuring approximately about 160 × 90 µm, with a thin (freshwater developed) or thick (marine developed) egg shell, operculated with a distinct operculum. As the eggs are laid, the development of the embryo/miracidium continues within the egg shell. The eggs develop and hatch under suitable conditions (of temperature and moisture) when the eggs have been freed from the faecal mass (Burgu 1981). The newly emerged miracidium finds a suitable intermediate aquatic snail host belonging to the families Lymnaeidae and Planorbidae. Its body shape is variable and is visible to the unaided eye. The miracidium show a 6:8:4:2 and 6:6:4:2 epidermal cell formula and is without pigment spots. The miracidium locates the molluscan host, the snail, either by innate behaviour, random chance or chemotaxis and enters its soft tissues. Generally, the younger snails are more susceptible to miracidium penetration. The miracidium makes its

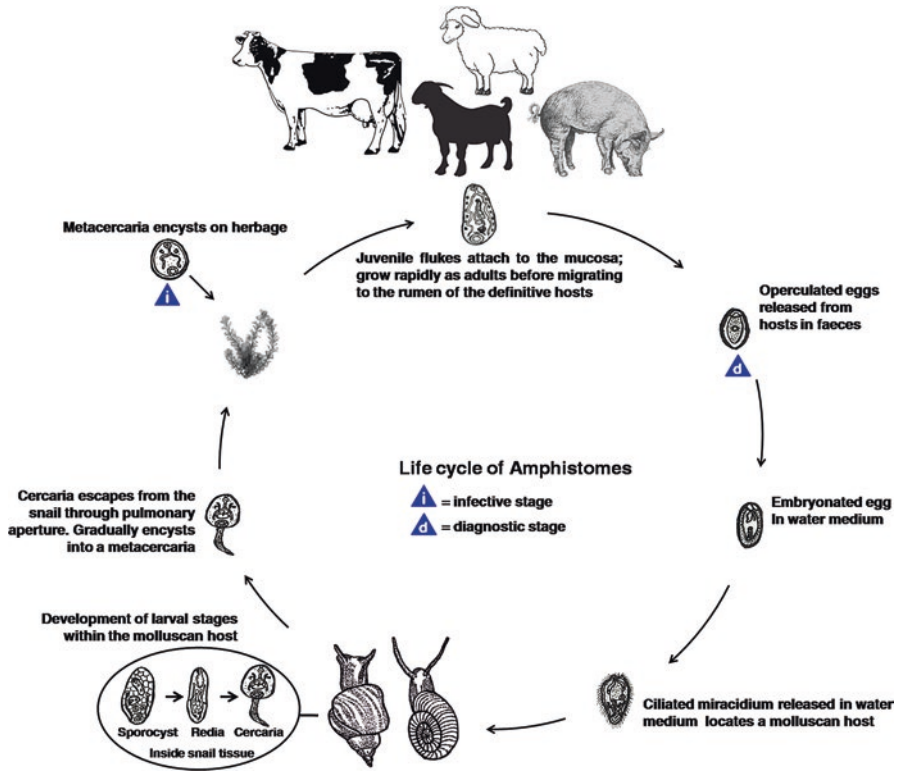

Fig. 9.3 Generalized life cycle of a typical amphistome fluke

way to the mantle cavity and thence to the heart, where the ciliated epidermal plates (cells) are shed after sometime and differentiates into sporocyst. A mature sporocyst is formed in the mantle tissue, sometimes in the digestive tissue and head-foot organ of the snail host. As the sporocyst grows the germ balls from the miracidium develop further and differentiate into the redia stage (Tandon 1957). A mature sporocyst contains developing rediae of different sizes and germ balls. Each developing redia possesses a pharynx, gut and three pairs of flame cells and some primordial germ cells. The rediae come out of the sporocyst by rupturing its anterior body wall or through a terminal opening in the sporocyst. After liberation from the sporocyst, the redia is still immature and continues its growth while in the digestive gland, ovotestis, mantle tissue or the alimentary tract. A mature redia is elongate and has neither collar nor procrusculi. The germ cells in the redia are exhausted in the formation of cercariae. Immature cercaria leaves the redia and completes development in tissue of the snail host (Malek 1971). A mature cercaria is released from the snail from the pulmonary aperture and swims actively in the water. The cercaria is large and of 'amphistome' type with an unforked tail; the acetabulum lies at the posterior end of its body; the body is either with dense pigmentation (*Cercaria pigmentata*) or with light pigmentation (*Cercaria intermedia*) or without pigmentation (*Cercaria diplocotylea*). A pair of eyespots and a lot of cystogenous cells is present (except in Heronimus). The protonéphridial system is well developed, where the excretory bladder is non-epithelial (Sey 1991; Gupta 1993). Infected snails can live and shed cercariae for up to 1 year. The free cercaria after its escape from the snail lives in that condition for a short period, from a few minutes to a few hours. Cercariae encyst to form metacercariae on the ventral surface of sub-aquatic plants where they can remain viable for up to 6 months. The metacercariae usually encyst freely. The final (definitive) host ingests the encysted metacercariae on the herbage. Excystment occurs in the small intestine and the newly hatched juvenile flukes attach to the mucosa in the first 3–6 months of the small intestine where they grow rapidly before migrating to the rumen after about 3–6 weeks. In the rumen the flukes attach to their predilection sites on the dorsal surface of the anterior ruminal pillar and dorsal and ventral aspects of the posterior ruminal pillar. Here they continue to grow to reach their maximum size 5–9 months after infection (Gupta 1993; Waal 2010).

9.4 Hosts and Distribution

Amphistomes are found as endoparasites in the digestive tracts, liver and bile ducts of many vertebrates, ranging from fishes to mammals. Majority of the species are found to parasitize mammalian livestock species such as cattle (cow, buffalo, mithun and yak), sheep, goats and pigs. However, representatives of this group are also found to infect a variety of other animal groups such as fishes (both freshwater and marine), amphibians (frogs, toads and salamander), reptiles (turtles, tortoises and snakes), aquatic birds and mammals other than livestock species (Table 9.1).

The distribution of amphistomes infecting cattle has been well documented owing to the economic importance of these parasites from veterinary standpoint. These parasites have been found to infect cattle globally, but the most severe effects occur in the tropical and subtropical region of the world including Australia, Asia, Africa, Eastern Europe and Russia (Boray 1959, 1969; Horak 1971; Singh et al. 1984; Phiri et al. 2007).

9.5 Epidemiology

9.5.1 Amphistomiasis in Ruminants

Amphistomes are known to be the causative agent of a debilitating disease 'Amphistomiasis' or 'stomach fluke disease' of domestic and wild animals, mainly ruminants. The disease is characterized by loss of appetite, foetid diarrhoea, dehydration, emaciation, extreme weakness, exhaustion, intermandibular oedema and subnor-

Table 9.1 Amphistome genera from vertebrates other than mammalian livestock (Yamaguti 1971; Gupta 1993; Jones et al. 2005; Sey 2005)

Host	Genera	Family	Distribution
Fish (freshwater and marine)	*Amurotrema, Bancroftrema, Basidiodiscus, Brevicaecum, Caballeroia, Cleptodiscus, Dadayius, Dadaytrema, Helostomatis, Kalitrema, Macrorchitrema, Microrchis, Neocladorchis, Nicollodiscus, Ophioxenos, Orientodiscus, Panamphistomum, Pisciamphistoma, Protocladorchis, Pseudocladorchis, Sandnoia, Travassosinia*	Cladorchiidae	Africa, Asia, Australia, North America, Caribbean, South America
	Pseudodiplodiscus	Displodiscidae	South America (Brazil)
Amphibians (frog, toad, newt and salamander)	*Allassostomoides, Megalodiscus, Ophioxenos, Opisthodiscus*	Cladorchiidae	Africa, Europe, North America
	Catadiscus, Diplodiscus, Progonimodiscus	Diplodiscidae	Africa (South Africa), Asia (India), South America, Central America, Europe (Germany)
Reptiles (snake, turtle and tortoise)	*Allassostoma, Allassostomoides, Halltrema, Nematophila, Ophioxenos, Orientodiscus, Parachiorchis, Pseudoallassostomoides, Pseudocleptodiscus, Quasichiorchis, Schizoamphistomoides, Schizoamphistomum, Stunkardia*	Cladorchiidae	Asia, North America, South America, Pacific, Atlantic, Mediterranean
	Catadiscus, Dermatemytrema	Diplodiscidae	North and South America
Rodents (agouti, muskrat, rat and mice)	*Chiostichorchis, Cladorchis, Taxorchis, Wardius*	Cladorchiidae	North America, South America (Brazil)
Aquatic birds	*Zygocotyle*	Zygotylidae	North America (USA), South America (Brazil)
Aquatic mammals (manatee and dugong)	*Chiorchis, Indosolenorchis, Solenorchis*	Cladorchiidae	Africa, Asia (Sri Lanka), North America (USA), Australia
Other mammals (wild dog, wild pig, tapir, deer, horse, bison, hippopotamus, elephant and rhinocerus)	*Buxifrons, Cotylophoron, Gigantocotyle, Macropharynx, Nilocotyle, Orthocoelium*,	Paramphistomidae	Africa, Asia, North America, Europe
	Paramphistomum, Ugandocotyle	Gastrodiscidae	Asia, Africa
	Choerocotyle, Gastrodiscoides, Gastrodiscus, Pseudodiscus	Choerocotyloididae	Africa (Zimbabwe)
	Choerocotyloides		
	Stephanopharynx	Stephanopharyngidae	Africa
	Cladorchis, Pfenderius, Stichorchis, Taxorchis	Cladorchiidae	Asia, Caribbean, South America
	Brumptia	Brumptidae	Africa
	Balanorchis	Balanorchiidae	South America
Primates (monkey)	*Gastrodiscoides, Watsonius*	Gastrodiscidae	Africa, Asia (Singapore and Thailand)
Human	*Gastrodiscoides (hominis)*	Gastrodiscidae	Africa, Asia
	Watsonius (watsoni)		

mal temperature (Chandrasekharan et al. 1982; Blood 1983). Though a neglected trematode infectious disease in ruminants, amphistomiasis is among the most pathogenic diseases of parasitic origin in domesticated livestock and has recently emerged as an important cause of productivity loss (Anuracpreeda et al. 2008). The significant damage caused by migrating flukes especially among young stock adds to great economic loss due to high morbidity and mortality (Lalitha and Anandan 1986). Thus, the disease is virulent when these amphistomes are immature and found in the small intestine eventually migrating to the rumen on maturity. In case of the genus *Explanatum*, the flukes may travel up the bile duct and clog it and the flow of the bile from the gall bladder to the duodenum is obstructed and the gall bladder becomes distended (Gupta 1993).

The outbreaks of amphistomiasis are usually restricted to the drier months of the year. This period, however, varies according to the climate of the region, and during rainy season ruminants have more access to graze freely over these areas; thus, the infection is easily acquired by ingesting vegetation that are metacercariae infested. The affected hosts usually inhabit water bodies due to anorexia, restlessness and polydypsis. The rate of infection also flares up due to heavily contaminated herbage. The rate of migration of these flukes from the intestine to the rumen is usually slow thereby inflicting grave intestinal damage in the hosts infected. In India, domestic ruminants are generally permitted to graze around small ponds or banks of streamlets during the months from September to January, which were previously swamped with water and are drying up. In the process, these ruminants ingest many metacercariae in a short period of time. Thus, the outbreaks may appear in the months that follow the monsoons. Amphistomiasis is thus a disease of domesticated animals that are forced by natural reasons or by farming practices to graze upon foliage that is infected by amphistome metacercariae (Gupta 1993).

A recent epidemiological survey in Central France demonstrated that the prevalence of natural infections with *Paramphistomum* in cattle significantly increased from 5.2% (in 1990) to 44.7% (in 1999). The apparent spread of the disease was attributed to the efficacy of fasciolosis control that leaves free the intermediate host (*Galba truncatula*); the development of extensive livestock farming or the propagation of metacercariae by mechanical vectors has also been incriminated (Mage et al. 2002). The disease is most rampant during monsoon and post monsoon period. Characterized by sporadic epizootics of acute gastroenteritis with high morbidity and mortality in domestic animals, amphistomiasis constitutes a major health hazard to ruminants particularly in low-lying areas where snails are found abundantly during monsoon and post monsoon season (Hassan and Juyal 2006).

Amphistomiasis has a wide geographical distribution in subtropical and tropical areas, where the infection leads to economic losses related to mortality and low productivity. The disease is distributed all around the world, but its highest prevalence has been reported in tropical and subtropical regions, particularly in Africa, Asia, Australia, Eastern Europe and Russia and also from various states of India (Horak 1971; Singh et al. 1984; Asanji 1989; Howlader et al. 1990; Mahato and Rai 1992; Rangel-Ruiz et al. 2003; Phiri et al. 2007). The disease is also increasingly being reported from Western Europe and is now being considered as an important emerging infection in the region (Huson et al. 2017).

The causative flukes mainly belong to different genera, viz. *Paramphistomum, Cotylophoron, Calicophoron, Explanatum, Orthoceolium, Gastrothylax* and *Fischoederius* (Lloyd et al. 2007; Hassan and Juyal 2006). The etiological agent implicated in the disease may be different species in specific regions (Ozdal et al. 2010). For example, in Europe, *Paramphistomum cervi* has been reported as the main causative agent of amphistomiasis, while it is *Paramphistomum microbothrium* in Sardinia, Yugoslavia and Hungary (Horak 1971). In Australia and New Zealand, species of amphistomes affecting cattle and sheep are *Paramphistomum ichikawai* and *Calicophoron calicophorum* (Brotowidjoyo and Copeman 1979); species responsible for infection in Turkey are mainly *P. cervi*, *P. ichikawai*

and *Calicophoron daubneyi* (Coskun 1988; Tinar et al. 1992). In Asia, *Paramphistomum explanatum, P. cervi, Gastrothylax crumenifer, Cotylophoron cotylophorum, Fischoederius elongatus* and *F. cobboldi* have been recorded in Ceylon and China (Boray 1959; Malek 1980; Hanna et al. 1988; Wang et al. 2006). In the northeastern region of India, as many as 25 amphistomid species representing 13 genera have been reported as occurring in livestock mammals (Roy 1990; Roy and Tandon 1990a, b, 1992, 1995; Shylla et al. 2011; Ghatani et al. 2012) (Fig. 9.4).

Across the world, effects of amphistomes are underestimated and they have to be considered, particularly when a risk of acute infection is present as the immature worms are very difficult to identify and there is no serological test available and during necropsy small lesions of abomasum and of ileum may be confused with other diseases. Adults are very prolific and many eggs are expelled: their number not being related to the parasitic burdens. Amphistomiasis is one of the most pathogenic diseases of domesticated animals, causing heavy losses to the livestock industry, amounting to several thousand crores of rupees annually (Khan et al. 2008; Hassan and Juyal 2006). It has been estimated that more than 500 million cattle worldwide are at risk due to parasitic infection (Ristic 1988). In some areas of India, the Republic of South Africa and Australia, the mortality of cattle has reached 80–90% in sheep and cattle (Castro-Trejo et al. 1990; Rangel-Ruiz et al. 2003; Juyal et al. 2003; Ilha et al. 2005; Khan et al. 2008). Economic loss caused by amphistome infections has not been estimated but may be greater than those caused by many other parasites; the cost of the parasite in terms of economic losses to human society is incalculable due to the high mortality and morbidity in young animals (Rangel-Ruiz et al. 2003; Kilani et al. 2003; Hassan and Juyal 2006; Phiri et al. 2007).

9.5.2 Human Amphistomiasis

Amphistomiasis is not restricted to ruminant hosts given that human infections have also been reported. Three species have been reported to infect man, namely, *Gastrodiscoides hominis, Watsonius watsoni* and *Fischoederius elongatus. G. hominis* is frequently found in pigs and accidentally in man as reported in Burma, China, India, Kazakhstan, the Philippines, Thailand and Vietnam (Ahluwalia 1960; Dutt and Srivastava 1972; Kumar 1980; Harinasuta et al. 1987). The exact life cycle is unknown but probably similar as in other species of Gastrodiscidae involving aquatic vegetations as the second intermediate environment that is used for the encystment of the metacercarial infective stage (Zablotski 1964; Dutt and Srivastava 1972; Mas-Coma et al. 2006). Human and animal contamination can take place when swallowing encysted metacercariae, by ingestion of vegetation (aquatic plants) or animal products, such as raw or undercooked crustaceans (crayfish), squid, molluscs or amphibians (frogs, tadpoles). Although it is mainly a parasite of pigs, sometimes high prevalence (41%) of *G. hominis* has also been detected in humans, in mainly children in Kamrup District in Assam, in northeastern India (Buckley 1939). *G. hominis* has a wide distribution throughout India including the states of Assam, Bengal, Bihar, Uttar Pradesh, Madhya Pradesh and Orissa (Shrivastav and Shah 1970; Murty and Reddy 1980). Apart from India, it is widely distributed in countries like Pakistan, Burma, Thailand, Vietnam, the Philippines, China, Kazakhstan and Russia (Ahluwalia 1960; Buckley 1939; Kumar 1980; Harinasuta et al. 1987; Yu and Mott 1994; Ivanov and Semenova 2000). In a later study carried out in Meghalaya (India), *G. hominis* was shown to have a pattern of seasonal prevalence (Roy and Tandon 1992).

Pathology and symptomatology of *G. hominis* infection are uncertain. In humans, the parasite causes inflammation of the mucosa of the caecum and ascending colon with attendant symptoms of diarrhoea. Ill health in a large number of individuals, and deaths among untreated patients, especially children, has been attributed to this infection (Kumar 1980). The specimens can be collected from the caecum, especially near the ileocaecal valve (Yu and Mott 1994). Human infection by *G. hominis* is easily recognizable by finding the characteristic eggs of this amphistome in faeces (Mas-Coma et al. 2005).

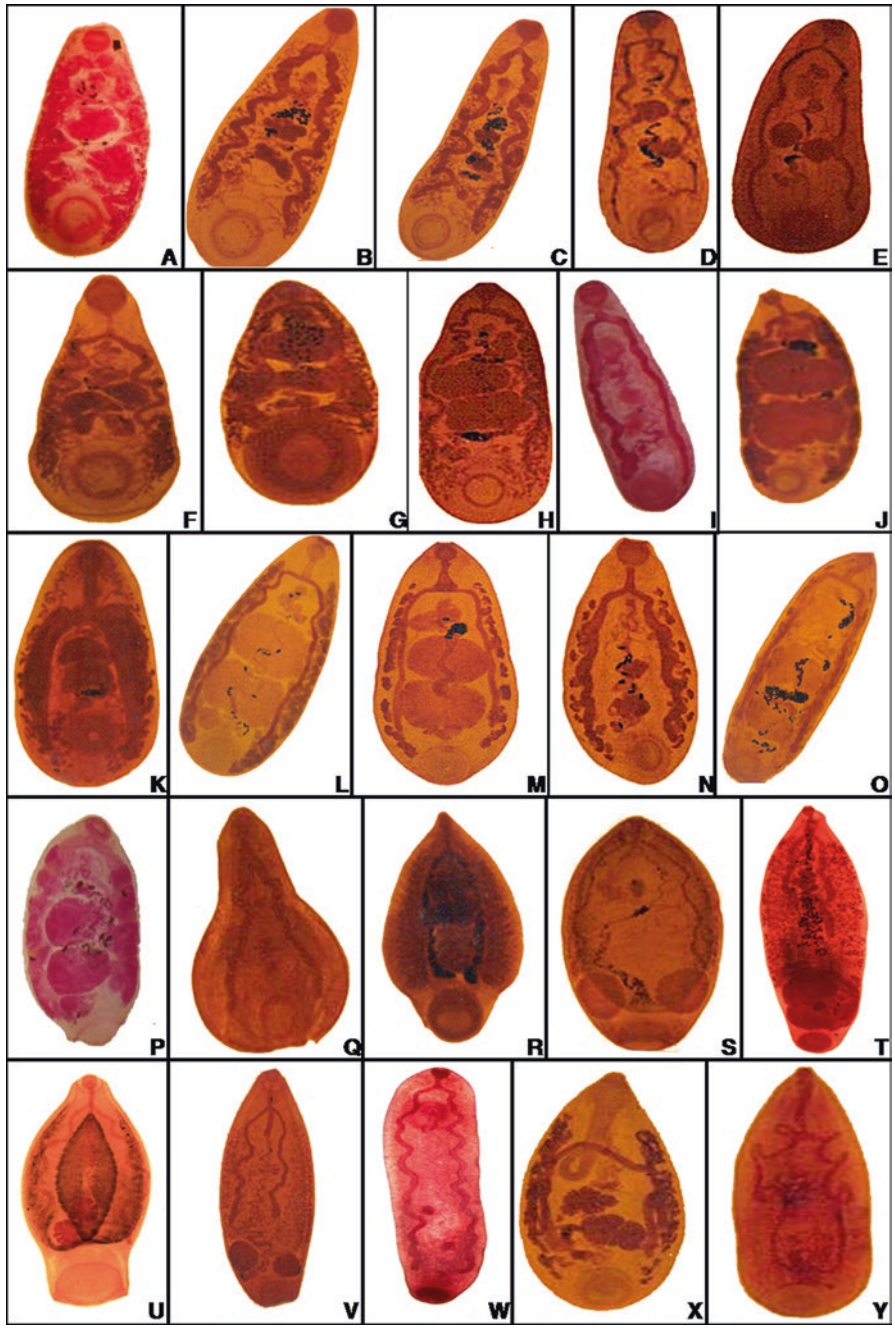

Fig. 9.4 Amphistome species of mammalian livestock reported from Northeast India (Roy and Tandon 1990a, b, 1992, 1995). (**a**) *Paramphistomum gracile*; (**b**) *P. epiclitum*; (**c**) *P. ichikawai*; (**d**) *Calicophoron papillosum*; (**e**) *C. calicophorum*; (**f**) *C. shillongensis*; (**g**) *Explanatum explanatum*; (**h**) *Cotylophoron cotylophorum*; (**i**) *Leiperocotyle meghalyensis*; (**j**) *Orthocoelium dinniki*; (**k**) *O. orthocoelium*; (**l**) *O. parpvipapillatum*; (**m**) *O. dawesi*; (**n**) *O. streptocoelium*; (**o**) *O. dicranocoelium*; (**p**) *O. scoliocoelium*; (**q**) *Gastrodiscoides hominis*; (**r**) *Homalogaster paloniae*; (**s**) *Gastrothylax crumenifer*; (**t**) *Fischoederius elongatus*; (**u**) *F. cobboldi*; (**v**) *Carmyerius spatiosus*; (**w**) *Velasquezotrema tripurensis*; (**x**) *Olveria indica*; (**y**) *Olveria bosi*

The other two species that have been reported from humans are *Watsonius watsoni* Stiles & Goldberger, 1910 (Gastrodiscidae) and *Fischoederius elongatus* (Poirier 1883) Stiles & Goldberger, 1910 (Gastrothylacidae) and are of lesser significance. *Watsonius watsoni* is a common paramphistome of various species of primates in eastern Asia and Africa. It has been reported only twice in humans in Africa (Pick 1964, 1967). *Fischoederius elongatus* is a parasite of ruminants with the only human infection reported from Guangdong, China (Li 1991).

9.5.3 Pathology and Clinical Aspects

There is little evidence regarding the pathogenesis of adult flukes to their hosts, but severe damage to the mucosa of the rumen was provoked in heavy infection in experimentally infected sheep (Rolfe et al. 1994). A high number of immature worms in the duodenum may affect production, since these parasites causing a lower feed conversion, rough hair coat, dullness, weakness, loss of appetite, intestinal haemorrhages, anaemia, reduced milk production, intermandibular swelling, a loss of weight and/or a decrease in milk production, are responsible for economic losses, morbidity and mortality (Chandrasekharan et al. 1982; Blood 1983; Rolfe et al. 1991; Ilha et al. 2005). Ruminants suffering from amphistomiasis exhibit a decrease in their appetite, resulting in complete anorexia (loss of appetite). Consumption of water is highly reduced and, therefore, sometimes they keep their muzzles dipped in water for long periods. Generally, it takes 2–4 weeks to develop diarrhoea in infected animals. The faeces are extremely fluid and foetid (an abnormally frequent discharge of fluid faecal matter from bowel). In severe cases, diarrhoea may be projectile, may be tinged with blood and may be present with some immature flukes. The latter present in the duodenum and ileum are plug feeders and cause haemorrhage, which leads to bleeding and diarrhoea; bleeding for a prolonged period may cause anaemia, which further weakens the host (Gupta 1993). Clinical amphistomiasis is usually diagnosed in cattle 4–18 months of age and is associated with invasion of the duodenum and upper jejunum by large numbers of immature flukes. Counts of up to 30,000 immature amphistomes may be associated with diarrhoea after 8 weeks grazing in tracer calves (Rolfe and Boray 1993).

The pathological and anatomical effects inflicted on the host can be divided into macropathological and micropathological effects. The macropathological effects include congestion of blood vessels on the peritoneal side of the affected intestine, hyperanaemia, haemorrhages and thickening of the mucosa giving the internal surface a corrugated appearance, cachexia, hydropericardium, hydrothorax, ascites and oedema of the mesentery, abomasum and submandibular space. The micropathological effects include oedema of the epithelial layer and lymphocytic infiltration in the propria and sometimes the submucosa layer; in the vicinity of the worms, the mucosa is necrosed and sloughs with some hypertrophy of the stratum corneum; the tips of the papillae equally degenerate and slough; and the worms also reach the submucosa and muscularis mucosa of the small intestine and this may result in the lymphocytic infiltration around the worms (Gupta 1993).

9.6 Clinical Signs

The occurrence of the disease depends both on the individual vulnerability and the species susceptibility, as well as on the number of ingested metacercariae. The susceptible categories mainly include young animals, as adults develop immunity easily for longer periods. However, adult cattle and sheep without previous exposure may produce clinical or subclinical conditions after the ingestion of high doses of metacercariae. It is evident that clinical outbreaks are associated to a high intake of metacercariae from grass, and this fact is related to a high rate of infection of the intermediate host. Doses of 5000 metacercariae of *C. cotylophorum* produced clinical signs at 116 days after dosage in a lamb, and death after day 124 post infection. Burdens of 40,000 flukes

gave rise to clinical signs and death in sheep, although field infections given by 2000 flukes were associated with the cause of death (Gupta 1993). Some cases of acute infection, the death of calves have been observed (Dorchies et al. 2000). The clinical signs include (1) characteristic and persistent fetid diarrhoea accompanied by weakness, depression, dehydration and anorexia, (2) submaxillary oedema, (3) visible paleness of the mucosa and (4) death which usually occurs 15–20 days after the first signs appear. The necropsy findings include subcutaneous oedema and accumulation of fluid in the body cavities; gelatinous fats depot; mucosa in the upper part of the duodenum thickened, covered with blood-stained mucus; patches of haemorrhage under the serosa; small, flesh-coloured flukes present; and, microscopically, immature flukes in the mucosal surface and deeper layers (Rolfe and Boray 1987, 1988; Rolfe et al. 1991, 1994).

9.7 Diagnosis

The clinical diagnosis of amphistomiasis remains difficult. Immunological methods and serum antibody detection are not conclusive. As a consequence the diagnosis of amphistomiasis in live animals still depends on faecal detection of eggs (Howell 2011; Vorster and Mapham 2012).

9.7.1 Faecal Egg Counts

Amphistome eggs are clear and measure 160–180 μm in size (Sanabria and Romero 2008). The use of either liquids of high density for floatation techniques or sedimentation techniques is required to detect eggs of these flukes since they are particularly heavy eggs, which do not float on water (Dorchies 2006). The filtration technique with sieves and sedimentation is the most accurate to identify eggs in faeces, producing clearer evidence in the sediment of the sample under study. In order to distinguish the differences between eggs more clearly, it is advisable to use contrast stains such as methylene blue or methyl green, instead of Lugol. The proportion of eggs recovered may vary between 18 and 75% (Conceicao et al. 2002; Suhardono et al. 2006).

McMaster technique is widely used but sometimes modified according to the parasites being investigated. Modifications are related to the weight of the faecal sample, the specific gravity and the volume of the liquid (more or less dense) and on the surface examined on McMaster slide: one or two chambers, the whole surface or not. The sedimentation technique appears to be more accurate and sensitive than flotation techniques. In some cases, cup sedimentation using tap water is the simplest and cheapest but more time consuming compared to flotation techniques (Dorchies 2006).

9.7.2 Immune Diagnosis

Prolonged prepatency often contributes to the difficulty for detection of mild infection by conventional methods such as coprological examination. Therefore, in addition to chemotherapeutic control, early and accurate immunodiagnosis of the disease is now a focus for study. ELISA has been used by few workers to detect anti-parasitic antibodies (Kaur et al. 2004). The majority of tests are applied to the detection of blood antibodies and the latest to the detection of antigen in faeces. Nevertheless, many cattle surveys are performed with the ELISA test alone without any confirmatory tests (Dorchies 2006). Whole adult somatic antigens of *Paramphistomum epiclitum, Cotylophoron cotylophorum* and *Gastrothylax crumenifer* were prepared; polyclonal antibodies were raised against *P. epiclitum* antigen in rabbits and were tested positive for antibody titre by Dot-ELISA and Plate-ELISA (Kaur et al. 2004, 2009). Polyclonal antibodies were also generated for the detection of coproantigens by ELISA and immunodot methods for early diagnosis of amphistomiasis in livestock (Saifullah et al. 2013). The use of immune diagnosis is, however, still in its rudimentary stage in case of amphistomiasis.

9.7.3 Molecular Diagnosis

The feasibility of developing PCR-based methods as diagnostic tools for helminth parasites has remarkably been enhanced with the introduction of genomic sequencing and the ample amount of data generated on a daily basis. The growing accumulation of mitochondrial and nuclear genome sequence data in public databases, including NCBI GenBank, facilitates the rapid development of PCR primer design. Comprehensive sequence information is, therefore, required to adapt methods for PCR-based diagnosis, and the remarkable increase of such information for the parasitic helminths over the past decade has facilitated the development, implementation and effectiveness of these types of diagnostic techniques (Gordon et al. 2011). Additionally variants of PCR like multiplex PCR (mPCR) and real-time PCR (qPCR) assays can aid in detection of multiple distinct helminth species in an individual host (Verweij et al. 2007; ten Hove et al. 2008; Basuni et al. 2011; Taniuchi et al. 2011). These diagnostic techniques are not only useful in human and veterinary medicine but also for environmental monitoring purposes.

Various workers have generated ample amount of data based on the exploration of genetic markers, viz. nuclear (18S RNA, 28S RNA, spacers, etc.) and mitochondrial DNA (cytochrome oxidase CO, NADH, etc.) which in the future may be used to yield species specific primers (Beer et al. 2010; Thaenkham et al. 2011, 2012; Ai et al. 2011; Amor et al. 2011; Atopkin 2011; Choe et al. 2011; de León et al. 2011; Domingues and Marques 2011; Prasad et al. 2011; Králová-Hromadová et al. 2011; Saijuntha et al. 2011; Vanhove et al. 2011; Brant et al. 2012). These primers may eventually be used to accurately identify this ambiguous group of parasites. PCR-based techniques providing rDNA ITS2 and mitochondrial COI sequences have proven to be a reliable tool to identify digenean species and to recover their phylogenetic relationships. These markers have been found to be useful for species identification of amphistomes as well (Blair et al. 1999; Goswami et al. 2009; Lotfy et al. 2010; Shylla et al. 2011; Ghatani et al. 2012; Ichikawa et al. 2013; Titi et al. 2014; Dube et al. 2016; Martinez-Ibeas et al. 2016; Sanguankiat et al. 2016). Moreover, the combination of PCR-RFLP analysis, which is a widely used method for the accurate determination of helminth parasites (Ichikawa and Itagaki 2010; Zaeemi et al. 2011) has been successfully used for characterizing some amphistome genera and species; Itagaki et al. (2003) characterized three different genera by ribosomal DNA (rDNA) internal transcribed spacer 2 (ITS-2), whereas Rinaldi et al. (2005) applied a similar technique using the ITS-2 of *Calicophoron daubneyi* from different definitive hosts and Sanabria et al. (2011) generated restriction profiles for *Paramphistomum* spp. Another variant of PCR, PCR-RAPD, has also been used for species identification of three amphistome flukes, viz. *Paramphistomum epiclitum*; *Orthocoelium streptocoelium* and *Fischoederius elongatus* 50 polymorphic markers were detected and 16 genetic markers were generated by the high annealing temperature-random amplified polymorphic DNA (HAT-RAPD) technique with OPA2, OPA4, OPB18, OPC9 and OPH11 primers (Sripalwit et al. 2007).

One of the latest techniques for species identification is DNA barcoding which is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species (Hebert et al. 2003). The DNA barcoding initiative is one prominent line of research within this field, coordinated by the Consortium for the Barcode of Life (CBoL, http://barcoding.si.edu) (Hebert et al. 2004). A desirable locus for DNA barcoding should be standardized so that large databases of sequences for that locus can be developed, present in most of the taxa of interest and sequenceable without species-specific PCR primers (CBOL Plant Working Group 2009) short enough to be easily sequenced with current technology (Kress and Erickson 2008), and provide a large variation between species, yet a relatively small amount of variation within a species (Lahaye et al. 2008). Although several loci have been suggested, a common choice is the mitochondrial

COI gene for animals and many other eukaryotes. Concerning the amphistomes found in Northeast India, DNA barcodes based on partial sequences of mitochondrial COI for 18 amphistomes were generated and deposited in the Barcode of Life Database (BOLD) (our unpublished results).

9.8 Control and Treatment

There are several methods, which can be employed for the control of amphistomes in ruminants. These can be categorized as follows:

9.8.1 Control in Grazing and Water Sources

Serious outbreaks may be prevented if pastures with a natural water supply are grazed only in spring and early summer. Late summer, autumn and early winter grazing should be avoided. If wet pastures are grazed continuously, young weaner calves and lambs should not be grazed together with adults. Affected areas must be drained properly and such areas should be fenced. Alternative water supply treated with a suitable molluscicide may be provided in these areas (Gupta 1993).

9.8.2 Chemical Control

In case of an outbreak, the whole flock or herd must be removed from the source of infection before treatment is instituted. All animals, irrespective of clinical conditions, must be treated, since an apparently healthy animal may die within few days of onset of symptoms (Gupta 1993). Although treatment for adult fluke has no direct benefit to the animal, it may reduce the source of infection for the snail intermediate host. This then reduces the size of the next generation of infective fluke larvae on pasture. Treatment with an appropriate drench should be timed for autumn and spring. Effective treatment of immature stomach fluke infection requires removal of stock from the source of infection, usually swampy land, as well as treatment with a drench which is effective against immature fluke. Supportive therapy to treat dehydration and any secondary infection may be needed (Lloyd et al. 2007).

There are several anthelminthics that are used for treatment of amphistomiasis in ruminants. These include terenol, vermitan, febantel, niclosamide, oxyclozanide, resorantel, bithionol, hexachloroethane and carbon tetrachloride (Sey 1989; Gupta 1993). Terenol is highly effective against both juvenile and adult flukes when applied at a dose of 65 mg/kg in cattle and sheep (Lämmler et al. 1969; Vujic et al., 1971; Kobulej and Udvarhelyi 1972; Chroust 1973; Wikerhauser et al. 1975). Vermitan is another anthelminthic which proved to be highly effective in sheep against artificial subclinical intestinal amphistomiasis with an efficacy of 99.2–99.5% at the dose rate of 20 mg/kg (Sey and Kassai 1984). Febantel was applied against ruminal and intestinal amphistomiasis in cattle; at a dose of 100 mg/kg, it had more than 90% efficacy (93.9% against juvenile and 94.9% against adult paramphistomes). Clinical symptoms were shown to disappear in 7–10 days after treatment (Corba 1981). Niclosamide is very efficient against immature amphistomes with efficiency of 92–99% observed against various species in sheep. A single dose of 160 mg/kg or two doses at 3 days apart is effective in cattle, and a dosage of 100 mg/kg is effective against immature amphistomes in sheep. Oxyclozanide is highly efficacious against adult as well as immature forms with two doses of 18.7 mg/kg 2 days apart and gave consistent result against immature amphistomes in cattle (Gupta 1993). A combination of levamisole and oxyclosanide is also very effective in treating both adult and immature forms (Rolfe and Boray 1987). Resorantel and bithionol have also been shown to be efficacious against both immature and mature forms (Gupta 1993).

9.8.3 Immunological Control

This method of control is by means of immunization and particularly in those areas where amphistomiasis is enzootic. Cattle, sheep and goats

have been shown to develop resistance after exposure to the parasite. This immunity protects the animal against the massive infections of immature fluke that cause the most problems. This property was utilized by Horak (1971) by successfully immunizing sheep, goats and cattle against massive artificial infections with *Calicophoron microbothrium*. The results indicated that cattle were the most suitable subjects for immunization. Immunity in adult cattle was attained within 4–6 weeks after immunization, and the immunity was effective for at least a year post-immunization.

9.8.4 Control of Intermediate Host

Snails are the intermediate hosts of amphistomes. If snails are controlled, the life cycles of these parasites become interrupted, and the disease is controlled automatically. Therefore, removal of snail from the chain of life cycle of amphistomes would be the most effective method in the control of amphistomiasis (Gupta 1993). This would involve fencing off of marshy areas, thus preventing cattle from grazing in those areas. Moreover, drainage of low lying areas that are the breeding places of snails is useful in controlling the snail population. It is also important to repair any leaks in dams and water troughs as the latter can create an ideal habitat for the survival of water snails. Use of molluscicides like copper sulphate, niclosamide, sodium pentachlorophenate is very effective in the elimination of snails. Reports from several parts of the world indicate that a number of plants have molluscicidal properties. Planting of these trees and shrubs along streams and irrigation channels can reduce the number of snails in a population. The efficacy of this method for control of flukes is yet to be assessed (Vorster and Mapham 2012).

The control measures discussed above are basically applied for amphistome infection in ruminants. However, a different control strategy has to be in place in controlling the infection of *Gastrodiscoides hominis* in man. Control methods including the following main axes have been proposed (Mas-Coma et al. 2006): (1) prevention of human contamination, (2) actions at human level to cut disease dissemination by humans, (3) control the disease at the animal reservoir level and (4) actions at the level of the intermediate molluscan host.

Trematode infections are some of the most economically important helminth diseases hampering the productivity of domestic ruminants worldwide. Amphistomiasis, at present emerging as an important livestock parasitosis, is still highly underestimated. It is often not diagnosed and the importance of subclinical infection has not been determined. The biodiversity of amphistomes is still highly underestimated both at the morphological and molecular level. Amphistomiasis being a debilitating disease of the livestock necessitates the importance of an in-depth study of the causative agents the amphistomes, which will, therefore, speed up the estimation and prompt the control of this group of parasites.

References

Ahluwalia SS (1960) *Gastrodiscoides hominis* (Lewis and McConnell, 1876) Leiper, 1913—the amphistome parasite of man and pig. Indian J Med Res 48:315–325

Ai L, Weng YB, Elsheikha HM, Zhao GH, Alasaad S (2011) Genetic diversity and relatedness of *Fasciola* spp. isolates from different hosts and geographic regions revealed by analysis of mitochondrial DNA sequences. Vet Parasitol 181:329–334

Amor N, Halajian A, Farjallah S, Merella P, Said K, Ben Slimane B (2011) Molecular characterization of *Fasciola* spp. from the endemic area of northern Iran based on nuclear ribosomal DNA sequences. Exp Parasitol 128:196–204

Anuracpreeda P, Wanichanon C, Sobhon P (2008) *Paramphistomum cervi*: antigenic profile of adults as recognized by infected cattle sera. Exp Parasitol 118:203–207

Asanji MF (1989) Paramphistomiasis of cattle in Sierra Leone and seasonal fluctuations in its prevalence. Bull Anim Health Prod Afr 37:327–331

Atopkin DM (2011) Genetic characterization of the *Psilotrema* (Digenea: Psilostomatidae) genus by partial 28S ribosomal DNA sequences. Parasitol Int 60:541–543

Baer JG, Joyeux C (1961) Classe des trematodes (Trematoda Rudolphi). In: Gassé PP (ed) Traité de Zoologie, Anatomie, Systématique, Biologie Premier Fascicule. Plathelminthes, Mésozoaires,

Acanthocéphales, Némertiens, vol 4. Masson et Cie, Paris, pp 561–677
Basuni M, Muhi J, Othman N, Verweij JJ, Ahmad M, Miswan N, Rahumatullah A, Aziz FA, Zainudin NS, Noordin R (2011) A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. Am J Trop Med Hyg 84:338–343
Beer SA, Voronin MV, Zazornova OP, Khrisanfova GG, Semenova SK (2010) Phylogenetic relationships among schistosomatidae. Med Parazitol (Mosk) 2:53–59
Blair D, Xu Z-B, Agatsuma T (1999) Paragonimiasis and the genus *Paragonimus*. Adv Parasitol 42:114–172
Blood DC (1983) Veterinary medicine, 6th edn. Bailliere Tindall, London
Boray JC (1959) Studies on intestinal amphistomosis in cattle. Aust Vet J 35:282–287
Boray JC (1969) Studies on intestinal amphistomosis in sheep due to *Paramphistomum ichikawai* Fukui, 1922. Vet Med Rev 4:290–308
Brant SV, Pomajbíková K, Modry D, Petrželková KJ, Todd A, Loker ES (2012) Molecular phylogenetics of the elephant schistosome *Bivitellobilharzia loxodontae* (Trematoda: Schistosomatidae) from the Central African Republic. J Helminthol 17:1–6
Brooks DR, O'Grady RT, Glen DR (1985) Phylogenetic analysis of the Digenea (Platyhelminthes: Cercomeria) with comments on their adaptive radiation. Can J Zool 63:411–443
Brotowidjoyo MD, Copeman DB (1979) Abattoir survey of bovine paramphistomiasis in North Queensland. Aust Vet J 55:402
Buckley JJC (1939) Observations on *Gastrodiscoides hominis* and *Fasciolopsis buski* in Assam. J Helminthol 17:1–12
Burgu A (1981) Studies on the biology of *Paramphistomum cervi* Schrank, 1790 in sheep in the district of Eskisehir Cifteler State farm. Ankara Üniv Vet Fakültesi Derg 28:50–71
CBOL Plant Working Group (2009) A DNA barcode for land plants. Proteins Nucleic acids 106: 12794–12797
Chandrasekharan K, Radhakrishnan K, Jacob VC (1982) Efficacy of Distodin in the treatment of amphistomiasis in Indian elephants. Kerala J Vet Sci 13:55–58
Choe SE, Nguyen TT, Kang TG, Kweon CH, Kang SW (2011) Genetic analysis of *Fasciola* isolates from cattle in Korea based on second internal transcribed spacer (ITS-2) sequence of nuclear ribosomal DNA. Parasitol Res 109:833–839
Chroust K (1973) Efficiency of the anthelmintic Terenol (Resorantel) against the fluke *Paramphistomum* (*Liorchis*) *scotiae* in cattle and against the tapeworm *Moniezia* spp. in lambs. Acta Vet Brno 42:281–286
Cohn L (1904) Helminthologische Mitteilungen. II. Arch Naturgesch 70:229–252
Conceicao MAP, Durao RM, Costa IH, da Costa JMC (2002) Evaluation of a simple sedimentation method (modified McMaster) for diagnosis of bovine fascioliosis. Vet Parasitol 105:337–343
Corba J (1981) Efficacy of Febantel (Rintal) against some important helminthiases of sheep and cattle. Vet Med Rev 1:15–24
Coskun SZ (1988) The prevalence of *Paramphistomum* spp. in ruminants. Turk J Vet Anim Sci 12:168–179
Creplin F (1847) Beschreibung zweier neuen Amphistomen- Artenaus dem zebu-ocher. Arch Naturgesch 13:30–35
de León GP, Mendoza-Garfias B, Razo-Mendivil U, Parra-Olea G (2011) A new genus and species of Brachycoeliidae (Digenea) from *Chiropterotriton* sp. (Caudata: Plethodontidae) in Mexico and its phylogenetic position within the Plagiorchiida based on partial sequences of the 28S ribosomal RNA gene. J Parasitol 97:128–134
De Waal T (2010) *Paramphistomum*—a brief review. Ir Vet J 63:313–315
Domingues MV, Marques FP (2011) Phylogeny and taxonomy of *Potamotrygonocotyle* Mayes, Brooks & Thorson, 1981 (Monogenoidea: Monocotylidae) with a description of four new species. J Helminthol 85:353–380
Dorchies, Ph (2006) Flukes: old parasites but new emergence. In: XXIV World Buiatrics Congress, Nice
Dorchies P, Levasseur G, Alzieu JP (2000) La Paramphistomose bovine: une pathologie d'actualité. Soc Franç Buiat:132–142
Dube S, Sibula MS, Dhlamini Z (2016) Molecular analysis of selected paramphistome isolates from cattle in southern Africa. J Helminthol 90:784–788
Dutt SC, Srivastava HD (1972) The life history of *Gastrodiscoides hominis* (Lewis and McConnel, 1876) Leiper, 1913, the amphistome parasite of man and pig. J Helminthol 46:35–46
Eduardo SL (1980) A new genus, *Leiperocotyle*, for *Cotylophoron okapi* Leiper, 1935 and *C. congolense* Baer, 1936 and rediscription of *C. okapi*. Syst Parasitol 1:255–263
Eduardo SL (1981) Fischoederius brevisaccus, a new species of pouched amphistome (Gastrothylacidae) from ruminants in the Philippines. Syst Parasitol 3:1–6
Eduardo SL (1982) The taxonomy of the family Paramphistomidae Fischoeder, 1901 with special reference to the morphology of species occurring in ruminants. II. General considerations. Syst Parasitol 4:7–57
Eduardo SL, Javellana CRH (1987) A new genus, Velasquezotrema, for Fischoederius brevisaccus Eduardo, 1981. Philipp J Vet Med 24:29–34
Fischoeder F (1901) Die Paramphistomidender Saugethiere. Zool Anz 24:367–375
Fischoeder F (1903) Die Paramphistomiden der Saugethiere. Zool Jahr Abteil Syst Geogr Biol Tiere 17:485–660
Fuhrmann O (1928) Zweite Klasse des Cladus Plathelminthes Trematoda. In: Kükenthal W, Krumbach T (eds) Handbuch der Zoologie, vol 2. Walter de Gruyter, Berlin, p 140
Fukui T (1929) Studies on Japanese amphistomatous parasites, with revision of the group. Japanese J Zool Trans Abstracts 2:219–351

Ghatani S, Shylla JA, Tandon V, Chatterjee A, Roy B (2012) Molecular characterization of pouched amphistome parasites (Trematoda: Gastrothylacidae) using ribosomal ITS2 sequence and secondary structures. J Helminthol 86:117–124

Gordon CA, Gray DJ, Gobert GN, McManus DP (2011) DNA amplification approaches for the diagnosis of key parasitic helminth infections of humans. Mol Cell Probes 25:143–152

Goswami LM, Prasad PK, Tandon V, Chatterjee A (2009) Molecular characterization of *Gastrodiscoides hominis* (Platyhelminthes: Trematoda: Digenea) inferred from ITS rDNA sequence analysis. Parasitol Res 104:1485–1490

Gupta NK (1993) Amphistomes: systematics and biology. Oxford and IBH Publishing, New Delhi

Hanna REB, Williamson DS, Mattison RG, Nizami WA (1988) Seasonal reproduction in *Paramphistomum epiclitum* and *Gastrothylax crumenifer*, rumen paramphistomes of the Indian water buffalo, and comparison with the biliary paramphistome *Gigantocotyle explanatum*. Int J Parasitol 18:513–521

Harinasuta T, Bunnag D, Radomyos P (1987) Intestinal fluke infections. In: Pawlowski ZS (ed) Balliere's clinical tropical medicine and communicable diseases. Intestinal helminthic infections. Balliere Tindall, London, pp 695–721

Hassan SS, Juyal PD (2006) Epidemiological observations of paramphistomosis in ruminants in endemic regions of Punjab and adjoining state (INDIA). Proceedings of the 11th International symposium on veterinary epidemiology and economics, 2006

Hassan SS, Juyal PD (2009) Towards epidemiology of paramphistomosis in domestic ruminants in Punjab. In: International symposia on veterinary epidemiology and economics proceedings, ISVEE 12: proceedings of the 12th symposium of the International Society for Veterinary Epidemiology and Economics, Durban, South Africa, theme 2—investigation of determinants and distribution of disease: poster session, disease distribution & determinants, p 658

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc R Soc B Biol Sci 270:313–321

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA barcodes. PLoS Biol 2:e312

Horak IG (1971) Paramphistomiasis of domestic ruminants. Adv Parasitol 9:33–72

Howell A (2011) Snail-borne diseases in bovids at high and low altitude in Eastern Uganda: integrated parasitological and malacological mapping. Liverpool School of Tropical Medicine M.Sc. Dissertation, p 77

Howlader MMR, Chowdhury SMZH, Taimur MJFA, Jahan S (1990) Fluke infestations of cattle in some selected villages of Bangladesh. Bangladesh Vet 7:45–47

Huson KM, Oliver NAM, Robinson MW (2017) Paramphistomosis of ruminants: an emerging parasitic disease in Europe. Trends Parasitol 33:836–844

Ichikawa M, Itagaki T (2010) Discrimination of the ITS1 types of *Fasciola* spp. based on a PCR-RFLP method. Parasitol Res 106:757–761

Ichikawa M, Kondoh D, Bawn S, Maw NN, Htun LL, Thein M, Gyi A, Sunn K, Katakura K, Itagaki T (2013) Morphological and molecular characterization of *Explanatum* from cattle and buffaloes in Myanmar. J Vet Med Sci 75:309–314

Ilha MR, Loretti AP, Reis AC (2005) Wasting and mortality in beef cattle parasitized by *Eurytrema coelamaticum* in the state of Parana, southern Brazil. Vet Parasitol 133:49–60

Itagaki T, Tsumagari N, Tsutsumi K, Chinone S (2003) Discrimination of three amphistome species by PCR-RFLP based on rDNA ITS2 markers. J Vet Med Sci 65:931–933

Ivanov VM, Semenova NN (2000) Parasitological consequences of animal introduction. Russ J Ecol 31:281–283

Jones A (2005a) Superfamily Paramphistomoidea Fischoeder, 1901. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI Publishing and The Natural History Museum, London, pp 221–227

Jones A (2005b) Introduction and key to superfamilies. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI Publishing and The Natural History Museum, London, pp 1–4

Jones A, Bray RA, Gibson DI (eds) (2005) Keys to the Trematoda, vol 2. CABI Publishing and The Natural History Museum, London

Juyal PD, Kasur K, Hassan SS, Kaur P (2003) Epidemiological status of paramphistomiasis in domestic ruminants in Punjab. J Parasit Dis:231–235

Kaur K, Hassan SS, Kaur P, Juyal PD (2004) Indirect Dot-ELISA for anti-Paramphistomum epiclitum antibodies detection. J Vet Parasitol 18:73–74

Kaur S, Singla LD, Hassan SS, Juyal PD (2009) Standardization and application of indirect plate ELISA for immunodiagnosis of paramphistomosis in ruminants. J Parasit Dis 33:70–76

Khan UJ, Tanveer A, Maqbool A, Masood S (2008) Epidemiological studies of paramphistomosis in cattle. Vet Arhiv 78:243–251

Kilani K, Guillot J, Chermett R (2003) Amphistomes digestive. In: Lefevre PC, Blanco J, Chermatt J (eds) Principales maladies infectieuses et parasitaires du betail, 1st edn. Editions Tec & Doc (Lavoisier), Paris, pp 1400–1410

Kobulej T, Udvarhelyi J (1972) Efficacy of Terenol in the treatment of cattle with natural ruminal paramphistome infections. Acta Vet Hung 22:219–223

Králová-Hromadová I, Bazsalovicsová E, Stefka J, Spakulová M, Vávrová S, Szemes T, Tkach V, Trudgett A, Pybus M (2011) Multiple origins of European populations of the giant liver fluke *Fascioloides magna* (Trematoda: Fasciolidae), a liver parasite of ruminants. Int J Parasitol 41:373–383

Kress WJ, Erickson DL (2008) DNA barcodes: genes, genomics, and bioinformatics. Proteins Nucleic Acids 105:2761–2762

Kumar V (1980) The digenetic trematodes, *Fasciolopsis buski*, *Gastrodiscoides hominis* and *Artyfechinostomum malayanum*, as zoonotic infections in South Asian countries. Ann Soc Belge De Med Trop 60:331–339

La Rue G (1957) The classification of digenetic Trematoda: a review and a new system. Exp Parasitol 6:306–349

Lahaye R, van der Bank M, Bogarin D, Warner J, Pupulin F, Gigot G, Maurin O, Duthoit S, Barraclough TG, Savolainen V (2008) DNA barcoding the floras of biodiversity hotspots. Proc Natl Acad Sci USA 105:2923–2928

Lalitha CM, Anandan R (1986) Survey of amphistomes parasites of ruminant. Cheiron 15:202–205

Lämmler G, Sahai BN, Herzog H (1969) Anthelmintic efficacy of 2, 6 -di-hydroxybenzoic acid-4′ -Bromanilide (HOE 296 V) against mature and immature *Paramphistomum microbothrium* in goats. Acta Vet Hung 19:447–451

Leiper RT (1913) Observations on certain helminths of man. Trans R Soc Trop Med Hyg 6:265–297

Li D (1991) A case of *Fischoederius elongatus* infection in China. Annu Bull Soc Parasitol Guangdong Prov 12:155–156

Lloyd J, Boray J, Love S (2007) Stomach fluke (paramphistomes) in ruminants. Primefact 452:1–4

Looss A (1896) Recherches sur les fauna parasitaire de Egypt. I. Mem Inst Egypt 3:1

Looss A (1899) Weitere Beiträge zur Kenntnis der Trematoden-Fauna Aegytens zugleich Versuch einer natürlichen Gleiderung des Genus *Distomum* Retzius. Zool Jahr Abteil Syst Geogr Biol Tiere 12:521–784

Looss A (1900) Nachträgliche Bemerkungen zu den Namen der von mir vorgeschlagenen Distomidengattungen. Zool Anz 23:601–608

Looss A (1902) Über neue and bekannte Trematoden aus Seeschildkröten. Erörterungen zur Systematik und Nomencltur. Zool Jahr Abteil Syst Okol Geogr Tiere 16:411–894

Looss A (1912) Über den Bau eineger anscheinend seltner Trematoden Arten. Zool Jahrb 15:3253–3366

Lotfy WM, Brant SV, Ashmawy KI, Devkota R, Mkojie GM, Loker ES (2010) A molecular approach for identification of paramphistomes from Africa and Asia. Vet Parasitol 174:234–240

Lewis TR, McConnell JFP (1876) Amphistoma hominis n. sp., a new parasite affecting man. Proc Asiat Soc 8:182–186

Lühe M (1909) Parasitische Plattwürmer. I: Trematodes. Süsswasserf Deutsch 17:1–217

Malek EA (1980) Snail-transmitted parasitic diseases, vol I. CRC Press, Florida, p 348

Mage C, Bourgne H, Toullieu JM, Rondelaud D, Dreyfuss G (2002) *Fasciola hepatica* and *Paramphistomum daubneyi:* changes in the prevalence of natural infections in cattle and Lymnaea truncatula from Central France over the past 12 years. Vet Res 33:439–447

Mahato SN, Rai K (1992) Prevalence of amphistomosis in cattle in the Koshi zone of Nepal. Vet Rev Kathmandu 7:63–64

Malek EA (1971) The life cycle of *Gastrodiscus aegypticus* (Cobbold, 1876) Looss, 1896 (Trematoda: Paramphistomatidae: Gastrodiscinae). J Parasitol 57:975–979

Maplestone PA (1923) A revision of the Amphistomata of mammals. Ann Trop Med Parasitol 17:113–205

Martinez-Ibeas AM, Munita MP, Lawlor K, Sekiya M, Mulcahy G, Sayers R (2016) Rumen fluke in Irish sheep: prevalence, risk factors and molecular identification of two paramphistome species. BMC Vet Res 12:143

Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278

Mas-Coma S, Bargues MD, Valero MA (2006) Gastrodiscoidiasis, a plant-borne zoonotic disease caused by the intestinal amphistome fluke Gastrodiscoides hominis (Trematoda: Gastrodiscidae). Rev Iber Parasitol 66:75–81

Mehra HR (1980) The fauna of India and adjacent countries. Manager of Publications, Government of India, Delhi

Monticelli FS (1892) *Cotylogaster michaelis* n. g., n. sp., e revisione degli Aspidobothriidae. In: *Festchrift Leuckart*, pp 166–214

Murty CV, Reddy CR (1980) A case report of *Gastrodiscoides hominis* infection. Indian J Pathol Microbiol 23:303–304

Näsmark KE (1937) Revision of the trematode family Paramphistomidae. Zool Bidrag Uppsala 16:301–565

Odening K (1974) Verwandtschaft, , System und zykloontogenetische Besonderheiten der Trematoden. Zool Jahr Abteil Syst Okol Geogr Tiere 101:345–396

Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int J Parasitol 33:733–755

Otto R (1896) Beitrge zur anatomie und histologie der Amphistomeen. Inaug Dissertation, Leipzig

Ozdal N, Gul A, Ilhan F, Deger G (2010) Prevalence of *Paramphistomum* infection in cattle and sheep in Van Province, Turkey. Helminthologia 47:20–24

Phiri AM, Chota A, Phiri IK (2007) Seasonal pattern of bovine amphistomosis in traditionally reared cattle in the Kafue and Zambezi catchment areas of Zambia. Trop Anim Health Prod 39:97–102

Pick F (1964) Informations nouvelles sur la distomatose a *Watsonius watsoni*. Bull Exotic Pathol Soc 57:502–510

Pick F (1967) Die tödliche terminale Watsonius-Ileitis. Wien Klin Wochenschr 79:666–667

Poirier MJ (1883) Description of d'helminths nouveaux du *Palonia frontalis*. Bull Soc Philomathique Paris 7:73–80

Prasad PK, Goswami LM, Tandon V, Chatterjee A (2011) PCR-based molecular characterization and insilico analysis of food-borne trematode parasites *Paragonimus westermani*, *Fasciolopsis buski* and *Fasciola gigantica* from Northeast India using ITS2 rDNA. Bioinformation 6:4–8

Rangel-Ruiz LJ, Albores-Brahms ST, Gamboa-Anguilar J (2003) Seasonal trends of *Paramphistomum cervi* in Tabasco, Mexico. Vet Parasitol 116:217–232

Rinaldi L, Perugini AG, Capuano F, Fenizia D, Musella V, Veneziano V, Cringoli G (2005) Characterization of the second internal transcribed spacer of ribosomal DNA of *Calicophoron daubneyi* from various hosts and locations in southern Italy. Vet Parasitol 131:247–253

Ristic M (1988) Babesiosis of domestic animals and man. CRC Press, Boca Raton

Rolfe PF, Boray JC (1987) Chemotherapy of paramphistomosis in cattle. Aust Vet J 64:328–332

Rolfe PF, Boray JC (1988) Chemotherapy of paramphistomosis in sheep. Aust Vet J 65:148–150

Rolfe PF, Boray JC (1993) Comparative efficacy of moxidectin, an ivermectin/clorsulon combination and closantel against immature paramphistomes in cattle. Aust Vet J 70:265–267

Rolfe PF, Boray JC, Nichols P, Collins GH (1991) Epidemiology of paramphistomosis in cattle. Int J Parasitol 21:813–819

Rolfe PF, Boray JC, Collins GH (1994) Pathology of infection with *Paramphistomum ichikawai* in sheep. Int J Parasitol 24:995–1004

Roy B (1990) Spectrum of digenetic trematode parasites of edible mammalian hosts in north-east India with some biochemical and ultrastructural studies. PhD thesis submitted to North-Eastern Hill University, Shillong

Roy B, Tandon V (1990a) On a new trematode belonging to the genus *Velasquezotrema* Eduardo, 1987 from *Bos indicus*. Zool Anz 224:106–112

Roy B, Tandon V (1990b) *Leiperocotyle meghalayensis* (Paramphistomidae: Orthocoelinae), a new species from cattle (*Bos indicus* L.) in India. Zool Anz 225:63–70

Roy B, Tandon V (1992) Trematodiasis in North-East India: a study on the spectrum of digenetic trematodes among pigs, buffaloes, cattle, goats and sheep. Indian J Anim Health 13:5–14

Roy B, Tandon V (1995) *Calicophoron shillongensis* sp. n. (Trematoda, Paramphistomidae), a new parasite from the goat (*Capra hircus* L.) in India. Acta Parasitol 40:193–197

Saifullah MK, Ahmad G, Abidi SM (2013) Immunodetection of coproantigens for the diagnosis of amphistomosis in naturally infected Indian Water Buffalo, *Bubalus bubalis*. Vet Parasitol 191:66–72

Saijuntha W, Tantrawatpan C, Sithithaworn P, Andrews RH, Petney TN (2011) Genetic characterization of *Echinostoma revolutum* and *Echinoparyphium recurvatum* (Trematoda: Echinostomatidae) in Thailand and phylogenetic relationships with other isolates inferred by ITS1 sequence. Parasitol Res 108:751–755

Sanabria R, Romero J (2008) Review and update of paramphistomosis. Helminthologia 45:64–68

Sanabria R, Gastón MC, Romero J (2011) Molecular characterization of the ITS-2 fragment of *Paramphistomum leydeni* (Trematoda: Paramphistomidae). Vet Parasitol 177:182–185

Sanguankiat S, Sato MO, Sato M, Maipanich W, Yoonuan T, Pongvongsa T, Boupha B, Chigusa Y, Moji K, Waikagul J (2016) First record of paramphistomes *Fischoederius cobboldi* and *Paramphistomum epiclitum* detected in bovine rumen from a local market of Savannakhet Province, Lao PDR. Korean J Parasitol 54:543–547

Sey O (1988) Scope of and proposal for systematics of the Amphistomida (Lühe, 1909) Odening, 1974 (Trematoda). Parasitol Hungarica 21:17–30

Sey O (1989) A review of chemotherapy of paramphistomosis of domestic ruminants in Europe. Parasitol Hungarica 22:51–55

Sey O (1991) CRC Handbook of the zoology of amphistomes. CRC Press, Boca Raton

Sey O (2005) Keys to the identification of the taxa of the amphistomes (Trematoda, Amphistomida). Regional Centre of the Hungarian Academy of Sciences, Veszprem (MTA VEAB) & University of Pecs, Hungary

Sey O, Kassai T (1984) Examination of some anthelmintics against intestinal paramphistomosis in sheep (in Hungarian). Reports of the Hungarian Academy of Sciences and Ministry of Food and Agriculture Budapest, p 12

Shrivastav HO, Shah HL (1970) On *Gastrodiscoides hominis* (Lewis and McConnell, 1876) Leiper, 1913 from pigs (*Sus scrofa domestica*) in Madhya Pradesh, its pathology and public health importance. Indian J Pathol Bacteriol 13:68–72

Shylla JA, Ghatani S, Chatterjee A, Tandon V (2011) Secondary structure analysis of ITS2 in the rDNA of three Indian paramphistomid species found in local livestock. Parasitol Res 108:1027–1032

Singh RP, Sahai BN, Prasad KD (1984) Haematological observations in goats experimentally infected with *Paramphistomum cervi*. Indian J Anim Sci 54:132–134

Skrjabin KI (1949) [Suborder Paramphistomata (Szidat, 1936) Skrjabin & Schulz, 1937]. In: Skrjabin KI (ed) Trematodes of animals and man. Osnovy Trematodologii 3:1–623

Skrjabin KI, Schulz RS (1937) Trematody krupnogo rogatogo skota i ego molodnyaka. Sel'hozgiz, Moscow

Southwell T, Kirshner A (1937) A description of a new species of amphistome, *Chiorchis purvisi*, with notes on the classification of the genera within the group. Ann Trop Med Parasitol 31:215–244

Sripalwit P, Wongsawad C, Wongsawad P, Anuntalabhochai S (2007) High annealing tempera-

ture—random amplified polymorphic DNA (HAT-RAPD) analysis of three paramphistome flukes from Thailand. Exp Parasitol 115:98–102
Stiles CW, Goldberger J (1910) A study of the anatomy of *Watsonius* (n. g.) *watsoni* of man and of nineteen allied species of mammalian trematode worms of the superfamily Paramphistomoidea. Bulletin of the Hygienic laboratory, Public Health and Marine-Hospital Service of the United States
Stunkard HW (1916) On the anatomy and relationships of some North American trematodes. J Parasitol 3:21–27
Stunkard HW (1917) Studies on North American Polystomidae, Aspidogastridae, and Paramphistomidae. Illinois Biol Monogr 3:285–395
Stunkard HW (1925) The present status of the amphistome problem. Parasitology 17:137–148
Suhardono, Roberts JA, Copeman DB (2006) Optimization of the procedure for counting the eggs of *Fasciola gigantica* in bovine faeces. Trop Anim Health Prod 38:383–387
Szidat L (1936) Über die Entwicklungsgeschichte und den ersten Zwischenwirt von *Paramphistomum cervi* Zeder, 1790, aus dem Magen von Wiederkäuern. Zietsch Parasitenk 9:1–19
Tandon RS (1957) Development of the miracidium and its morphology in *Olveria indica* Thapar & Sinha, 1945, an amphistome (Trematoda), parasite of cattle and buffalo in India. Trans Am Microsc Soc 76:353–358
Taniuchi M, Verweij JJ, Noor Z, Sobuz SU, Lieshout L, Petri WA Jr, Haque R, Houpt ER (2011) High throughput multiplex PCR and probe-based detection with luminex beads for seven intestinal parasites. Am J Trop Med Hyg 84:332–337
ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Dieye L, van Lieshout L (2008) Multiplex real-time PCR for the detection and quantification of *Schistosoma mansoni* and *S. haematobium* infection in stool samples collected in northern Senegal. Trans R Soc Trop Med Hyg 102:179–185
Thaenkham U, Nawa Y, Blair D, Pakdee W (2011) Confirmation of the paraphyletic relationship between families Opisthorchiidae and Heterophyidae using small and large subunit ribosomal DNA sequences. Parasitol Int 60:521–523
Thaenkham U, Blair D, Nawa Y, Waikagul J (2012) Families Opisthorchiidae and Heterophyidae: are they distinct? Parasitol Int 61:90–93
Thapar GS, Sinha BB (1945) On the morphology of a new genus of amphistomes from the rumen of cattle in the United provinces. Indian J Vet Sci Anim Husbandry 15:219–222
Tinar R, Coskun SZ, Dogan H, Demir S, Akyol ÇV (1992) The prevalence of *Amphistomum* spp. in ruminants of South Marmara region. Turk J Vet Anim Sci 16:187–197
Titi A, Mekroud A, Chibat Mel H, Boucheikhchoukh M, Zein-Eddine R, Djuikwo-Teukeng FF, Vignoles P, Rondelaud D, Dreyfuss G (2014) Ruminal paramphistomosis in cattle from northeastern Algeria: prevalence, parasite burdens and species identification. Parasite 21:50
Travassos L (1934) Synopse dos Paramphistomoidea. Mem Inst Oswaldo Cruz 29:19–178
Travassos L, Freitas JF, Kohn A (1969) Trematodeos do Brasil. Mem Inst Oswaldo Cruz 67:1–886
Vanhove MP, Snoeks J, Volckaert FA, Huyse T (2011) First description of monogenean parasites in Lake Tanganyika: the cichlid *Simochromis diagramma* (Teleostei, Cichlidae) harbours a high diversity of *Gyrodactylus* species (Platyhelminthes, Monogenea). Parasitology 138:364–380
Verweij JJ, Brienen EAT, Ziem JB, Polderman AM, Van Lieshout L (2007) Simultaneous detection and quantification of *Ancylostoma duodenale, Necator americanus*, and *Oesophagostomum bifurcum* in fecal samples using multiplex realtime PCR. Am J Trop Med Hyg 77:685–690
Vorster JH, Mapham PH (2012) Conical flukes in ruminants. http://www.cpdsolutions.co.za/Publications/article_uploads/Paramphistomiasis.pdf, pp 1–13
Vujic B, Petrovic K, Palic D, Peric Z (1971) A case of acute paramphistomiasis in cattle. Vet Glas 4:265–271
Wang CR, Qiu JH, Zhu XQ, Han XH, Ni HB, Zhao JP, Zhou QM, Zhang HW, Lun WR (2006) Survey of helminthes in adult sheep in Heilongjiang Province, People's Republic of China. Vet Parasitol 140:378–382
Ward HB (1917) On the structure and classification of north American parasitic worms. J Parasitol 4:1–12
Wikerhauser T, Brglez J, Kuticic V (1975) Anthelmintic efficacy of Terenol (resorantel) against *Paramphistomum microbothrium* in cattle. Acta Parasitol Lugoslavica 6:25–29
Yamaguti S (1934) Studies on the helminth fauna of Japan. Part 2. Trematodes of fishes, I. Jpn J Zool 5:249–541
Yamaguti S (1958) Systema helminthum. Volume I. The digenetic trematodes of vertebrates. Parts I & II. Interscience, New York
Yamaguti S (1971) Synopsis of digenetic trematodes of vertebrates. Volumes I and II. Keigaku Publishing, Tokyo
Yu SH, Mott KE (1994) Epidemiology and morbidity of food-borne intestinal trematode infections. Trop Dis Bull 91:R125–R152
Zablotski VI (1964) Early development stages of *Gastrodiscoides hominis* Lewis et McConnell, 1876. Sb Parazit Rab Trudy Astrskh Zapovedn 9:119–126
Zaeemi M, Haddadzadeh H, Khazraiinia P, Kazemi B, Bandehpour M (2011) Identification of different *Theileria* species (*Theileria lestoquardi, Theileria ovis* and *Theileria annulata)* in naturally infected sheep using nested PCR-RFLP. Parasitol Res 108:837–843

Dicrocoeliidae Family: Major Species Causing Veterinary Diseases

10

M. Yolanda Manga-González
and M. Carmen Ferreras

10.1 Introduction

Family Dicrocoeliidae Odhner, 1911, includes more than 400 species of Digenea parasites (Pojmanska 2008) which infect the liver, bile ducts, gall bladder, intestine or pancreas (amongst others) of amphibians, reptiles, birds and mammals (Yamaguti 1958). The type genus is *Dicrocoelium* Dujardin, 1845.

10.2 Taxonomy of Dicrocoeliidae Family

There is no unanimous criterion regarding the inclusion of family Dicrocoeliidae in higher taxa. Some authors (Yamaguti 1958; Dawes 1968) include it in the order Digenea, suborder Prosostomata, although some of them also consider the superfamily Plagiorchiida (Dawes 1968). The order Dicrocoeliata was created later (Panin 1971). However, other authors (Mehlhorn and Walldorf 1988) include family Dicrocoeliidae in the order Plagiorchiida.

Family Dicrocoeliidae falls within the following taxonomic levels (Fauna Europaea 2012):

Kingdom Animalia
Subkingdom Eumetazoa
Phylum Platyhelminthes
Subphylum Neodermata
Class Trematoda
Subclass Digenea
Order Plagiorchiida
Infraorder Plagiorchioidea
Family Dicrocoeliidae

Nor is there any agreement amongst authors regarding the name and number of lower taxa of family Dicrocoeliidae, as follows:

TRAVASSOS (1944) (Travassos 1944):
Subfamilies:
Dicrocoeliinae Looss, 1899
Infidinae Travassos, 1944
Mesocoeliinae

YAMAGUTI (1958) (Yamaguti 1958):
Subfamilies:
Anchitrematinae Mehra, 1935
Leipertrematinae Yamaguti, 1958
Dicrocoeliinae Looss, 1899
Tribes: Euparadistomini Yamaguti, 1899

M. Y. Manga-González (✉)
Departamento de Sanidad Animal, Instituto de Ganadería de Montaña, Consejo Superior de Investigaciones Científicas (CSIC-ULE), León, Spain
e-mail: y.manga@csic.es

M. C. Ferreras
Departamento de Sanidad Animal, Facultad de Veterinaria, Instituto de Ganadería de Montaña (CSIC-ULE), Universidad de León, León, Spain
e-mail: mcfere@unileon.es

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_10

Controrchiini Yamaguti, 1958
Brodeniini Yamaguti, 1958
Eurytrematini Yamaguti, 1958
Lypersomini Yamaguti, 1958
Athesmiini Yamaguti, 1958
Brachylecithini Yamaguti, 1958
Dicrocoeliini Yamaguti, 1958

PANIN (1981) (Panin 1981):
Subfamilies:
Dicrocoeliinae Looss, 1899
Eurytrematinae Panin, 1971
Proacetabulorchinae Odening, 1964
Infidinae Travassos, 1944

AGRAWAL and SHARMA (1990) (Agrawal and Sharma 1990):
Subfamily:
Neodicrocoeliinae Agrawal and Sharma (1990)

The following genera, amongst others, are included in the tribe Dicrocoeliini (Yamaguti 1958) and the subfamily Dicrocoeliinae (Panin 1981) of the family Dicrocoeliidae.

Athesmia Looss, 1899
Brachylecithum Strom, 1940
Brodenia Gedoelst, 1913
Canaania Travassos, 1944
Conspicuum (Bhalerao, 1936) Travassos, 1944
Controrchis Price, 1928
Dicrocoelium Dujardin, 1845
Dictyonograptus Travassos, 1919
Eurytrema Looss, 1907
Leipertrema Sandosham, 1951
Lyperosomum Looss, 1899
Metadelphis Travassos, 1944
Platynosomum Looss, 1907
Pseudathesmia Travassos, 1942

The following genera are mentioned within family Dicrocoeliidae (Fauna Europaea 2012):

Athesmia Looss, 1899
Brachydistomum Travassos, 1944
Brachylecithum Shtrom, 1940
Brodenia Gedoelst, 1913
Conspicuum Bhalerao, 1936
Corrigia Shtrom, 1940
Dicrocoelium Dujardin, 1845
Euparadistomum Tubangui, 1931
Eurytrema Looss, 1907
Lutztrema Travassos, 1941
Lyperosomum Looss, 1899
Paradistomum Kossack, 1910
Platynosomum Looss, 1907
Prosolecithus Yamaguti, 1971
Pseudoparadistomum Roca, 2003
Skrjabinus Bhalerao, 1936
Stromitrema Skrjabin, 1944
Unilaterilecithum Oschmarin in Skrjabin and Evranova (1952)
Zonorchis Travassos, 1944

10.2.1 Major Dicrocoeliidae Genera and Species Causing Veterinary Diseases

After analysing the taxonomic position of family Dicrocoeliidae, whose morphological traits have been indicated (Travassos 1944) and outlined (Mapes 1951), we will now concentrate on the most important genera and species in veterinary parasitology, mainly those affecting ruminants.

Genus *Dicrocoelium* Dujardin, 1845
D. dendriticum Rudolphi 1819
D. chinensis Tang & Tang, 1978
D. hospes Looss, 1907
D. orientale Sudarikov & Ryjikov, 1951
D. petrowi Kassimov, 1952
These species of genus *Dicrocoelium* have been mentioned as valid (Fauna Europaea 2012).

Genus *Eurytrema* Looss, 1907
E. cladorchis (Chin, Li & Wei, 1965)
E. coelomaticum (Girad & Billet, 1892)
E. pancreaticum (Janson, 1889)
E. procyonis Delton, 1942
Genus *Platynosomum* Looss, 1907
P. fastosum Kossack, 1910

10.3 Biology of the *Dicrocoelium* Species

Dicrocoelioses are parasite infections caused by different species of the type genus *Dicrocoelium* Dujardin, 1845. The diagnosis of the adult parasites of this genus is as follows (Pojmanska 2008): Body elongate, lanceolate or fusiform. Tegument unspined. Oral sucker usually slightly larger than ventral. Pharynx small. Oesophagus relatively long. Caeca do not reach posterior extremity. Testes large, immediately posterior to ventral sucker, diagonal, may be lobed. Cirrus-sac elongate, may reach anterior border of ventral sucker. Genital pore median, just posterior to intestinal bifurcation. Ovary smaller than testes, close to posterior testis. Uterus occupies entire hindbody, intercaecal. Vitellarium in two bands is composed of small follicles, limited to the middle third of body. Excretory vesicle I- or Y-shaped; stem may reach close to ovary; pore terminal. The adult parasites live in the bile ducts and gall bladder of mammals and birds; cosmopolitan.

The most important species of this genus which infects ruminants are *Dicrocoelium dendriticum* (Rudolphi 1819) Looss, 1899; *Dicrocoelium hospes* Looss, 1907; and *Dicrocoelium chinensis* Tang and Tang, 1978.

10.3.1 *Dicrocoelium dendriticum*

This species is one of the most widespread trematodes in mammals, mainly ruminants (ovine, bovine, cervide, elks, camelids) in many countries of Europe, Asia, North Africa and North America (Malek 1980b; Soulsby 1982; Manga-González et al. 1991; Campo et al. 2000; Otranto and Traversa 2002, 2003; Manga-González and González-Lanza 2005; Goater and Colwell 2007; Otranto et al. 2007; Senlik et al. 2006; Colwell and Goater 2010; Manga-González et al. 2010; Sargison et al. 2012; Beck et al. 2015; Lambacher et al. 2016; Mitchell et al. 2017, amongst others). Moreover, *D. dendriticum* has been found in the European brown hare (*Lepus europaeus*) in Macedonia (Greece) (Diakou et al. 2014) and in rabbits grazing on Machair pastures (Mitchell et al. 2017). This trematode could affect humans (Mohamed and Mummery 1990; Cengiz et al. 2010; Gualdieri et al. 2011; Jeandron et al. 2011; Pepe et al. 2015).

This parasite was confused with an immature form of *Fasciola hepatica* for a long time, because both trematodes are frequently found together in the liver of ruminants at the same time. Its description thus came late. The synonymy of this parasite is complex, due to the different generic and specific denominations received (Mapes 1951; Schuster 1987). This could be due primarily to the fact that Rudolphi described the same species twice: once as *Fasciola lanceolata* and again as *Distoma dendriticum* in 1803 and 1819, respectively. On re-examining the specimens studied by Rudolphi on both occasions, it was concluded that *Distoma dendriticum* was identical to *Fasciola lanceolate* (Odhner 1910). Nevertheless, since the name *lanceolata* had previously been invalidated, the law of priority meant that *dendriticum* was the correct species denomination. *Dicrocoelium dendriticum* (Rudolphi 1819) Looss, 1899, appears as a valid name on the "Official list of specific names in zoology", whilst *Dicrocoelium lanceatum* and *Dicrocoelium lanceolatum* are mentioned in the "Official index of rejected and invalid specific names in zoology" (Schuster 1987).

10.3.1.1 Life Cycle of *Dicrocoelium dendriticum*

The life cycle of *Dicrocoelium dendriticum* is extremely complex (Fig. 10.1) because land molluscs and ants are required as first and second intermediate hosts, respectively (Manga-González et al. 2001; van Paridon et al. 2017a, b). It was completed for the first time (Krull and Mapes 1952a, 1953) after numerous studies were carried out over a century (Mapes 1951; Del-Rio 1967). This research showed that the eggs were fully mature when laid but did not hatch in the bile duct (Moulinié 1856) or in water (Von Willemoes-Suhm 1871) or in aquatic molluscs (Leuckart 1886-1901; Henkel 1931). Later a long-tailed cercariae was found which was associated with *D. dendriticum*, in land molluscs which live in enzootic zones of the parasite (Von

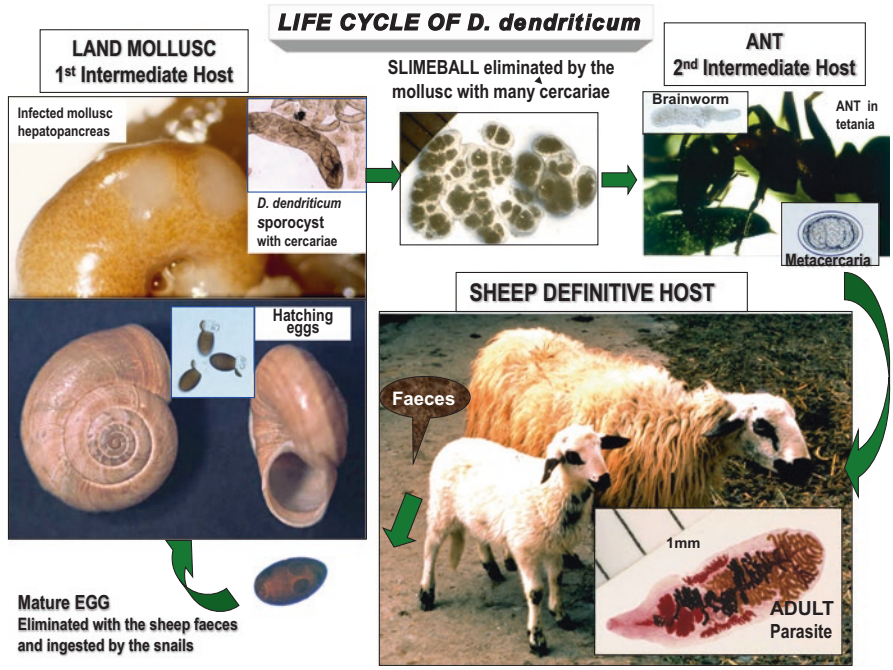

Fig. 10.1 Life cycle of *Dicrocoelium dendriticum*. (1) The sheep definitive host which harboured the adult parasite in the liver. (2) The land molluscs first intermediate host eat the parasite eggs which when they hatch occur the miracidium liberation; finally the parasite evolved to daughter sporocyst harbouring mature cercariae. (3) Many of these mature cercariae go to the respiratory chamber of the mollusc and are covering in slime forming small spherules that constitute a slimeball, which is eliminated by pneumostoma of the molluscs. (4) The ants, second intermediate host, became infected when they ingest these slimeballs; the parasite that go to the brain "brainworm" causes tetania to the ant; the rest "metacercariae" go to the abdomen. The ruminant became infected when ingest the infected ants

Linstow 1887; Nöller 1929; Vogel 1929; Piana 1882). Based on this, and after finding two small tailed forms found in a sheep severely parasitized with *D. dendriticum*, it was suggested that the definitive host was infected via drinking water (Zarnik 1910). After various failures on attempting to infect definitive hosts by feeding them with infected molluscs and cercariae, the possible existence of a second intermediate or auxiliary host was considered. Given the failure to detect any infection in fly larvae, parasitic nematodes and beetle larvae (Neuhaus 1936) or to make the long-tailed cercariae penetrate ants, slugs or earthworms (Nöller 1932), it was questioned whether that parasite belonged to *D. dendriticum*. However, this doubt disappeared when the full development of the parasite was followed in snails and they were seen to shed slimeballs containing cercariae (Mattes 1936; Neuhaus 1936, 1938; Krull and Mapes 1952b). Some authors state that they have been able to infect sheep with slimeballs (Mattes 1936; Neuhaus 1936, 1938) but must have made a mistake, as all the later attempts to repeat the experiment failed (Krull and Mapes 1952c). Due to this a second intermediate host was looked for again and the conclusion that ants were the hosts of the *D. dendriticum* metacercariae and they were used to successfully infect lambs in an experiment (Krull and Mapes 1952a, 1953). These authors established that three hosts are required for the complete cycle of *D. dendriticum* and that, in New York state, the first intermediate host was the mollusc *Cionella lubrica* (=*Cochlicopa lubrica*) and the second one was the ant species *Formica fusca* and that the definitive hosts could be mammals such as sheep, oxen, deer, marmots or rabbits.

The first ants found infected with metacercariae of *D. dendriticum* in Europe (Florsheim region, Germany) were *Formica fusca* too (Vogel and Falcao 1954). These authors also followed the experimental metacercaria development in *Formica fusca*, *Formica rufibarbis* var. *fuscorufibarbis* and *Formica gagates* ants feeding them with slimeballs containing *D. dendriticum* cercariae. Likewise, for the first time they completed the life cycle of an experimentally infected one rabbit, two sheep and single mouse by administering mature metacercariae from natural or experimentally infected ants.

Considering the mentioned research and that carried out later by several authors, the development of the complex life cycle of *D. dendriticum* can be summarized as follows.

10.3.1.2 Adult *D. dendriticum* in Definitive Hosts

The adult parasites, distome type, measuring 5–12 mm in length by 1.5–2.5 mm in width live in the mammal's definitive hosts, have a pale body, flattened in a dorsal-ventral direction with the maximum width about halfway along and more pointed at the front than at the back (Manga-González 1999; Manga-González and Quiroz-Romero 1999; Campo et al. 2000). The smooth tegument allows the uterus and vitellaria, which are reddish-brown, to become transparent. These adults, which live in the bile ducts (Fig. 10.2) and gall bladder, deposit the embryonated eggs in the bile ducts after fertilization. The eggs then pass through the common bile duct to the intestine to be shed with the host's faeces. The 36–50 × 22–30 μm eggs have an operculum, a thick wall and are dark brown in colour, with two marks which correspond to the germinal masses (Manga-González 1999; Manga-González and Quiroz-Romero 1999; Sandoval et al. 2013).

10.3.1.3 Larval Stages in the Mollusc First Intermediate Hosts

Egg hatching and live miracidium liberation only occur in the digestive tract of mollusc intermediate hosts due to various stimuli, presumably physical-chemical (Timon-David 1965; Ractliffe 1968; Mitterer 1975). Numerous species belonging to different families, genera and species of land molluscs act as first intermediate hosts (Del-Rio 1967; Denev et al. 1970a; Alunda and Rojo-Vázquez 1984; Schuster 1992; Manga-González et al. 2001; Otranto and Traversa 2002; Martínez-Ibeas et al. 2011; Gürelli 2017; van Paridon et al. 2017a). The miracidium (20 by 25 μm) has cilia at the front and a papilla endowed with a stylet, but no ocular markings. The free miracidium crosses the intestinal wall of the mollusc, loses the cilia and settles in the interlobular spaces of the hepatopancreas near the heart and kidney (observations not yet published). There it becomes a mother or first-generation sporocyst which, as it has no wall of its own, takes on the form of the space it occupies. The daughter or second-generation sporocysts are differentiated from the mother sporocyst germinal masses as

Fig. 10.2 Several *D. dendriticum* adults inside of one lamb hepatic bile duct. Bar = 0.5 cm

their size varies between 140 and 4160 μm, mainly according to their maturity and the mollusc species hosting them (Manga-González 1987, 1999; Manga-González and Quiroz-Romero 1999; Manga-González et al. 2001). The daughter sporocysts are sacciform and have their own wall. They migrate until they settle in different parts of the mollusc hepatopancreas and, in the case of intense infections, they can also be found in the reproductive apparatus. The germinal masses, which the immature daughter sporocysts fill, are transformed into Distoma and Xiphidiocercaria type cercariae. The body of these cercariae that measures 360–760 μm in length by 50–165 μm in width has the oral sucker on its front which includes the mouth and perforator stylet and has the ventral sucker on the central section. In addition the cercaria has a 200–1000-μm-long tail. The time between egg ingestion by the snail until the cercaria is mature, a minimum of 3–6 months, varies mainly with the mollusc species and temperature (Svadzhyan 1951; Krull and Mapes 1952c; Tarry 1969; González-Lanza et al. 1997). The mature cercariae leave the sporocysts and go to the mollusc's respiratory chamber through the circulatory system. There they are covered in slime forming small spherules (Fig. 10.3) measuring 1–2 mm, which contain a generally high number of cercariae (108–600, mean 364) (Del-Rio 1967).

Various of these spherules (from 3 to 13) form a bunch and constitute a slimeball (Del-Rio 1967), which is brilliant white when newly emitted and can be as large as 1 centimetre in diameter and host from 300 to 7800 cercariae (Neuhaus 1936; Del-Rio 1967; Alzieu and Ducos De Lahitte 1991). These slimeballs are expelled by the breathing movements of the snail through the pneumostome (respiratory orifice) and remain attached to the mantle edge of the mollusc until they are deposited on plants or other supports as the snail moves. Whilst some authors state that temperature and humidity are the factors with the greatest influence on slimeball emission, others consider that light has the greatest influence. In a few hours, the sun affects appearance, size and consistency of the slimeballs and thus the viability of the cercariae. The infective capacity of these for ants is prolonged by at least 18 h if the slimeballs are stored at 4 °C. At this temperature a very small number of cercariae maintain all their mobility after 72 h (Del-Rio 1967).

10.3.1.4 Larval Stages in Ant Second Intermediate Hosts

When the slimeballs are ingested under suitable conditions by different species of ants of the family Formicidae (*Formica fusca*, *Formica rufibarbis*, *Formica pratensis*, *Formica sanguinea*, *Formica aserva*, amongst others) which act as

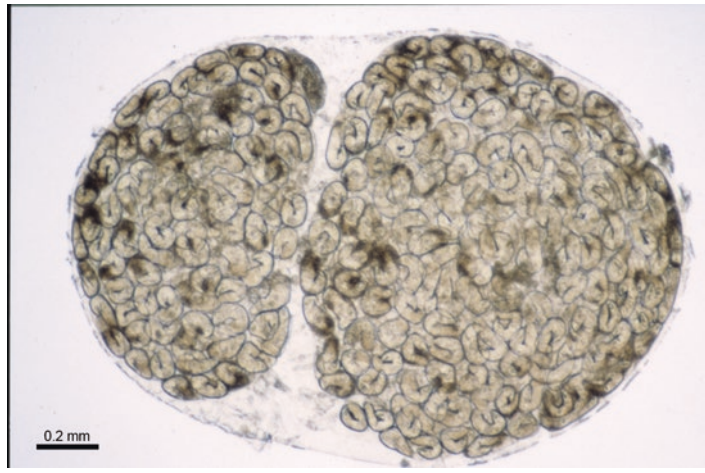

Fig. 10.3 Photomicrography of a spherule from a slimeball emitted by a *D. dendriticum*-infected mollusc, which contain a high number of *D. dendriticum* cercariae. Bar = 0.2 mm

second intermediate hosts (Schuster 1991; Manga-González et al. 2001; Martínez-Ibeas et al. 2011; van Paridon et al. 2017a), the cercariae cross the craw of the ants where they leave scars (Hohorst and Graefe 1961) with the help of the stylet, lose their tail in the first few hours after ingestion and migrate to their final position. One of these (sometimes two or three), called a "brainworm", settles in the suboesophageal ganglia of the ant (Hohorst and Graefe 1961; Hohorst 1962; Roming et al. 1980; Manga-González et al. 2001) (Fig. 10.4), and the rest become metacercariae, with a more consistent cystic wall, which settle in the abdomen in numbers varying (from 1 to 580) according to the ant species and different authors (Manga-González et al. 2001) (Fig. 10.5). However, metacercariae have also been found in the trachea and muscles of these Formicidae (Pegreffi 1957) and in the thorax (Hohorst and Lämmler 1962). According to the studies on the movement of the cercariae, once they had been ingested by the ants until they settled in the brain and became encysted in the abdomen, it seems that 30% of the ingested cercariae become active and begin their migration from the abdomen to the head (Schneider 1971; Schneider and Hohorst 1971; Martín-Vega et al. 2018). When one penetrates and settles in the ant's brain—the brainworm—the rest of the cercariae initiate their return to the abdomen where they become encysted. The time needed for this process to be completed varies according to the temperature. So the final distribution of the metacercariae occurs after 3 days at 26 °C, after 3½ days at

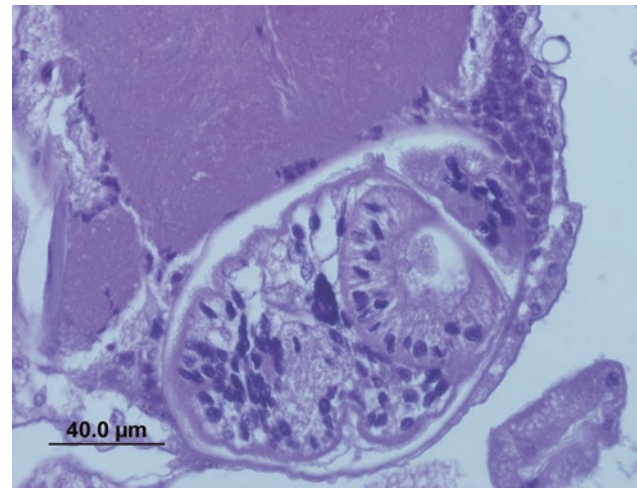

Fig. 10.4 Infected ant head (*Formica rufibarbis*) harbouring a *D. dendriticum* brainworm. Note the parasite in contact with the host nervous tissue. H-E. Bar = 40 µm

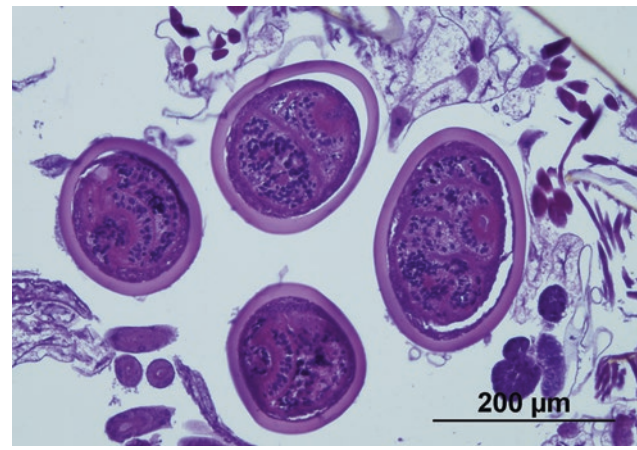

Fig. 10.5 Ant gaster (*Formica rufibarbis*) harbouring four *D. dendriticum* encysted metacercariae. H-E. Bar = 200 µm

24 °C and after 8 days at 16 °C. A correlation between the number of scars on the craw and that of metacercariae in the abdominal cavity has been observed (Kalkan 1976). Nevertheless, it seems that the presence of a high number of metacercariae in the abdomen produces very few harmful effects (Srivastava 1975).

The size of the *D. dendriticum* encysted metacercariae obtained from the abdomen of different species of ants by several authors (Manga-González et al. 2001) varies according to their species and the number of metacercariae per ant. The largest size of the metacercariae (481–596 μm length, 260–311 μm width) was obtained in *Formica pratensis* (Paraschivescu 1981). Moreover the cyst wall thickness was also very variable (5–96 μm) according to the species and authors (Manga-González et al. 2001).

When the temperature falls, the brainworm (or worms) in the suboesophageal ganglion changes the ant's behaviour by causing tetany of its mandibular muscles. This makes it attach to the upper part of the plants (Hohorst and Graefe 1961; Hohorst 1962; Manga-González et al. 2001) which facilitates ingestion of the ant with metacercariae by the definitive host when grazing. The work of some authors (Botnevik et al. 2016) demonstrated a significant effect of temperature on tetany in *Formica polyctena* infected with *D. dendriticum*, whilst neither light nor relative humidity influenced this behaviour. Recently, physical contact between the parasite and the ant brain tissue at the anterior part of the suboesophageal ganglion (SOG) has been studied by non-invasive micro-CT scanning (Martín-Vega et al. 2018). These authors demonstrated for the first time that there is physical contact between the parasite and the ant brain tissue at the most anterior part of the SOG, including a case of multiple brain infection where only the parasite lodged in the most anterior part of the SOG was in contact with the ant brain tissue.

The time needed for the metacercariae, once ingested by the ants, to become infective for the definitive host varies according to the ant species and the temperature: 38–63 days in *Formica fusca* (Krull and Mapes 1952a, 1953); 38–56 days at 26 °C in *Formica fusca*, *Formica rufibarbis* var. *fuscorufibarbis* and *Formica gagates* (Vogel and Falcao 1954); 43 days in *Formica rufibarbis* (Grigoryan and Akopyan 1960); 35–38 days at 28–32 °C and 40–62 days at 19–20.5 °C in *Formica rufibarbis* and *Proformica nasuta* (Svadzhyan 1960); 45 days in *Formica sanguinea* (Groschaft 1961); 40 days or less at 25 °C (Timon-David 1962); and 40 days at 25 °C in *Formica rufibarbis* (Hohorst and Lämmler 1962).

10.3.1.5 Detection of *Dicrocoelium dendriticum* in the Molluscs and ants IH by PCR Techniques

A DNA probe specific for the detection of *D. dendriticum* in ants of *Formica* spp. and *Lasius* spp. has been generated and used by some authors (Heussler et al. 1998). The probe hybridizes to abundant sequences and is therefore very sensitive and capable of detecting single metacercariae.

In the last few years, some studies to identify *D. dendriticum* in the intermediate hosts have been carried out by PCR (polymerase chain reaction) techniques which would, on the one hand, allow precise identification of *D. dendriticum* in mollusc and ant intermediate hosts and, on the other, allow early detection of their infection in order to avoid false negatives, using the mitochondrial (mt DNA) and ribosomal internal transcribed spacer (ITS-2) sequence (Martínez-Ibeas et al. 2011; Martínez-Ibeas 2013). First, these authors collected specimens of several species of molluscs and ants, both naturally infected and uninfected, *D. dendriticum* adult parasites from experimentally infected lambs and other species of flukes for specificity tests in different parts of the province of León (Spain). Furthermore, experimental infections of a batch of 80 mollusc specimens of *Cernuella (Xeromagna) cespitum arigonis* and *Cernuella (Cernuella) virgata* were carried out with *D. dendriticum* eggs. Faced with the lack of *D. dendriticum* sequences, five pairs of degenerate oligonucleotide were designed and tested by aligning mitochondrial sequences of phylogenetically close parasites available in databases. A primer pair that amplified a 1034 bp of mitochondrial DNA fragment was chosen, which

was submitted to the GenBank database with Accession No. JF690758. From this sequence, a second pair of specific primers was designed, which amplified a 169 bp fragment. The first primer permitted the detection of even a single *D. dendriticum* metacercaria from the *Formica rufibarbis* and *Formica pratensis* abdomen, as well as the detection of the brainworm in the heads of the ants collected in tetania. Although these primers did not amplify *Dicrocoelium chinensis* DNA and permitted *D. dendriticum* to be detected in the molluscs, they did not discriminate Brachylaimidae metacercariae found in the same mollusc.

Due to the lack of discrimination between *D. dendriticum* and Brachylaimidae sp., a new pair of primers was designed to amplify a 93 bp fragment of the nuclear region ITS-2 (Martínez-Ibeas et al. 2011; Martínez-Ibeas 2013). The PCR designed is *D. dendriticum* specific as it did not amplify *D. chinensis*, Brachylaimidae, *F. hepatica*, *C. daubneyi*, Plagiorchiidae or Notocotylidae. Besides, this technique is very sensitive since it permitted *D. dendriticum* to be detected in the molluscs from the first day post-infection (Fig. 10.6) as well as the brainworm in the heads of the ants and only one *D. dendriticum* metacercaria from the abdomens of the ants (Fig. 10.7). Natural infection by *D. dendriticum* was confirmed for the first time in ten species of naturally infected molluscs.

Recently, some authors (van Paridon et al. 2017b) used the sequence analysis of the cytochrome oxidase 1 (cox1) mitochondrial gene to identify the terrestrial snail *Oreohelix subrudis* and the ant *Formica aserva* as first and second intermediate hosts, respectively, in Cypress Hills Provincial Park, Alberta, Canada. These results are the first to describe the complete life cycle of emerging lancet fluke in western North America.

Moreover, the evaluation of molecular methods for the field study of the natural history of *D. dendriticum* was carried out by other authors (Mitchell et al. 2017) on Machair pastures, on the Inner Hebridean Isle of Coll. This was the same study site in which the only previous historic field study of *D. dendriticum* in the British Isles had been undertaken (Tarry 1969). The authors (Mitchell et al. 2017) carried out coprological analysis by filtration and sedimentation methods for the identification of *Dicrocoelium* eggs in faeces and also morphological analysis of the snails and ants by stereomicroscopy to detect the infection by larval stage of *D. dendriticum*. Moreover, they (Mitchell et al. 2017) also carried out molecular analysis for the detection of the infection by *D. dendriticum* in snails and ants using PCR techniques, by amplification of a mitochondrial DNA (mtDNA) fragment previously published (Martínez-Ibeas et al. 2011). No amplification of a 169 bp mtDNA fragment was seen by these authors (Mitchell et al. 2017) in any species other than *D. dendriticum*. When used on snail and ant sample DNA, the primers were successful in amplifying 135–145 bp mt DNA fragments, which BLAST analysis determined to be *D. dendriticum* (98–99% score, 99.2%) identity to JF690758 (Martínez-Ibeas et al. 2011). The authors (Mitchell et al. 2017) studied the specificity and sensitivity of mtDNA primers they used for the detection of *D. dendriticum* in snails and ants.

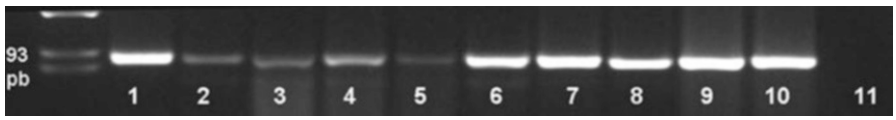

Fig. 10.6 Products of PCR amplification of *Dicrocoelium dendriticum* in agarose gel with ethidium bromide, using specific ITS-2 primers. (1) *D. dendriticum* adult. (2–10) *Cernuella (X.) cespitum arigonis* mollusc specimens, experimentally infected with *D. dendriticum* and slaughtered 1, 3, 6, 10, 22, 27, 34, 41 and 50 days p.i., respectively, when the parasite could not yet be seen under the microscope. (11) Negative control (From Martínez-Ibeas et al. 2011, *Parasitology* 138:1916–1923, with permission of the Journal Editor)

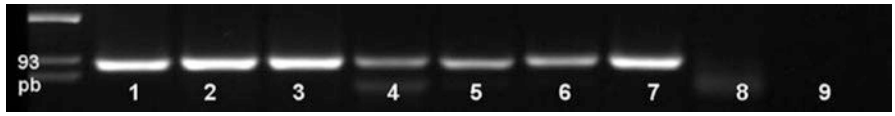

Fig. 10.7 Products of PCR amplification of *Dicrocoelium dendriticum* in agarose gel with ethidium bromide, using specific ITS-2 primers. (1) *D. dendriticum* adult. (2 and 3) Complete bodies of *Formica rufibarbis* collected in tetania containing 52 and 45 metacercariae, respectively, in the abdomen. (4 and 5) Head of *F. rufibarbis* and *Formica pratensis*, respectively, infected with brainworm of *D. dendriticum*. (6 and 7) One and two metacercariae of *D. dendriticum*, respectively, extracted from the *Formica rufibarbis* abdomen. (8) Non-infected abdomen of *Formica rufibarbis*. (9) Negative control (From Martínez-Ibeas et al. 2011, *Parasitology* 138:1916–1923, with permission of the Journal Editor)

10.3.1.6 Infection of the Definitive Hosts

When the definitive hosts ingest these infected ants, the mature metacercariae excyst in the digestive tube and the young flukes go through the opening of the common bile duct (Krull 1958; Svadzhyan 1959; Srivastava et al. 1978) and sometimes the portal circulation (Polozhentseva 1968), to the liver, settling in the bile ducts where they become adult worms. When these are mature, they lay eggs which are shed in the faeces of the host and allow the life cycle of *D. dendriticum* to begin again. The prepatent period in sheep varies according to different authors: 96 days p.i. (Hohorst and Lämmler 1962), 56 p.i. (Tarry 1969) 48–49 p.i., (Chandra 1973) and 49–79 p.i. (Campo et al. 2000). The elimination of *D. dendriticum* eggs by sheep varies according to the hour of the day (Senlik et al. 2006; Campo et al. 2000). So egg elimination was higher in the faeces samples taken in the afternoon (Campo et al. 2000). The Trematode *D. dendriticum* was introduced into Cypress Hills Provincial Park in South-eastern Alberta, Canada, in the mid-1990s and now occurs in 60–90% of co-grazing elk and beef cattle examined at necropsy (Beck et al. 2014). These authors carried out a study on the liver of 173 elk, which were made available by hunters during the 1997–2011 hunting seasons, and the liver from 35 cattle were purchased from ranchers. The study on the parasites found in the liver of each animal showed that the distribution of liver flukes was highly aggregated within the sample of cattle and elk. The authors analysed the association between host age, liver weight and the fluke abundance focused on 61 elk liver collected during 2009–2011. According to these authors, the distribution of liver flukes was highly aggregated within the sample of cattle and elk.

Moreover, the decline in fluke burden with host age is stated to not be consistent with an age-related decline in exposure to metacercariae in intermediate host and high rates of fluke-introduced host mortality. Rather the pattern of peak fluke burdens in elk calves and juveniles followed by a decline in older animals is consistent with the development of a protective immune response in older hosts. This characteristic pattern means that a restricted proportion of both populations of hosts will be responsible for the contamination of pasture in CHP with liver fluke eggs. The results obtained by these authors (Beck et al. 2014) indicate that host age, at least for elk, contributes to this pattern of aggregation.

A comparison between data from experimental infection by *D. dendriticum* in cattle and sheep and by natural infections in elk in Cypress Hills Provincial Park, Alberta (Canada), was carried out (Beck et al. 2015). All flukes reached reproductive maturity, and the degree of reproductive inequality between individual flukes within each infrapopulation was moderate and approximately equal amongst the three host species.

10.3.2 *Dicrocoelium hospes*

Some authors (Macko and Pacenovsky 1987), based on the morphological variability of Dicrocoeliidae adults from the former Soviet Union and Czechoslovakia, concluded that *Dicrocoelium hospes* Looss, 1907, was synonymous to *D. dendriticum*. However, other authors

(Bourgat et al. 1976; Kajubiri and Hohorst 1977; Lucius and Frank 1978; Malek 1980a, b; Lucius 1981; Fashuyi and Adeoye 1986) considered that *D. hospes* was a valid species. Molecular characterization of the 28S region and the ITS-2 of this species have been carried out (Maurelli et al., 2007). This liver fluke species is widely distributed in ruminants (cattle, sheep, goats and buffaloes) from the savanna areas of Africa south of the Sahara (Lucius 1981). It uses Stylommatophora molluscs, from the genus *Limicolaria* (family Achatinidae), in its biological cycle (Bourgat et al. 1976; Lucius 1981), which act as first intermediate hosts. It also uses family Formicidae ants, belonging to species of genus *Camponotus* (Formicinae), *Crematogaster* (Myrmicinae) and *Dorylus* (Dorylinae) (Bourgat et al. 1976; Roming et al. 1980; Lucius 1981).

10.3.3 *Dicrocoelium chinensis*

Dicrocoelium chinensis (Sudarikov and Ryjikov 1951) has been mainly found in ruminants from East Asia (Sudarikov and Ryjikov 1951; Tang et al. 1983, 1985; Taira et al. 2006) and in sika deer (*Cervus nippon*) from different countries of Europe, probably imported from Asian countries (Hinaidy 1983; Poglayen et al. 1996; Otranto et al. 2007). The adults of this species are morphologically larger than those of *D. dendriticum*, and both species are molecularly different (Otranto et al. 2007). *Dicrocoelium chinensis* uses Stylommatophora molluscs, genus *Bradybaena* (Bradybaenidae), *Xeropicta* (Helicidae) and *Ganesella* (Pleurodontidae) as first intermediate hosts (Tang et al. 1983; Gu et al. 1990). The ants mentioned as second intermediate hosts of *Dicrocoelium chinensis* belong to genera *Formica* and *Camponotus* (Tang et al. 1983; Gu et al. 1990).

Recently, the phylogenetic relationships between *Dicrocoelium chinensis* populations in Japan and China were studied based on mitochondrial "*nad1* gene" sequences (Hayashi et al. 2017). These authors identified the parasites taking into account the testis orientation and the nucleotide sequences of the ribosomal ITS-2. Moreover they carried out one phylogenetic analyses using mitochondrial "*nad1* gene" sequences. An analysis of molecular variance showed that the percentage of variation between the countries was extremely high. This fact indicates that *D. chinensis* populations in Japan and China are genetically different. The hypothesis of these authors is that *D. chinensis* might have been introduced into Japan along with the migration of infected wild ruminants in the Pleistocene glaciations, and then the population became differentiated from the Chinese population.

10.4 Epidemiology of Dicrocoeliosis

Although there are various papers on the different aspects of the complex life cycle of *D. dendriticum*, some of which were mentioned above, integrated studies of the epidemiology of dicrocoeliosis—mutual relationships between the parasite, the definitive and intermediate hosts and the environment—are scarce. These studies will allow discovery of the source of contamination and the periods with a risk of infecting ruminants, essential information for applying effective strategic treatments against the parasite in a specific zone (Manga-González et al. 2010).

Regarding the complex epidemiology of dicrocoeliosis, the following aspects, amongst others, should be considered:

1. Diagnosis of infection by the *D. dendriticum* parasite of the definitive host mammals, mainly domestic and wild ruminants
2. Kinetics of trematode egg elimination in the faeces of the definitive host and contamination of pasture by these eggs
3. Times of year and environmental factors which can affect the survival and viability of the parasite eggs
4. Presence in the zone of appropriate species of land molluscs, the first intermediate hosts which ingest the eggs and permit the miracidium to hatch, followed by larval development of the parasite inside them

5. Life cycle of those molluscs, periods when adult or young molluscs are predominant and their activity
6. Influence of the environmental conditions on the development of the parasite in the different mollusc species
7. Appropriate meteorological characteristics and time of year for the slimeballs to be shed with cercariae inside
8. Ingestion of these balls by specific species of ants, the second intermediate hosts, which live in the same biotopes
9. Times of ant activity and inactivity throughout the year
10. Behaviour of the ants infected by *D. dendriticum*, with reference to the time, temperature and light intensity
11. Times when the definitive hosts are infected by ingesting infected ants with mature metacercariae
12. Transformation of the ingested metacercariae into adult worms which, after the prepatent period, shed the parasite egg, thus contaminating the pasture

10.4.1 Prevalence of Infection in Livestock

Various coprological studies have been carried out to discover the prevalence (%) of *D. dendriticum* infection in livestock in several, mainly European and Asian, countries. Nevertheless, the data provided vary according to the host species, geographical area and ecology, time of year, time when the samples are taken and methodology used.

The data on infection prevalence (%) by *D. dendriticum* in ovines, obtained via conventional coprological studies, vary amongst countries and within the same country (Manga-González et al. 1991, 2007; Manga-González and González-Lanza 2005). Prevalences of between 60% and 100% have been recorded in animals from Azerbaijan (Sadykhov and Melikov 1980), Bulgaria (Pavlov et al. 1965), China (Tang et al. 1981), France (Calamel 1976), Greece (Liakos 1985), Italy (Ambrosi and Principato 1981; Quesada et al. 1991), Macedonia (Angelovski et al. 1970), Russia (Vershinin 1957), Spain (Del-Rio 1967; Uriarte et al. 1985; Manga-González et al. 1991) and Turkey (Kalkan 1971), amongst others. Other lower prevalence % rates have also been recorded. Animal age clearly affects egg elimination, as the highest infection rates are in the oldest animals (Al-Khalidi and Al-Bayati 1989; Manga-González et al. 2007).

The prevalence rates obtained by coprology in cattle were generally lower than those in sheep: 38% in Spain (González-Lanza et al. 1993), 0.5–21% in Bulgaria (Denev et al. 1970b), 5% in Italy (Batelli et al. 1987), 1.51% in Pakistan (Afzal et al. 1981), amongst others. Slaughterhouse studies have revealed 27–35% infection in cattle in Libya (Ben Amer and Ahmed 1980). The percentage amongst infected caprines (via bile ovoscopy) was 45% in Spain (Mañas et al. 1978). Examination of the liver of slaughtered goats (n = 228) from two regions of India showed 18.9% of the goats were infected with *D. dendriticum* (Godara et al. 2014). Moreover, a survey of liver flukes in livestock based on abattoir data in Kermanshah was carried out in the west of Iran (Shahbazi et al. 2016). This study showed that liver condemnation due to dicrocoeliosis was common in autumn for sheep and cattle and in winter for goats.

The epidemiological studies carried out in southern Alberta (Canada) (Goater and Colwell 2007) on the invading parasite *D. dendriticum* in sympatric wapiti (*Cervus elaphus*) and beef cattle showed that 83% of the ungulates examined between October and December were infected with this parasite. The prevalence of infection by *D. dendriticum* in wapiti (*Cervus elaphus*) was 96% in calves, 60% in yearlings and 71% in adults. As regards beef cattle, the infection was 100% in yearlings and adults and 83% in calves. The higher intensity of parasite was observed in calf wapiti (4343 worms) and in calf (983) and adult (711) of beef cattle.

In a study carried out in Switzerland (Hilbe et al. 2015) on the infection of New World camelids—llamas (*Lama guanicoe glama*) and alpacas (*Vicugna pacos*)—with *D. dendriticum* and *F. hepatica*, it was observed that dicrocoeliosis was the most significant parasitic infection.

According to the study carried out on faecal samples collected from healthy South American camelids (llamas and alpacas), kept in the southern and western regions of Austria, the number of *D. dendriticum* eggs eliminated was higher in the animals from the southern than in those from the western region (Lambacher et al. 2016).

Examination of the livers of slaughtered goats (=228) from two regions of India showed 18.9% of the goats were infected with *D. dendriticum* (Godara et al. 2014).

Moreover, studies carried out on a total of 300 gall bladder from cattle, which included 155 males and 145 females collected from the slaughterhouses in Kogi State (Nigeria), gave a result of 39% infection prevalence by *D. dendriticum* (Iyaji et al. 2018).

Liver condemnation due to dicrocoeliosis was common in slaughtered animal in Iran (Jahed Khaniki et al. 2013). Moreover, the highest condemnation was observed in spring for both sheep and goats in Lorestan, Iran (Ezatpour et al. 2015). On the other hand, a prevalence rate of 20% has been obtained by necropsy in roe deer (*Capreolus capreolus*) from Northern Turkey (Bolukbas et al. 2012).

Egg elimination of *D. dendriticum* by chamois, mountain goats and mouflons was observed in the Catalonian Pyrenees (Gutiérrez et al. 1993). In addition, various species of wild Leporidae, mainly rabbits, have been found infected with *D. dendriticum* in the lower reaches of the French Pyrenees and in Northwest Spain (Bailenger et al. 1965; Blasco et al. 1993).

Moreover, eggs of *Dicrocoelium dendriticum* were found in the faeces of the three species of squirrel: *Callosciurus finlaysonii*, *Callosciurus prevosti* and *Tamias striatus* in the southern Italy (d'Ovidio et al. 2014).

10.4.2 Periods of Pasture Contamination and Egg Survival

The kinetic of *D. dendriticum* egg shedding by ruminants varies from one country to another. Therefore in countries with a continental climate within the Mediterranean Atlantic transition, like Spain (León Province), with cold winters and hot summers, the shedding of *D. dendriticum* eggs in the faeces of ruminants occurs uninterruptedly throughout the year in both ovines and bovines (Manga-González et al. 1991; González-Lanza et al. 1993; Manga-González and Quiroz 1999; Manga-González and González-Lanza 2005; Manga-González et al. 2007, 2010), although the highest values are recorded at the end of the autumn and mainly in the winter. In Germany the highest elimination of eggs by sheep was observed in the spring and by cows in the autumn (Kopp 1975). In Italy, the highest rate for egg deposition in sheep was observed between February and May (Ambrosi and Principato 1981). In addition changes have been recorded in the faecal egg counts of *D. dendriticum* elimination according to the different hours of the day (Senlik et al. 2006).

The viability of *D. dendriticum* eggs in the field depends to a great extent on the consistency of the faeces shed by the definitive host and the environmental conditions they are exposed to. The resistance of *D. dendriticum* eggs to environmental conditions means that pasture contamination continues for long periods of time. Viable parasite eggs have been obtained after 5 years from dried sheep faeces and after 16 months on grass in the former Soviet Union (Ershov 1964). *D. dendriticum* eggs are more resistant to low temperatures than high ones (Alzieu and Ducos De Lahitte 1991). They have been experimentally proven to resist temperatures between −20 and −50 °C (Boray 1985). Studies carried out under controlled field conditions in León (Spain) (Alunda and Rojo-Vázquez 1983a) have shown that egg mortality in faeces exposed to the environment is a phenomenon regardless of the time they have been outside and, in contrast, show marked seasonality. Thus, whilst the eggs deposited in the environment between January and July have high resistance (75–85%), those shed between July and August suffer extremely high mortality (almost 100%). A significant survival rate is observed from September to December, as in the first half of the year, so the eggs from that period are not destroyed until the

hot months of the following year (Alunda and Rojo-Vázquez 1983a). In conclusion, the cause of *D. dendriticum* egg mortality under natural conditions seems to be exclusively due to the lethal effect of the high temperatures reached inside the faeces directly exposed to the sun, which causes miracidium death. Thus in the colder period of autumn-winter, when the greatest egg elimination takes place in the province of León (Spain), the survival of *D. dendriticum* eggs is very high. Due to this, pasture contamination by viable eggs is considerable in the spring, when molluscs (mainly the young ones) become active and are very abundant (Manga-González et al. 2001; Manga-González and González-Lanza 2005).

10.4.3 Mollusc First Intermediate Hosts

The role played by land molluscs in the epidemiology of dicrocoeliosis is very important as they are generally coprophagous and easily ingest the faeces of the definitive hosts containing parasite eggs. Egg hatching and miracidium release appear to occur due to several physical-chemical stimuli of the mollusc intestine, low pH and reducing conditions (Ractliffe 1968; Mitterer 1975). Recently *D. dendriticum* eggs have been hatched by freezing (at −80 °C or in liquid nitrogen) and then thawing, extracting the miracidium DNA and its molecular identification (Sandoval et al. 2013).

Dicrocoelium dendriticum egg hatching and miracidium liberation only occur in the intestine of the appropriate molluscs that act as intermediate hosts. Inside the mollusc the parasite multiplies enormously by asexual reproduction (numerous cercariae can be formed from one ingested egg). This increases the possibilities of parasite transmission. Since the long-tailed cercaria in *Helix carthusiana* (=*Monacha (M.) cartusiana*) was encountered for the first time (Piana 1882) and later described as *Cercaria vitrina* (from *Zebrina detrita*) (Von Linstow 1887) and associated with *D. dendriticum*, many studies have been carried out to discover the mollusc species which act as first intermediate hosts for this parasite. More than 100 mollusc species (Gastropoda, Pulmonata, Stylommatophora) have been found receptive to *D. dendriticum* under natural and laboratory conditions They mainly belong to the following families: Bradybaenidae, Buliminidae, Clausiliidae, Cochlicopidae, Helicidae, Hygromiidae (previously included in Helicidae) and Zonitidae (Del-Rio 1967; Denev et al. 1970a; Kalkan 1971; Rozman et al. 1974, Alunda and Manga-González 1982; Boray 1982; Manga-González 1983; Alunda and Rojo-Vázquez 1984; Bocharova 1984; Boray 1985, Manga-González 1987; Badie et al. 1992, Schuster 1993; Manga-González et al. 2001; Otranto and Traversa 2003; Manga-González and González-Lanza 2005; Gürelli 2017), amongst others.

This parasite shows markedly little specificity as regards its first intermediate host. In addition the parasite can develop in various species of mollusc in the same area. In Spain the most important species of mollusc in the epidemiology of dicrocoeliosis are *Cernuella (Xeromagna) cespitum arigonis* on plateaux and plains and *Helicella (Helicella) itala* mainly in the northern mountains (Del-Rio 1967; Manga-González et al. 2001; Alunda and Rojo-Vázquez 1982). The latter species mentioned has also been found naturally infected with *D. dendriticum* in the UK (Tarry 1969) and Bosnia and Herzegovina (Rozman et al. 1974). Other significant species recorded in the transmission of *D. dendriticum* are *Helicella (Helicella) obvia* in Germany, Bosnia and Herzegovina, Bulgaria and Turkey; *Zebrina (Zebrina) detrita* in Germany, Bosnia and Herzegovina, Bulgaria, France, Italy, Russia and the USA; and *Cochlicopa lubrica* (=*Cionella lubrica*) in the USA, Spain, France, Russia and Ukraine. Many other species of molluscs have also been recorded as infected with *D. dendriticum* in different countries and with infection percentages varying between 0.5 and 62% (Manga-González et al. 2001). Nevertheless, the prevalence data indicated by the different authors must be higher, as the first larval stages of the parasite are not detected under the stereomicroscope until at least 50

days p.i. (González-Lanza et al. 1997) in molluscs experimentally infected and maintained in the laboratory and until a maximum of 9 months in molluscs experimentally infected and maintained in a natural environment (Manga-González et al. 1995). Therefore, histological (Álvarez-Nogal et al. 1992), isoenzymatic or molecular biology (Martínez-Ibeas et al. 2011; Martínez-Ibeas 2013) techniques are necessary to detect the first larval stages. It must also be borne in mind that the infection prevalence increases with the age of the molluscs (Schuster 1993; Manga-González 1987; Manga-González et al. 2001).

The life history of the mollusc intermediate hosts is of great epidemiological interest, as regards the abundance, age, activity for both the ingestion periods of *D. dendriticum* eggs and the survival of the parasite in them. The abundance and activity of *H. itala* in Spain were greater in spring and autumn, with young specimens being more abundant in spring, whereas adults were more abundant in autumn (Manga-González 1987; Manga-González et al. 2001). The same is true for *H. obvia* in Germany (Schuster 1993). Age and nutritional state of the molluscs, infective dose, ambient temperature and relative humidity, amongst other aspects, influence the long development of larval stages of this digenean in the first intermediate hosts (Schuster 1993; Gómez et al. 1996; González-Lanza et al. 1997; Manga-González et al. 2001). Both *H. itala* and *H. obvia* adults were more abundant in autumn whilst the young ones were in spring (Schuster 1993; Manga-González et al. 2001).

The egg ingestion periods of *D. dendriticum* are dependent on mollusc activity, and the survival and development of the larval parasites are important in the transmission of dicrocoeliosis. The sporocysts require a temperature of over 4 °C to mature, with development increasing with a rise in temperature (Schuster 1993). Under controlled field conditions, in hot months, larval development of *D. dendriticum* is accelerated within the mollusc host (Manga-González et al. 1995; Manga-González and González-Lanza 2005). The mollusc species can also influence *D. dendriticum* development (Gómez et al. 1996).

The infection prevalence increased with mollusc age (Alunda and Rojo-Vázquez 1983b; Schuster 1993; Manga-González et al. 2001). In general, the highest rate of infection in molluscs occurred in the autumn and spring. Moreover, immature daughter sporocysts (with germinal masses) predominated in adult and young molluscs collected in the autumn in León (Spain), whilst sporocysts with mature or nearly mature cercariae predominated in adults collected in the winter and spring (Manga-González et al. 2001). In the Marmara (Turkey) region, the lowest parasite percentages were generally detected at the end of the summer and in the autumn (Kalkan 1971). In France the infection of the molluscs occurs between the end of one hibernation period and the beginning of the next (Badie 1978). The results obtained in León (Spain) seem to indicate that young molluscs, which are more abundant in spring, are infected between the beginning of spring and the beginning of summer (Manga-González et al. 2001; Manga-González and González-Lanza 2005). So the first infected ones can shed slimeballs with cercariae in the summer or at the beginning of the autumn, whilst those infected later can shed slimeballs (as adults) in the first half of the following year, provided they survive the harsh winter. In Germany most specimens of *H. obvia* are infected in the autumn of their second year of life, when the shell sizes are intermediate (Schuster 1993). In addition, the percentage of molluscs with daughter sporocysts is higher in the spring, and the shedding of slimeballs occurs in May and June. Likewise the first slimeballs shed by *Z. detrita* were also observed in Germany at the beginning of May and the last ones in mid-October (Hohorst and Lämmler 1962). The sun affects the appearance, size and consistency of slimeballs in only a few hours and thus influences the viability of the cercariae. Their infective capacity for ants is prolonged at least 18 h, if slimeballs are stored at 4 °C, although a very low number of cercariae maintain mobility after 72 h at this temperature (Del-Rio 1967).

10.4.4 Ant Second Intermediate Hosts

When slimeballs are ingested by appropriate species of ants, they become infected. The "brainworms" settle in the suboesophageal ganglion of the ant whilst the rest of the metacercariae are located in the abdomen. The infection prevalence obtained by the authors varied amongst the different species (*Formica pratensis*, *Formica rufibarbis*, *Formica fusca*, amongst others), but mainly due to the type of sampling (Manga-González et al. 2001). The higher infection percentage is obtained when the ants are collected in tetany. So the infection of *F. rufibarbis* and *F. pratensis* collected directly from the anthills in León (Spain) were 6.59% and 4.05%, respectively, whilst the infection percentage recorded in the specimens of the same ant species collected in tetany was 95.39% and 100%, respectively (Manga-González et al. 2001). Similar results were obtained in *F. pratensis* (1.1% and 97%, respectively) from Bosnia and Herzegovina (Jonlija et al. 1973) and in *Formica lugubris* (0.14% and 70%, respectively) collected in the same country (Rozman et al. 1974). The number of *D. dendriticum* metacercariae given by different authors (from 1 to 580) varied amongst the different species and even within the same one (Manga-González et al. 2001). The variability could be due to the time of year, as it is higher in summer (Paraschivescu et al. 1976); the different affinity of the ant species for slimeballs (Loos-Frank 1978); the type of vegetation and the ant species (Paraschivescu 1978); the size of the abdomen (Kalkan 1976); and the different species, size and possible ecological and behavioural causes (Schuster 1991). It seems that *F. rufibarbis* and *F. pratensis* are the most important species in the epidemiology of dicrocoeliosis in Spain and in many other countries (Manga-González et al. 2001).

The importance of ants as second intermediate hosts is mainly due to their abundance, wide distribution and alteration (tetany) in their behaviour produced by the "brainworm", especially when temperatures and solar intensity decrease, which makes infection of the definitive hosts by ingestion of the parasitized ants easier. The tetany stage of infected ants occurs mainly in the early hours of the morning and late in the afternoon, although on cloudy or warmer days it is also possible to detect ants in tetany at the end of the morning and the beginning of the afternoon (Spindler et al. 1986; Manga-González et al. 2001). The highest temperatures at which *F. pratensis* and *F. rufibarbis* were observed in tetany in León (Spain) were 26.9 and 28 °C, respectively (Manga-González et al. 2001). This temperature was higher than the 18–21 °C reported by other authors (Jonlija et al. 1972; Paraschivescu 1983; Spindler et al. 1986; Schuster 1991). On the other hand, active ants were observed between March and November and infected ones from April to November in those collected in León from the nest and in tetany between May and October (Manga-González et al. 2001). Authors from other countries have also observed infected ants between the spring and autumn in Kazakhstan (Dementev and Karabaev 1968), Bosnia and Herzegovina (Jonlija et al. 1972), France (Badie 1978) and Germany (Schuster 1991). Transmission to definitive hosts only occurs at times when the ants are not hibernating. Nevertheless, some infected ants survive in their nests during the winter, and they are responsible for infection of the definitive host on becoming active at the beginning of spring (Tarry 1969; Badie 1978). In the following months until November, when ant hibernation starts again, the ingestion of ants containing infective metacercariae by the definitive hosts and the number of adult worms of *D. dendriticum* in their liver increase. As a consequence, parasite egg elimination also increases during this period, reaching its highest values at the end of the autumn but mainly in winter, the cold period when egg survival is at its highest. Recently a multiple regression model has been used to predict risk habitats for the transmission of *D. dendriticum* to grazing ruminants (Ekstam et al. 2011).

10.5 Pathology and Clinical Aspects of Dicrocoeliosis

10.5.1 Clinical Signs

Dicrocoeliosis is considered asymptomatic (Otranto and Traversa 2003; Rojo-Vázquez et al. 2012). The fluke usually produces mild non-specific clinical symptoms (Skálová et al. 2007; Bártíková et al. 2011) that are not usually manifested, even in heavy infections with doses of 3000 metacercariae (Wolff et al. 1984; Campo et al. 2000). Nevertheless, diarrhoea and a decrease in growth in lambs have been observed in sheep tested with 3905 metacercariae (Hohorst and Lämmler 1962). According to previous reports, parasitic burdens of 1000 or less flukes do not result in clinical manifestations (Rojo-Vázquez et al. 1981; Campo et al. 2000). Animals with dicrocoeliosis may show eosinophilia, anaemia, icterus, oedema, weight loss, reduced meat, milk and wool production as well as impairment of the liver in severely infected cases (Otranto and Traversa 2002, 2003; Sánchez-Andrade et al. 2003; Bártíková et al. 2011). In experimentally infected lambs, the lowest weight increase was observed 60 days p.i., both in the lambs infected with 3000 metacercariae (−15%) and those tested with 1000 (−12%) (Manga-González et al. 2004). In this study the highest reduction (−18%) was detected in the group of lambs with 401–600 worms, and no other clinical signs were observed. Previous reports (Salimova 1972) considered that the reduced weight gain was more marked until the parasites reached sexual maturity. Poor growth and body condition and severe photosensitization characterized by oedema of the unpigmented areas of the face and ears, yellow crusts and skin necrosis were identified in 14-month-old Texel cross Cheviot ewe lambs, probably associated with dicrocoeliosis (Sargison et al. 2012). The levels of infection in this case were unusually high, as indicated by their faecal *D. dendriticum* egg counts (909 eggs per gram—epg).

10.5.2 Biochemical Parameters

In lambs experimentally infected with 1000 and 3000 metacercariae, respectively, which harboured between 30 and 2063 adult worms in their necropsy carried out at 2 and 6 months p.i., a slight increase in the serum levels of total bilirubin (7%) and albumin (3%) was observed (Manga-González et al. 2004). The hepatic enzyme values: aspartate aminotransferase, AST(19%), and alanine aminotransferase, ALT (22%), increased, mainly in lambs tested with 3000 metacercariae. The highest increase in these enzyme values observed at the early stages of the infection (60 days) could be related to the hepatic damage produced by the migration of the worms before reaching sexual maturity, since the first *D. dendriticum* egg elimination was observed in the faeces of the lambs between 49 and 79 days p.i. (Campo et al. 2000). No clear relationship was observed between the increased hepatic enzyme values and the parasite burden, although the highest AST, ALT and gamma-glutamyl transpeptidase (GGT) values were detected in the animal with the greatest burden. The GGT values were higher (11%) in the lambs slaughtered on day 180 p.i., and the increased activity of this enzyme seems to be directly related to the parasite burden (Manga-González et al. 2004). It may also be related to the more severe bile duct lesions observed in this animal group. In cattle infected with *F. hepatica*, the increase in GGT serum values coincided with the penetration of the bile ducts by migrating flukes (Ranucci et al. 1981).

Serum liver glutamate dehydrogenase (GDH), GGT and bile acid concentrations were markedly elevated, whilst serum albumin concentration was low, indicative of severe liver parenchymal and biliary disease, in lambs with dicrocoeliosis and hepatogenous photosensitization (Sargison et al. 2012). Oxidative liver damage with increased ALT and AST activity as well as bile duct proliferation and chronic cholangitis have been described in experimentally infected hamsters (Sánchez-Campos et al. 1996, 1999, 2000). A significant elevation in bile flow (+20%) and in the

biliary output of glutathione (+34%), bile acid (+59%), cholesterol (+108%), phospholipids (+99%) and alkaline phosphatase (+36%) was observed in these infected animals (Sánchez-Campos et al. 1998). Biochemical evidence for the generation of oxidative stress by enhancement of lipid peroxidation (higher malondialdehyde concentration) and reduction of the enzymatic antioxidant ability (significant decrease of superoxide dismutase and glutathione peroxidase) has been detected in grazing sheep naturally infected by *D. dendriticum* (Bahrami et al. 2015). The oxidative stress may play an important role in the erythrocyte destruction in sheep naturally infected with *D. dendriticum*, although no clear relationships were observed between the oxidative stress, hepatic damage and parasite burden (Samadieh et al. 2017).

10.5.3 Gross Lesions

In the final hosts, as it was already discussed in the "Biology of the *Dicrocoelium* species" section, the ingested metacercariae excyst within the proximal intestinal tract and migrate through the small intestinal lumen and common bile duct to the liver, maturing to monoecious adults within the bile ducts (Sargison et al. 2012).

In natural and experimental dicrocoeliosis, a different degree of hepatic induration and the presence of whitish, firm, thickened and distended (cholangiectasia) bile ducts were a constant finding. These bile ducts were visible as branching cords on the visceral surface of the liver, mainly on the left and right hepatic lobes. Frequently, the damaged bile ducts were observed dorso-laterally at the renal depression area of the right hepatic lobe. The incised liver surface revealed numerous enlarged bile ducts containing the parasites and bile. In heavy infections a variable number of parasites are also seen inside the extrahepatic bile ducts and gall bladder. The hepatic lymph nodes increased in size (Wolff et al. 1984; Manga-González et al. 2004). These macroscopic lesions seem to be directly related to the parasite burden (Jithendran and Bhat 1996; Manga-González et al. 2004).

10.5.4 Microscopic Lesions

Pathological changes in the liver (cholangiohepatitis) and gall bladder (cholecystitis) are probably caused not only by the action of toxic products formed by the parasite but also by the mechanical irritation of the walls of the bile ducts by the fluke (Fig. 10.8). Due to its buccal

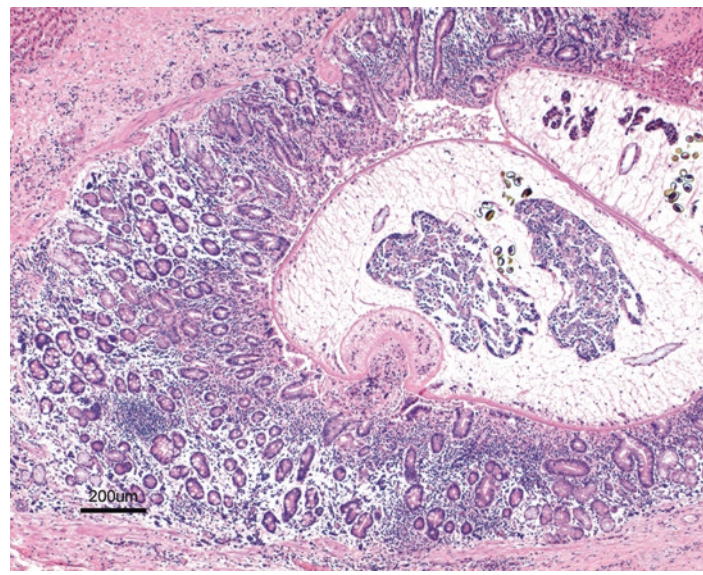

Fig. 10.8 Sheep liver naturally infected with *D. dendriticum*. Dilated septal bile duct harbouring two adult *D. dendriticum* worms. Note the hyperplasia of bile duct epithelium, leukocytic infiltration and periductal fibrosis (chronic cholangitis). H-E. Bar = 200 μm

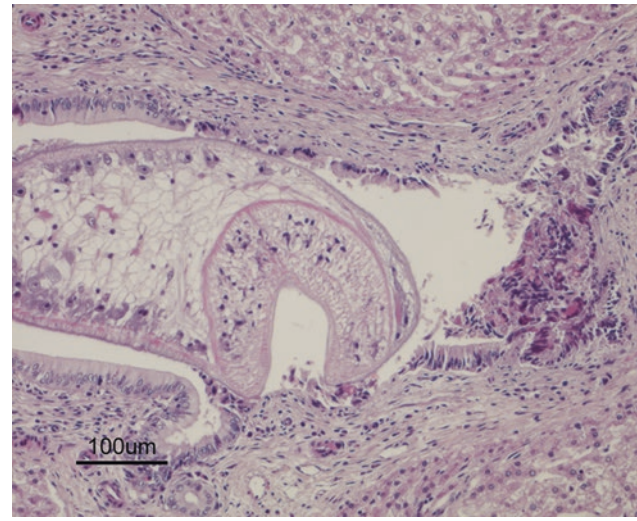

Fig. 10.9 Erosive effect of a *D. dendriticum* adult sucker in the lining epithelial cells of a septal bile duct from an experimentally infected lamb slaughtered 60 days p.i. H-E. Bar = 100 μm

stylets, *D. dendriticum* irritates the bile duct surface, causing changes in the septal bile ducts (Rahko 1972a; Camara et al. 1996). This parasite is devoid of spines on the tegument, so the hepatic lesions it causes are less significant than those caused by *F. hepatica*, except in the case of massive chronic infection (Camara et al. 1996). The adult parasites cause damage to the lining of the bile ducts (Fig. 10.9); however, in comparison with *F. hepatica*, *D. dendriticum* does not cause extensive hepatocellular damage. Hence its potential pathogenicity is frequently underestimated (Otranto and Traversa 2002). In experimental dicrocoeliosis in sheep, lesions mainly affecting the biliary system but also hepatocytes were associated with the parasite burden (Manga-González et al. 2004). The septal bile ducts showed extensive hyperplasia, desquamation and necrosis of mucosal epithelium and goblet cell differentiation (Rahko 1972a; Manga-González et al. 2004). The hyperplastic biliary epithelial cells stain positively for alcian blue-periodic acid-Schiff and silver methenamine stains. Thus both neutral mucins and acidic mucins, concentrated close to the apical surface in the epithelial cells of septal bile ducts, could be demonstrated in the same type of cell in natural and experimental dicrocoeliosis (Rahko 1972b; Manga-González et al. 2004). However, the proportion of sialomucins and sulphomucins seen increases in this parasitic cholangitis (Rahko 1972b). A variable number of intra-epithelial globule leukocytes and lymphocytes were also observed (Rahko 1972a; Manga-González et al. 2004). The origin and function of globule leukocytes are not fully understood. These cell populations are considered to be derived from mucosal mast cells or from large granular lymphocytes that migrate into the epithelial compartment. Dicrocoeliosis and fasciolosis in goats showed an increase in the total number of hepatic mast cells with sulphomucins in their granules, whilst neutral mucins and carboxy-mucins were revealed in the globules of globule leukocytes (Rahko 1972c). Similar findings have been recorded in other trematodosis such as paramphistomosis by *Calicophoron daubneyi* (Fuertes et al. 2015). In this study it has been proved that the histochemical properties of these two cell populations are different. Whilst the mast cells showed metacromatic granules that were positive for acid mucosubstances and negative for neutral mucins, the granules of the globule leukocytes that showed no metachromasy were positive by neutral mucins and negative for acid mucosubstances. The globule leukocytes seem to be significantly correlated to tissue eosinophils and may play an important role in the immune response to some parasitic infections (Robinson et al. 2010). The lumen of these bile ducts frequently contained some worms, and a superficial erosive effect of the parasite sucker

was seen on the lining of epithelial cells (Rahko 1972a; Manga-González et al. 2004). Leukocytic infiltration (macrophages, eosinophils, lymphocytes and plasma cells) and periductal fibrosis were also observed. Similar histopathological findings were detected in the hepatic and cystic ducts. Their simple columnar epithelium showed numerous globet cells, and the mucous glands present in the lamina propria are very hyperplastic (Manga-González et al. 2004; Cullen and Stalker 2016). The presence of abundant mucinous secretion (principally acidic mucins) in the epithelial cells of major bile ducts, confirmed by histochemical staining and electron microscopy, may be a defensive mechanism in the host response against the parasite (Manga-González et al. 2004) and has been associated with proliferating cholangitis (Katayama 1996). A periductal lymphocytic infiltration (diffuse or forming lymphoid aggregates and follicles) is frequently present in both extrahepatic and septal bile ducts. Abundant fibrous tissue, often with smooth muscle cell differentiation, surrounds the extrahepatic bile ducts. The presence of fibrosis of the bile duct walls throughout the liver is a pathological characteristic in severely affected animals with several thousand *D. dendriticum* in the bile ducts (Sargison et al. 2012). Portal and septal fibrosis were always seen in lambs experimentally infected with *D. dendriticum*, but perisinusoidal and centrilobular fibrosis were also found in lambs with the most parasites (Manga-González et al. 2004). Severe extensive biliary fibrosis was a constant finding in heavy infections with both *F. hepatica* and *D. dendriticum* in naturally infected sheep (Cullen and Stalker 2016). Liver fibrosis develops as a long-term consequence of chronic liver injury. The hepatic stellate cells, which transdifferentiate into myofibroblasts upon liver injury, are the main fibrogenic cells in the liver (Mederacke 2013). In experimental dicrocoeliosis in sheep (Manga-González et al. 2004), cirrhosis was never encountered, in contrast to the studies on natural infections in sheep (Jithendran and Bhat 1996) and on experimentally induced dicrocoeliosis in hamsters (Prunescu et al. 1979).

The interlobular bile ducts in the portal tracts were surrounded by an inflammatory infiltrate and showed nuclear pyknosis, necrosis and desquamation of epithelial cells. Parasite eggs or flukes were never seen in these ducts in experimental conditions (Manga-González et al. 2004). Occasional ectopic worms were detected inside the septal hepatic veins in natural and experimental dicrocoeliosis (Manga-González et al. 2004) probably due to migration of *D. dendriticum* through the portal circulation.

The gall bladder in experimental dicrocoeliosis in sheep showed focal loss of epithelial cells, subepithelial oedema with lymphatic distension, occasional globule leukocytes in the mucosal epithelium as well as diffuse or nodular lymphoid infiltrates. Parasitosis with *D. dendriticum* is a significant risk factor for cholelithiasis. Cholesterol and pigment stones were found in the major bile ducts and the gall bladder in 18 out of 254 livers in sheep naturally infected with *D. dendriticum*. The assumed mechanism of stone formation was unknown, but it seems that the presence of parasites in the bile ducts and the gall bladder impairs bile flow (Katsoulos et al. 2011).

In the liver parenchyma, vacuolar (or hydropic) degeneration, glycogen infiltration (mainly in periportal areas), compression atrophy of liver cell cords, sinusoidal leukocytosis and wall thickening of the portal veins were also observed in experimental dicrocoeliosis (Manga-González et al. 2004). The degenerate hepatocytes from cattle (Dhar and Shing 1963) and goats (Rahko 1972a) naturally infected with *D. dendriticum* suffer glycogen depletion, although this varied from zone to zone of the liver lobuli. These facts may indicate that the toxic metabolites released by the adult flukes induce hepatic injury both in portal tracts and hepatocytes as has been documented in experimental dicrocoeliosis (Sánchez-Campos et al. 2000; Manga-González et al. 2004). The hepatic lymph nodes showed an increase in cellularity of B- and T-cell areas, plasmacytosis and histiocytosis.

Severe intimal and adventitial thickening and mild-to-moderate medial thickening of the lung arteries consistent with hypertension have been

described in New World camelids in association with *D. dendriticum* infection. These arterial lesions showed a strong association with the typical liver changes due to *D. dendriticum* (Hilbe et al. 2015).

10.5.5 Immunohistochemical Studies

Immunohistochemical studies focused on characterizing the inflammatory infiltrates associated with hepatic lesions in experimental dicrocoeliosis were carried out on two groups of lambs infected with doses of 1000 and 3000 metacercariae (Ferreras et al, 2007). In all infected animals, CD3+ T lymphocytes were scattered diffusely or forming lymphoid aggregates and follicles in septal bile ducts affected by proliferative cholangitis. Most CD3+ cells also expressed CD4+ T cells, but CD8+ T lymphocytes were scattered surrounding damaged septal bile ducts. Biliary intra-epithelial lymphocytes positive for CD3, CD4 and CD8 were frequently identifiable in the septal and interlobular bile ducts, suggesting the participation of these cells in the host's local defence against the parasite or its products. Numerous CD3+ lymphocytes were present around the interlobular bile ducts and in the hepatic sinusoids. Scarce WC1 γδ-T cells were observed throughout periductal areas in septal and interlobular bile ducts. CD79 αcy and CD45R-positive B lymphocytes were frequently demonstrated in relation to lymphoid aggregates and follicles seen around the large bile ducts. Mainly at 180 days p.i., these bile ducts showed a central core of B lymphocytes CD79 αcy+ surrounded by numerous CD45R and CD4+ lymphocytes. IgG+ plasma cells, with a diffuse pattern or forming clusters near hyperplastic bile epithelium, were numerous in septal bile ducts (Fig. 10.10). These cells may be involved in the local humoral immune response, probably induced by the antigen load released by the parasites. The maximum serum IgG antibody level against excretory-secretory and somatic antigens of *D. dendriticum* in these lambs was reached on day 60 p.i. and remained high until the experiment ended 180 days p.i. (González-Lanza et al. 2000). In the portal tracts, CD79 αcy, CD45R and IgG-positive cells were intermingled with C3+, CD4+ and CD8+ T cells. Macrophages positive for VPM32 and CD14 antibodies were observed scattered or in groups around septal bile ducts but also near the liver sinusoids and in the portal spaces. With the anti-MHC class IIβ monoclonal antibody, a large population of cells were positive

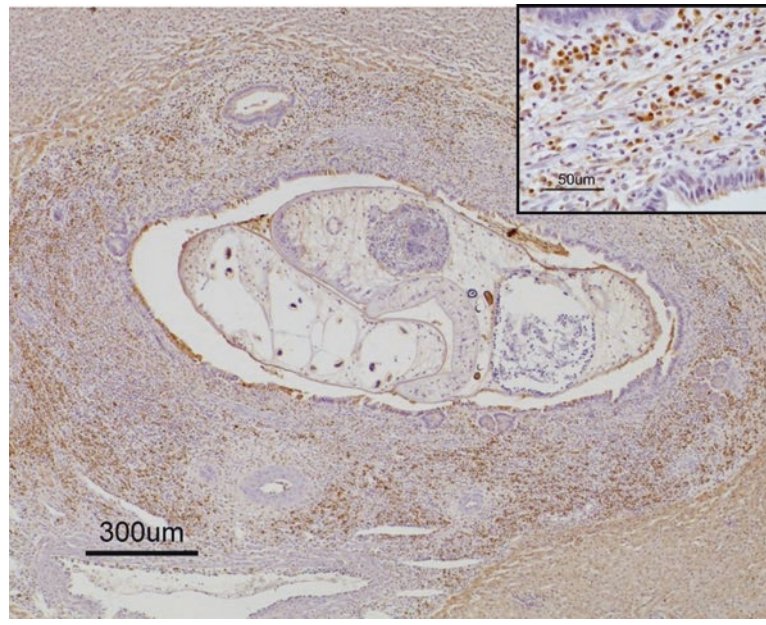

Fig. 10.10 Lamb liver. IgG-producing plasma cells with a diffuse pattern around a septal bile duct in which two worms of *D. dendriticum* are observed. Lamb infected with 1000 metacercariae (72 worms recovered) slaughtered 60 days p.i. ABC system. Haematoxylin counterstain. Bar = 300 μm. Inset: Higher magnification of positive IgG plasma cells. Bar = 50 μm

(B and T cells and activated macrophages), especially around septal bile ducts but also in the portal tracts and liver parenchyma. There was no correlation between the parasitic burden and the cell distribution and intensity of labelling patterns observed.

The role and immunophenotypic characteristics of myofibroblasts in sheep liver naturally infected by *D. dendriticum* have also been analysed. It has been proven that activated hepatic stellate cells, interface myofibroblasts and portal/septal myofibroblasts express α-smooth muscle actin (α-SMA). The increase in the intensity of α-SMA expression in livers with fibrosis emphasizes the importance of these cells in the development of fibrosis in sheep naturally infected with the lancet liver fluke (Kukolj et al. 2015).

10.6 Diagnosis of Dicrocoeliosis

The diagnosis of ruminant dicrocoeliosis is mainly based on the post-mortem examination of the animal liver and gall bladder (Braun et al. 1995), on collecting the parasite found in these organs and detection of the eggs in the host faeces (Campo et al. 2000), as well as on immunological methods (González-Lanza et al. 2000).

Dicrocoeliosis is usually diagnosed during post-mortem examination of the liver or by coproscopic techniques, although faecal examination has a low sensitivity when compared with those of liver necropsy. So the percentage of sheep positive to egg count was only 26.9% in an agricultural region of Sardinia (Italy); nevertheless, 50.3% of the sheep livers examined had flukes of *D. dendriticum* (Scala et al. 1991).

10.6.1 By Conventional Coprological Methods

Coprological examination is currently based on quantitative coprological techniques carried out by sedimentation and McMaster (Campo et al. 2000; Otranto and Traversa 2002; Manga-González and González-Lanza 2005) and by flotation and McMaster, using in this case the high-density solution as zinc sulphate (specific gravity 1.18) and potassium iodomercurate (at dilution 1:10), amongst others (Rehbein et al. 1999; Otranto and Traversa 2002; Sargison et al. 2012). These coprological techniques allow the number of *D. dendriticum* eggs per gram (epg) of faeces from infected animals to be counted. The potassium iodomercurate solution gave the highest rate of egg recovery from faeces (91.2 ± 9.4%). The detection of the infection using this coprological technique has the disadvantage of the animals not having eliminated eggs of *D. dendriticum* until the prepatent period is reached. This period varies in lambs from 48 to 79 days p.i. according to the different authors (Tarry 1969; Chandra 1973; Campo et al. 2000). Due to this, most of the hepatic damage has already been done (Manga-González et al. 2004), and pasture contamination has started when the eggs of the parasite are detected. Moreover, false-negative results concerning egg detection could be obtained because only a small sample of the animal faeces is examined with coprological techniques.

10.6.2 By Immunological Methods

Because of this, serological methods such as enzyme-linked immunosorbent assays (ELISAs) have been used to detect anti-*D. dendriticum* antibodies, using somatic (SO) and excretory-secretary (ES) products as antigens (Baldelli et al. 1981; Bode and Geyer 1981; Jithendran and Bhat 1996; Wedrychowicz et al. 1997; González-Lanza et al. 2000; Otranto and Traversa 2002; Sánchez-Andrade et al. 2003; Şimşek et al. 2006). Nevertheless, this indirect test is not specific (Fagbemi and Obarisiagbon 1991), because it does not allow exposure to the parasite and active infection to be distinguished. Moreover, the antigens normally used to detect antibodies are highly complex mixtures of different protein compounds, which induce different immune responses and cause problems of cross-reactivity. These circumstances make them practically inadequate for diagnosing the infection in hosts with multiple parasites, as occurs in ruminants kept on pasture.

On the other hand, SO antigen appears to stimulate a greater antibody response in cattle, but this is of low specificity. Slightly less IgG response was detected using SO antigens rather than ES products in experimentally infected sheep (González-Lanza et al. 2000). Moreover, both antibody titres (peaked 60 days p.i.) were no correlated to the parasitic burden in this experiment. The antibody response in the bile of naturally infected cattle with worm burdens ranging from 120 to 280 was characterized using ELISA, and it was seen that IgA, IgM and IgG1 isotypes were predominant and there was little IgG2 reactivity (Wedrychowicz et al. 1995). Similarly not evident IgG2 reactivity was seen in Western blots of sera from the naturally infected cattle (Colwell and Goater 2010). In this study immunoblots were positive for IgG1 and IgM antibodies, and the IgG1 antibody response to proteins at 86 kDa was particularly prominent. The profile of reactive proteins seen in naturally infected sheep was in the range of 130 kDa (Revilla-Nuín et al. 2005). Differences between cattle and sheep humoral responses to trematode infections provide a possible explanation.

As was reported, 43.5% of the 26 calves studied had *D. dendriticum* eggs present in their faeces, and immunoblots performed with sera from these animals were all positive for antibodies (Colwell and Goater 2010). All of these calves were serologically positive despite more than half being coprologically negative, showing the effectiveness of ELISA using E/S products for the diagnosis of dicrocoeliosis in cattle. Moreover, seropositivity in sheep was detected by indirect ELISA in 86.2% tested animals, whereas faecal prevalence was 6.7%; all those faecal-positive were also ELISA-positive (Sánchez-Andrade et al. 2003). This fact (high percentage of sheep positive to the ELISA test and negative to egg output) may indicate that the migratory phase of *D. dendriticum* was occurring in these sheep but could also indicate previous exposure to the parasite without current infection (Sánchez-Andrade et al. 2003).

The indirect ELISA using excretory-secretory antigens from *D. dendriticum* to evaluate humoral immune responses was accurate in detecting infected cattle and sheep (González-Lanza et al. 2000; Colwell and Goater 2010). With this method *D. dendriticum* infection can be detected in experimental lambs as early as 30 days after infection, although the maximum level of antibodies was obtained 60 days p.i., after which it decreased slightly but remained high until the end of the experiment 180 days p.i. (González-Lanza et al. 2000).

To avoid the lack of indirect ELISA technique specificity, in order to be sure that the infection is active, it would be very important to develop a direct ELISA sandwich technique to detect *D. dendriticum* antigens in the faeces as well as the serum of infected animals. Nevertheless, to develop this technique first the *D. dendriticum* specific antigens must be identified, isolated, purified and characterized (Revilla-Nuín et al. 2005). These antigens could also serve as vaccine if they can be shown to protect the animals against the parasite (González-Lanza et al. 2006).

10.6.3 By Proteomic Analysis of the TG and ES Antigens of Adults of *D. dendriticum*

The first study on the identification of the major antigen proteins in the adult *D. dendriticum* tegument (TG) and excretion-secretion (ES) extracts, using bidimensional electrophoresis (2D PAGE) and mass spectrometry (MS) techniques (MALDI-TOF/TOF), has been carried out over the last few years (Martínez-Ibeas 2013; Martínez-Ibeas et al. 2013a). The best results in terms of spot resolution and reproducibility were obtained treating the samples with "ReadyPrep 2D clean-up" kit, 3–10 IPG non-linear strips and colloidal Coomassie staining. Under those conditions, 332 spots were detected in the TG and 284 in the ES. Both extracts showed a similar distribution in the number of spots as well as in the ranges of molecular weights and isoelectric points. Immunodetection of antigen proteins onto nitrocellulose membranes was carried out with sera from experimentally infected sheep. Majority and antigenic proteins were subsequently excised from Coomassie stained gels

and identified by mass spectrometry. A quantity of 29 proteins in the excretion-secretion products and 43 in the tegument were identified first in *D. dendriticum*, 23 of them antigenic, involved in various processes such as metabolism, detoxification, chaperone, transport or structural molecules. The absence of information about *D. dendriticum* in the databases has been highly inconvenient in identifying the proteins found.

10.6.4 By Molecular Biology Techniques

10.6.4.1 Construction of an Expression Library and EST Analyses

The immunological diagnosis of dicrocoeliosis remains unsatisfactory due to the lack of recombinant proteins that could be tested in the diagnosis. For this reason a double-strand cDNA library of *D. dendriticum* was synthesized from mRNA and ligated with Uni-ZAP XR vector, and then *E. coli* XL1-Blue MRF' cells were transformed with the ligation mixture and used for library propagation and maintenance (Martínez-Ibeas 2013; Martínez-Ibeas et al. 2013b). After construction and titration, the cDNA library was amplified for greater stability. The amplified cDNA library had 2×10^7 plaque forming units (pfu)/mL, the cDNA insertion rate was 90%, and the length of the cDNAs ranged from 120 to 1300 pb. The library was of good quality, because less than 20% useless sequences were found and 80% of the total sequenced genes were different ones. A random screening of 230 phage plaques was carried out by plaques PCR and then cloned in the pGEM-T easy vector. After edition and analysis sequences, the trimmed ESTs were assembled into 158 clusters, the most abundant of which was identified as mitochondrial DNA. All other ESTs were registered in GenBank under accession number JZ330400-JZ330572. Those EST sequences that displayed significant similarities with known sequences were categorized by their biological process, molecular function and cellular component according to information obtained from the Gene Ontology database. Many of the molecules described (Martínez-Ibeas et al., 2013b) could carry out important functions such as penetration and migration in host tissues, immunoevasion, digestion, redox homeostasis or cellular stress response that could serve as a starting point for the further research in disease control.

10.6.4.2 Obtaining and Evaluating of Recombinant Protein in Dicrocoeliosis Diagnosis

In order to identify the antigenic molecules of *D. dendriticum* that can be used in the serological diagnosis of dicrocoeliosis, three clones were selected to be expressed in recombinant form and evaluated in disease diagnosis (Martínez-Ibeas 2013; Martínez-Ibeas et al. 2013b). Proteins were selected based on the literature and the solubility and antigenicity results obtained through "in silico" sequence studies. Thus, the clones selected were (a) Clone No. 179 myoglobin, (b) Clone No. 182 8 kDa protein and (c) Clone No. 151 cystatin. Bioinformatic analysis of the three proteins was performed to ensure that they had a complete ORF and the presence of a possible signal peptide and to predict their secondary structure. Three cDNAs were subcloned into both the glutathione-*S*-transferase (GST) pGEX6P vector (Health Care) and the His6-tag pRSET vector (Life Technologies). A PCR assay was carried out using specific primers, including the restriction enzyme sites. Subsequenctly, the cDNAs were cloned into both vectors, and different IPTG expression conditions were tested in the transformed *E. coli*. The best results were obtained with pGEX-6P vector. In the case of the pRSET-A vector, it was only possible to induce cystatin expression in the insoluble fraction, and this hampers the subsequent purification process.

With the mentioned library, the authors (Martínez-Ibeas et al. 2013b) studied and published 173 ESTs. The results are very useful in the research and characterization of new genes and novel molecules that could help to carry out an early, sensitive and specific diagnosis of dicrocoeliosis and to develop vaccines if their

protection against the parasite is demonstrated. Furthermore, a cDNA encoding an 8-kDa protein was cloned, expressed in *E. coli* and evaluated as a potential diagnostic antigen, using Western blot and ELISA techniques. The results obtained by these authors indicate that it may be a valuable candidate in the serological diagnosis of dicrocoeliosis, although further study is needed to discover their true immunodiagnostic potential on dicrocoeliosis and to overcome the observed drawbacks of cross-reactions.

10.6.4.3 Characterization of Nine Polymorphic Microsatellite Loci for *D. dendriticum* Adults

A very interesting and detailed study has been carried out on the characterization of nine polymorphic microsatellite loci for *Dicrocoelium dendriticum* adults, collected from sympatric elk and cattle in the Cypress Hills Interprovincial Park, Alberta, Canada (van Paridon et al. 2016). Library construction and sequencing were carried out in the Cornell University (USA), using the methods described in Nali et al. (2014). Potential microsatellite loci sized 150–450 bp were selected for primer design. Testing and optimization of microsatellite loci were performed on a dataset of 66 adult worms collected from 4 elk livers and 3 cattle livers from Cypress Hills Provincial Park. The authors described the protocol followed by them to obtain a library construction. Moreover, they gave the characteristics of nine polymorphic loci isolated from *D. dendriticum* based on dataset with clones reduced to one copy, annealing temperature, number of alleles, gene diversity and inbreeding coefficient. The authors state that the nine polymorphic microsatellite loci will be of value when studying the population genetics, life cycle and global spread of this emerging trematode and could be used in assignment tests to potentially identify the source population(s) of the invasion of North America. The authors (van Paridon et al. 2016) comment that amongst the nine loci described, four deviated significantly from Hardy-Weinberg equilibrium (HWE) due to technical artefacts.

A very interesting paper was carried out on the population genetic analysis reports on the invasion history of the emerging trematode *D. dendriticum* in Canada (van Paridon et al. 2017b). According to these authors, parasite distributions are constantly changing due to climate change, local and global movement of animals and humans as well as land use and habitat change. Concerning *D. dendriticum* was reported for the first time in Eastern Canada in the 1930s and Western Canada in the 1970s, although historical records are scarce and its emergence is poorly understood. In order to explore the use of population genetic approaches to help elucidate the invasion history of a relatively recently established helminth parasites, the authors compare the genetic diversity and population structure of a number of *D. dendriticum* populations from Western and Eastern Canada and compare these with much longer established European populations. Two independent genetic marker systems were used: a microsatellite marker panel and a cytochrome c oxidase 1 (cox1) mitochondrial (mt) DNA sequence marker. The authors found differences in both genetic diversity and population structure of the different Canadian populations that provide insights into their invasion histories compared with the European ones. Two populations from British Columbia, Canada—Salt Spring and Vancouver Islands—are of low diversity, show evidence of a population bottleneck and are closely related to each other, suggesting a shared recent history of establishment. These west coast populations are otherwise most closely related to those from Eastern Canada and Western Europe and, in contrast, are genetically divergent from those in Cypress Hills, Alberta, Canada. Although the Alberta parasite population is the most recently reported in Canada, it was the most genetically diverse of those examined and showed a strong pattern of admixture of genotypes present in Western and Eastern Europe. The results obtained by these authors are consistent with a model in which Western Europe is likely the source of flukes on the east coast of Canada, which were then subsequently translocated to the west coast.

10.6.4.4 Molecular Characterization of *D. dendriticum* Exosomes

In the last few years, some papers on the molecular characterization of *D. dendriticum* exosomes have revealed the presence of miRNA (Bernal et al. 2014). In order to identify potential targets for intervention against parasitic helminths, the surface of the parasitic *D. dendriticum* was analysed by the mentioned authors. They identified 182 parasite proteins on the outermost surface of *D. dendriticum*. The presence of exosome-like vesicles in the ESP (excretory/secretory products) of *D. dendriticum* and their components has also been characterized. Using proteomic approaches, 84 proteins have been characterized in these vesicles. This work represented the first report of miRNAs in parasitic exosomes of *D. dendriticum* in which the authors performed a proteomic analysis of the external surface of the non-model organism *D. dendriticum* using the currently available datasets.

Furthermore, a method to hatch *D. dendriticum* eggs, obtain the miracidium DNA and develop a PCR molecular tool for detecting the presence of *D. dendriticum* eggs in the animal faeces has been developed (Sandoval et al. 2013). Moreover, some studies on morphological (Manga-González et al. 2001) and molecular detection by PCR (Martínez-Ibeas 2013; Martínez-Ibeas et al. 2011) of *D. dendriticum* larval stages in molluscs and ants, first and second intermediate hosts of this parasite, have been carried out. On the other hand, studies on the adult parasite have been carried out concerning isoenzymatic characterization (Campo et al. 1998), genetic variability (Sandoval et al. 1999; Morozova et al. 2002), molecular identification using the partial sequencing of 18S rDNA and the internal transcribed spacer nuclear (ITS-2) of ribosomal DNA (Otranto et al. 2007), the 28S and ITS-2 (Maurelli et al. 2007) and the ITS-2 (Bazsalovicsová et al. 2010). The interspecific variations comparing *D. dendriticum* and *D. hospes* (Maurelli et al. 2007) and *D. dendriticum* and *D. chinensis* (Otranto et al. 2007) have been shown. The ITS-2 rDNA provided a suitable marker to infer phylogenetic relationships of *Dicrocoelium* species (Biant et al. 2015).

Moreover, the characterization of *D. dendriticum* haplotypes from sheep, goat and cattle in Iran, based on the internal transcribed spacer 2 (ITS-2) and NADH dehydrogenase gene (nad1), has been carried out (Gorjipoor et al. 2015). These authors deposited them in GenBank under accession numbers JX050110-134 and JQ966972-3. Recently the molecular (28srDNA) characterization of *D. dendriticum* isolates from sheep, goat and cattle in Mazandaran Province (Iran) has been carried out (Bari et al. 2018). The authors of this paper concluded that, taking into account the molecular and morphometric results, *D. dendriticum* is the only species infecting sheep, goat and cattle in the province of Mazandaran.

10.7 Treatment, Control and Prevention of Dicrocoeliosis

Applying prophylactic and control measures to dicrocoeliosis caused by *D. dendriticum* is difficult due to the complexity of its biological life cycle; the low specificity of the parasite with reference to the numerous species of mammals, molluscs and ants, definitive and intermediate hosts; as well as the complexity of its epidemiology.

As there is no vaccine against *D. dendriticum*, the most effective control method is to administer an efficacious anthelminthic to the ruminants, taking into account the dicrocoeliosis epidemiological model in a specific area (Manga-González et al. 2001, 2010 Manga-González and González-Lanza 2005).

Although various anthelminthics have been used against *D. dendriticum* (mainly in ovine) by different authors, like albendazole, fenbendazole, luxabendazole, thiophanate, netobimin and diamphenethide, amongst others; nevertheless none are effective against the juvenile and immature stages of *D. dendriticum*, or if they are effective, as is diamphenethide, the dose must be high (240 mg/kg: 93–95% efficacy), and serious side effects appear after administration (Stratan 1986). This lack of effectiveness against juveniles

must be taken into account when applying a strategic control (Manga-González et al. 2010).

One of the more common and important anthelminthics used to control *D. dendriticum* infection is albendazole (ABZ) (Bártíková et al. 2011). This drug is a benzimidazole compound, possessing high activity against the lancet fluke. In the host ABZ is rapidly oxidized to ABZ sulphoxide (ABZ.SO), an active metabolite present in the bloodstream. Passive transport is the main mechanism for the entry of benzimidazole anthelminthics (ABZ and ABZ.SO) through the trematode tegument. However, active ABZ uptake and active efflux of ABZ and ABZ.SO also participate in the transport of these drugs in *D. dendriticum* (Bártíková et al. 2011). In mouflons with dicrocoeliosis, there is a significant increase in thiobenzamide-S-oxidase (TBSO) activity, mainly ascribed to flavine monooxygenases (FMO), and a significant decrease in glutathione-*S*-transferase (GST) has been documented (Skálová et al. 2007). Some substances of parasitic excreta or inflammation mediators may cause these changes in the expression of these liver biotransformation enzymes. In this study dicrocoeliosis in mouflons significantly increased ABZ.SO formation, probably because of the enhancement of FMO activity, but only caused mild changes in ABZ hepatic biotransformation, so the use of ABZ in the therapy of dicrocoeliosis can be recommended.

ABZ administered at a single dose of 10 or 15 mg/kg per os decreased parasitic burden by 92.22% (Cordero-del-Campillo et al. 1982) and by 99.6% (Himonas and Liakos 1980), respectively, in sheep naturally infected with *D. dendriticum*. A 94.05% reduction in *D. dendriticum* egg shedding was obtained with two doses of 10 mg/kg, 7 days apart (Cordero-del-Campillo et al. 1982). When the ABZ treatment was administered to sheep as intraruminal boluses (dose 42 mg/day), an efficacy of 91.89% was obtained (Corba and Krupicer 1992). Moreover efficacies from 92.9 to 94% were also obtained with doses of 15 and 20 mg/kg of albendazole in ovine in Germany (Schuster and Hiepe 1993). Ewes and ewe lambs treated with ABZ at an oral dose rate of 15 mg/kg (three times that recommended for the treatment of gastrointestinal nematode parasites) achieved a 79.2% reduction in *D. dendriticum* egg count 21 days post-treatment (Sargison et al. 2012).

Orally administered thiophanate treatment in ovine produced 100% efficacy with a 50 mg/kg (Ambrosi 1989) and a 200 mg/kg (Ambrosi et al. 1986) dose. Other authors (Brugêre-Picoux et al. 1986) recorded efficacies above 99% using doses of 50, 100 and 200 mg/kg.

When a dose of 10 mg/kg of luxabendazole was administered to sheep, the worm reduction was 59 and 99.1% according to different authors (Corba et al. 1987; Schuster and Hiepe 1993). Moreover, doses of 10 and 12.5 mg/kg reduced 63.2% and 83.3% of worms, respectively (Kassai et al. 1988).

The efficacy of netobimin administered at doses of 20 mg/kg was 98.8%, 97.9% and 89.1%, respectively, according to some authors (Sanz et al. 1987; Corba et al. 1993; Schuster and Hiepe 1993). When a dose of 15 mg/kg was administered to sheep (Rojo-Vázquez et al. 1989), the efficacy obtained was 91.9%. The efficacy of the two netobimin oral suspensions of 5% and 15% in sheep naturally infected with *D. dendriticum* was 90.80% and 91.50%, respectively (Senlik et al. 2008). On the other hand, the efficacy of mebendazole was 93–99.4% when a dose of 40–80 mg/kg was administered (Ambrosi and Grenolli 1991). The efficacy of fenbendazole varied from 99.9 to 100% when a dose of 100 mg/kg was administered (Stratan 1986). In an experiment carried out with a 50 mg/kg dose of praziquantel efficacies from 89 to 98% were obtained (Wolff et al. 1984). Praziquantel can be used to treat dicrocoeliosis in other species. It has been documented that 50 mg/kg oral praziquantel is required for efficacious dosing and that this dose rate is safe in llamas and thus is recommended for the treatment of camelids naturally infected with *D. dendriticum* (Dadak et al. 2013).

Taking into account the approach to dicrocoeliosis epidemiology described in Sect. 10.3, as well as in previous studies carried out in the North West of Spain (Alunda and Rojo-Vázquez 1983a; Manga-González et al. 2001; Manga-González and González-Lanza 2005) together

with an experiment on strategic control of *D. dendriticum* egg excretion in sheep carried out using an anthelminthic (ABZ) only effective against adult parasites (Manga-González et al. 2010), the following conclusion can be reached: In a zone with a continental climate within the Mediterranean Atlantic transition, the most effective control model consists of applying two treatments: one at the beginning of November (when ant hibernation starts) to eliminate the adult worms, and another treatment in January when, without reinfection (because ant hibernation does not finish until about the end of March), most of the metacercariae ingested by the animals until November have become adults capable of shedding eggs. By applying these two treatments, the biggest reduction in egg excretion—mainly in the cold period when elimination is highest and egg survival greatest—is obtained (Manga-González et al. 2010). Nevertheless, due to the complexity of dicrocoeliosis epidemiology, strategic treatments need to be repeated over several years to reduce the parasite load in ruminants, the contamination of pastures with viable parasite eggs and the infection rate in the mollusc and ant intermediate hosts.

Other authors also carried out their studies on the efficacy of some benzimidazoles against *D. dendriticum* (in Germany) in winter, mainly to research their effect on mature worms (Schuster and Hiepe 1993).

Restrictive husbandry practices like no grazing early in the morning or late in the evening when the highest number of ants in tetany is present in the herbage may be an option for dicrocoeliosis control. Methods against the intermediate host snails (molluscicide) and ants are not feasible due to their cost and for ecological reasons (Otranto andTraversa 2003).

10.8 Economic Impact of Dicrocoeliosis

The economic and health significance of dicrocoeliosis is partly due to the direct looses occasioned by the confiscation of altered livers (Del-Rio 1967; Lukin 1980; Karanfilovski 1983) and also the indirect ones caused by the digestive disorders derived from the hepatobiliary alterations caused by these parasites, such as decreased animal weight (Campo et al. 2000; Boray 1985), growth delay (Hohorst and Lämmler 1962), reduced milk production (Cavani et al. 1982), amongst others. Moreover, the additional costs incurred by the application of anthelminthic treatments, to which the animals must be subjected, have to be considered.

10.9 Other Genera of Interest

10.9.1 Genus *Eurytrema*: *Eurytrematodosis*

Dicrocoeliidae flukes of the genus *Eurytrema* Looss, 1907, responsible of mammal Eurytrematodosis, are common parasites which adults live in bile ducts, gall bladder, pancreatic ducts and intestine of cattle, buffaloes, camels, deer, goats, sheep, pigs and human beings from Europe, Madagascar, Asia and South America (Soulsby 1982; Graydon et al. 1992; Ilha et al. 2005; Pojmanska 2008). These parasites use in their life cycle land snails (genus *Bradybaena*) and grasshoppers (genus *Conocephalus*) as first and second intermediate hosts, respectively (Soulsby 1982). The most important species of the genus is the type species *Eurytrema pancreaticum* (Jubb and Stent 2016). Moreover, *Eurytrema coelomaticum* is also common in Brazilian cattle and in China ruminants (Ilha et al. 2005; Jubb and Stent 2016); *Eurytrema cladorchis* is found in cattle in Bangladesh (Mohanta et al. 2016), as well as *Eurytrema procyonis* in raccoons in the USA (Jubb and Stent 2016). In the final host metacercariae de-encyst in the duodenum and migrate to the pancreas. Infection of the pancreatic ducts leads to chronic interstitial pancreatitis (Jubb and Stent 2016). The fluke body of *E. cladorchis* is flattened, elongated to oval with smooth margins. The oral sucker is subterminal and smaller than the ventral sucker, which is located at the middle to anterior one third of the body. The pharynx is small, the oesophagus is short, and the caeca are narrow and

bifurcated at the level of the genital pore reaching posteriorly along the body margin beyond the posterior limit of the vitelline glands but remaining short from the posterior extremity of the body. The testes are large. The cirrus-sac is elongated, containing the seminal vesicle inside and situated between the caecal bifurcation and acetabulum. The genital pore is median. The ovary is lobulated (4–10 lobes). The vitelline glands are long. Most of the hind body is occupied by the uterus which contains dark brown operculated eggs (Mohanta et al. 2016).

The infection prevalence by *E. coelomaticum* in cattle was 47.8% in Brazil (Bassani et al. 2006). Progressive emaciation was the most common clinical sign, and high plasma amylase concentration has been observed suggesting exocrine pancreatic insufficiency (Ilha et al. 2005). Grossly there were multiple dilated pancreatic ducts (mainly in the left pancreatic lobe for *E. pancreaticum*) with thickened whitish walls containing trematodes. Histologically, extensive pancreatic parenchymal loss with replacement fibrosis and interstitial inflammatory infiltrate, predominantly lymphocytic, as well as ectatic ducts with hyperplastic epithelium and periductal fibrosis were found (Fávero et al. 2016; Jubb and Stent 2016; Schwertz et al. 2016). A granulomatous inflammatory reaction around the trematode eggs was also observed (Ilha et al. 2005). Positive correlation between *E. coelomaticum* burden and histopathological changes of the pancreas has been observed (Schwertz et al. 2016). The occurrence of oxidative stress caused by *E. coelomaticum* in naturally infected cattle has also been investigated. A positive and significant correlation has been proved between levels of thiobarbituric acid reactive substances (TBARS) in the pancreas with serum, suggesting that lipid peroxidation products produced in the pancreas during infection by the trematode have systemic oxidizing effects on the host organism, which can affect other systems, organs and cells (Schwertz et al. 2016). NTPDase enzyme is responsible for the hydrolysis of the ATP into ADP and AMP. This enzyme acts as an anti-inflammatory agent, since it controls the levels of nucleotide ATP, a pro-inflammatory molecule; 5′ nucleotidase enzyme hydrolyzes AMP to adenosine, an important endogenous anti-inflammatory molecule. NTPDase enzyme showed high activity in *E. coleomaticum* naturally infected cattle, probably acting in the removal of excess ATP nucleotide released by pancreatic damage due to the presence of the parasite in the tissue. The positive correlation between NTPDase and 5′ nucleotidase enzymatic activities and the degree of parasitism and histopathological lesions that has been observed suggest that these enzymes are involved in the modulation of inflammation, and they can act as markers of inflammatory response (Fávero et al. 2016). In a later study, using the same groups of animals and the same protocol as Fávero et al. (2016), a reduction of the enzyme adenosine deaminase (ADA) activity was observed in the pancreas in *E. coelomaticum* naturally infected cattle (Grosskopf et al. 2017). ADA is critical for modulating the immune system. The reduction in ADA activity in the pancreas allows adenosine to exert a possible anti-inflammatory effect by reducing the immune response against the parasite and favouring infection, since a negative correlation between ADA activity and cellular damage has been observed (Grosskopf et al. 2017). Adenosine, a purine nucleoside, is elaborated at injured and inflamed sites by parasite infections and has a central role in the regulation of inflammatory responses and in limiting inflammatory tissue destruction such as in *E. coelomaticum* infections (Grosskopf et al. 2017). In sheep severe parasitism by *E. pancreaticum* was characterized by progressive weight loss, chronic pancreatitis, fibrosis and atrophy of the pancreatic parenchyma (Graydon et al. 1992). Marked glucosuria and diabetes mellitus have been described in cases of eurytrematodosis in sheep (Graydon et al. 1992).

Little genomic information is available in the public databases about any members of family Dicrocoeliidae. A novel transcriptome of the adult stage of *E. pancreaticum* had been produced that should provide a useful resource for designing new strategies against pancreatic flukes of human and animal health significance (Liu et al. 2016). The complete mitochondrial (mt) genome of *E. pancreaticum* is 15,031 bp in size

and encodes 36 genes: 12 protein-coding genes, 2 ribosomal RNA genes and 22 transfer RNA genes (Chang et al. 2016). This phylogenetic analysis shows that *E. pancreaticum* is more closely related to *D. chinensis* and *D. dendrititum* (Chang et al. 2016).

Interspecific variation between 18S rRNA sequence of *E. cladorchis* and *E. pancreaticum* (0.4–0.6%) as well as between 18S rRNA sequences of *E. cladorchis* and *E. coelomaticum* (1.0–1.1%) has been established (Mohanta et al. 2016).

Recently the complete nuclear ribosomal DNA (rDNA) sequences of *E. pancreaticum* in five individuals were determined and have been deposited in GenBank under accession numbers KY490000–KY490004. The 18S, ITS1, 5.8S, ITS-2 and 28S rDNA regions were all quite strongly conserved, with no length variations, and the length variation in the IGS rDNA region was only 4 bp (Su et al. 2018).

10.9.2 Genus *Platynosomum*: Platynosomiosis

The adult parasites of genus *Platynosomum* Looss, 1907 (Dicrocoeliidae), responsible for platynosomiosis in birds and mammals in Europe, Africa, Asia, North, Central and South America, live in the liver, gall bladder and pancreas of these animals. Known life cycles of *Platynosomum* type use land molluscs, arthropods as second and lizards as paratenic (or third intermediate) hosts (Pojmanska 2008).

Platynosomum fastosum has been detected in the liver and bile ducts of domestic cats in Malaysia, Northern Vietnam as well as Central and South America, the Caribbean and southern USA (Soulsby 1982; Nguyen et al. 2017). This parasite, considered synonymous of *Platynosomum illiciens* found in birds (Pinto et al. 2016), is of veterinary significance and evolves in snails (*Subulina octona*) and lizards (*Anolis cristatellus*). Mice infection with encysted metacercariae of *P. illiciens* (=*P. fastosum*) recovered from terrestrial isopods (*Oniscidea* spp. and *Nagurus nanus)* and lizards (*Hemidactylus mabouia*), and the recovery of adult parasites in this experimental definitive host demonstrates that lizards are not the obligatory third intermediate hosts involved in the transmission of *P. illiciens* (=*P. fastosum*) (Pinto et al. 2014). It has also been demonstrated that *Subulina octona* is the first intermediate host of *P. illiciens* (=*P. fastosum*) in South America, and terrestrial isopods are new natural second intermediate hosts of the parasite (Pinto et al. 2014). A recent study has provided the first molecular evidence that *P. illiciens* can naturally infect felids and nonhuman primates and infect rodents in experiments (Pinto et al. 2018). Moreover, ITS sequences proved that the same *Platynosomum* species infects cats in the Americas and Asia, thus supporting the synonymy of *P. illiciens* and *P. fastosum*. Thus these authors (Pinto et al. 2018) proposed that *P. illiciens* should be used as the correct name of the causative agent of feline platynosomosis in the Americas and Southeast Asia.

The cats become infected by eating metacercariae from naturally infected lizards. The metacercariae migrate from the common bile duct to the gall bladder and bile ducts, where they develop into adult trematodes (Ferreira et al. 1999). The adults are characterized by ellipsoidal, flattened and brownish bodies, 2–6 mm long and 0.5–2 mm wide, with the anterior end rounded and the posterior end tapered; thin smooth cuticle; oral sucker; acetabulum in the anterior half of the body; short oesophagus; small intestinal caeca; testicles symmetrical; ovary located under the right testicle; uterus, in the posterior part of the body, containing small dark brown eggs; and bilaterally vitelline glands located in the middle third of the body, cirrus-sac, that may reach the anterior border of ventral sucker and common genital pore, antero-sinistral to intestinal bifurcation (Andrade et al. 2012; Nguyen et al. 2017). According to Nguyen et al. (2017), *P. fastosum* can be distinguished from *P. illiciens* by only two criteria: the class of hosts (mammals and birds, respectively) and the testis shape. The partial region of rDNA, namely, the ITS1 and the 5.8S from 3 *P. fastosum* specimens,

was studied in order to establish the phylogenetic position of this trematode (Nguyen et al. 2017). The smallest interspecific distance amongst dicrocoeliid species was 20.9%.

The infection prevalence by *P. fastosum* in free-roaming domestic cats was 42.6% in Brazil (Braga et al. 2016). In cats the disease is generally asymptomatic, but in some cases this trematode causes vomiting, diarrhoea and jaundice (Soulsby 1982). Clinical signs and abnormalities such as hepatomegaly, coagulation disorders, hypoalbuminemia, abnormal levels of liver-linked serum enzymes, anaemia, eosinophilia and thrombocytopenia have been observed in *P. fastosum*-infected black-tufted marmosets (*Callithrix penicillata*) (Mattioli et al. 2016).

Dilation and thickening of the gall bladder wall, common bile duct and bile ducts were macroscopic findings associated with the presence of this parasite in cats (Andrade et al. 2012; Headley et al. 2012; Braga et al. 2016). Histologically a non-suppurative fibrosing cholangiohepatitis, characterized by bile duct fibrosis, hyperplasia of the ductal epithelium and infiltrate (mainly lymphocytes, some macrophages and eosinophils) as well as cholecystitis, has been observed (Andrade et al. 2012; Headley et al. 2012; Braga et al. 2016**;** Cullen and Stalker 2016). A relationship between the *P. fastosum* infection and the appearance of cholangiocarcinomas has been reported in cats (Andrade et al. 2012). At least five methods using faecal samples have been described to diagnose the presence of *P. fastosum*. They include direct smear, zinc sulphate, sucrose and modified detergent floatation and formalin-ether sedimentation techniques (Basu and Charles 2014). The FLOTAC technique is also considered as an efficient copromicroscopic technique that may be used to enhance the diagnosis of *P. fastosum* in cats and may be extended to the diagnosis of platynosomiasis in other vertebrate host species (Nascimento Ramos et al. 2016).

The control measures include faecal screening, controlling the feral cat population and incorporating a trematode treatment drug into a parasite control programme (Warren et al. 1998). Two praziquantel treatment regimens have been evaluated using *post-mortem* fluke counts for *P. fastosum* infections in cats: a high-dose treatment of 20 mg/kg body weight (BW) administered intramuscularly (IM) once a day for 3 consecutive days and a low-dose treatment of 5 mg/kg BW administered once (IM) and repeated 14 days later. Neither treatment was 100% effective (Lathroum et al. 2018).

This parasite has also been diagnosed in orangutans (*Pongo pygmaeus*) with clinical signs of inappetence and chronic diarrhoea and lesions in bile ducts and liver tissue.

The species *Platynosomum proxillicens* (Warren et al. 1998) caused bile duct hyperplasia and fibrosis as well as hepatic inflammation in cockatoos (*Cacatua sulfurea*) (Kazacos et al. 1980).

10.9.3 Genus *Brachylecithum*

A molecular taxonomic phylogenetic study was carried out for the first time on the genus *Brachylecithum* Shtrom, 1940 (Dicrocoeliidae) (Hildebrand et al. 2016). The adult specimens of *Brachylecithum* used by the authors in their studies were collected from birds that came from Czech Moravia, with some additional birds sampled in Poland (Mazovia District, Baltic Coast). The authors used two markers: the nuclear ribosomal 28S DNA (rDNA) gene and the mitochondrial *cox1* gene, for six species of the genus. They described the DNA extraction, gene amplification and sequencing. Eleven partial 28S rDNA sequences (1198 bp) and partial *cox1* sequences (388 bp) were obtained by them from adult trematodes of the genus *Brachylecithum*, *B. capilliformis*, *B. glareoli*, *B. kakea*, *B. laniicola*, *B. lobatum* and *B strigis*, and from larvae obtained from snails. They carried out the first phylogenetic analysis based on partial 28S rDNA gene with the newly generated sequences of *Brachylecithum* and selected sequences of dicrocoeliids from the GenBank.

The authors carried out Bayesian analysis of the partial sequences of the 28S rDNA gene of 16 members of Dicrocoeliidae and, in addition, the

same type of analysis of the partial mitochondrial protein-coding gene *cox1* derived from nine isolates of *Bachylecithum* spp. Moreover, they carried out a Bayesian analysis of partial sequence 28S rDNA+parcial sequence *cox1* data of nine members of the *Brachylecithum* genus.

References

Afzal M, Shafique M, Hussain A, Saeed M (1981) A study of helminths of cattle and buffaloes in Lahore. Pakistan J Sci 33:14–20

Agrawal SC, Sharma SK (1990) On a new trematode: neodicrocoeliinae subf. n., *Neodicrocoelium gayaparsadai* gen. n. et sp. n. (Trematoda, Dicrocoeliidae) from the airbreathing fish, *Channa marulius* (Ham.) from Jhansi (India). Helminthologia 27:91–98

Al-Khalidi NW, Al-Bayati MMA (1989) The epidemiology of dicrocoeliasis in sheep in Mosul (Iraq). J Vet Parasitol 3:21–23

Alunda JM, Manga-González MY (1982) Susceptibility of some species of the genus *Helicella* Férussac, 1821 (Gastropoda, Helicidae) to the infection by *Dicrocoelium dendriticum* (Trematoda). Malacologia 22:51–54

Alunda JM, Rojo-Vázquez FA (1982) Susceptibility of some populations of *Cernuella (Xeromagna) cespitum arigonis* (Schmidt, 1875) (Gastropoda, Helicidae) from the Douro basin (Iberian Peninsula) to the infection by *Dicrocoelium dendriticum* (Trematoda). Malacologia 22:39–43

Alunda JM, Rojo-Vázquez FA (1983a) Survival and infectivity of *Dicrocoelium dendriticum* eggs under field conditions in NW Spain. Vet Parasitol 13:245–249

Alunda JM, Rojo-Vázquez FA (1983b) Effect of infection rate and host age on the intramolluscan development of *Dicrocoelium dendriticum*. Helminthologia 20:251–258

Alunda JM, Rojo-Vázquez FA (1984) Some new molluscan hosts of *Dicrocoelium dendriticum* in Spain. Ann Parasitol Hum Comp 59:57–62

Álvarez-Nogal R, Manga-González MY, González-Lanza C, Del Pozo Carnero P (1992) Preliminary study on *Dicrocoelium dendriticum* (Trematoda) first larval stages in experimentally infected *Cernuella (Xeromagna) cespitum arigonis* (Mollusca). In: Abstracts of the VIth European Multicolloquium of Parasitology, The Hague

Alzieu JP, Ducos De Lahitte J (1991) La dicrocoeliose chez les bovins. Bull GTV 6:135–146

Ambrosi M (1989) Efficacia del tiofanato a dose normale contro *Dicrocoelium dendriticum*. Il Nuovo Prog Vet 15–16:1–7

Ambrosi M, Grenolli V (1991) Prove di campo sull'uso del mebendazole nel poliparassitismo ovino. Ob Doc Vet 12:89–91

Ambrosi M, Principato M (1981) Dicroceliosi ovina: variazioni stagionali della positività coproscopica in animali spontaneamente parassitati. Ob Doc Vet 2:55–56

Ambrosi M, Morettini B, Ranucci S, Tesei B (1986) Efficacia della somministrazione orale di tiofanato (1-bis(3-etossicarbonil-2-tioureido)benzene) per la terapia dell'infestione da *Dicrocoelium dendriticum* negli ovini. Riv Zootec Vet 14:25–31

Andrade RIFS, Dantas AFM, Pimentel LA, Galiza GJN, Carvalho FKL (2012) *Platynosomum fastosum*-induced cholangiocarcinomas in cats. Vet Parasitol 190:277–280

Angelovski T, Iliev A, Madzirov Z (1970) Nasi noviji rezultati istrazivanja epizootiologije dikrocelioze u ovaca. Vet Glasnik 24:995–999. En: *Helm. Abstr*, 42, 1126

Badie A (1978) La dicroceliose ovine: incidence des facteurs climatiques et contribution à la mise au point d'une méthode de prévision. Ann Parasitol Hum Comp 53:373–385

Badie A, Hourdin P, Rondelaud D (1992) L'infestation de *Cochlicopa lubrica* Müller par *Dicrocoelium lanceolatum* Rudolphi. A propos de quelques observations histophatologiques. Bull Soc Fr Parasitol 10:51–57

Bahrami S, Jalali MHR, Jafari A (2015) Evaluation of hepatic antioxidant changes in ovine dicrocoeliasis. J Parasit Dis 39:766–769

Bailenger J, Tribouley J, Amyot B, Duret J (1965) Importance des Léporides comme réservoirs sauvages dans l'épidemiologie des distomatoses a *Fasciola hepatica* et *Dicrocoelium dendriticum*. Ann Parasitol Hum Comp 40:51

Baldelli B, Piergili-Fioretti D, Ambrosi M, Polidori GA, Grelloni V, Moretti A (1981) Dicroceliosi ovina: studio degli anticorpi verso *Dicrocoelium dendriticum* in agnelli nati da pecore spontaneamente infertte. Riv Parassitol 42:23–30

Bari S, Arbabi M, Gill P, Sharif M, Hezarjaribi HZ, Dodangeh S, Alizadeh A, Hedayati Z, Sarvi S (2018) Morphological and molecular (28s RDNA) characterization of *Dicrocoelium dendriticum* isolates from sheep, goat and cattle in Marzandaran province, Iran. J Mazandaran Univ Med Sci 27:38–45

Bártíková A, Vokřál I, Forstová-Křížová V, Skálová L, Lamka J, Szotákova B (2011) The transport of albendazole and albendazole sulphoxide in the lancet fluke (*Dicrocoelium dendriticum*). Vet Parasitol 176:27–33

Bassani CA, Sangioni LA, Saut JPE, Yamamura MH, Headley SA (2006) Epidemiology of eurytrematosis (*Eurytrema* spp. Trematoda: Dicrocoeliidae) in slaughtered beef cattle from the central-west region of the State of Paraná, Brazil. Vet Parasitol 141:356–361

Basu AK, Charles RA (2014) A review of the cat liver fluke *Platynosomum fastosum* Kossack, 1910 (Trematoda: Dicrocoeliidae). Vet Parasitol 200:1–7

Batelli G, Poglayen G, Martini M (1987) Indagine sulla diffusione della fascioliasi bovina in provincia di Firenze. Summa 4:45–49

Bazsalovicsová E, Králová-Hromadová I, Spakulová M, Reblánová M, Oberhauserová K (2010)

Determination of ribosomal internal transcribed spacer 2 (ITS-2) interspecific markers in *Fasciola hepatica*, *Fascioloides magna*, *Dicrocoelium dendriticum* and *Paramphistomum cervi* (Trematoda), parasites of wild and domestic ruminants. Helminthologia 47:76–82

Beck MA, Goater CP, Colwell DD, van Paridon BJ (2014) Fluke abundance versus host age for an invasive trematode (*Dicrocoelium dendriticum*) of sympatric elk and beef cattle in southeastern Alberta, Canada. Int J Parasitol Parasites Wildlife 3:263–268

Beck MA, Goater CP, Colwell DD (2015) Comparative recruitment, morphology and reproduction of a generalist trematode, *Dicrocoelium dendriticum*, in three species of host. Parasitology 142:1297–1305

Ben Amer KF, Ahmed Z (1980) Studies on the prevalence of liver flukes with special reference to *Dicrocoelium dendriticum* (Rudolphi, 1891) Looss 1899 in sheep and cattle from Tripoli region Libya. Riv Parassitol 41:15–18

Bernal D, Trelis M, Montane S, Cantalapiedra F, Galiano A, Hackenberg M, Marcilla A (2014) Surface analysis of *Dicrocoelium dendriticum*. The molecular characterization of exosomes reveals the presence of miRNAs. J Proteomics 105:232–241

Biant QQ, Zhao GH, Jia YQ, Fang YQ, Cheng WY, Du SZ, Ma XT, Lin Q (2015) Characterization of *Dicrocoelium dendriticum* isolates from small ruminants in Shaanxi Province, North-Westem China, using intemal transcribed spacers of nuclear ribosomal DNA. J Helminthol 89:124–129

Blasco S, Feliu C, Torres J, Casanova JC, Miguel J, Ruiz-Olmo J (1993) *Oryctolagus cuniculus* (Linnaeus, 1758) hospedador de *Dicrocoelium dendriticum* (Rudolphi, 1819) en Cataluña. Acta Parasitol Port 1(2):171

Bocharova MM (1984) (Zonal distribution of foci of *Dicrocoelium* infection on the northern slopes of the Central Caucasus and the eastern pre-Caucasus region (USSR)). Materialy Nauchnoi Konferentsii Vsesoyuznogo Obshchestva Gel'mintologov (Biologiya y taksnomiya gel'mintov zhivotnykh y cheloveka) 34:97–105

Bode L, Geyer E (1981) Experimental dicrocoeliasis: the humoral immune response of gladden hamsters and rabbits to primary infection with *Dicrocoelium dendriticum*. Z Parasitenkd 66:167–178

Bolukbas CS, Gurler AT, Beyhan YE, Acici M, Umur S (2012) Helminths of roe deer (*Capreolus capreolus*) in the Middle Black Sea Region of Turkey. Parasitol Int 61:729–730

Boray JC (1982) Molluscan hosts and trematodes. In: Symons LEA, Donald AD, Dineen JK (eds) Biology and control of endoparasites. Academic Press, Sydney

Boray JC (1985) Flukes of domestic animals. In: Gaafar SM, Howard WE, Marsh RE (eds) Parasites, pests and predators. World Animal Science. B Disciplinary approach. Elsevier Science Publishers B.V, Amsterdam

Botnevik CF, Malagocka J, Jensen B, Fredensborg BL (2016) Relative effect of temperature, light, and humidity on clinging behaviour of metacercariae infected ants. J Parasitol 102:495–500

Bourgat R, Séguin D, Bayssade-Dufour C (1976) Données nouvelles sur *Dicrocoelium hospes* Looss, 1907: Anatomie de l'adulte et cycle évolutif. Ann Parasitol Hum Comp 50:701–714

Braga RR, Teixeira AC, Oliveira JAA, Cavalcanti LPG (2016) Prevalence of *Platynosomum fastosum* infection in free roaming cats in northeastern Brazil: fluke burden and grading of lesions. Vet Parasitol 227:20–25

Braun U, Wolfensberger R, Hertzbrg H (1995) Diagnosis of liver flukes in cows—a comparison of the findings in the liver, in the faeces, and in the bile. Schweiz Arch Tierheilkd 137:438–444

Brugêre-Picoux J, Tafani JP, Chirol C, Morand M (1986) Efficacité du thiophanate sur *Dicrocoelium lanceolatum* chez le mouton. Rec Méd Vét 162:973–978

Calamel M (1976) La dicrocoeliose ovine et caprine dans le Sud-Est de la France. Rev Med Vet 127:1529–1536

Camara L, Pfister J, Aeschlimann A (1996) Analyse histopathologique de foie de bovin infesté par *Dicrocoelium dendriticum*. Vet Res 27:87–92

Campo R, Manga-González MY, González-Lanza C (1998) Characterization of adult *Dicrocoelium dendriticum* by isoelectric focusing. J Helminthol 72:109–116

Campo R, Manga-González MY, González-Lanza C (2000) Relationship between egg output and parasitic burden in lambs experimentally infected with different doses of *Dicrocoelium dendriticum* (Digenea). Vet Parasitol 87:139–149

Cavani C, Losi G, Manfredini M, Pavoncelli RM, Pietrobelli M, Restani R (1982) Ricerche sull'influenza della dicroceliasi sulle caratteristiche quantitative e qualitative della produzione di latte in pecore. Ob Doc Vet 3:59–63

Cengiz ZT, Yilmaz H, Dulger AC, Cicek M (2010) Human infection with *Dicrocoelium dendriticum* in Turkey. Ann Saudi Med 30:59–161

Chandra G (1973) First detection of *Dicrocoelium* eggs in the faeces of experimentally infected lambs, Guinea pigs and pigs. HAU J Res 3:31–34

Chang QC, Liu GH, Gao JF, Zheng X, Zhang Y, Duan H, Yue D-M, Fu X, Su X, Gao Y, Wang C-R (2016) Sequencing and characterization of the complete mitochondrial genome from the pancreatic fluke *Eurytrema pancreaticum* (Trematoda: Dicrocoeliidae). Gene 576:160–165

Colwell DD, Goater CP (2010) *Dicrocoelium dendriticum* in cattle from Cypress Hills, Canada: humoral response and preliminary evaluation of an ELISA. Vet Parasitol 174:162–165

Corba J, Krupicer I (1992) Efficacy of intraruminal albendazole boluses against *Dicrocoelium dendriticum* in sheep. Parasiol Res 78:640–642

Corba J, Hovorka J, Spaldonova R, Stoffa P, Legeny J, Andrasko H (1987) Efficacy of luxabendazole (hoe 216 V) sup. 5% in sheep naturally infected with the most important helminths. Helminthologia 24:227–235

Corba JA, Krupicer L, Petko B, Varady M (1993) Efficacy of netobimin against liver flukes, gastrointesinal and lungworms in naturally infected sheep. Helminthologia 30:57–61

Cordero-del-Campillo M, Rojo-Vázquez FA, Díez-Baños P, Chaton-Schaffner M (1982) Efficacité de l'albendazole contre une infestation naturelle a *Dicrocoelium dendriticum* chez le mouton. Rev Med Vet 133:41–49

Cullen JM, Stalker MJ (2016) Liver and biliary system. In: Maxie MG (ed) Jubb, Kennedy, and Palmer's pathology of domestic animals, 6th edn. Elsevier, St. Louis

D'Ovidio D, Rinaldi L, Ianniello D, Donnelly TM, Pepe P, Capasso M, Cringoli G (2014) FLOTAC for diagnosis of endo-parasites in pet squirrels in southern Italy. Vet Parasitol 200:221–224

Dadak AM, Wiesera C, Joachimb A, Franz S (2013) Efficacy and safety of oral praziquantel against *Dicrocoelium dendriticum* in llamas. Vet Parasitol 197:122–125

Dawes B (1968) The Trematoda. Cambridge University Press, Cambridge

Del-Rio J (1967) Epizootiología de la dicroceliosis en la provincia de León. An Fac Vet León 13:211–253

Dementev IS, Karabaev DK (1968) (Infection of terrestrial molluscs and ant by *Dicrocoelium dendriticum* larvae in southeastern Kazakhstan). Vestik sel'-skokhozyaistvennoi Nauki, Alma-Ata 122:51–55

Denev I, Savova S, Stoimenov K, Tahirov B, Donev A, Kassabov R, Petrov P (1970a) [An investigation into the dicrocoeliosis in north-eastern Bulgaria. II. On the intermediary and additional hosts of *Dicrocoelium lanceatum* (Stiles et Hassal, 1896)]. Vet Med Nauki 7:23–31

Denev I, Stoimenov K, Donev A, Petrov P, Kasabov R (1970b) Dicrocoeliasis in North-Eastern Bulgaria. I. Geographical and seasonal incidence. Vet Med Nauki Sofia 7:21–29

Dhar DN, Shing KS (1963) Histochemical studies on liver in dicrocoeliasis. Indian J Vet Sci 33:142–151

Diakou A, Sokos C, Papadopoulos E (2014) Endoparasites found in European brown hares (*Lepus europaeus*) hunted in Macedonia, Greece. Helminthologia 51:345–351

Ekstam B, Johansson B, Dinnétz P, Ellström P (2011) Predicting rik habitats for the transmission of the small liver fluke, *Dicrocoelium dendriticum* to grazing ruminants. Geospat Health 6:125–131

Ershov VS (1964) Textbook of veterinary helminthology. Izdatelstvo Kolos, Moscow

Ezatpour B, Hasanvand A, Azami M, Anbari K, Ahmadpour F (2015) Prevalence of liver fluke infections in slaughtered animals in Lorestan, Iran. J Parasit Dis 39:725–729

Fagbemi BO, Obarisiagbon IO (1991) Common antigens of *Fasciola gigantica, Dicrocoelium hospes* and *Schistosoma bovis* and their relevante to serology. Vet Q 13:81–87

Fashuyi SA, Adeoye GO (1986) The possible snail intermediate hosts of *Dicrocoelium hospes* in Nigeria. Acta Vet Brno 55:85–88

Fauna Europaea (2012) Fauna Europaea version 2.5 web service available on line at http://www.faunaeur.org/full

Fávero JF, Schwertz CL, Doleski PH, Leal DBR, Machado G, Manzoni AG, de Silva ES, Gabriel ME, Stedilleb FA, Christ R, Stefanía LM, Mendes RE, da Silva AS (2016) NTPDase and 5′-nucleotidase as inflammatory markers in cattle naturally infected by *Eurytrema coelomaticum*. Comp Immunol Microbiol Infect Dis 48:48–53

Ferreira AMR, Almeida ECP, Labarthe NV (1999) Liver fluke infection (*Platynosomum concinnum*) in Brazilian cats: prevalence and pathology. Feline Pract 27:19–22

Ferreras MC, Campo R, González-Lanza C, Pérez V, García-Marín JF, Manga-González MY (2007) Immunohistochemical study of the local immune response in lambs experimentally infected with *Dicrocoelium dendriticum* (Digenea). Parasitol Res 101:547–555

Fuertes M, Pérez V, Benavides J, González-Lanza MC, Mezo M, González-Warleta M, Giráldez FJ, Fernandez M, Manga-González MY, Ferreras MC (2015, l209) Pathological changes in cattle naturally infected by *Calicophoron daubneyi* adult flukes. Vet Parasitol:188–196

Goater CP, Colwell DD (2007) Epidemiological characteristics of an invading parasite: *Dicrocoelium dendriticum* in sympatric wapiti and beef cattle in southern Alberta, Canada. J Parasitol 93:491–494

Godara R, Katoch R, Anish Y, Borah MK (2014) Dicrocoeliosis in goats in Jammu, India. J Parasit Dis 38:201–204

Gómez BJ, Manga-González MY, Angulo E, González-Lanza C (1996) Alteraciones histológicas producidas por *Dicrocoelium dendriticum* (Trematoda) en dos especies de *Cernuella* (Mollusca) sacrificadas a los tres meses post-infestación. Iberus 14:189–195

González-Lanza C, Manga-González MY, Del-Pozo P (1993) Coprological study of the *Dicrocoelium dendriticum* (Digenea) egg elimination by cattle in highland areas in León Province, Northwest Spain. Parasitol Res 79:488–491

González-Lanza C, Manga-González MY, Campo R, Del-Pozo MP (1997) Larval development of *Dicrocoelium dendriticum* in *Cernuella (Xeromagna) cespitum arigonis* under controlled laboratory conditions. J Helminthol 71:311–317

González-Lanza C, Manga-González MY, Campo R, Del-Pozo MP, Sandoval H, Oleaga A, Ramajo V (2000) IgG antibody response to ES or somatic antigens of *Dicrocoelium dendriticum* (Trematoda) in experimentally infected sheep. Parasitol Res 86:472–479

González-Lanza C, Manga-González MY, Revilla-Nuín B (2006) Preliminary protective capacity study of a *Dicrocoelium dendriticum* antigenic protein in hamsters. Parasitol Res 99:749–752

Gorjipoor S, Moazeni M, Sharifiyazdi H (2015) Characterization of *Dicrocoelium dendriticum* haplotypes from sheep and cattle in Iran based on the

internal transcribed spacer 2 (ITS-2) and NADH dehydrogenase gene (*nad*1). J Helminthol 89:158–164
Graydon RJ, Carmichael IH, Sánchez MD, Weidosari E, Widjayanti S (1992) Mortalities and wasting in Indonesian sheep associated with the trematode *Eurytrema pancreaticum*. Vet Rec 131:443
Grigoryan GA, Akopyan VD (1960) [Experimental data on the biology of *Dicrocoelium lanceolatum* Stiles & Hassall, 1896]. Trudi Armyanskogo Nauchno Issledovatelskogo Instituta Zhivotnovodstva i Veterinarii 4:247–254
Groschaft J (1961) [Mravenci doplnkoví mezihostitelé motolice (*Dicrocoelium dendriticum* Rudolphi 1819)]. Česk Parasitol 8:151–165
Grosskopf HM, Schwertz CL, Machado G, Bottari NB, da Silva ES, Gabriel ME, Lucca NJ, Alves MS, Schetinge MRC, Morsch VM, Mendes RE, da Silva AS (2017) Cattle naturally infected by *Eurytrema coelomaticum*: relation between adenosine deaminase activity and zinc levels. Res Vet Sci 110:79–84
Gu JT, Liu RK, Li QF, Wang XM, Da LT, Tang CT, Tang ZZ (1990) Epidemiological survey on *Eurytrema pancreaticum* and *Dicrocoelium chinensis* in sheep in the southern area of Daxinganling Mountain of Inner Mongolia. Chin J Vet Sci Technol 3:15–16
Gualdieri L, Rinaldi L, Petrullo L, Morgoglione ME, Maurelli MP, Musella V, Piemonte M, Caravano L, Coppola MG, Cringoli G (2011) Intestinal parasites in the city of Naples (southern Italy). Acta Trop 117:196–201
Gürelli G (2017) Dicrocoeliosis Epidemiyolojisinde Kara Salyangozlarinin Önemi. Turk Parazitol Dernegi 41:169–172
Gutiérrez JF, Castellá J, Muñoz E, Lacasa MI, Ferrer J, Meya J (1993) Parásitos de rumiantes salvajes em Cataluña (NE de España). Acta Parasitol Port 1(2):196
Hayashi K, Tang WQ, Ohari Y, Ohtori M, Mohanta UK, Matsuo K, Sato H, Itagaki T (2017) Phylogenetic relationships between *Dicrocoelium chinensis* populations in Japan and China based on mitochondrial *nad1* gene sequences. Parasitol Res 116:2605–2609
Headley SA, Gillen MA, Sanches AW (2012) *Platynosomum fastosum*-induced chronic intrahepatic cholangitis and *Spirometra* spp infections in feral cats from Grand Cayman. J Helminthol 86:209–214
Henkel H (1931) Untersuchungen zur Ermittlung des Zwischenwirtes von *Dicrocoelium lanceatum*. Z Parasitenkd 3:664–712
Heussler V, Kaufmann H, Glaser I, Ducommun D, Müller C, Dobbelaere D (1998) A DNA probe for the detection of *Dicrocoelium dendriticum* in ants of *Formica* spp. and *Lasius* spp. Parasitol Res 84:505–508
Hilbe M, Robert N, Pospischil A, Gerspach C (2015) Pulmonary arterial lesions in new world camelids in association with *Dicrocoelium dendriticum* and *Fasciola hepatica* infection. Vet Pathol 52:1202–1209
Hildebrand J, Sitko J, Zalesny G, Jezewski W, Laskowski Z (2016) Molecular characteristics of representatives of the genus *Brachylecithum* Shtrom, 1940 (Digenea, Dicrocoeliidae) with comments on life cycle and host specificity. Parasitol Res 115:1417–1425
Himonas CA, Liakos V (1980) Efficacy of albendazole against *Dicrocoelium dendriticum* in sheep. Vet Rec 197:288–289
Hinaidy HK (1983) *Dicrocoelium suppereri* nomen novum (syn. *D. orientalis* Sudarikov et Ryjikov 1951), a new trematode for the parasite fauna of Austria. Zentralbl Veterinarmed B 30:576–589
Hohorst W (1962) Die rolle der Ameisen im Entwicklungsgang des Lanzettegels (*Dicrocoelium dendriticum*). Z Tropenmed Parasitol 13:377–397
Hohorst W, Graefe G (1961) Ameisen-obligatorische Zwischenwirte des Lanzettegels (*Dicrocoelium dendriticum*). Naturwissenschaften 48:229–230
Hohorst W, Lämmler G (1962) Experimentelle Dicrocoeliose-Studien. Z Tropenmed Parasitol 3:377–397
Ilha MRS, Loretti AP, Reis ACF (2005) Wasting and mortality in beef cattle parasitized by *Eurytrema coelomaticum* in the State of Paraná, southern Brazil. Vet Parasitol 133:49–60
Iyaji FO, Yaro CA, Peter MF, Abutu AEQ (2018) *Fasciola hepatica* and associated parasite, *Dicrocoelium dendriticum* in slaughter houses in Anyigba, Kogi State, Nigeria. Adv Infect Dis 8:1–9
Jahed Khaniki GR, Kia EB, Raei M (2013) Liver condemnation and economic losses due to parasitic infections in slaughtered animals in Iran. J Parasit Dis 37:240–244
Jeandron A, Rinaldi L, Abdyldaieva G, Usubalieva J, Steinmann P, Cringoli G, Utzinger J (2011) Human infections with *Dicrocoelium dendriticum* in Kyrgyzstan: the tip of the iceberg? J Parasitol 97:1170–1172
Jithendran KP, Bhat TK (1996) Prevalence of dicrocoeliosis in sheep and goats in Himachal Pradesh, India. Vet Parasitol 61:265–271
Jonlija R, Cankovic M, Rozman M (1972) Examination of ants the second intermediary hosts of *Dicrocoelium lanceatum* in Bosnia and Hercegovina, I. Veterinaria 21:317–322
Jonlija R, Milka R, Cankovic M (1973) Ispitivanje infestiranosti puzeva y mrava razvojnim oblicima *Dicrocoelium lanceatum* u Bosni y Hercegovini. l. Veterinaria 22:449–470
Jubb KVF, Stent AW (2016) Pancreas. In: Maxie GM (ed) Jubb, Kennedy and Palmer' Pathology of domestic animals, 6th edn. Elsevier, St. Louis
Kajubiri V, Hohorst W (1977) Increasing incidence of *Dicrocoelium hospes* (LOOSS, 1907) (Trematoda: Digenea) in Uganda. J Helminthol 51:212–214
Kalkan A (1971) *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 in Turkey. l. Field studies of intermediate and final hosts in the South Marmara region, 1968. Br Vet J 127:67–75
Kalkan A (1976) *Dicrocoelium dendriticum* in Turkey. 2. Observations on second intermediate hosts (ants) in the South Marmara region. Etlik Veteriner Bakteriyoloji Enstitüsü Dergisi 4:11–37
Karanfilovski G (1983) Economic losses caused by fascioliasis and dicrocoeliasis to livestock production in

Pljevlja and surrounding area. Veterinaria Yugoslavica 32:253–260

Kassai T, Takats C, Fok E, Redl P (1988) Activity of luxabendazole against liver flukes gastrointestinal roundworm and lungworm in naturally infected sheep. Parasitol Res 75:14–18

Katayama F (1996) Alterations of the mucin components in bile duct epithelium associated with proliferating cholangitis. An experimental study. Hokkaido Igaky Zasshi 71:585–598

Katsoulos PD, Christodoulopoulos G, Karatzia M, Pourliotis K, Minas A (2011) Liver flukes promote cholelithiasis in sheep. Vet Parasitol 179:262–265

Kazacos KR, Dhillon AS, Winterfield RW, Thacker HL (1980) Fatal hepatic trematodiasis in cockatoos due to *Platynosomum proxillicens*. Avian Dis 24:788–793

Kopp H (1975) Untersuchungen über die Eiausscheidung von *Fasciola hepatica* und *Dicrocoelium dendriticum* bei Schaf und Rind im Verlauf eines Jahres. Inaugural-Disertation zur Erlangung der tiermedizinischen Doktorwürde des Fachbereichs Tiermedizin der Ludwig- Maxinillians-Universität, München

Krull WH (1958) The migratory route of the metacercaria of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 in the definitive host: Dicrocoeliidae. Cornell Vet 48:17–24

Krull WH, Mapes CR (1952a) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). VII. The second intermediate host of *Dicrocoelium dendriticum*. Cornell Vet 42:603–604

Krull WH, Mapes CR (1952b) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). III. Observations on the slimeballs of *Dicrocoelium dendriticum*. Cornell Vet 42:253–276

Krull WH, Mapes CR (1952c) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). IV. Infection experiments involving definitive hosts. Cornell Vet 42:277–285

Krull WH, Mapes CR (1953) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). IX. Notes on the cyst, metacercaria, and infection in the ant, *Formica fusca*. Cornell Vet 43:389–410

Kukolj V, Aleksic-Kovacevic S, Katic-Radivojevic S, Knezevic DJ, Jovanovic M (2015) The role and immunophenotypic characteristics of myofibroblasts in liver of sheep naturally infected with the lancet liver fluke (*Dicrocoelium dendriticum*). Vet Parasitol 208:181–189

Lambacher B, Wittek T, Joachim A, Dadak A, Stanitzning A, Hinney B, Tichy A, Duscher G, Franz S (2016) From the New World to the Old Word: endoparasites of South American Camelids in Austria. Wien Tierarztl Monatsschr 103:33–42

Lathroum CN, Shell L, Neuville K, Ketzis JK (2018) Efficacy of praziquantel in the treatment of *Platynosomum fastosum* in cats with natural infection. Vet Sci 5, 7 pp

Leuckart R (1886-1901) Die Parasiten des Menschen und die von ihnen herrührenden Krankheiten, vol 2. C.F. Winter, Leipzig, pp 379–399

Liakos DV (1985) Epidemiological survey of parasitoses of sheep and goats. Etlen Kteniat Hellen Vet Med 28:271–278

Liu GH, Xu MJ, Song HK, Wang CR, Zhu XQ (2016) *De novo* assembly and characterization of the transcriptome of the pancreatic fluke *Eurytrema pancreaticum* (Trematoda: Dicrocoeliidae) using Illumina paired-end sequencing. Gene 576:333–338

Loos-Frank B (1978) Zum Verhalten von Ameisen der Gattung *Formica* (Hymenoptera: Formicidae) gegenüber Schleimballen des Kleinen Leberegels *Dicrocoelium dendriticum* (Digenea: Dicrocoeliidae) und über infektionsbedingte Veränderungen ihrer Hämolymphe. Entomol Germ 4:12–23

Lucius, R (1981) Untersuchungen zur Biologie, Pathologie und Ökologie von *Dicrocoelium hospes* Looss, 1907 (Trematode, Dicrocoeliidae). Dissertation zur Erlangung des Grades eines Doktors der Naturwissenschaften vorgelegt der Fakultät II (Biologie) der Universität Hohenheim

Lucius R, Frank W (1978) Beitrag zur Biologie von *Dicrocoelium hospes* Looss, 1907 (Trematodes, Dicrocoeliidae). Acta Trop 35:161–181

Lukin AK (1980) The prevalence and economic loss due to dicrocoeliasis in ruminants. Trudy Saratovskoi Nauchno-Issledovatel'skoi veterinamoi Stantsii 14:76–79

Macko JK, Pacenovsky J (1987) On the variability of *Dicrocoelium dendriticum* (Rudolphi, 1819) in domestic and free-living animals. II. On individual variability of the cattle (*Bos taurus* -race locale) dicrocoeliids in Algeria. Helminthologia 24:111–118

Malek EA (1980a) Ocurrence of *Dicrocoelium hospes* in Mali and Senegal, West Africa. J Helminthol 54:45–46

Malek EA (1980b) Snail-transmitted parasitic diseases, vol I & II. CRC Press, New York

Mañas I, Gómez V, Lozano J, Rodríguez M, Campos M (1978) Estudio de la frecuencia de la dicroceliosis en el ganado de la provincia de Granada. Rev Iber Parasitol 38:751–773

Manga-González, MY (1983) Los Helicidae (Gastropoda, Pulmonata) de la Provincia de León. Institución "Fray Bernardino de Sahagún", Excma. Diputación Provincial de León, Consejo Superior de Investigaciones Científicas (CECEL), León, Spain

Manga-González MY (1987) Some aspects of the biology and helminthofaune of *Helicella (Helicella) itala* (Linnaeus, 1758) (Mollusca). Natural infection by Dicrocoeliidae (Trematoda). Rev Iber Parasitol 1987:131–148

Manga-González MY (1999) Trematodos. In: Cordero del Campillo MC, Rojo-Vázquez FA, Martínez-Fernández AR, Sánchez-Acedo C, Hernández-Rodríguez S, Navarrete I, Díez-Baños P, Quiroz-Romero H, Carvalho-Varela M (eds) Parasitología Veterinaria. McGraw-Hill. Interamericana, Madrid, pp 79–104

Manga-González MY, González-Lanza C (2005) Field and experimental studies on *Dicrocoelium dendriticum* and dicrocoeliosis in northern Spain. J Helminthol 79:291–302

Manga-González MY, Quiroz-Romero H (1999) Dicroceliosis. In: Cordero del Campillo MC, Rojo-Vázquez FA, Martínez-Fernández AR, Sánchez-Acedo C, Hernández-Rodríguez S, Navarrete I, Díez-Baños P, Quiroz-Romero H, Carvalho-Varela M (eds) Parasitología Veterinaria. McGraw-Hill. Interamericana, Madrid, pp 260–283

Manga-González MY, González-Lanza C, Del-Pozo-Carnero P (1991) Dynamics of the elimination of *Dicrocoelium dendriticum* (Trematoda, Digenea) eggs in the faeces of lambs and ewes in the Porma Basin (León, NW Spain). Ann Parasitol Hum Comp 66:57–61

Manga-González MY, González-Lanza C, Del-Pozo-Carnero MP & Campo R (1995) Studies on experimental infection by *Dicrocoelium dendriticum* (Trematoda) of *Cernuella (Xeromagna) cespitum arigonis* (Mollusca) specimens in natural environmnet. In: Abstracts of 12th internacional malacological congress, Vigo, Spain

Manga-González MY, González-Lanza C, Cabanas E, Campo R (2001) Contributions to and review of dicrocoeliosis, with special reference to the intermediate hosts of *Dicrocoelium dendriticum*. Parasitology 123:S91–S114

Manga-González MY, Ferreras MC, Campo R, González-Lanza C, Perez V, García-Marín JF (2004) Hepatic marker enzymes, biochemical parameters and pathological effects in lambs experimentally infected with *Dicrocoelium dendriticum*. Parasitol Res 93:344–355

Manga-González MY, González-Lanza C, Cabanas E (2007) *Dicrocoelium dendriticum* primoinfection and kinetic egg elimination in marked lambs and ewes from León mountains (Spain). Rev Iber Parasitol 67:15–25

Manga-González MY, Quiroz-Romero H, González-Lanza C, Miñambres B, Ochoa P (2010) Strategic control of *Dicrocoelium dendriticum* (Digenea) egg excretion by naturally infected sheep. Vet Med 55:19–29

Mapes CR (1951) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Loos, 1899 (Trematoda, Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). I. A study of *Dicrocoelium dendriticum* and *Dicrocoelium* infection. Cornell Vet 41:382–432

Martínez-Ibeas, AM (2013) Nuevos enfoques para el diagnóstico y el control de la Dicrocoeliosis, importante parasitosis hepática de los rumiantes. Tesis Doctoral, Universidad de León (España), 267 Pages

Martínez-Ibeas AM, Martínez-Valladares M, González-Lanza C, Miñambres B, Manga-González MY (2011) Detection of *Dicrocoelium dendriticum* larval stages in mollusc and ant, intermediate hosts by PCR, using mitochondrial and ribosomal internal transcribed spacer (ITS-2) sequences. Parasitology 138:1916–1923

Martinez-Ibeas AM, González-Lanza C, Manga-González MY (2013a) Proteomic analysis of the tegument and excretory-secretory products of *Dicrocoelium dendriticum* (Digenea) adult worms. Exp Parasitol 133:411–420

Martínez-Ibeas AM, Perteguer MJ, González-Lanza C, Garate T, Manga-González MY (2013b) Analysis of an expressed sequence tag library from *Dicrocoelium dentriticum*. Exp Parasitol 135:287–296

Martín-Vega D, Garbout A, Ahmed F, Wicklein M, Goater CP, Colwell DD, Hall MJR (2018) 3D virtual histology at the host/parasite interface: visualization of the master manipulator, *Dicrocoelium dendriticum*, in the brain of its ant host. Sci Rep 8:8587

Mattes O (1936) Der Entwicklungsgang des Lanzettegels *Dicrocoelium lanceolatum*. Z Parasitenkd 8:371–430

Mattioli MP, Batista JS, Ferrari M, Paludo GR, Dias CA, Hoppe EG, da Rocha GC, Monteiro RV (2016) Clinical, hematological, biochemical, and ultrasonographic aspects of *Platynosomum* sp. (Trematoda: Dicrocoeliidae) infection of captive *Callithrix penicillata*. Primates 57:279–287

Mattioli MP, dos Santos Batista J, Ferrari M, Paludo GR, Dias CA, Hoppe EGL, Chaves da Rocha G, Monteiro RV (2016) Clinical, hematological, biochemical, and ultrasonographic aspects of *Platynosomum* sp. (Trematoda: Dicrocoeliidae) infection of captive *Callithrix penicillata*. Primates 57:279–287

Maurelli MP, Rinaldi L, Capuano F, Perugini AG, Veneziano G, Cringoli G (2007) Characterization of the 28S and the second internal transcribed spacer of ribosomal DNA of *Dicrocoelium dendriticum* and *Dicrocoelium hospes*. Parasitol Res 101:1251–1255

Mederacke I (2013) Liver fibrosis-mouse models and relevance in human liver. Z Gastroenterol 51:55–62

Mehlhorn, H & Walldorf, V (1988) Life cycles. In: Parasitology in focus: facts and trends. Springer, Berlin

Mitchell G, Cuthill G, Haine A, Zadoks R, Chaudhry U, Skuce P, Sargison N (2017) Evaluation of molecular methods for the field study of the natural history of *Dicrocoelium dendriticum*. Vet Parasitol 235:100–105

Mitterer KE (1975) Untersuchungen zum Schlüpfen der Miracidien des Kleinen Leberegels *Dicrocoelium dendriticum*. Z Parasitenkd 48:35–45

Mohamed ARE, Mummery V (1990) Human dicrocoeliosis. Report on 208 cases from Saudi Arabia. Trop Geogr Med 42:1–7

Mohanta UK, Ichikawa-Seki M, Hayashi K, Itagaki T (2016) Morphological and molecular characterization of *Eurytrema cladorchis* parasitizing cattle (*Bos indicus*) in Bangladesh. Parasitol Res 114:2099–2105

Morozova EV, Ryskov AP, Semyenova SK (2002) RAPD variation in two trematode species (*Fasciola hepatica* and *Dicrocoelium dendriticum*) from a single cattle population. Russ J Genet 38:977–983

Moulinié JJ (1856) De la reproduction chez les trématodes endoparasites. Mémoires de l'Institut de Genève 3:31–44

Nali RC, Zamudio KR, Prado CPA (2014) Microsatellite markers for Bokermannohyla species (Anura, Hylidae) from the Brazilian cerrado and Atlantic forest domains. Amphibia-Reptilia 35:355–360

Nascimento Ramos RA, Santana Lima VF, Melo Monteiro MF, de Andrade SM, Lepold R, da Gloria Faustino MA, Rinaldi L, Cringoli G, Camara Alves L (2016) New insights into diagnosis of *Platynosomum fastosum* (Trematoda: Dicrocoeliidae) in cats. Parasitol Res 115:479–482

Neuhaus W (1936) Untersuchungen über bau und entwicklung der Lanzettegel-Cercarie (*Cercaria vitrina)* und Klarstellung des infektionsvorganges beim endwirt. Z Parasitenkd 8:431–473

Neuhaus W (1938) Die Invasiosnswege der Lanzettegelcercarie bei der Infektion des Endwirtes und ihre Entwicklung zum *Dicrocoelium lanceatum*. Z Parasitenkd 10:476–512

Nguyen HM, Hoang HV, Ho LT (2017) *Platynosomum fastosum* (Trematoda: Dicrocoeliidae) from cats in Vietnam: morphological redescription and molecular phylogenetics. Korean J Parasitol 55:39–45

Nöller W (1929) Befunde bei Schnecken von Thüringer Schafweiden in einem Lanzettegelgebiete. Tierärztliche Rundschau 35:485–489

Nöller W (1932) Über die Rolle der Wildkaninchen als Lanzettegelträger in einem Thüringer Lanzettegelbiete. Tierärztliche Rundschau 38:190–191

Odhner T (1910) *Dicrocoelium dendriticum* (Rud.) der "richtige" Name des kleinen Leberegels. Zool Anz 35:317–318

Otranto D, Traversa D (2002) A review of dicrocoeliosis of ruminants including recent advances in the diagnosis and treatment. Vet Parasitol 107:317–335

Otranto D, Traversa D (2003) Dicrocoeliosis of ruminants: a little known fluke disease. Trends Parasitol 19:12–15

Otranto D, Rehbein S, Weigl S, Castacessi C, Parisi A, Lia RP, Olson PD (2007) Morphological and molecular differentiation between *Dicrocoelium dendriticum* (Rudolphi, 1819) and *Dicrocoelium chinensis* (Sudarikov and Ryjikov, 1951) Tang and Tang, 1978 (Platyhelminthes: Digenea). Acta Trop 104:91–98

Panin, VY (1971) (A new trematode Suborder, Dicrocoeliata n. suborder). Izvestiya Akademii Nauk *Kazakhskoi* SSR 6:60–66. En: Helminthological Abstract (1974) 44:270

Panin, VY (1981) (Principles of the taxonomy and the system of trematodes of the family Dicrocoeliidae Odhner, 1911). Parazity komponenty vodnykh i nazemnykh biotsenozov Kazakhstana 123–133

Paraschivescu, D (1978) Dynamics of formicids (Hym., Formicidae) in tetany of some meadows with dicrocelioza in Romania. Travaux du Museúm d'Histoire Naturell "Grigore Antipa" 19:321–323

Paraschivescu D (1981) Etho-ökologische Untersuchungen an einigen Formiciden-Arten als Zwischenwirte von *Dicrocoelium dendriticum*. Waldhygiene 14:65–72

Paraschivescu D (1983) Untersuchungen zur Temperaturabhangigkeit der Tetaniephase von *Formica pratensis* bei einer Infektion mit Stadien des Kleinen Leberegels *Dicrocoelium dendriticum*. Waldhygiene 15:21–24

Paraschivescu D, Hurghisiu I, Popescu S (1976) Bioecologic and biochemical research upon Formicidae complementary hosts of the *Dicrocoelium lanceatum* fluke (Stiles and Hassall, 1896). Arch Vet 11(12):159–178

Pavlov P, Popov A, Bankov D, Bratanov V, Dimitrov G (1965) Untersuchungen über die Wirksamkeit von Hetolin® bei der Infektion der Schafe mit *Dicrocoelium lanceolatum*. Tierarztl Umsch 20:574–575

Pegreffi G (1957) La distomatosi epatica da *Dicrocoelium dendriticum*. Vet Ital 8:123–135

Pepe P, Castellano M, Alfano S, Della Pepa ME, Tirino V, Piemonte M, Desiderio V, Zappavigna S, Galdiero M, Caraglia M, Cringoli G, Rinaldi L, Galdiero M (2015) *Dicrocoelium dendriticum* induces autophagic vacuoles accumulation in human hepatocarcinoma cells. Vet Parasitol 212:175–180

Piana P (1882) Le cercarie nei molluschi studiate in rapporto colla presenza del *Distoma epatico* e del *Distoma lanceolato* nel fegato del ruminanti domestici. Clin Vet (Milan) 5:306–314

Pinto HA, Mati VLT, de Melo AL (2014) New insights into the life cycle of *Platynosomum* (Trematoda: Dicrocoeliidae). Parasitol Res 113:2701–2707

Pinto HA, Mati VLT, de Melo AL (2016) Can the same species of *Platynosomum* (Trematoda: Dicrocoeliidae) infect both mammalian and avian hosts? J Helminthol 90:372–376

Pinto HA, Pulido-Murillo EA, Braga RR, Mati VLT, Melo AL, Tkach VV (2018) DNA sequences confirm low specificity to definitive host and wide distribution of the cat pathogen *Platynosomum illiciens (=P. fastosum)* (Trematoda: Dicrocoeliidae). Parasitol Res 117:1975–1978

Poglayen G, Roda R, Tona A, Mulciri A, Venturi G (1996) Endoparasites of the muflon (*Ovis ammon musimon*) from the Belluno province, Italy. Ricerche di Biologia della Selvaggina 4:73–77

Pojmanska T (2008) Family Dicrocoeliidae Looss, 1899. In: Bray RA, Gibson DI, Jones A (eds) Keys to the Trematoda, vol 3. CAB, London

Polozhentseva AA (1968) (Observations of *Dicrocoelium* in the hepatic veins of sheep). *Veterinariya*, Moscow 45:59–60

Prunescu C, Prunescu P, Fromunda V, Paraschivescu D, Popescu S (1979) Histopathological changes of the liver in hamsters with experimental dicroceliasis. Arch Vet 14:83–90

Quesada A, Russillo A, Rosa P, Cringoli G (1991) Diffusione di *F. hepatica* e *D. dendriticum* in greggi ovini allevati in un distretto alto-collinare delle Basilicata. Acta Med Vet 37:295–304

Ractliffe LH (1968) Hatching of *Dicrocoelium lanceolatum* eggs. Exp Parasitol 23:67–78
Rahko T (1972a) Studies on the pathology of dicrocoeliasis and fascioliasis in the goat. I. The histopathology of the liver and bile ducts. Acta Vet Scand 13:554–562
Rahko T (1972b) Studies on the pathology of dicrocoeliasis and fascioliasis in the goat. II. The histochemistry of bile-duct mucosubstances. Acta Vet Scand 13:563–574
Rahko T (1972c) Studies on the pathology of dicrocoeliasis and fascioliasis in the goat. III. The histochemistry of mast cells and globule leucocytes. Acta Vet Scand 13:575–584
Ranucci S, Mughetti L, Ambrosi M, Grelloni V (1981) Rilievi ematochimici, anatomo-istopatologici e parassitologici in ovini infetti da dicroceliosi. Riv Zoot Vet 9:173–175
Rehbein S, Kokott S, Lindner T (1999) Evaluation of thecniques for the enumeration of *Dicrocoelium* eggs in sheep faeces. Zentralbl Veterinarmed 46:133–139
Revilla-Nuín B, Manga-González MY, Miñambres B, González-Lanza C (2005) Partial characterization and isolation of 130kDa antigenic protein of *Dicrocoelium dendriticum* adults. Vet Parasitol 134:229–240
Robinson N, Piedrafita D, Snibson K, Harrison P, Meeusen E (2010) Immune cell kinetics in the ovine abomasal mucosa following hyperimmunization and challenge with *Haemonchus contortus*. Vet Res 41:37
Rojo-Vázquez FA, Cordero-del-Campillo M, Díez-Baños P, Chaton-Schaffner M (1981) Relation existant entre le nombre d'oeufs dans les feces et la charge parasitaire lors des infestations naturelles a *Dicrocoelium dendriticum* chez les ovins. Rev Med Vet 132:601–607
Rojo-Vázquez FA, Meana A, Tarazona JM, Duncan JL (1989) The efficacy of netobimin, 15 mg/kg, against *Dicrocoelium dendriticum* in sheep. Vet Rec 124:512–513
Rojo-Vázquez FA, Meana A, Valcárcel F, Martínez-Valladares M (2012) Update on trematode infections in sheep. Vet Parsitol 189:15–38
Roming T, Lucius R, Frank W (1980) Cerebral larvae in the second intermediate host of *Dicrocoelium dendriticum* (Rudolphi, 1819) and *Dicrocoelium hospes* Looss, 1907 (Trematodes, Dicrocoeliidae). Z Parasitenkd 63:277–286
Rozman S, Gradjanin M, Cankovic M (1974) The transitory hosts of *Dicrocoelium dendriticum* in the mountain area of Bosnia and Herzegovina. In: Proceedings of the third international congress of parasitology, Munich, 1974
Sadykhov IA, Melikov YuF (1980) (Features of the distribution of *Fasciola* and *Dicrocoelium* in farming complexes in the Sheki-Zakataly area of the Azerbaidzhan SSR). Izvestiya Akademii Nauk Azerbaidzhan SSR, Biol Nauki 3:75–79. En: Helminthological Abstract (1981) 50:5810
Salimova M (1972) (Experimental dicrocoeliasis of Karakul lambs). Trudy VII Vsesoyuznoi Konferentsii po Prirodnoi Ochagovosti Boleznei i Obshchim Voprosam Parazitologii Zhivotnykn, 14–18 Oktyabrya 1969, Samarkand, 6. Tashkent, USSR: Izdatel'stvo "FAN" Uzbekskoi SSR:155–157
Samadieh H, Mohammadi G-R, Malaki M, Borji H, Azizzadeh M, Heidarpour M (2017) Relationships between oxidative stress, liver, and erythrocyte injury, trace elements and parasite burden in sheep naturally infected with *Dicrocoelium dendriticum*. Iran J Parasitol 12:46–55
Sánchez-Andrade R, Paz-Silva A, Suárez JL, Arias M, López C, Morrondo P, Scala A (2003) Serum antibodies to *Dicrocoelium dendriticum* in sheep from Sardinia (Italy). Prev Vet Med 57:1–5
Sánchez-Campos S, Tuñón MJ, González P, Campo R, Ferreras MC, Manga-González MY, González-Gallego J (1996) Effects of experimental dicrocoeliosis on oxidative drug metabolism in hamster liver. Comp Biochem Physiol 115C:55–60
Sánchez-Campos S, Tuñón MJ, González P, Marín JJG, González-Gallego J (1998) Enhanced bile formation induced by experimental dicrocoeliosis in the hamster. Life Sci 63:1963–1974
Sánchez-Campos S, Tuñón MJ, González P, González-Gallego J (1999) Oxidative stress and changes in liver antioxidant enzymes induced by experimental dicrocoeliosis in hamsters. Parasitol Res 85:468–474
Sánchez-Campos S, González P, Ferreras MC, García-Iglesias MJ, González-Gallego J, Tuñón MJ (2000) Morphologic and biochemical changes caused by experimentally induced dicrocoeliosis in hamsters *(Mesocricetus auratus*). Comp Med 50:147–152
Sandoval H, Manga-González MY, Campo R, Garcia P, Castro JM, Pérez de la Vega M (1999) Preliminary study on genetic variability of *Dicrocoelium dendriticum* determined by random amplified polymorphic DNA. Parasitol Int 48:21–26
Sandoval H, Manga-González MY, Castro JM (2013) A tool for diagnosis of *Dicrocoelium dendriticum* infection: hatching eggs and molecular identification of the miracidium. Parasitol Res 112:1589–1595
Sanz F, Tarazona JM, Jurado R, Frías J, Tarazona JV, Duncan JL (1987) An evaluation of the efficacy of netobimin against *Dicrocoelium dendriticum* in sheep. Vet Rec 17:57–58
Sargison ND, Baird GJ, Sotiraki S, Gilleard JS, Busin V (2012) Hepatogenous photosensitisation in Scottish sheep caused by *Dicrocoelium dendriticum*. Vet Parasitol 189:233–237
Scala A, Coda S, Ximenes LA, Cavedagna G (1991) Dicroceliasi ovina in Sardegna: Tassi di prevalenza, lesioni anatomo-istopatologiche e tests ematochimici. Atti Federazione Mediterranea Sanità e Produzione Ruminanti 1:209–214
Schneider, J (1971) Die Wanderung der Metacercarien von *Dicrocoelium dendriticum* (Rud. 1819), Looss, 1899 (Digenea, Trematoda) im Ameisen Zwischen wirt und ihre Steuerung durch physiologisch-ökologische Faktoren. Inaügural Dissertation Naturwiss. Fakultat der Johaim Wolfgang for the Universita, Frankfurt/M
Schneider G, Hohorst W (1971) Wanderung der Metacercarien des Lanzett-Egels in Ameisen. Naturwissenschaften 58:327–328

Schuster R (1987) Ein geschichtlicher Überblick zur Namensgebung des Lanzettegels *Dicrocoelium dendriticum*. Angew Parasitol 28:205–206

Schuster R (1991) Factors influencing the metacercarial intensity in ants and the size of *Dicrocoelium dendriticum* metacercarial cyst. J Helminthol 65:275–279

Schuster R (1992) Zur Beeinflussung von *Helicella obvia* durch *Dicrocoelium* Parthenitae. Angew Parasitol 33:61–64

Schuster R (1993) Infection patterns in the first intermediate host of *Dicrocoelium dendriticum*. Vet Parasitol 47:235–243

Schuster R, Hiepe T (1993) Bekämpfung der Dicrocoeliose beim Schaf. Monatsh Veterinarmed 48:657–661

Schwertz CI, Gabriel ME, Henker LC, Bottari NB, do Carmo G, Guarda Ndos S, Moresco RN, Machado G, Morsch VM, Schetinger MRC, Stedille FA, Baska P, Mattei V, da Silva AS, Mendes RE (2016) Oxidative stress associated with pathological changes in the pancreasof cattle naturally infected by *Eurytrema coelomaticum*. Vet Parasitol 223:102–110

Senlik B, Cirak VY, Muz M, Tinar R (2006) Changes in faecal egg counts at different hours of the day and relationship between faecal egg count and parasite burden in sheep naturally infected with *Dicrocoelium dendriticum*. Turk J Vet Anim Sci 30:107–111

Senlik B, Cirak VY, Tinar R (2008) Field efficacy of two netobimin oral suspensions (5% and 15%) in sheep naturally infected with *Dicrocoelium dendriticum*. Small Rumin Res 80:104–106

Shahbazi Y, Hashemnia M, Safavi EAA (2016) A retrospective survery of liver flukes in livestock based on abattoir data in Kermanshah, west of Iran. J Parasit Dis 40:948–953

Şimşek S, Köroğlu E, Ütük AE (2006) Application of Western blotting and enzyme linked immunosorbent assay (ELISA) for the diagnosis of *Dicrocoelium dendriticum* in sheep using excretory secretory (E/S) antigens. Turk J Vet Anim Sci 30:113–119

Skálová L, Forstová-Křížová V, Civilink V, Szotáková B, Storkánová L, Velík J, Lamka J (2007) Mouflon (*Ovis musimon*) dicrocoeliosis: effects of parasitosis on the activities of biotransformation enzymes and albendazole metabolism in liver. Vet Parasitol 146:254–262

Soulsby EJL (1982) Helminths, arthropods and protozoa of domesticated animals, 7th edn. Baillère Tindal, London

Spindler EM, Zhaler M, Loos-Frank B (1986) Behavioural aspects of ants as second intermediate hosts of *Dicrocoelium dendriticum*. Z Parasitenkd 72:689–692

Srivastava GC (1975) The intensity of infection in naturally infected *Formica pratensis* with the metacercariae of *Dicrocoelium dendriticum* in relation to their size. J Helminthol 49:57–64

Srivastava GC, Kolev G, Georgiev M (1978) A surgical approach to trace the route of migration of *Dicrocoelium dendriticum* in the laboratory animals. Riv Parassitol 39:131–137

Stratan NM (1986) Efficacy of Acemidophen against *Dicrocoelium dendriticum* in sheep. Byulleten' Vsesoyuznogo Instituta Gel'mintologii im. K.I Skryabina 42:64–66

Su X, Zhang Y, Zheng X, Wang XX, Li Y, Li Q, Wang CR (2018) Characterization of the complete nuclear ribosomal DNA sequences of *Eurytrema pancreaticum*. J Helminthol 92:484–490

Sudarikov VE, Ryjikov KM (1951) On the helminth fauna of ungulates of the Baikal region. Trudy Gelmintologicheskoi- Laboratorii Akaemiya Nauk SSSR 5:53–58

Svadzhyan PK (1951) [Contribution to the study of the snails serving as intermediate hosts of *Dicrocoelium lanceatum*]. Izvestiya Akademii Nauk Armyanskoi SSR 4:649–656

Svadzhyan PK (1959) Puti migracii metacerkarii *Dicrocoelium lanceatum* Stiles et Hassall, 1896 v organizme okonchatel'nogo joziaina. Veterinariya 36:45–48

Svadzhyan PK (1960) [The development of metacercariae of *Dicrocoelium dendriticum* in the second intermediate host- the ant]. Zool Zhurnal 39:1568–1571

Taira K, Shirasaka S, Taira N, Ando Y, Adachi Y (2006) Morphometry on lancet flukes found in Japanese sika deer (*Cervus nippon centralis*) captured in Iwate Prefecture, Japan. J Vet Med Sci 68:375–377

Tang C, Tang Z, Qi P, Duo C, Li Q, Cao H, Pan C (1981) Studies on the biology of *Dicrocoelium lanceatum* in Xin Jiang Automonous Region. Acta Zool Sin 27:265–273

Tang C, Tang Z, Tang L, Cui Q, Lu H, Qian Y (1983) Studies on the biology and epizooties of *Dicrocoelium chinensis* in the eastern Inner Mongol Autonomous region. Acta Zool Sin 29:340–349

Tang C, Tang L, Wang F, Shi H, Sai Q, Wen Z, Luo Y (1985) Investigations on the biology of a few *Dicrocoelium* species of sheep on Qinghai Plateau. Acta Zool Sin 31:245–262

Tarry DW (1969) *Dicrocoelium dendriticum*: the life cycle in Britain. J Helminthol 43:403–416

Timon-David J (1962) Fourmis et "Petite Douve". Nouvelle découvertes et progrès de la question. L'Entomologiste 18:69–72

Timon-David J (1965) Infestation expérimentale d'une hélicelle par huit espèces de trématodes digénétiques appartenant à quatre familles différentes. Ann Parasitol Hum Comp 40:149–154

Travassos L (1944) Revisao da Familia Dicrocoeliidae Odhner, 1910. Monografías do Instituto Oswaldo Cruz 2: vii+357 pp

Uriarte J, Cabaret J, Tanco JA (1985) The distribution and abundance of parasitic infections in sheep grazing on irrigated or non-irrigated pastures in north-eastern Spain. Ann Rech Vet 16:321–325

van Paridon BJ, Goater CP, Gilleard JS, Criscione CD (2016) Characterization of nine microsatellite loci for *Dicrocoelium dendriticum*, an emerging liver fluke of ungulates in North America, and their use to detect clonemates and random mating. Mol Biochem Parasitol 207:19–22

van Paridon BJ, Guilleard JS, Colwell DD, Goater CP (2017a) Life cycle, host utilization, and ecological fitting for invasive lanced liver fluke, *Dicrocoelium dendriticum*, emerging in southern Alberta, Canada. J Parasitol 103:207–212

van Paridon BJ, Colwell DD, Goater CP, Gilleard JS (2017b) Population genetic analysis informs the invasion history of the emerging trematode *Dicrocoelium dendriticum* into Canada. Int J Parasitol 47:845–856

Vershinin II (1957) (Epizootiology of *Dicrocoelium* infections of sheep and its biology in the Kaluga region). Trudy Moskovskoy Veterinarnoi Akademii 19:3–15

Vogel H (1929) Beobachtungen über *Cercaria vitrina* und deren Beziehung zum Lanzettegelproblem. Arch Schiffs Tropen Hyg 33:474–489

Vogel H, Falcao J (1954) Über den Lebenszyklus des Lanzettegels, *Dicrocoelium dendriticum*, in Deutschland. Z Tropenmed Parasitol 5:275–296

Von Linstow D (1887) Helminthologische Untersuchungen, *Cercaria vitrina* n.sp. *Zoologische Jahrbücher*. Abteil Syst Oekolgie Geograph Tiere (Jena) 3:105–106

Von Willemoes-Suhm R (1871) Zur Entwicklungsgeschichte des kleinen Leberegels. Z Wiss Zool 21:175–179

Warren KS, Swan RA, Hobbs RP, Heriyanto, Kuhn EM, Heeney JL (1998) *Platynosomum fastosum* in ex-captive orangutans from Indonesia. J Wildl Dis 34:644–646

Wedrychowicz H, Ducommun D, Górski P, Pefister K (1995) Somatic antigens of adult *Dicrocoelium dendriticum* recognized by bile antibodies of naturally infected cattle. Vet Parasitol 56:47–56

Wedrychowicz H, Ducommun D, Bambara OD, Pefister K (1997) Local and systematic antibody response against surface, somatic and ES antigens of *Dicrocoelium dendriticum* in sheep naturally infected with the trematode. Acta Parasitol 42:248–253

Wolff K, Hauser B, Wild P (1984) Dicrocoeliose des Schafes: Untersuchungen zur Pathogenese und zur Regeneration des Leber nach Therapie. Berl Munch Tierarztl Wochenschr 97:378–387

Yamaguti S (1958) Systema Helminthum. I. Digenetic Trematodes of Vertebrates. Interscience, New York

Zarnik B (1910) Über den Entwicklungszyklus von *Dicrocoelium lanceatum* (*Distomum lanceolatum*). Sitzungsberichte der Physikalisch-medizinischen Gesellschaf zu Würzburg 2:27–31

Selected Wildlife Trematodes

11

Matthew G. Bolek, Jillian T. Detwiler,
and Heather A. Stigge

11.1 Introduction

In the broadest sense, over the last 20 years, few areas of wildlife biology have experienced such an enormous increase in scientific interest as host–parasite interactions (Schmidt-Hempel 2011). This surge in research has flooded the literature with papers on these interactions and their influence on studies in behavioral ecology, genetics, population biology, eco-immunology, and molecular biology. In terms of host–parasite interactions, the digenetic trematodes have received more attention in the scientific literature than most other parasite groups infecting wildlife (Prudhoe and Bray 1982; Gibson and Bray 1994; Choudhury et al. 2016; Scholz et al. 2016; Madhavi and Bray 2018). This is not surprising since the digenetic trematodes are a species-rich group of parasites. Some estimates suggest there are more than 24,000 species, with at least one species of digenetic trematode reported from most groups of wildlife (Poulin 2000). However, compared to the trematodes of humans and domestic animals, for which the biology has been relatively well studied, the majority of trematode species infecting wildlife have not received as much attention (Detwiler et al. 2012). Most of the early work on trematodes of wildlife has concentrated on species descriptions and elucidating their life cycles. For example, following a flurry of species descriptions in the 1900s, there was great interest and effort dedicated to describing life cycles of many trematodes of wildlife that commonly include 2–3 or even 4 hosts (Krull 1930, 1935a, b). These studies typically involved field surveys of hosts, and in some cases infections in the laboratory were done to confirm host specificity and ontogenetic development of the specific trematode species in its hosts (Krull 1930, 1935a, b; Thomas 1939; Rankin 1944). However, few people ever questioned the validity of a life cycle once it was reported. Subsequent work on many digenean species of wildlife primarily involved expanding new host records and making taxonomic revisions when warranted rather than assessing the credibility of the original report on the life cycle (see Snyder and Janovy 1996; Bolek and Janovy 2008; Bolek et al. 2009a, 2010, 2016; Detwiler et al. 2010, 2012; Stigge and Bolek 2015).

M. G. Bolek (✉)
Department of Integrative Biology, Oklahoma State University, Stillwater, OK, USA
e-mail: bolek@okstate.edu

J. T. Detwiler
Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada
e-mail: Jillian.Detwiler@umanitoba.ca

H. A. Stigge
Department of Biology, College of Saint Mary, Omaha, NE, USA
e-mail: hstigge@csm.edu

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_11

More recently, a renewed interest in host–parasite interactions, including cataloging the global biodiversity of parasites, understanding parasite transmission strategies in nature, studies on the evolution of parasite life cycles, and the diseases trematodes cause in their wildlife hosts, has resulted in new research on many wildlife trematodes that were first described in the early 1900s (Poulin 2000; Poulin and Cribb 2002; Detwiler et al. 2010; Kasl et al. 2018). With this revived focus on the biology of trematodes of wildlife, it has become apparently clear that the taxonomic status and nomenclature of many of the previous species descriptions of trematodes throughout the world are confusing and difficult to decipher. For example, identifying trematodes of wildlife can be troublesome because the majority of trematode species were inadequately described and many species have been synonymized over time (McAlpine 2006; Bolek and Janovy 2007a, b; Detwiler et al. 2012). Additionally, the expansive literature can be daunting and difficult to follow because many groups of trematodes infecting wildlife have been redescribed, including systematic revisions, repeatedly over time (Kennedy 1981; Kanev et al. 1998, 2008; Dronen 2009). Reexamining specimens from past studies has often revealed that identifications were based on few specimens, including specimens of poor quality or lacking adequate life history and/or host information (Beaver 1937; Rankin 1939; Kennedy 1981; Kanev et al. 2008; Dronen 2009; Bolek and Janovy 2007a, b; Bolek et al. 2009a, b; Detwiler et al. 2012).

With the development of molecular barcoding techniques for trematodes, more recent studies on the identification, systematic position, host use, and transmission strategies of trematodes of wildlife are providing new insights into their taxonomy, biology, and host associations (Tkach et al. 2002; Olson and Tkach 2005; Moszczynska et al. 2009; Locke et al. 2010; Routtu et al. 2014; Chibwana et al. 2015; Blasco-Costa et al. 2016). Assessing biodiversity using DNA barcodes has provided many advantages for trematodes that are species rich, difficult to morphologically identify, and poorly cataloged in all their hosts. Over the last 25 years, the results of some of those studies suggest that many cryptic species of trematodes are being discovered (Leung et al. 2009; Pérez-Ponce de León and Nadler 2010; Pérez-Ponce de León et al. 2016). More importantly, this technique has provided a tool for assessing host use, and host specificity for many species of trematodes of wildlife (Kraus et al. 2014; Locke et al. 2010; Pérez-Ponce de León et al. 2016). Species identification is critical for understanding host–parasite interactions including host specificity and transmission of trematodes in wildlife populations and varies tremendously across species of trematodes (Detwiler et al. 2012). Additionally, species identification is also critical for evaluating the ecological factors driving trematode abundance and distribution in their hosts. Below, we review some of our recent work on trematodes of amphibians, birds, mammals and their snail intermediate hosts, in the hope of providing a tool kit on how to study the biodiversity, life cycles, host–parasite interactions, and transmission of trematodes of wildlife. In our review, we provide a brief introduction to each group of wildlife trematodes, followed by some examples of the challenges each group of trematodes has relative to the goal of understanding of the biology and host–parasite interactions of these organisms. Finally, we conclude with some potential solutions to overcome the difficulties of studying the taxonomy, life cycles, and transmission of wildlife trematodes.

11.2 Frog Lung Flukes: The Problem of Species Identification of Adult Worms: Examples from Classical Morphology and Life Cycle Studies

One of the major difficulties of studying trematodes of wildlife is their identification. To illustrate the obstacles involved with identifying trematodes of wildlife, we describe some of the convoluted taxonomic history for frog lung flukes from the United States and Canada. We chose to focus on this group because their history

demonstrates the difficulties of dealing with the obscure literature and identification of trematodes of wildlife in general. Below, we review the group, their life cycles, associate pathology with their amphibian definitive hosts, and provide examples of the confusion in species identifications for this group of wildlife trematodes. Finally, we provide some examples of solutions to these problems, which are necessary for dealing with most trematodes of wildlife.

The first frog lung fluke was described from the edible frog, *Pelophylax esculentus*, collected in Berlin, Germany, as *Distomum variegatum* by Rudolphi (1819), but was transferred to the newly erected genus *Haematoloechus* by Looss (1899), who described two additional European species (Stafford 1902). Over the last 120 years, the genus has been revisited numerous times. In 1902, Looss renamed the genus to *Pneumonoeces*, whereas Harwood (1932) and Ingles (1932) reinstated it back to *Haematoloechus* (Harwood 1932; Ingles 1932). Other members of this genus were placed in other genera (*Metahaematoloechus, Neohaematoloechus, Ostioloides, Ostiolum*, *Peumobitens*, and *Skrjabinoeces*) based on morphological differences in the location of their uterine loops and/or vitelline follicles (Pratt 1903; Ward 1917; Sudarikov 1950; Odening 1958; Skrjabin and Antipin 1962; Yamaguti 1971). However, recent morphological, molecular, and life cycle studies on various frog lung flukes in some of these erected genera, including *Haematoloechus*, indicated that they were polyphyletic (Bolek and Janovy 2007a, b; León-Règagnon et al. 1999, 2001; León-Règagnon and Paredes-Calderón 2002; León-Règagnon and Brooks 2003; León-Règagnon 2010; Snyder and Tkach 2001; Zamparo et al. 2011; León-Règagnon and Topan 2018). As a result, one species belongs to the genus *Ostioloides* and all remaining species were placed into the genus *Haematoloechus* by León-Règagnon and Topan (2018).

The genus *Haematoloechus* (Looss 1899) currently includes 70 valid species of amphibian flukes which are distributed worldwide except for Antarctica (León-Règagnon and Brooks 2003; León-Règagnon and Topan 2018). Ten species have been described from Africa, one from Australia, 24 from Eurasia, and 35 from the Americas (see León-Règagnon and Topan 2018). Most species have been reported from the lungs of anuran definitive hosts, with a few exceptions from the lungs of salamanders (see León-Règagnon and Topan 2018). Frog lung flukes are elongate, flat, transparent flukes approximately 3–10 mm long, with a ventral sucker that can be either well developed or reduced in size. The testes can be tandem, oblique, or opposite and are located in the posterior half of the body; the ovary is ovoid or lobed and pretesticular. A cirrus sac is present and the genital pore is located ventrally to the pharynx or the esophagus, and the vitelline follicles are arranged in many separate clusters throughout the body. The uterus is very long, consisting of many folds and loops which fill most of the area around the other internal organs, and the excretory vesicle is Y-shaped with the arms extending anteriorly in the body (Krull 1933; Bolek and Janovy 2007a; Fig. 11.1).

The reported life cycles of frog lung fluke species are quite similar to one another (Krull 1930, 1931, 1932, 1933, 1934; van Theil 1930; Ingles 1932; Grabda-Kazubska 1960; Dollfus 1961; Schell 1965; Combes 1968; Dronen 1975; Bourgat and Kulo 1979; Snyder and Janovy 1994, 1996; Bolek and Janovy 2007a, b), and they are typical of many trematodes in that they involve obligatory molluscan, arthropod, and vertebrate hosts (Fig. 11.2). Adult flukes are found in the lungs of various species of frogs, toads, and salamanders where they feed on blood (Krull 1930; León-Règagnon and Topan 2018). Within the lungs of their amphibian host and after mating, adult worms release eggs that contain fully formed miracidia. The eggs are then swallowed, pass through the digestive tract, and are voided in the feces of their amphibian host. If the eggs are deposited in water, the miracidia remain alive within the eggs for weeks and some of those eggs are ingested by their molluscan first intermediate hosts (Fig. 11.3). First intermediate hosts for frog lung flukes include various species of freshwater snails in the family Planorbidae and Physidae (see Snyder and Janovy 1996; Bolek and Janovy 2007b). Once eggs are ingested by the snail first intermediate host, the eggs hatch in

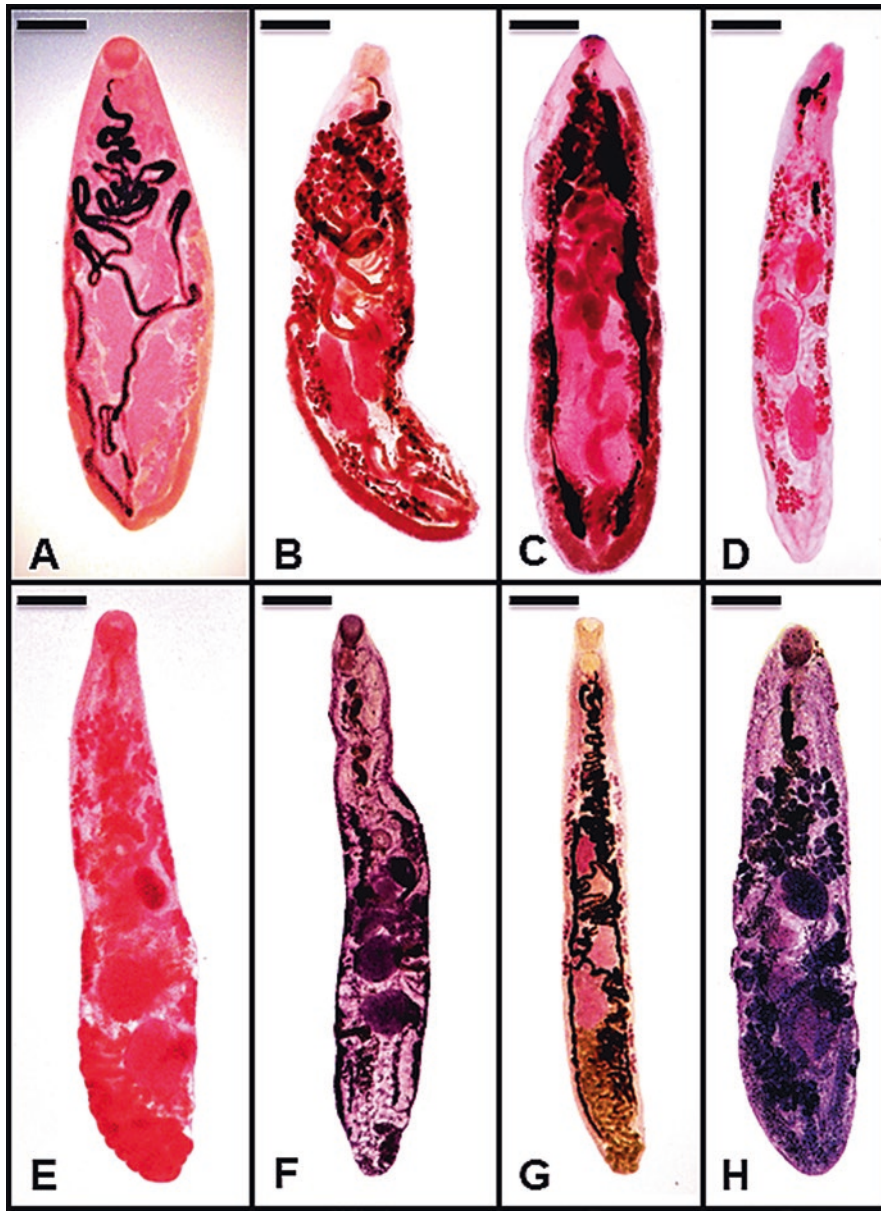

Fig. 11.1 Examples of some common North American *Haematoloechus* species showing the variation in the shape and locations of internal organs. (**a**) *Haematoloechus breviplexus* from a mink frog, *Rana septentrionalis*. (**b**–**d**) *Haematoloechus floedae*, *H. longiplexus*, and *H. parviplexus* from a bullfrog, *Rana catesbeiana*. (**e**) *Haematoloechus complexus* from a plains leopard frog, *Rana blairi*. (**f**–**h**) *Haematoloechus coloradensis, H. medioplexus*, and *H. varioplexus* from the northern leopard frog, *Rana pipiens*. Note the variation in testes shape and position and the length and position of the uterine loops. Scale bars = 0.75 mm

the snail's intestine, and the miracidia penetrate the snail's intestine (Fig. 11.4). Thirty days post-infection, mother and daughter sporocysts develop between the epithelium and basement membrane of the snail's intestine and produce xiphidiocercariae of the ornate group (Fig. 11.3). Cercariae are released from the snail host into the water column, and they infect various types of

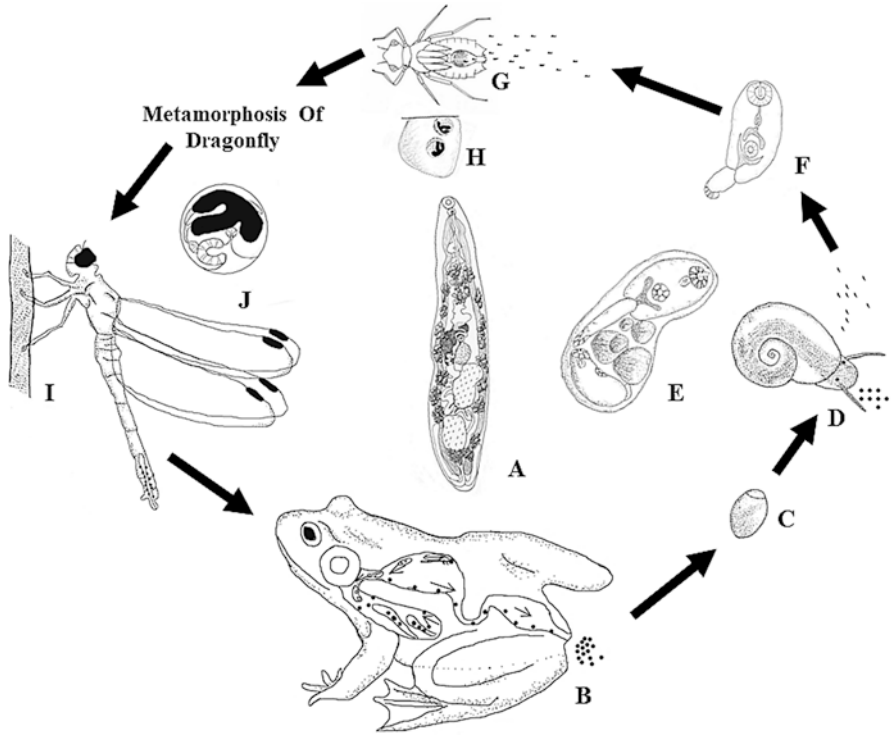

Fig. 11.2 Life cycle of *Haematoloechus parviplexus*. (**a**) Adult *H. parviplexus* from the lungs of the bullfrog, *Rana catesbeiana*. (**b**) Bullfrog showing the escape of the metacercariae from the dragonfly in the stomach and their migration to the lungs; adult worms depositing eggs and the route of the eggs to the external environment. (**c**) Egg. (**d**) *Gyraulus parvus* eating eggs and releasing cercariae. (**e**) Daughter sporocyst from the surface of the intestine of *Gyraulus parvus* with cercariae in various stages of development. (**f**) Cercaria showing body, tail, stylet, oral sucker, pharynx and cecae, ventral sucker, and excretory bladder. (**g**) Larva of the eastern pondhawk dragonfly, *Erythemis simplicicollis*, showing the swimming cercariae being taken into the branchial basket respiratory organ of the larva. (**h**) Lamella from the branchial basket of eastern pondhawk dragonfly larva containing two encysted metacercariae. (**i**) Teneral eastern pondhawk dragonfly with encysted metacercariae within the vestige of the branchial basket of the larva. (**j**) Encysted metacercaria. Modified from Bolek et al. (2016)

aquatic arthropod second intermediate hosts (Schell 1965; Dronen 1975; Bolek and Janovy 2007b). Within the arthropod second intermediate host, the cercariae develop to the metacercaria stage. Depending on the species of frog lung flukes involved, the metacercariae are either encysted or unencysted and are found in the branchial basket of dragonflies or the body cavity of other aquatic arthropod second intermediate hosts (Fig. 11.3). Frogs become infected when they ingest second intermediate hosts with metacercariae, and worms migrate to the lung where they begin feeding on blood and mating before they become gravid in approximately 30 days (Krull 1933). Although amphibian definitive hosts can become heavily infected (Fig. 11.3), surprisingly, little pathology is observed in infected amphibians other than some chronic inflammation in the connective tissue under the lung epithelium (Shields 1987).

Although most species of frog lung flukes have similar life cycles, recent studies on arthropod host specificity indicate that distinct patterns of cercarial behavior patterns and arthropod second intermediate host specificity, ranging from generalists to specialists, have evolved in European and North American frog lung flukes (Snyder and Janovy 1994, 1996; Bolek and Janovy 2007a, b; Bolek et al. 2016). Molecular phylogenetic studies by Snyder and Tkach (2001) and Bolek (2006) on 12 species of European and North American frog lung flukes indicate that

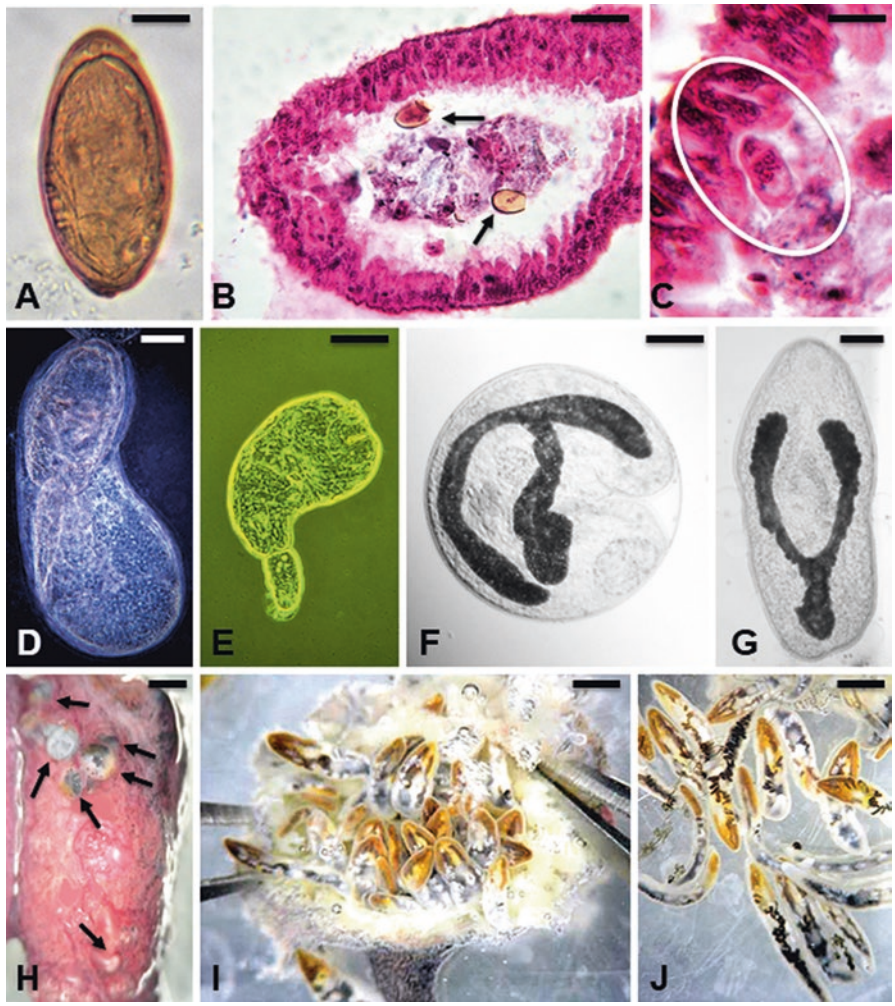

Fig. 11.3 Life stages of *Haematoloechus* species in various hosts in the life cycle. (**a**) Typical egg of *Haematoloechus coloradensis*. Note the golden brown color, indistinct operculum and fully formed miracidium. Scale bar = 5 μm. (**b**) H&E stained cross section of the intestine of the snail, *Gyraulus parvus*, showing two ingested eggs of *Haematoloechus longiplexus* (black arrows) in the lumen in process of hatching. Scale bar = 10 μm. (**c**) H&E stained cross section of the intestine of the snail, *Gyraulus parvus*, showing the miracidium in the lumen of the intestine in the process of penetrating the intestinal epithelium. Scale bar = 15 μm. (**d**) A daughter sporocyst with developing cercariae of *Haematoloechus complexus* removed from the basement membrane of the first intermediate host *Physa acuta*. Scale bar = 20 μm. (**e**) A xiphidiocercariae of the ornate group of *Haematoloechus complexus*. Note the stylet and the dorso-ventral finfold on the tail. Scale bar = 25 μm. (**f**) A typical encysted metacercaria of *Haematoloechus coloradensis* removed from the branchial basket of a dragonfly second intermediate host. Note the distinct Y-shaped excretory vesicle. Scale bar = 25 μm. (**g**) An unencysted metacercaria of *Haematoloechus longiplexus* removed from the body cavity of a damselfly second intermediate host. Note the characteristic Y-shaped excretory vesicle. Scale bar = 25 μm. (**h**, **i**) A removed lung from a heavily infected bullfrog showing nodules on the surface of the lungs (arrows) and *Haematoloechus parviplexus* worms within the lumen of the lung. Scale bars = 2 mm. (**j**) Adult gravid *Haematoloechus parviplexus* in tap water releasing eggs. Note the transparent nature of the flukes. Scale bar = 2 mm

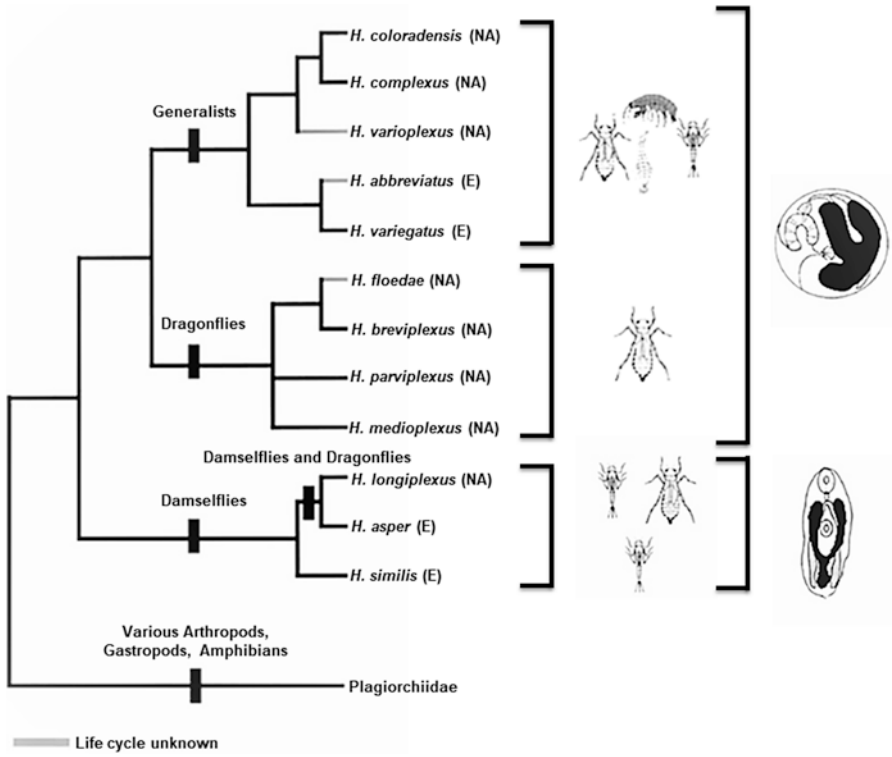

Fig. 11.4 Arthropod host specificity, metacercaria type, and geographical distribution among species of *Haematoloechus* indicated on a phylogenetic tree derived from internal transcribed spacer rDNA data by Snyder and Tkach (2001) and Bolek (2006). Generalist parasites have the ability to form metacercariae in odonate and non-odonate arthropods. Species in gray indicate that the life cycle is unknown. *NA* North America, *E* Europe. Modified from Bolek et al. (2016)

some clades are comprised of both European and North American species, suggesting that these lineages arose before the breakup of Laurasia and then radiated after Eurasia and North America separated (Fig. 11.4). Not surprisingly, members of each evolutionary lineage share similar patterns of arthropod host specificity and metacercaria morphology that are distinct from patterns found in the other lineages (Fig. 11.4).

Some species of frog lung flukes only infect dragonfly species. In these cases, the cercariae of these trematodes are passively infecting their odonate host. For a dragonfly larva to be infected, the cercariae must be drawn into the dragonfly's branchial basket where they encyst as metacercariae (Snyder and Janovy 1996; Fig. 11.4). In contrast, other species are generalists at the second intermediate host level and can infect any aquatic arthropod. The cercariae of these species are actively infecting their hosts. Upon contact with an arthropod host, the cercariae attach with their suckers to any region of an arthropod host and search for an inter-segmental membrane on the exoskeleton where they penetrate using their stylet (Snyder and Janovy 1994, 1996; Bolek and Janovy 2007a). Finally, some frog lung flukes are intermediate in their arthropod host specificity and can infect both dragonflies and damselflies, but no other arthropods. In these species, dragonflies become infected when they draw cercariae into their branchial basket. However, when these cercariae encounter a larval damselfly, they attach to the caudal gills with their suckers, actively move to the base of the

damselfly's caudal gills, and enter through the anus where the metacercariae develop to an unencysted form (Snyder and Janovy 1996; Bolek and Janovy 2007b; Fig. 11.4).

Although frog lung flukes are fascinating from a life cycle evolution perspective, their identification is one of the difficulties of studying these flukes, as is the case with most trematodes of wildlife. The labyrinthine nature of species identification of frog lung flukes of amphibian hosts from the United States and Canada dates back to their original descriptions (Stafford 1902; Seely 1906; Cort 1915; Harwood 1932; Ingles 1932, 1936; Irwin 1929; Prokopič and Křivanec 1974; Kennedy 1980a, b, 1981). This situation led to a major revision of North American representatives of the genus by Kennedy (1981) who declared only 6 of the 15 species known at the time to be valid. His major argument for the revision was that the morphological variation among the 15 species of frog lung flukes he examined was host induced. However, more recent morphological and life cycle works, in combination with molecular data on many of the frog lung fluke species synonymized by Kennedy clearly indicate that his synonymies were not valid (Bolek 2006; Bolek and Janovy 2007a, b; León-Règagnon and Topan 2018).

Since that revision, most North American workers dealing with *Haematoloechus* species have used Kennedy's (1981) descriptions for *Haematoloechus* species identifications, and as a result, these reports cannot be trusted. For example, Kennedy (1981) synonymized *H. parviplexus* with *H. varioplexus* and three other *Haematoloechus* species, based on his own specimens and vouchers deposited by Brooks (1976) and others. Since Kennedy's revision, Bolek and Janovy (2007b) reviewed all the voucher specimens of *Haematoloechus parviplexus* and *H. varioplexus* from amphibians in the United States, and this work indicated that most of these voucher specimens were misidentified as a result of workers identifying frog lung flukes with Kennedy's key. Additionally, Bolek and Janovy (2007b) reexamined some of the voucher specimens used by Kennedy (1980b, 1981) and found that these specimens did not match the illustrations presented in the revision and the resulting key (Kennedy 1980b, 1981). In fact, it appears that the drawings provided by Kennedy in his 1981 revision and the resulting key for North American frog lung flukes were based on composite drawings from multiple species of *Haematoloechus*! Below, we evaluate the taxonomical issues and convoluted literature for *H. varioplexus* as an example of the difficulties in placing names of trematodes of wildlife.

As with many trematodes of wildlife, the original description and taxonomy of *H. varioplexus* has been troublesome. In the original description paper, Stafford (1902) described *H. varioplexus* from bullfrogs from Toronto and Montreal, Canada, and *H. similiplexus* from northern leopard frogs and American toads, *Bufo americanus*, from numerous locations in Canada but did not deposit any type specimens in an accredited museum. Cort (1915), in a later study on North American frog lung flukes, emended the description of *H. similiplexus* and considered *H. varioplexus* a species inquirenda. Irwin (1929) described *H. parviplexus* from the green frog, *R. clamitans*, from Minnesota, but it is not clear why she did not compare her specimens with the descriptions of *H. varioplexus* and *H. similiplexus* by Stafford (1902). From Irwin's (1929) description, and from the description and drawing of *H. varioplexus* by Stafford (1902), it is clear that both authors were dealing with the same species of frog lung fluke. A few years later, Manter (1938), in a review of amphibian trematodes, synonymized Stafford's (1902) *H. varioplexus* from bullfrogs with Stafford's (1902) *H. similiplexus* from northern leopard frogs and American toads. He did not justify this synonymy, but most likely he made this decision because of the synonymy of this species by Travassos and Darriba (1930). Brooks (1976) used Manter's synonymy and reported *H. varioplexus* (originally described as *H. similiplexus*) from northern leopard frogs, plains leopard frogs, and Woodhouse's toads, and *H. parviplexus* from bullfrogs and Woodhouse's toads in Nebraska, and deposited voucher specimens of both species. Finally, Kennedy (1981) synonymized *H. parviplexus* with *H. varioplexus* and three other *Haematoloechus* species based on his own specimens and vouchers deposited by Brooks (1976)

and others. However, since Kennedy (1981) synonymy of *H. varioplexus* and *H. parviplexus* from leopard frogs and bullfrogs, *H. varioplexus* has not been found in northern leopard frogs and plains leopard frogs when collected from the same locations as bullfrogs that harbor this species (Brooks 1976; Snyder 1996; Bolek and Janovy 2007a, b). To Bolek and Janovy (2007b), the absence of infection in co-occurring potential hosts suggested that *H. parviplexus* and *H. varioplexus* were most likely distinct species.

To test their hypothesis, Bolek and Janovy (2007b) conducted field surveys and anuran host specificity experiments by completing the life cycle of *H. parviplexus* in the laboratory. They exposed laboratory-reared bullfrogs, northern leopard frogs and plains leopard frogs with metacercariae of *H. parviplexus*. Then, after 30–35 days after exposure, all exposed frogs along with control unexposed frogs were killed and examined for frog lung flukes. Their results indicated that all exposed bullfrogs became infected and contained gravid worms of *H. parviplexus*, whereas none of the exposed northern leopard frogs, plains leopard frogs, or unexposed control frogs became infected. They then evaluated 24 morphological characters of museum voucher specimens and field-collected worms recovered from five species of true frogs that they identified based on the original descriptions of *H. varioplexus* (synonym of *H. similiplexus*) by Stafford (1902) and *H. parviplexus* by Irwin (1929). Their analysis indicated that there were significant differences in 13 of the 24 morphological characters among worms identified as *H. varioplexus* from northern leopard frogs, plains leopard frogs and wood frogs, and *H. parviplexus* from bullfrogs and green frogs. Although these differences were statistically significant, there was overlap among these characteristics in all cases except for the acetabulum length and width, oral sucker/acetabulum width ratio, and egg length. Additionally, *H. parviplexus* had a distinctly lobed ovary, while the ovary was never lobed in *H. varioplexus*. To confirm these differences in morphology and life history, Bolek (2006) sequenced the complete internal transcribed spacer (ITS) region of rDNA from worms identified as *H. parviplexus* and *H. varioplexus* along with other species of frog lung flukes collected from various hosts and locations across the United States. Sequence divergence for ITS sequences obtained from *H. parviplexus* and *H. varioplexus* indicated that there was little to no intraspecific variation between *H. parviplexus* and *H. varioplexus*, regardless of the species of host or geographical location the worms were collected from. In contrast, the interspecific ITS divergence among *H. parviplexus* and *H. varioplexus* was quite high and similar to previous studies on the intraspecific and interspecific variation of the ITS barcoding region in this group of parasites (Snyder and Tkach 2001). The resulting phylogeny clearly indicated that *H. parviplexus* and *H. varioplexus* belonged to different clades of frog lung flukes (Fig. 11.4).

Similar results have been obtained for other species of North American frog lung flukes including *H. complexus* and *H. coloradensis*, and *H. breviplexus* and *H. floedae* (León-Règagnon and Brooks 2003; León-Règagnon et al. 2005; Bolek 2006; Bolek and Janovy 2007a). In all of those cases, observations of inconsistencies in host use, evaluations of the literature and museum voucher specimens, as well as re-evaluations of the original species descriptions were all crucial steps in making sense of the identification of trematodes of various amphibian hosts in nature. In addition, careful life cycle studies along with molecular sequence data were critical in the placement of those trematode species in a phylogenetic and evolutionary context (Bolek 2006; Bolek and Janovy 2007a, b; León-Règagnon and Topan 2018).

11.3 Molecular Techniques in Elucidating Cryptic Species and Host Use of Wildlife Trematodes: Examples from Echinostomatid Trematodes in Intermediate Hosts

One of the major interests in studying wildlife trematodes is to evaluate if parasites have negative effects on their hosts. However, few surveys

of parasite communities identify the species of parasites, but instead most studies identify the parasites only to genus. This loss of detail makes it difficult to tease apart the effects of individual parasite species on the host from the effects of the entire parasite community on the host since the species of parasites from the same genus are grouped (Poulin and Leung 2010). Historically, researchers used differences in morphology, host use, infection site within hosts, and geographic information to delineate species of trematode (see Detwiler et al. 2012). However, genetic barcoding data indicate that many wildlife helminths are cryptic (Poulin 2011; Pérez-Ponce de León and Poulin 2018). Crypsis appears to be especially problematic and widespread in adult trematodes recovered from definitive hosts. For example, 10 genetic studies of adult trematodes reported 43 cryptic species of trematode from various vertebrate definitive hosts (Poulin 2011). Additionally, 13 cryptic species were reported from 5 genetic studies of larval trematodes (rediae, cercariae, and metacercariae) collected from intermediate hosts (Poulin 2011). The difficulties of identifying adult and larval trematodes to the species level have important implications for wildlife disease (Poulin 2011).

Due to the abundance of cryptic species of trematodes, it is likely that variation in pathogenicity across cryptic trematode species may be missed when the cryptic species are identified solely using morphological characters and/or host use (e.g. cryptic species grouped as congeners). One exciting outcome of recognizing cryptic species using genetic approaches is the ability to examine differences in pathology among their wildlife hosts, resulting in a better understanding of host–parasite interactions of wildlife trematodes. For example, cryptic species make it difficult to determine the source of transmission for wildlife diseases (i.e., foci of transmission to the vertebrate definitive host). Furthermore, the occurrence of cryptic species makes it difficult to recognize which species of trematodes are responsible for specific pathology in intermediate hosts, especially those of conservation concern like some mollusc and amphibian hosts.

This section focuses on larval echinostomes because they illustrate the problems that erroneous identifications of cercaria and metacercariae stages of trematodes can cause when trying to understand the effects of particular trematode species on wildlife. Applying the barcoding approach to studying larval trematodes has shown that echinostome species diversity has been underestimated (e.g., Detwiler et al. 2010; Georgieva et al. 2013). Furthermore, it is clear that some agents of wildlife disease, especially in amphibian hosts, were incorrectly identified (Detwiler et al. 2010). Barcoding techniques have also shown that host use by larval echinostomes is more variable than previously thought. For example, this approach has demonstrated that some echinostome species use more than one molluscan species as the first and/or second intermediate host in their life cycles (Donald et al. 2004; Detwiler et al. 2010), despite the paradigm in parasitology that a trematode species uses only one species of mollusc as a first intermediate host. This may have implications for prior studies that used first intermediate host specificity as a trait to identify species of larval echinostomes.

Echinostomes are one of the most taxonomically diverse groups of trematodes. For instance, there are 91 nominal genera in the family Echinostomatidae Looss, 1899 (Kostadinova 2005). These trematodes are primarily parasites of wildlife. For example, a large range of bird and mammal species are infected by 11, 3, and 3 genera from three subfamilies of echinostomes (Echinostomatinae, Echinochasminae, and Himasthlinae) in North America (Kostadinova 2005). However, due to their complex life cycle, other wildlife may be affected by echinostomes. In North America, there has been focus particularly on testing for the negative effects of larval echinostomes on amphibian second intermediate hosts (Koprivnikar et al. 2002).

As with many trematodes, the typical life cycles of echinostomes include three hosts. Adult worms reside in the intestines and bile ducts of reptiles, birds, or mammals which serve as definitive hosts (Fig. 11.5; Schell 1985; Toledo et al. 2007). Within the definitive host, worms mate

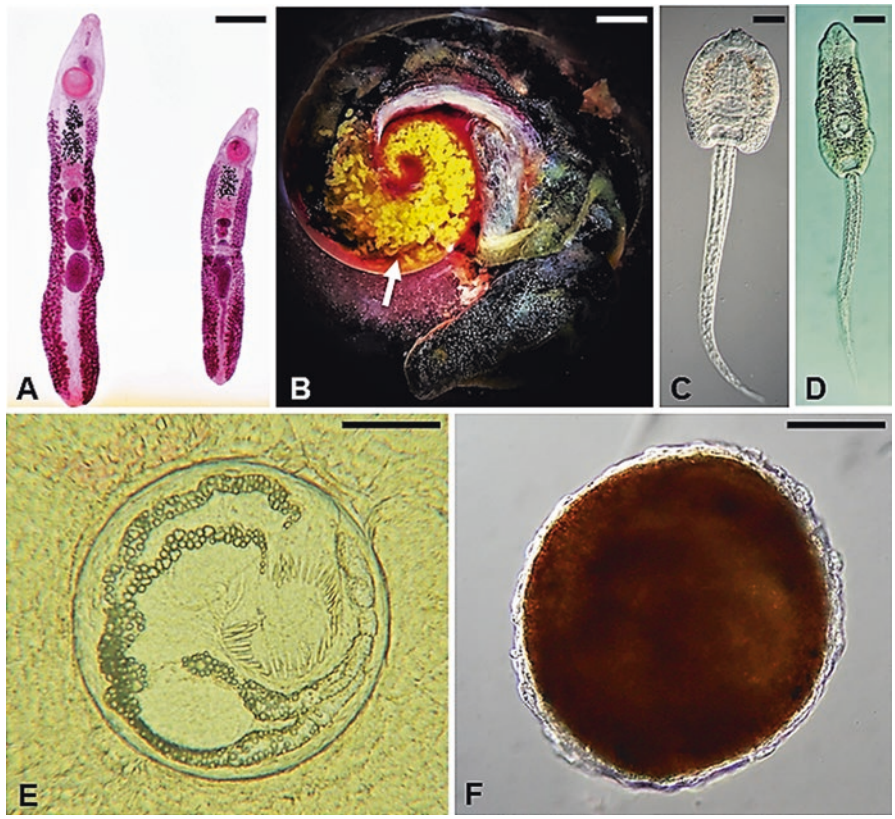

Fig. 11.5 Examples of adult and larval stages of North American echinostomes. (**a**) Adults of *Echinostoma revolutum* sensu stricto recovered from an experimentally infected chicken using metacercariae from infected snails collected in Germany. Scale bar = 2.2 mm. (**b**) A *Helisoma trivolvis* snail first intermediate host collected from Oklahoma and with its shell removed showing rediae stages throughout the digestive gland–ovotestis complex. Scale bar = 3.6 mm. (**c**, **d**) Typical echinostome cercariae released from *Helisoma trivolvis* collected in Oklahoma. Note that the collar and spines on the oral sucker are difficult to see when the cercarial body is compressed in (**c**). Scale bar = 85 μm. (**e**) An echinostome metacercaria encysted in a kidney of a tadpole stage of the bullfrog, *Rana catesbeiana*. Note the collar of spines around the oral sucker. Scale bar = 35 μm. (**f**) A melanized and dead echinostome metacercaria removed from the kidney of a tadpole of the bullfrog, *Rana catesbeiana*. Scale bar = 35 μm

and produce large unembryonated eggs which are void in the host feces. If the eggs are deposited in water, miracidia develop within the eggs in 2–3 weeks, hatch, and actively search and penetrate aquatic snail first intermediate hosts. Several species of physid, planorbid, lymnaeids, and bulinid snails serve as first intermediate hosts for echinostomes. In a typical life cycle and within the snail first intermediate host, a sporocyst develops in the heart of the snail first intermediate host and gives rise to two generations of redia stages. Daughter rediae develop in the digestive gland–ovotestis complex, and in many species cercariae are released into water within 4–6 weeks post infection. A number of echinostome species show a low degree of second intermediate host specificity and metacercariae encyst in various aquatic second intermediate hosts including planarians, molluscs, fish, and amphibians and on aquatic vegetation in some genera (e.g., *Himasthla*, Schell 1985). Of the known life cycles, the metacercariae become infective to the definitive host within 1–3 weeks of encystment, and adult worms mature in the intestinal track and/or bile ducts of the definitive host after being ingested (Schell 1985).

Echinostomes are known to cause substantial pathology, including host death, in first and second intermediate hosts as well as definitive hosts (Toledo et al. 2006, 2007). For example, laboratory studies illustrate that extensive damage occurs to the snail first intermediate host when miracidia penetrate and migrate through the snail host tissues (Toledo et al. 2007). Additionally, redial stages within the snail first intermediate host consume gonadal tissue and lead to parasitic castration (Sorensen and Minchella 1998). Laboratory studies on the metacercaria stages located in the kidneys of tadpoles of amphibian second intermediate hosts can cause pathology and even host death. Experimental studies on tadpoles of northern leopard frogs indicate that tadpoles in the early stages of development (Gosner stages 25–26) died within a few days of being infected. Additionally, many of these tadpoles experienced generalized edema during infection. In contrast, as tadpoles aged and their kidneys developed, they became less susceptible to echinostome infections and older tadpoles (Gosner stages 27–39) did not die or experience edema from infections (Schotthoefer et al. 2003). Supporting field studies on tadpoles of bullfrogs, *Rana catesbeiana*, indicate that larger and older tadpoles had significantly fewer echinostome metacercariae than did smaller and younger tadpoles (Rhoden and Bolek 2012). Furthermore, many echinostome metacercariae located in the kidneys of older bullfrog tadpoles were melanized and dead (Fig. 11.5), suggesting that these larger and older tadpoles were eliminating established echinostome infections (Rhoden and Bolek 2012).

Many of the studies on the effects of larval echinostomes on amphibian second intermediate hosts are based on morphological identifications of echinostome larval stages from field-collected amphibians and/or experimental infections of tadpoles in the laboratory using naturally infected and field-collected snail first intermediate hosts (Schotthoefer et al. 2003; Johnson and McKenzie 2008). As with adult echinostomes, the cercaria and metacercaria stages of echinostomes have distinct morphotypes that include an oral sucker surrounded by a spiny collar (Fig. 11.5; Schell 1985). This characteristic, along with the number and orientation of the collar spines, is shared by the adult stage in species' life cycles (Kanev et al. 2009). However, other traits can vary, especially among genera. For example, "magnacauda" refers to echinostome cercaria with a head collar and spines as well as an unusually large tail (e.g., *Petasiger* and *Neopetasiger*, Gordy et al. 2016). However, more problematic is the fact that some genera and even subfamilies of echinostomes can share the same cercarial morphotype. Therefore, identifying which species of echinostome is responsible for any pathological consequences to the amphibian host can be difficult because the same larval morphotype is shared among many genera. For example, in the subfamily Echinostomatinae, seven genera have the echinostomid cercarial morphotype (*Drepanocephalus*, *Paryphostomum*, *Echinoparyphium*, *Hypoderaeum*, *Echinostoma*, *Isthmiophora*, *Euparyphium*), and one genus has the magnacauda cercarial morphotype (*Petasiger*). In addition, the cercarial morphotype is unknown for three genera (*Lyperorchis*, *Longicollia*, *Bashkirovitrema*).

Because larval echinostome morphotypes can be conserved within genera and even within the family, identification of cercarial and metacercarial stages based on morphology must include measuring discrete morphological traits. The number, size, shape, and arrangement of the collar spines in cercariae can differentiate among species (Kanev et al. 2009). These traits can be measured from live or preserved specimens. Measuring several of these traits, rather than just relying on spine counts, is important because congeners as well as different echinostome genera have the same number of spines (e.g., 27 total in *Bashkirovitrema*, *Paryphostomum*, *Drepanocephalus*, and *Euparyphium*). However, all these characters may not always be present in every specimen as collar spines can be retracted or fall off (Kanev et al. 2009). As a result, multiple individuals should be evaluated. Additionally, other echinostome cercariae traits need to be critically evaluated, including the presence or absence of finfolds on the tail and characteristics of the para-esophageal gland cells (Szuroczki and Richardson 2009). The number of finfolds

and their sizes (length, width) is best measured in live specimens, while para-esophageal gland cells require vital staining with Neutral Red and Nile Blue sulfate (Georgieva et al. 2013). Thus, morphological identification of echinostome larval stages requires significant time and expertise but may be critical to differentiate among species. The insights from the recent and rapidly exploding literature on the effects of larval echinostomes on wildlife could be even more insightful if more details were provided on the morphological diversity of parasites utilized. For example, if host phenotype and personality affect disease outcomes (Koprivnikar et al. 2002), may the same be true for parasites? Without more detailed morphological analysis of parasites, this type of question cannot be addressed. Further, it should be noted that even with careful morphological studies, echinostome larval stages may include cryptic species, and thus, the morphological practices should be combined with the barcoding approach.

It is particularly important to test the hypothesis of crypsis when different species of larval trematodes use the same first intermediate host species. In fact, several echinostome species use the same first intermediate hosts in the same geographic area. For example, for the larval trematodes in Central Europe, there are keys that distinguish among seven echinostome species that all parasitize the snail *Lymnaea stagnalis* (Faltynková et al. 2007). Also in Europe, the planorbid snail, *Planorbdella planorbis*, serves as the first intermediate host for three echinostome species (Faltynková et al. 2008). Unfortunately, there are no similar keys available for species that occur in other parts of the world such as North America. Such a key may help wildlife disease ecologists and conservation biologists identify echinostome cercariae to species so that their role in regulating host populations and communities can be evaluated (Koprivnikar et al. 2012).

Laboratory studies show that larval echinostomes negatively impact their first and second intermediate hosts at the individual level. The cercarial stages have been demonstrated to negatively impact larval amphibians, especially younger stages, by decreasing survival of lab-exposed and field-exposed hosts (Beaver 1937; Fried et al. 1997; Schotthoefer et al. 2003; Holland et al. 2007; Holland 2010; Belden and Wojdak 2011; Johnson and Buller 2011; Raffel et al. 2010). Although these effects have been demonstrated in the laboratory and in semi-natural experiments, the effects on natural host populations are less clear. This is an area of critical research especially considering the number of other factors, such as climate change and human land use, that negatively impact amphibian populations.

However, the ability to accurately identify echinostome species is key to accomplishing this work. Unlike controlled laboratory conditions, there could be several species co-occurring in the same habitat that may or may not be cryptic (Detwiler et al. 2012). DNA barcoding has helped address this issue, as this approach does not require expertise in cercarial or mollusc taxonomy. However, one notable problem with barcoding is that not all researchers are using the same gene (e.g., nad1 or CO1) or the same part of the gene (e.g., Folmer region vs. other parts of CO1). Further, some of the species records in GenBank need to be updated to prevent misidentifications. For instance, Holland (2010) identified the echinostome in their study as *Echinostoma revolutum* based on sequence similarity to a record in GenBank. However, in a more comprehensive survey of echinostomes from North America, it became clear that the specimen was misidentified (Detwiler et al. 2010). To prevent others from making the same mistake, the best approach would be to update the GenBank submission.

The accurate identification of larval trematodes is crucial to understanding the transmission of parasites to wildlife. Trematode larval stages (rediae, cercariae, and metacercariae) tend to have fewer morphological features than adults. They are also smaller sized, making it more difficult for non-experts to photograph, preserve, and stain properly for morphological analysis. In addition, some features are best observed live (i.e., flame cells on cercariae, number and distribution of tail flaps), and so the best preparations include measurements from live and preserved specimens. This process can be very time-consuming

especially considering that it may just answer the question of: What is it? Despite these hurdles, it is advantageous to work with the larval trematode stages, especially in infected molluscs, because these hosts are much easier to gain access to than vertebrates. Furthermore, with barcoding, it is easy to identify them to different taxonomic levels and link these sequences to those of adults to complete life cycles (Locke et al. 2010). This type of work is key to understanding the basic biology of wildlife trematodes (geographic distribution, host specificity), as well as the epidemiology of wildlife disease. For echinostomes, future efforts should focus on developing keys to larval identification in particular areas such as North America and Asia. Further, additional molecular tools could be developed, such as PCR multiplexes, to help a broad array of researchers identify larval echinostome species with more accuracy.

11.4 Life Cycle Plasticity of Wildlife Trematodes: Examples from Frog Bladder Flukes and Amphibian Hemiurid Trematodes

Wildlife biologists and parasitologists routinely use host species as a mode for identifying trematodes as the result of a common assumption that most trematode species of wildlife are host specific, infecting only one host or a few closely related host species. Although many trematodes of wildlife have been reported from only a single host or from a few closely related hosts, this specificity can be an artifact of sampling effort among different potential host species and few studies have examined the distribution of trematodes across the geographic range of one or multiple host species (Detwiler et al. 2012). As a result, we know very little about host specificity at each of the life cycle stages for most trematodes of wildlife (Snyder and Janovy 1996; Detwiler et al. 2012; Kraus et al. 2014). More importantly, recent work on helminth life cycles strongly suggests that life cycles are more variable than the typical textbook life cycle diagrams depict them to be, and transmission strategies have a strong ecological component (Snyder and Janovy 1996; Brooks et al. 2006; Bolek and Janovy 2008; Bolek et al. 2009a, 2010).

The portrayal of life cycles as fixed and invariable units is a consequential error because our knowledge of life histories of parasites is the foundation for our understanding of host–parasite interactions, parasite community and population ecology, life cycle evolution, and the epidemiology of diseases. Therefore, understanding life cycle plasticity and variability is crucial. Despite the importance of this work, few biologists focus on life cycles of parasites as the center of their research. Furthermore, unfortunately most parasitologists that have studied life cycles of parasites only did so until the life cycle could be completed. Once a solution of suitable host combinations was found, most investigators did not continue to search for other hosts through which the life cycle may be completed in nature. Finally, published trematode life cycles tend to be accepted as absolute truth, and their validity is rarely questioned (Krull 1952; Bolek and Janovy 2008; Bolek et al. 2009a, 2010).

Possibly even more problematic, parasitologists have made generalizations about the life cycles of groups of trematodes from the described life cycles of only one or few species from within that group; therefore, much of the variation that likely exists in life cycles of different trematode species is lost. As a result, the common perception of parasite life cycles is that of rigid iron wheels with defined parameters and little or no room for variability (Bolek et al. 2016). However, life cycles of many trematodes are not as simple as they appear in textbook diagrams because in most cases, the variability in life cycles, especially among closely related species, has been largely overlooked or simply ignored (Snyder and Janovy 1994, 1996; Bolek and Janovy 2007a, b, 2008; Bolek et al. 2009a, 2010; Stigge and Bolek 2015, 2016a, b). Below, we provide examples on how studies on life cycles of two groups of trematodes, frog bladder flukes and frog hemiurid trematodes, can be useful in helping us understand the relationships of wildlife trematodes with their hosts.

The first example we discuss below demonstrates that life cycle variation and host usage can exist within a single species of trematode. The conceptual strength of the following study rests with the examination of alternative routes of infections by a single species of frog bladder fluke to different species of definitive amphibian hosts that vary in their life histories and phylogenetic relationships (Bolek et al. 2009a).

Amphibian bladder flukes belong to the family Gorgoderidae Looss, 1901. They are translucent, flat, non-spinous flukes that are broadly pyriform, banjo, or lanceolate in body shape. They have well-developed suckers with the acetabulum usually projecting prominently. The pharynx is absent and the cecae are long, sinuous, and end blindly. The cirrus sac is absent and the genital pore is media. The testes can vary from two to nine depending on the genus, the ovary is intertesticular or pretesticular and the vitelline glands are in the form of two opposite clusters of follicles anterior to the testes (Prudhoe and Bray 1982). Additionally, adult flukes contain various papillae on the tegumental surface which appear to be species specific (Mata-López and León-Règagnon 2006). Four genera of bladder flukes have been reported from amphibian hosts including *Phyllodistomum*, *Progorgodera*, *Gorgoderina*, and *Gorgodera*. However, of these, the genera *Gorgoderina* and *Gorgodera* (Fig. 11.6) are the most species rich in amphibian hosts (Prudhoe and Bray 1982). In general, most amphibian gorgoderid species are difficult to identify because the testis and ovary can fragment over time as worms age, and the uterus obstructs most of the internal organs (Cort 1912). Additionally, host-induced morphological variation is commonly reported in members of this family (Bolek et al. 2009b; Cutmore et al. 2010).

Currently, there are 53 species of *Gorgoderina* Looss, 1902 reported to infect amphibian bladders and they are found worldwide (Mata-López 2006). Five species have been described from Africa, 17 from Eurasia and 31 from the Americas (see Mata-López et al. 2005; Mata-López 2006). In contrast, there are approximately 16 species of

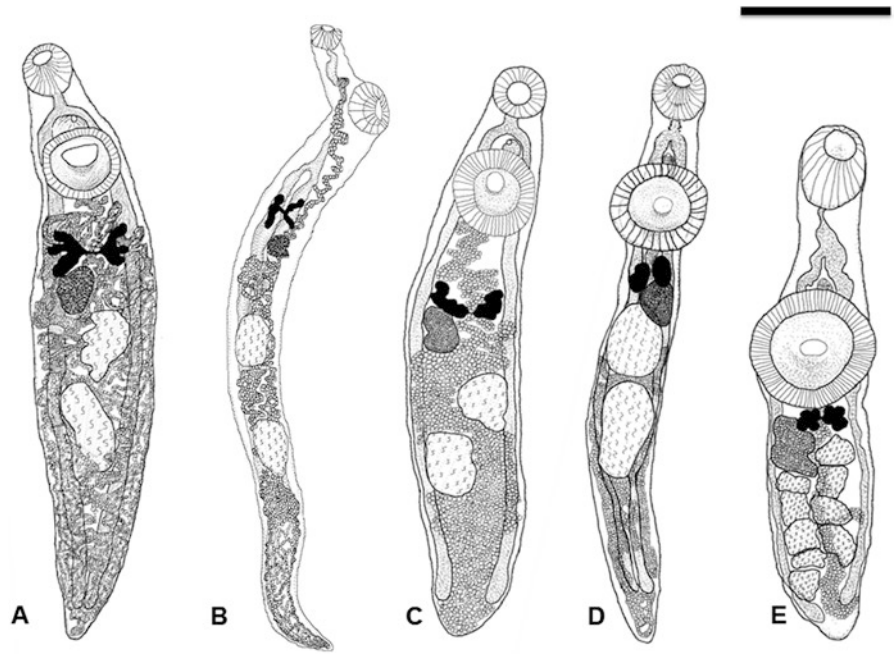

Fig. 11.6 Examples of common North American frog bladder flukes. (**a**) *Gorgoderina translucida* from the urinary bladder of the American toad *Bufo americanus*. (**b**, **c**) *Gorgoderina simplex* from the urinary bladder of *Rana clamitans*. (**d**) *Gorgoderina attenuata* from the urinary bladder of *Rana pipiens*. (**e**) *Gorgodera amplicava* from the urinary bladder of *Rana catesbeiana*. Scale bar = 0.9 mm. All drawings are original

bladder flukes in the genus *Gorgodera* Looss, 1899 described from amphibians from North America, Eurasia, and New Zealand. However, most of the descriptions for species of *Gorgodera* are based on few specimens collected from a single host without considering host-induced morphological variation as has been reported for many of the bladder fluke species infecting fish definitive hosts (Cutmore et al. 2010). As a result, it is not clear if these descriptions represent distinct species, and the genus is in dire need of revision (Cort 1912; Yamaguti 1971; Dale 1967).

Of the 69 amphibian bladder flukes reported from around the world, 12 species of *Gorgoderina* and 3 species of *Gorgodera* have been described from anuran and caudatan hosts from Canada and the United States (Yamaguti 1971; Mata-López et al. 2005; Mata-López 2006; Bolek et al. 2009b). Stafford (1902) described three species of *Gorgodera* Looss 1899, now considered to be members of *Gorgoderina*: *Gorgoderina attenuata* (Stafford 1902) Stafford, 1905; *G. opaca*, (Stafford 1902) Stafford, 1905; and *G. translucida* (Stafford 1902) Stafford, 1905. Stafford (1902) also reported on *Gorgoderina simplex* (Looss 1899) Looss, 1902 and *Gorgodera amplicava* Looss, 1899 from anurans assumed to be collected in Toronto, Canada. As typical of many species descriptions from the earlier part of the twentieth century, none of these specimens were deposited in any museum, and to our knowledge, no type specimens exist for any species of *Gorgoderina* or *Gorgodera* described or reported on by Stafford (1902) in museum collections. As a result of the lack of reliable descriptions, voucher specimens and host records, most of the species of North American *Gorgoderina* and *Gorgodera* species are reported from one or a few closely related amphibian definitive hosts (Mata-López et al. 2005; Mata-López and León-Règagnon 2005; Mata-López 2006), and so they appear to conform to the foundational assumption about host specificity. In contrast, *G. attenuata* is the most commonly reported bladder fluke in North American anurans and caudatans, and it has been reported from 24 amphibian and reptilian hosts (see Brooks 1976; Mata-López et al. 2002; Bolek and Coggins 2003; Bolek et al. 2009b), suggesting that *G. attenuata* is commonly misidentified, the life cycle is more variable than previously assumed or a combination of these factors.

The typical life cycle for amphibian bladder flukes involves bivalves in the family Sphaeriidae as first intermediate hosts, various aquatic snails, arthropods, and larval and adult amphibians as second intermediate hosts and metamorphosed amphibians as definitive hosts (Bolek et al. 2009b). Embryonated eggs containing miracidia are released in the urine and feces of amphibian hosts (Fig. 11.7) and hatch when they enter water. The miracidia enter the incurrent siphon of fingernail clams, where they develop through two generations of larval sporocysts within the gill chamber of fingernail clams. Within the daughter sporocyst, cysticercus cercariae (Fig. 11.7) develop and are released through the excurrent siphon 38–48 days post infection (Krull 1935a; Rankin 1939; Ubelaker and Olsen 1972). After leaving the fingernail clam, the cercariae are ingested by the appropriate host for the gorgoderid species such as various aquatic snails, arthropods, or tadpoles (Prudhoe and Bray 1982). Depending on the gorgoderid species and/or second intermediate host species, the metacercariae either can be encysted or remain unencysted within the second intermediate hosts (Fig. 11.7). Commonly, gorgoderid metacercariae encyst in the hemocoel of arthropod and snail second intermediate hosts or the body cavity of amphibians (Krull 1935a; Rankin 1939; Goodchild 1948; Ubelaker and Olsen 1972). In addition, unencysted gorgoderid metacercariae have been reported from the kidneys of tadpoles (Goodchild 1950; Bolek et al. 2009a). To further increase the complexity of variation, progenetic metacercariae have also been reported from at least one amphibian gorgoderid species (Ubelaker and Olsen 1972). Once ingested by the definitive host, encysted metacercariae will excyst in the small intestine, and all types of metacercariae, regardless if they were once encysted or not, migrate along the mucosa of the digestive track, enter the cloaca and invade the urogenital ducts (Fig. 11.7) where worms develop in the Wolffian ducts (Goodchild 1950). Eventually, worms

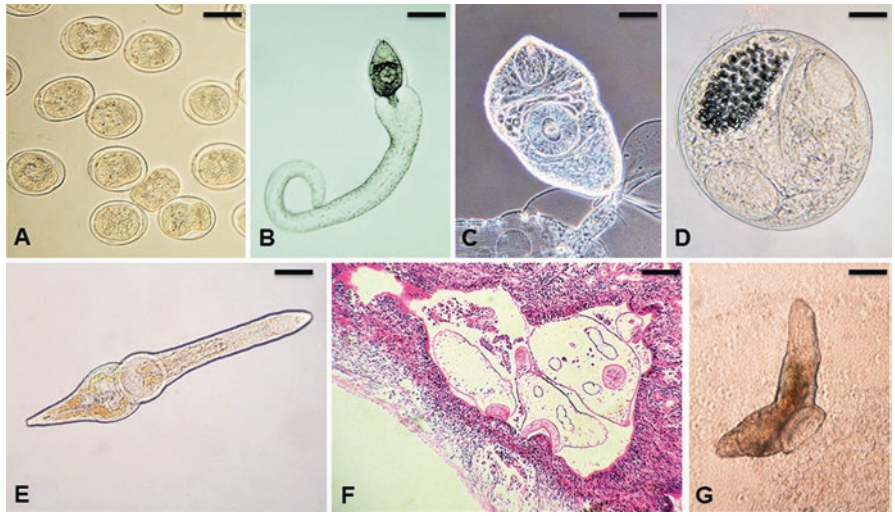

Fig. 11.7 Life cycle stages of *Gorgoderian attenuata*. (**a**) Typical eggs of gorgoderids. Note that the eggs are unoperculated and not golden brown as is typical for many trematodes of wildlife. Scale bar = 20 μm. (**b**) Cysticercus cercaria. Note the long and thick tail. Scale bar = 0.1 mm. (**c**) The cercaria body emerging from the tail. Scale bar = 30 μm. (**d**) Encysted metacercariae from a larval damselfly, *Ischnura verticalis*. Note the granule excretory vesicle. Scale bar = 25 μm. (**e**) Unencysted metacercaria removed from the kidneys of a tadpole of a northern leopard frog, *Rana pipiens*. Scale bar = 30 μm. (**f**) Cross section of juvenile worms in the kidney of a metamorphosed bullfrog, *Rana catesbeiana*. Scale bar = 500 μm. (**g**) Adult worm attached to the wall of the urinary bladder of an adult northern leopard frog *Rana pipiens*. Scale bar = 500 μm

migrate to the urinary bladder where they attain sexual maturity (Fig. 11.7), feed on blood and mucus secretions, mate and release eggs in the urine of their amphibian host (Krull 1935a; Goodchild 1950).

In some amphibian species, such as bullfrogs, *Rana catesbeiana*, adult worms never enter the urinary bladder and can be trapped in the urogenital ducts where they cause considerable pathology (Goodchild 1950). Studies indicate that the invasion of the interstitial mesonephric tissue or mesonephric tubules by juvenile worms escalates a host reaction including hyperemia and encapsulation of each worm by granulocytes (Goodchild 1950). High worm burdens in the kidneys and ureters are harmful to the host, and heavily parasitized frogs are sluggish (Goodchild 1950). Heavy worm burdens can cause the kidneys to be hyperemic, purplish in color and result in death of the frog. Studies by Goodchild (1950) suggest that death may be due to injurious histolytic wastes and uremia caused by kidney failure. As a result, gorgoderid trematodes may have negative consequences on native frog population (Goodchild 1950).

Over the years, work on the life cycles and transmission strategies of amphibian bladder flukes indicates a remarkable plasticity in the use of second intermediate hosts in their life cycles. Species of *Gorgodera* and *Gorgoderina* primarily infect tadpoles as second intermediate hosts but can also infect odonates and molluscs. However, species of *Phylodistomum* use arthropods as second intermediate hosts, while adult amphibians become infected by ingesting arthropods, snails, tadpoles, or other frogs infected with metacercariae (Krull 1935a; Rankin 1939; Crawford 1940; Goodchild 1943, 1948; Ubelaker and Olsen 1972; Bolek et al. 2009a). Field studies also indicate that within individual anuran species, newly metamorphosed and juvenile anurans are less commonly infected with bladder flukes than are larger adult frogs because of small gape size, which affects the size of potential intermediate hosts that can be ingested by amphibian definitive hosts (Bolek et al. 2009a). However, and contrary to these studies, observations from Nebraska by Bolek et al. (2009b) indicate that newly metamorphosed northern leopard

frogs are commonly infected with *G. attenuata*, with prevalence reaching 80%. Even more surprising is that newly metamorphosed toads (1 cm snout vent length) that never feed on tadpoles, other anurans, or odonates are commonly infected with *G. attenuata* (Bolek and Janovy 2007a; Bolek et al. 2009a).

Previous studies by Rankin (1939) on the life cycle of *G. attenuata* from the northeastern region of the United States indicated that adult anurans become infected after ingesting a tadpole or a metamorphosed frog infected with encysted metacercariae of *G. attenuata*. This discrepancy in field observations from anurans collected from Nebraska by Bolek et al. (2009a) suggested that either the life cycle of *G. attenuata* was more variable than previously thought or they were dealing with cryptic species of bladder flukes in Nebraskan anurans. As a result of these contradictions, Bolek et al. (2009a) questioned the validity of Rankin's (1939) "iron wheel" and re-evaluated the life cycle.

To investigate the plasticity in the life cycle of *G. attenuata*, Bolek et al. (2009a) examined field-collected tadpoles, newly metamorphosed leopard frogs and toads, as well as adult leopard frogs, Woodhouse's toads, and bullfrogs for metacercariae and adult stages of *G. attenuata* from a single location in western Nebraska. To their surprise, no encysted gorgoderid metacercariae were found! Instead, their field data indicated that all infected tadpoles contained non-gravid worms in their kidneys. In contrast, only a single metamorphosed leopard frog and none of the metamorphosed toads had worms in the kidneys, but all contained adult worms in their bladders. These field observations suggested that the lack of worms in the kidneys of metamorphosed leopard frogs and toads indicated that both of these anuran species acquire infections with *G. attenuata* primarily during the tadpole stage and that worms migrated from the kidneys to the urinary bladder as soon as tadpoles metamorphose into froglets or toadlets. Additionally, because tadpoles of bullfrogs were less commonly infected with gorgoderids in the kidneys, but adult bullfrogs and some adult leopard frogs contained non-gravid worms in their kidneys, they hypothesized that adult frogs were also actively recruiting *G. attenuata* after metamorphosis.

To test their hypotheses, Bolek et al. (2009a) examined the life cycle in the laboratory. They collected naturally infected fingernail clams with what they assumed were the cercariae of *G. attenuata* and sequenced the complete ITS region of nuclear rDNA from the cercariae and juvenile and adult worms recovered from the kidneys and urinary bladders of field-collected tadpoles and metamorphosed anurans, respectively. The sequence data confirmed that all stages belonged to *G. attenuata*. Next, they exposed tadpoles of leopard frogs with the cercariae of this species and examined them for the locations of worms at different stages through metamorphoses. Their results indicated that tadpoles became infected by accidentally ingesting cercariae, and juvenile worms entered tadpole kidneys, eventually migrating to the urinary bladder as tadpoles metamorphosed into froglets. The combination of field and laboratory data indicated that *G. attenuata* had a truncated two-host life cycle that includes fingernail clams and leopard frogs and toads.

Bolek et al. (2009a) expanded their study to examine the variability in life cycle when frogs are infected with *G. attenuata* as adults. Because leopard frogs commonly feed on odonates and bullfrogs often consume both odonates and tadpoles, arthropods were surveyed for the presence of gorgoderid metacercariae and adult leopard frogs and adult bullfrogs for the presence of worms in their kidneys. The fieldwork surveys indicated that gorgoderid metacercariae were present in larval odonates and non-gravid worms were present in the kidneys of adult leopard frogs and bullfrogs. To confirm their field observations, Bolek et al. (2009a) first exposed uninfected damselfly larvae with the cercariae of *G. attenuata* and recovered encysted metacercariae from these hosts. Second, they exposed laboratory-reared frogs with metacercariae recovered from damselflies and found non-gravid *G. attenuata* worms in the kidneys of their exposed frogs. With that experiment, they were able to show a second transmission strategy where adult leopard

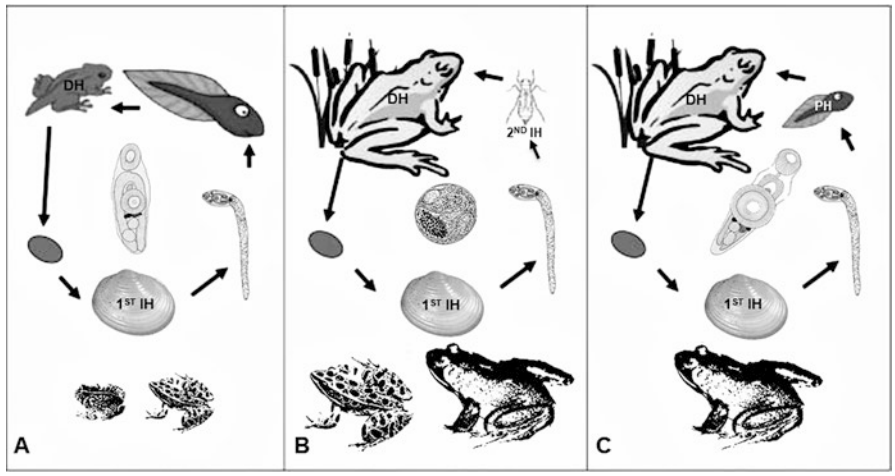

Fig. 11.8 Variations on the life cycle of *Gorgoderina attenuata* from western Nebraska, United States, and the anuran hosts which are infected. (**a**) Two host life cycle where cercariae develop within the Sphaerid pea clams (*Pisidium compressum*) are released into the water column and ingested by tadpoles of *Rana pipiens* and *Bufo woodhousii*. Unencysted metacercariae (drawing in center of life cycle) are found in tadpole kidneys and migrate to the urinary bladder once tadpoles metamorphose. (**b**) Three host life cycle where the cercaria is ingested by an odonate second intermediate host (second IH) and metacercariae encyst in the hemocoel (drawing in center of life cycle). This transmission strategy is used to infect adult leopard frogs and adult bullfrogs. (**c**) Three host life cycle using a tadpole as a transport host (PH) with unencysted metacercariae in the kidneys of tadpoles (drawing in center of life cycle). This transmission strategy is used to infect adult bullfrogs. Adapted from Bolek et al. (2016)

frogs and adult bullfrogs can become infected by *G. attenuata* when they ingest metacercariae from damselfly second intermediate hosts. Finally, laboratory-reared bullfrogs were fed leopard frog tadpoles infected with non-gravid *G. attenuata* worms in their kidneys. Surprisingly, the results of those infections revealed a third transmission strategy, where *G. attenuata* can also infect adult bullfrogs when bullfrogs feed on other infected anurans possessing worms in their kidneys! More importantly, this comparative approach allowed Bolek et al. (2009a) to demonstrate that in nature different life cycle strategies were occurring simultaneously, allowing for infections of different life stages and different species of anurans (Fig. 11.8). Conclusively, the bladder fluke example clearly demonstrates that some trematode life cycles are more plastic than previously thought and identification of adult worms based on definitive host species may not always be appropriate.

The second example we discuss below demonstrates that life cycle variations in trematode site fidelity in the definitive host can also exists within a single species of trematode. Over the years, variation in site specificity has caused major confusion on the identification among some members of the genus *Halipegus* (Stigge and Bolek 2016a). The conceptual strength of this work rests with the use of life cycle studies to elucidate the complex and confusing taxonomical history on trematodes that have been used as model systems for ecological studies on trematode metapopulations and transmission dynamics (Goater et al. 1990a, b; Wetzel and Esch 1996; Zelmer and Esch 1998a, b).

Species of *Halipegus* occur in amphibian definitive hosts through Asia, Europe, North America, and South America (Bolek et al. 2010; Stigge and Bolek 2015; McAlpine 2006; Stigge and Bolek 2016a, b). Members of the genus include hemiurid trematodes that occur in the stomach, mouth, or eustachian tubes of amphibian definitive host (Bolek et al. 2010). Adult worms are elongate, fusiform, or oval in shape, approximately 4–9 mm in length and 1.5–3 mm in width (Fig. 11.9). The tegument is non-spinous and they lack a sinus organ, sinus sac, or her-

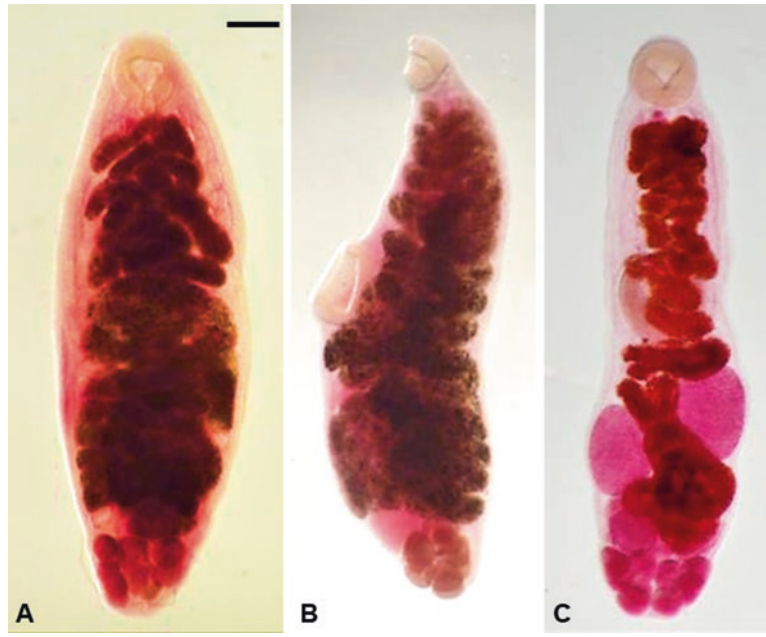

Fig. 11.9 Examples of North American *Halipegus* species. (**a**) *Halipegus eccentricus* from a green frog, *Rana clamitans*. (**b**, **c**) Lateral and ventral view of *Halipegus occidualis* from a bullfrog, *Rana catesbeiana*. Scale bar = 360 μm

maphroditic duct (Kohn et al. 1990; Zelmer and Esch 1999). Six species are currently recognized in the genus, two of which (*Halipegus eccentricus* and *Halipegus occidualis*) have been reported from various species of frog and salamander definitive hosts from the United States (León-Règagnon and Romero-Mayén 2013).

Arguably, trematodes in the genus *Halipegus* have some of the most bizarre and complex trematode life cycles and include four hosts in their life cycles (Krull 1935a, b; Bolek et al. 2010; Stigge and Bolek 2015, 2016a). Depending on the species, adult worms reside in the stomach, mouth, or eustachian tubes of amphibian definitive host, where they mate and release embryonated eggs with long abopercular filaments. After being ingested by aquatic snails in the families Physidae and Planorbidae first intermediate hosts, eggs hatch and the non-ciliated miracidium uses a crown of pen-like spines to penetrate the snail's intestine (Fig. 11.10). A generation of sporocyst development is followed by a generation of rediae which develop in the digestive gland of the snail first intermediate host. Depending on the species, and within 30–60 days post infection, cystophorus cercariae (Fig. 11.10) are released from the snail first intermediate host. The cercariae are non-motile and reside on the bottom of the pond after being released from the snail host. The cercaria body, along with a delivery tube, is tightly coiled and maintained under great pressure within the double-walled tail, which encloses the two as a cyst. On one side of the cercarial tail is a structure known as a handle or caudal appendage. When various species of ostracod and copepods second intermediate hosts attempt to ingest the cercaria, the delivery tube is everted through the caudal appendage, the cercarial body travels through the delivery tube and is injected into the hemocoel of the microcrustacean second intermediate host (Fig. 11.10). Over a period of 2 weeks, the unencysted metacercaria develops an everted bladder with distinct villi (Fig. 11.10), which is shed when the metacercaria becomes infective to the next host in the life cycle (Bolek et al. 2010). Because metamorphosed frogs do not commonly ingest microcrustaceans but instead commonly feed on odonate larvae which themselves are veracious predators of microcrustaceans, damselflies, and dragonflies are utilized as paratenic host in the life cycle (Bolek et al. 2010; Stigge and Bolek 2015, 2016b). Metacercariae reside in the gut of aquatic damselfly and dragonfly hosts and survive

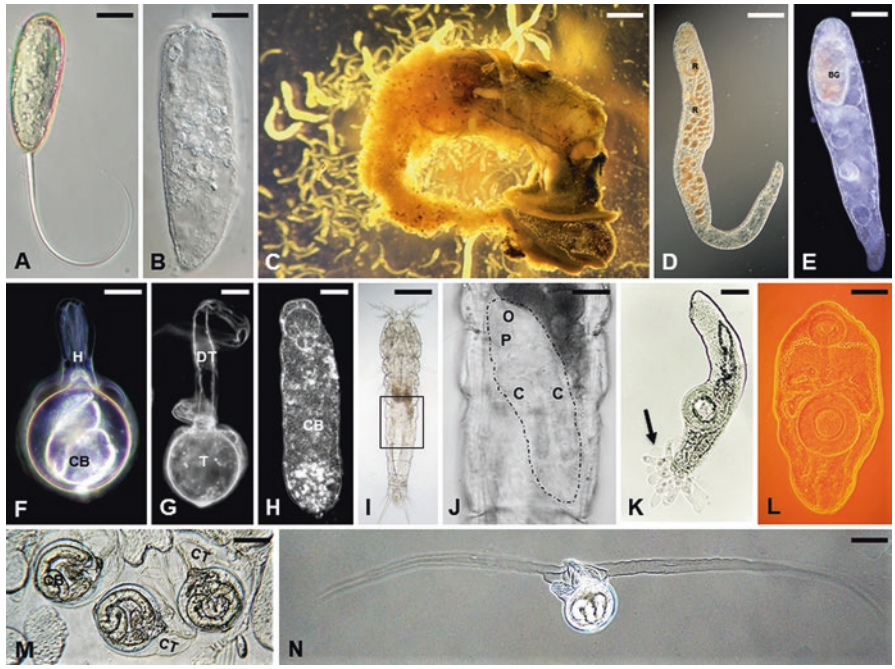

Fig. 11.10 Non-adult life cycle stages of *Halipegus* species. (**a**) Typical egg showing abopercular filament. Scale bar = 20 µm. (**b**) Non-ciliated miracidium. Note the crown of pen-like spines. Scale bar = 10 µm. (**c**) *Helisoma trivolvis* snail first intermediate host with the shell removed and infected with *Halipegus occidualis* showing numerous rediae released from the digestive gland. Scale bar = 2 mm. (**d**, **e**) Sporocyst with developing rediae (R) and daughter redia with developing cercariae. Note the blind gut (BG) in the redia. Scale bars = 100 µm. (**f**) An undischarged cystophorous cercaria of *H. occidualis* showing the cercarial body (CB) and handle (H). Scale bar = 25 µm. (**g**, **h**) A discharged cystophorous cercaria of *H. occidualis* showing the delivery tube (DT) and empty tail (T) and the released cercarial body (CB). Scale bars = 20 and 15 µm. (**i**, **j**) A harpacticoid copepod *Phyllognathopus* sp. second intermediate host, showing developing metacercaria in the hemocoel. Note the oral sucker (O), pharynx (P), and ceca (C) in the outlined worm. Scale bars = 0.2 mm and 25 µm. (**k**) Metacercaria removed from the hemocoel of a copepod second intermediate host. Note the everted bladder with distinct villi (arrow). Scale bar = 200 µm. (**l**) Infective metacercaria removed from the intestine of a damselfly paratenic host. Note the lack of everted bladder. Scale bar = 150 µm. (**m**, **n**) Developing and released cystophorous cercaria of *H. eccentricus.* Note the cercaria body (CB) and tail (CT) in (m) and two lateral streamers in (**n**). Scale bars = 38 µm

through metamorphoses (Fig. 11.10). When frogs ingest infected odonates, the worms reside in the stomach for up to 40–50 days and depending on the species eventually migrate to the eustachian tubes or to the lingual vein under the tongue of their frog definitive host, where they mate and produce eggs. In terms of pathology, studies by Wetzel and Esch (1996) indicated that large number of worms under the tongue of frogs can build over time and then suddenly drop to zero. They hypothesized that the large number of worms under the tongue of frogs can stimulate a strong inflammatory response and this caused worms to be sloughed off.

As a general rule, adult worms of species of *Halipegus* have few morphological characteristics to distinguish between species (Zelmer and Brooks 2000; León-Règagnon and Romero-Mayén 2013). As a result, many investigators working on this group of trematodes have used non-adult characters such as the egg and/or cercarial morphology for species identification, but most commonly the site fidelity of the adult worms in the amphibian definitive host was used to distinguish between species (Figs. 11.9 and 11.10; Goater et al. 1990a, b; Zelmer and Esch 1999; Zelmer and Brooks 2000; Bolek et al. 2010; León-Règagnon and Romero-Mayén

2013). However, the identification of *Halipegus* species has been questionable as a result of inadequate species descriptions and various life cycle work on worms originally recovered from different locations (eustachian tubes, under the tongue or stomach) in different species of amphibian definitive hosts collected from different geographical locations across the United States (Stafford 1905; Krull 1935a, b; Thomas 1939; Rankin 1944; Macy et al. 1960).

As is the case for many wildlife trematodes discussed in this chapter, the nomenclatural history of North American species of *Halipegus* is extremely confusing due to a lack of appropriate holotypes and incomplete species descriptions, thus resulting in many misidentifications within the literature (McAlpine 2006; León-Règagnon and Romero-Mayén 2013). In 1905, Stafford described *Halipegus occidualis* based on 37 worms he recovered from the mouth of the bullfrog, *R. catesbeiana*, collected in eastern Canada. Specifically, he indicated that the worms were recovered from the mouth and included the eustachian tubes, in the fold along the inner edge of the jaw bone, at the entrance of the posterior nares and round the entrance to the esophagus (Stafford 1905). In addition, he indicated that egg filaments he was able to measure from nine eggs varied from 47 to 53 μm. Recognizing Stafford's (1905) inadequate description, Krull (1935b) elucidated the life cycle and redescribed this species based on what he assumed was *H. occidualis* from worms he recovered from under the tongue of green frogs, *Rana clamitans*, collected from Maryland, United States. Later, Thomas (1939) elucidated the partial life cycle and described *Halipegus eccentricus* from the eustachian tubes of northern leopard frogs, *R. pipiens*; green frogs, *R. clamitans*; and bullfrogs, *R. catesbeiana*, collected in Michigan, United States. Thomas (1939) based his differentiation of *H. eccentricus* from *H. occidualis* based on Krull's (1935b) life cycle work. More specifically, he used the differences in first intermediate snail host use, cercarial morphology, egg filament length (56–58 μm for *H. eccentricus* versus 160–200 μm for Krull's description of *H. occidualis*), and the location of adult worms in the eustachian tubes of frogs for *H. eccentricus* versus under the tongue of frog hosts for *H. occidualis* (Fig. 11.11). Finally, to add to the confusion, Macy et al. (1960) reported on the life cycle of worms identified as *Halipegus occidualis* with egg filament lengths of 61–100 μm but recovered from the esophagus and upper stomach of the northern red-legged frog, *Rana aurora*, and two salamander species, the rough-skinned newt *Taricha granulosa* and the California giant salamander, *Dicamptodon ensatus*, collected from Oregon.

More recently, using genetic evidence, along with egg filament size and habitat of adult worms, Goater et al. (1990b) indicated that worms known as *H. eccentricus* with short egg filaments and located in the eustachian tubes of frogs versus worms with long egg filaments redescribed by Krull (1935b) as *H. occidualis* from under the tongue of frogs were distinct species. From that work, McAlpine and Burt (1998) suggested that Krull's (1935b) *H. occidualis* redescription based on worms with long egg filaments (160–200 μm) and recovered from under the tongue of frogs was in error and represented a distinct species from Stafford's original, 1905, description of *H. occidualis* which had short egg filaments (47–53 μm) and was not reported from under the tongue of frogs. In addition, they argued that the short egg filament length of Thomas' (1939) description of *H. eccentricus* (56–58 μm) was similar to the egg filament length of *H. occidualis* described by Stafford (1905). As a result, they suggested that Thomas' (1939) description of *H. eccentricus* from the eustachian tubes of frogs in fact represented Stafford's *H. occidualis*. Finally, because the worms identified as *H. occidualis* by Macy et al. (1960) occurred in the esophagus and upper stomach of amphibian definitive hosts, and the egg filaments were intermediate in size (61–100 μm) compared to the descriptions provided by Stafford (1905), Krull (1935b), and Thomas (1939), they suggested this *Halipegus* species could represent an undescribed species.

Since the publication of McAlpine and Burt (1998) and the original confusion as to the location of the worms described by Stafford (1905) from the mouth of the bullfrog, there have been disagreements as to the nomenclature of North

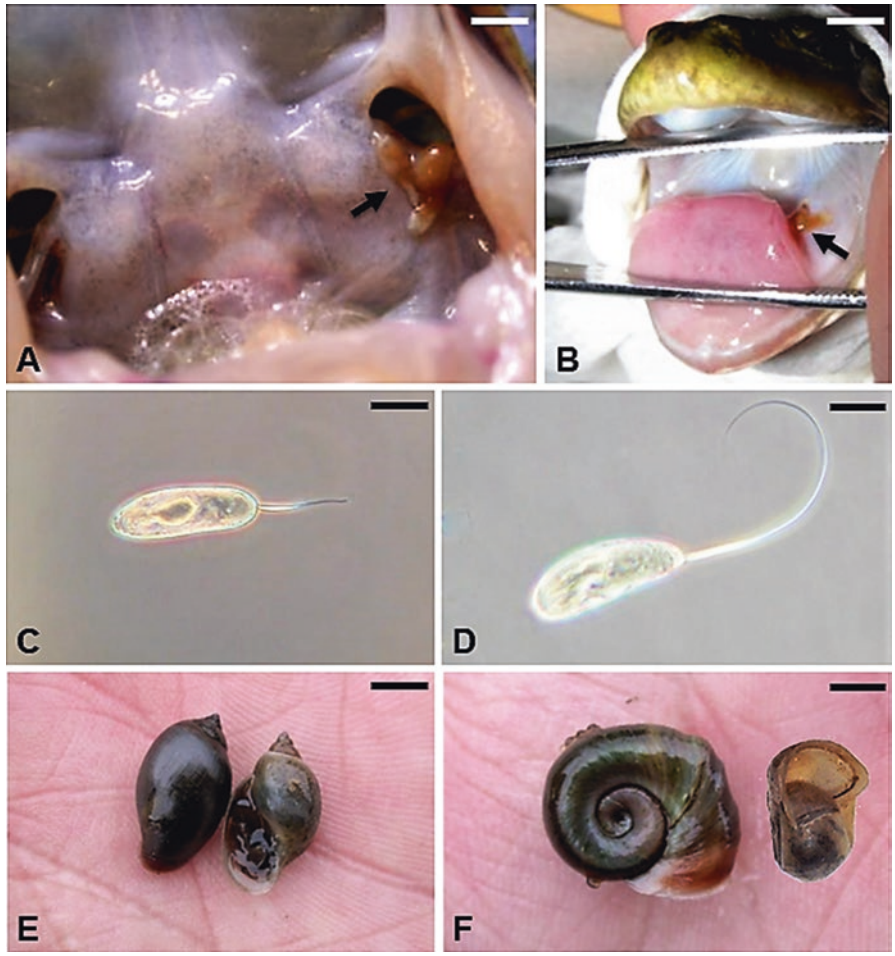

Fig. 11.11 Hosts and eggs of *Halipegus eccentricus* and *Halipegus occidualis*. (**a**, **b**) Site fidelity of *H. eccentricus* in the eustachian tube of a bullfrog, *Rana catesbeiana*, and *H. occidualis* under the tongue and attached to the lingual vein of a green frog, *Rana clamitans*. Scale bars = 5 and 10 mm. (**c**, **d**) Comparison of egg filaments of *H. eccentricus* and *H. occidualis*. Note the much longer egg filament of *H. occidualis* in (**d**). Scale bars = 24 μm. (**e**, **f**) Typical first intermediate snail hosts for *H. eccentricus* (*Physa acuta*) and *H. occidualis* (*Helisoma trivolvis*). Scale bars = 5 mm

American species of *Halipegus* residing under the tongue or the eustachian tubes of frog definitive hosts (Zelmer and Esch 1999; McAlpine 2006). The resulting consensus reached was that worms residing under the tongue of frogs should be referred to as *H. occidualis* and the worms occurring in the eustachian tubes of frogs as *H. eccentricus* (McAlpine 2006). As a result, site fidelity of amphibian hemiurid trematodes in their amphibian hosts has become the gold standard for species identification and descriptions (León-Règagnon and Romero-Mayén 2013).

However, over the last 110 years, and since Stafford's (1905) original description of *H. occidualis* from the mouth of the bullfrog, there are no credible reports of bullfrogs being infected with worms under the tongue (Bolek et al. 2010). More interestingly, Bolek et al. (2010) observed that bullfrogs were infected with gravid worms that resided in the upper stomach and contain eggs with long filaments (112–175 μm) that were more similar in size to the egg filaments of *H. occidualis* from under the tongue of frogs than *H. eccentricus* from the eustachian tubes of frogs.

Considering the work of Macy et al. (1960) and since all other reports of *H. occidualis* were from under the tongue of green frog definitive hosts, Bolek et al. (2010) hypothesized that either worms residing in the stomach of bullfrogs represented an undescribed species of *Halipegus*, or *H. occidualis* might reside under the tongue and/or the upper stomach of anurans.

To test this hypothesis, Stigge and Bolek (2015, 2016a, b) brought the life cycle into the laboratory. Their rationale was that if individuals of *H. occidualis* occupy two different habitats within different species of amphibian definitive hosts, then the site fidelity of *Halipegus occidualis*, and potentially other species of *Halipegus*, was not as strongly conserved as previously thought. On the contrary, it was also possible, as suggested by McAlpine and Burt (1998), that trematodes recovered from the stomach of amphibians were misidentified as *H. occidualis*, and therefore they could represent a distinct species of *Halipegus* that exhibit specificity for the stomach. To complete the life cycle in the laboratory, eggs were collected from gravid worms recovered from the stomach of naturally infected bullfrogs to be used for laboratory-reared snail infections. Once cercariae were produced, laboratory-reared microcrustacean ostracod second intermediate hosts were then fed the laboratory-reared cercariae. Anurans were exposed to *H. occidualis* metacercariae by pipetting 20 exposed ostracods infected with 20-day-old metacercariae into the stomach of multiple individuals of seven amphibian species. Importantly, the seven species of anurans included green frogs and bullfrogs which have been reported to contain worms under their tongue or the upper stomach, respectively, as well as other anuran species that were only occasionally (leopard frogs) or never (toads and tree frogs) reported to be infected with *Halipegus* species in nature (Stigge and Bolek 2016a). After exposure, the buccal cavity of each anuran was checked daily for worms by placing a flat blunt probe into the edge near the corner of the mouth and gently applying force until the mouth opened. Once individuals of *H. occidualis* appeared under the tongue, and with great care not to damage the worms, worms were individually removed from the mouth using rounded and blunt forceps and examined for the presence of eggs as a wet mount of each worm with compound microscope. After worms were examined for the presence of eggs, all worms were placed back into the mouth of the anuran from which they were removed. Finally, after 95 days post exposure all anurans were euthanized and examined for worms in the stomach.

Their results indicated that *H. occidualis* appeared in the buccal cavities of all anuran species except bullfrogs. Worms that infected bullfrogs were never observed in the mouth during the 95 days that anuran mouths were examined. However, when all seven anuran species were dissected 95 DPE, gravid worms were found in the stomach of only bullfrogs, and all other worms were located under the tongue of all infected individuals of the six other amphibian species. Additionally, they observed significant differences in the average time it took worms to migrate from the stomach to the buccal cavity among the six anuran species with worms under their tongues. For example, worms took a significantly longer time to migrate to the buccal cavity of leopard frogs, whereas the average time of migration from the stomach to the buccal cavity in all other possible host species combinations was not significantly different from each other. To confirm that worms in the stomach of bullfrogs had enough time to migrate to the buccal cavity, Stigge and Bolek (2016a) conducted additional observation on two naturally infected bullfrogs collected from a local pond and maintained in the laboratory for up to a year. Their observations indicated that individuals of *H. occidualis* never migrated into the mouth of these frogs. However, upon necropsy, five and three individuals of *H. occidualis* (identified based on egg morphology) were recovered from the stomach of these bullfrogs 95 and 365 days after frogs were collected from the pond, respectively.

To confirm these differences in host-induced site fidelity, Stigge and Bolek (2016a) performed additional worm transplant experiments in gray tree frogs, *Hyla versicolor*, green frogs, and bullfrogs. Gravid worms transplanted from under the tongue of gray tree frogs remained under the tongue of gray tree frogs and green frogs for the

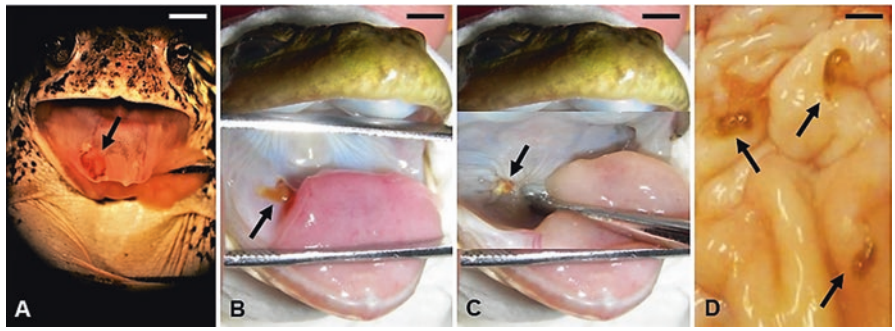

Fig. 11.12 (**a**) Two gravid *Halipegus occidualis* (arrow) attached to the lingual vein of a Woodhouse's toad, *Bufo woodhousii*. Scale bar = 10 mm. (**b**, **c**) Migration of transplanted *Halipegus occidualis* from the mouth into the stomach of a bullfrog. Note the worm (arrow) attached to the lingual vein in (**b**) and a worm (arrow) migrating down the esophagus in (**c**). Scale bar = 10 mm. (**d**) Three gravid *H. occidualis* (arrows) attached to the stomach lining of a bullfrog, *Rana catesbeiana*. Scale bar = 5 mm

entire duration of the experiment (58 days). None of the worms in these two anuran species were observed migrating into different habitats during this time, and, importantly, all worms remained attached to the same lingual vein under the tongue where they originally attached after being transplanted. In contrast, all gravid worms that were transplanted from under the tongue of a gray tree frog to under the tongue of bullfrogs moved from their site of transplant within the first 24 h. Over a period of 6–8 days, worms were observed in different locations within the mouths of bullfrogs, including being found on different lingual veins under the tongue as well as the margins and roof of the mouth. After being transplanted, worms remained in the mouth of bullfrogs for 6–8 days, and at 7 days post transplantation some worms were observed migrating down the esophagus of bullfrogs (Fig. 11.12). Two weeks after being transplanted, necropsies revealed that transplanted worms were attached to the anterior half of the stomachs of each bullfrog, and no other worms were found in any other location. Similarly, gravid worms transplanted from the stomach of a bullfrog to under the tongue of a gray tree frog remained under the tongue of the gray tree frog for 58 days. In contrast, gravid worms that were transplanted from the stomach of a bullfrog into the mouth of a second bullfrog migrated to the stomach within 6 days of being transplanted, and both worms were recovered in the stomach 58 days after transplant. Finally, gravid worms that were transplanted from the stomach of a bullfrog into the stomach of a gray tree frog migrated into the mouth of the tree frog and attached to the lingual veins under the tongue within 5–7 days after being transplanted. In contrast, gravid worms removed from the stomach of a bullfrog and transplanted into the stomach of a second bullfrog never migrated from the stomach to the buccal cavity and remained attached to the stomach 58 days after being transplanted.

This comparative approach allowed Stigge and Bolek (2016a) to demonstrate that in some species of *Halipegus* site fidelity is variable especially when those worms infect different amphibian species. More importantly, it demonstrates that as parasitologists and wildlife biologists, we must be careful in accepting gold standards for the identification of trematodes of wildlife, and whenever possible we need to use multiple approaches for these endeavors.

11.5 Implications of Cryptic Hosts on Identifying and Studying Life Cycles of Wildlife Trematodes: Examples from Snail First Intermediate Hosts

As described for *Halipegus* species and other trematodes, host species used by trematodes have been used routinely to confirm the species of trematode; however, host usage can be a precarious and erroneous taxonomic character, even for

larval trematodes. Historically, parasitologists have assumed that trematodes are highly host specific, infecting one or two closely related molluscan species at the first intermediate host level of the life cycle (Adamson and Caira 1994). Given the recent reports of common cryptic trematode species, it may also be important to examine the occurrence of cryptic host species and revisit our reliance on trematode first intermediate host specificity for identifying and studying trematodes of wildlife.

Trematodes are not the only taxa with cryptic species. Barcoding has shown that the species diversity of molluscs, which serve as first intermediate hosts for many trematodes, may also be underestimated due to cryptic species (e.g., Standley et al. 2013). In other cases, cryptic species of snails may confuse patterns of susceptibility and host specificity. For example, barcoding studies identified that the freshwater snail, *Lymnaea schirazensis*, was genetically distinct from other morphologically cryptic/similar freshwater snail species from the genera *Galba* and *Fossaria* spp. In laboratory exposures with miracidia of *Fasciola* species, the cryptic snail species, *L. schirazensis*, was not a viable host because the cercariae could not develop even though miracidia stages could penetrate the snails (Bargues et al. 2011). Because this newly recognized species was mistaken for the morphologically similar *Galba* species, patterns of susceptibility, specificity, and the geographical distribution of *Fasciola* may need to be revisited (Bargues et al. 2011).

Similar to wildlife trematodes discussed previously, studies of crypsis in snails demonstrate that using morphology is unreliable for species identification. Given that molluscs are required first hosts, it is important to consider the implications of cryptic mollusc species in wildlife trematode research. There is a growing number of barcoding studies demonstrating crypsis in molluscs suggesting that species diversity can be underestimated. For example, a Web of Science search resulted in a total of 42 studies that had investigated gastropod groups for cryptic species (search terms: "snails," "cryptic species," 9/18/2018). Among these studies, the most common gene regions used to discriminate among species were cytochrome c oxidase subunit 1 (CO1) and 16S rDNA in the mitochondrial genome. Compared to the choice of mitochondrial marker, there was less agreement in the choice of nuclear marker(s) though more commonly chosen regions were the internal transcribed spacer subunit 2 (ITS2) and histone3. Based on these genetic studies, marine and brackish water snails within ten genera have been reported to contain cryptic species (Table 11.1). For land snails, cryptic species have been suggested in 13 genera (Table 11.1). In addition, one group was evaluated at the family level, and although crypsis was not found, further sampling was recommended before making firm conclusions about crypsis (Table 11.1). For freshwater snails, 11 of 12 genera were suggested to have cryptic species (Table 11.1). For the exception, *Goniobasis*, more intraspecific genetic variation was reported for *G. proximus* compared to interspecific variation between other nominal conspecifics. Although this was not interpreted as evidence for cryptic species, it was recommended that the biological basis for this species should be re-evaluated (Dillon and Frankis 2004). However, these conclusions are only preliminary and suggest that the diversity of intermediate hosts for trematodes is gravely underestimated, given the tremendous species diversity of molluscs that have not been examined for cryptic species. Recent estimates suggest the existence of 45–50,000 marine, 25,000 terrestrial, and 5000 freshwater species of molluscs (Appeltans et al. 2012; Rosenberg 2014). Thus, the existence of cryptic intermediate host species could have far-reaching implications on our understanding of host specificity of trematodes. Therefore, the host specificity of terrestrial and freshwater trematodes may not be as clear as we think and needs to be reexamined.

Freshwater echinostomes are one example of wildlife trematodes that include cryptic parasite species (see the previous section) and also infect cryptic snail first intermediate hosts. Across the world, echinostomes (superfamily Echinostomatoidea) use snail species in the families Lymnaeidae, Planorbidae, and Physidae (Kostadinova 2005). Yet, there is a lot of confusion regarding the taxonomy within these snail

Table 11.1 Studies examining crypsis in marine, terrestrial, and freshwater gastropods

Habitat	Gastropod group	Citations
Marine		
	Alviniconcha	Johnson et al. (2015)
	Crepipatella	Nuñez et al. (2012)
	Gemmuloborsonia	Puillandre et al. (2010)
	Hexaplex	Marzouk et al. (2017)
	Hydrobia	Wilke and Pfenninger (2002)
	Leptochonchus	Gittenberger and Gittenberger (2011)
	Littorina	Azuma et al. (2011)
	Melampus	Dennis and Hellberg (2010)
	Nassarius	Couceiro et al. (2012)
	Stramonita	De Biasi et al. (2016), El Ayari et al. (2017)
Terrestrial		
	Amplirhagada	Koehler and Johnson (2012)
	Cyclophorus	Nantarat et al. (2014)
	Damochlora	Criscione and Kohler (2015)
	Diplommatinidae	Rundell (2008)[a]
	Iberus	Elejalde et al. (2008)
	Jaminia	Modica et al. (2016)
	Mandarina	Chiba and Davison (2008)
	Monacha	Pienkowska et al. (2018)
	Pyramidula	Razkin et al. (2016, 2017)
	Rhiostoma	Prasankok et al. (2011)
	Rumina	Prévot et al. (2015)
	Trochulus	Depraz et al. (2009)
	Tropidophora	Emberton (1995)
	Xanthomelon	Koehler and Burghardt (2016)
Freshwater		
	Ancylus	Macher et al. (2016), Weiss et al. (2018)
	Ferissisia	Walther et al. (2010)[b], de Lacerda et al. (2015)
	Galba	Standley et al. (2013), Alda et al. (2018)
	Goniobasis	Dillon and Frankis (2004)[c]
	Heleobia	Collado et al. (2013, 2016)
	Hemistomia	Haase and Zielske 2015
	Juga	Lee et al. 2006
	Leiorhagia	Haase and Zielske (2015)
	Lymnaea	Durand et al. (2002), Bargues et al. (2011)
	Physa	Collado (2017)
	Pomacea	Rama Rao et al. (2018)
	Radix	Patel et al. (2015)

All cited studies suggested there was crypsis except for the three citations denoted with superscript numbers ($n = 42$ from Web of Science search on 9/18/2018).

[a]Did not identify to genus; no crypsis found, but authors suggested more sampling is needed

[b]Concluded that several species should be collapsed into fewer species rather than considered cryptic

[c]Reported high genetic intraspecific variation (maximum *p*-distance: 14.7% cytochrome oxidase 1, 17.1% 16S) but did not suggest cryptic species. Authors recommended that in light of this genetic variation, the biological species taxonomy needed to be assessed

families (Bargues et al. 2001; Taylor 2003). Among some species of snail, there are minimal interspecific differences in morphology and anatomy (Bargues et al. 2001; Collado 2017). Barcoding would be useful to apply to these species to avoid misidentification and likely resulting in underestimation of gastropod species diversity. In contrast, mollusc species diversity has also

been overestimated. Lymnaeid and physid shell morphology can be strongly influenced by the environment resulting in a high degree of phenotypic plasticity that could be misinterpreted as different species (e.g., Burch 1968; Gustafson et al. 2014; Gustafson and Bolek 2016). Indeed, the degree of taxonomic confusion for molluscs is greatest at the species level. For example, in regard to Physidae, Taylor (2003) states: "Lack of shell characters means that it is not only previous classifications, entirely or largely based on shells, that are deficient. Identification of species, except in rare cases, has been practically impossible. In the thousands of local lists, keys, handbooks, and other citations published during the last, 200 years, species identifications are simply untrustworthy."

More recently, Gustafson et al. (2014) and Gustafson and Bolek (2016) evaluated snail shell morphology for the freshwater snail *Physa acuta* collected from wetlands and streams and the relationship of those snail morphotypes to their larval trematode communities. To accomplish this, 4183 physid snails were collected from four wetlands (non-flow environments) and 4 streams (flow environments) and a subset of individuals from each population were identified as *P. acuta* based on their penial complex morphology and/or sequencing the cytochrome oxidase *c* subunit I (COI) mitochondrial gene. Shell morphology was then evaluated for *P. acuta* using geometric morphometrics, and the larval trematodes snails from each habitat were identified to group based on cercarial morphology. Finally, snails from flow and non-flow populations were reared and maintained in laboratory aquaria under non-flow and flow conditions and their shell morphology was evaluated using geometric morphometrics.

Their results indicated that habitat was the only significant factor affecting snail shell shape and size of field-collected snails. Additionally, using standard morphological keys for freshwater snails and based on shell morphology alone, snails from stream habitats and wetland habitats were identified as two distinct species of *Physa*. Stream snails had more narrow bodies with tall spires and more acute spire angles. In contrast, wetland snails were larger and exhibit wide apertures, wide bodies, short spires, and wide spire angles. Overall, seven cercaria types infecting *P. acuta*, with an overall low prevalence (4.4%), and no multiple infections were detected. Although snails from wetlands and streams shared all but one of the trematode types, their relative combinations and frequencies differed between the two habitats, resulting in significant differences in community structure. More importantly, when snails collected from wetlands and streams were reared in the laboratory under different flow and non-flow environments, the shapes of flow and non-flow tank snails significantly differed where non-flow tank snails resembled wetland snails and flow tank snails resembled stream snails. The lesson from these studies suggests that when snail first intermediate hosts are misidentified based on shell morphology this can lead to erroneous conclusions on trematode snail host specificity associations.

The uncertainty in mollusc species identification by experts in molluscan taxonomy should be concerning for non-experts, like many wildlife disease biologists and parasitologists. If the first intermediate host is not correctly identified, then we cannot be certain about the fundamental biology of wildlife trematodes including their geographic range, host specificity, and life cycles. This will affect our ability to understand their ecology and evolution as well as our ability to study them from an epidemiological perspective. We suggest that studies of larval trematodes should begin barcoding their snail hosts. Shell characteristics as well as whole bodies (for later examination of anatomy) should be deposited in museums for future reference. If barcoding is not preferred, multiplex PCR assays could be developed and used to aid in species identification of intermediate hosts to help monitor the mollusc vectors of wildlife trematode diseases (Alda et al. 2018).

11.6 Concluding Remarks

In recent years, there has been increased interest in studying the host–parasite interactions between trematodes and their wildlife hosts. With this surge in research efforts, it has become

obvious that the taxonomic status and nomenclature of many of the previous species descriptions of trematodes throughout the world are confusing and difficult to decipher. This clearly hinders the ability to study trematodes of wildlife and the diseases that they cause since the ability to identify the parasite is foundational to these studies. This chapter illustrates the problems involved with identifying trematodes of wildlife including their convoluted taxonomic history that is often confusing and difficult to follow; the morphology of many trematode species is inadequately described especially considering the many life cycle stages encountered in wildlife; cryptic species of trematodes appear to be ubiquitous; identification of some trematode species requires the troublesome assumption of host specificity or site specificity within their hosts; lastly, identifying the hosts can be problematic because cryptic species of hosts affect a wildlife biologist's ability to detect differences in host specificity and pathology in these hosts, and the host morphology can be variable within a single host species. We recommend that, in order to thoroughly and accurately study trematodes of wildlife, researchers conduct both field and experimental studies on the life cycles of the trematodes. We also urge wildlife biologists to include genetic analyses, such as DNA barcoding, of both the trematodes and the hosts being examined to be able to recognize and account for cryptic species. Lastly, we stress that it is important to deposit specimen vouchers into a museum so that future researchers can reexamine the trematodes in the future.

References

Adamson ML, Caira JN (1994) Evolutionary factors influencing the nature of parasite specificity. Parasitology 109:S85–S95

Alda P, Lounnas M, Vázquez AA, Ayaqui R, Calvopiña M, Celi-Erazo M, Dillon RT Jr, Jarne P, Loker ES, FCM P, Muzzio-Aroca J, Nárvaez AO, Noya O, Robles LM, Rodríguez-Hidalgo R, Uribe N, David P, Pointier JP, Hurtez-Boussès S (2018) A new multiplex PCR assay to distinguish among three cryptic *Galba* species, intermediate hosts of *Fasciola hepatica*. Vet Parasitol 251:101–105

Appeltans W, Ahyong ST, Anderson G, Angel MV, Artois T, Bailly N, Hopcroft RR (2012) The magnitude of global marine species diversity. Curr Biol 22:2189–2202

Azuma N, Yamazaki T, Chiba S (2011) Mitochondrial and nuclear DNA analysis revealed a cryptic species and genetic introgression in *Littorina sitkana* (Mollusca, Gastropoda). Genetica 139:1399–1408

Bargues MD, Vigo M, Horak P, Dvorak J, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, Mas-Coma S (2001) European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. Infect Genet Evol 1:85–107

Bargues MD, Artigas P, Khoubbane M, Flores R, Glöer P, Rojas-García R, Ashrafi K, Falkner G, Mas-Coma S (2011) *Lymnaea schirazensis*, an overlooked snail distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PLoS One 6:e24567

Beaver PC (1937) Experimental studies on *Echinostoma revolutum* (Froelich), a fluke from birds and mammals. Biol Monogr 15:1–96

Belden LK, Wojdak JM (2011) The combined influence of trematode parasites and predatory salamanders on wood frog (*Rana sylvatica*) tadpoles. Oecologia 166:1077–1086

Blasco-Costa I, Cutmore SC, Miller TL, Nolan MJ (2016) Molecular approaches to trematode systematics: "best practice" and implications for future study. Syst Parasitol 93:295–306

Bolek MG (2006) The role of arthropod second intermediate hosts as avenues for and constraints on the transmission of frog lung flukes (Digenea: Haematoloechidae). PhD thesis, University of Nebraska–Lincoln, Lincoln

Bolek MG, Coggins JR (2003) Helminth community structure of sympatric eastern American toad, *Bufo americanus americanus*, northern leopard frog, *Rana pipiens*, and blue-spotted salamander, *Ambystoma laterale*, from southeastern Wisconsin. J Parasitol 89:673–680

Bolek MG, Janovy J Jr (2007a) Small frogs get their worms first: The role of non-odonate arthropods in the recruitment of *Haematoloechus coloradensis* and *Haematoloechus complexus* in newly metamorphosed northern leopard frogs, *Rana pipiens*, and Woodhouse's toads, *Bufo woodhousii*. J Parasitol 93:300–312

Bolek MG, Janovy J Jr (2007b) Evolutionary avenues for and constraints on the transmission of frog lung flukes (*Haematoloechus* spp.) in dragonfly second intermediate hosts. J Parasitol 93:593–607

Bolek MG, Janovy J Jr (2008) Alternative life cycle strategies of *Megalodiscus temperatus* in tadpoles and metamorphosed anurans. Parasite 15:396–401

Bolek MG, Snyder SD, Janovy J Jr (2009a) Alternative life-cycle strategies and colonization of young anurans by *Gorgoderina attenuata* in Nebraska. J Parasitol 95:604–615

Bolek MG, Snyder SD, Janovy J Jr (2009b) Redescription of the bladder fluke *Gorgoderina attenuata* from the northern leopard frog, *Rana pipiens*. J Parasitol 95:665–668

Bolek MG, Tracy HR, Janovy J Jr (2010) The role of damselflies (Odonata: Zygoptera) as paratenic hosts in the transmission of *Halipegus eccentricus* (Digenea: Hemiuridae) to anurans. J Parasitol 96:724–735

Bolek MG, Stigge HA, Gustafson KD (2016) The iron wheel of parasite life cycles: then and now. In: Janovy J Jr, Esch GW (eds) A century of parasitology: discoveries, ideas and lessons learned by scientists who published in the Journal of Parasitology, 1914–2014. Wiley, London, pp 131–147

Bourgat R, Kulo SD (1979) Cycle biologique d' *Haematoloechus johnsoni* Bourgat, 1977, (Trèmatode) parasite pulmonaire de *Dicroglossus occipitalis* (Günther, 1858) (Amphibien, Anoure) au Togo. Rev Suisse Zool 86:467–472

Brooks DL (1976) Parasites of amphibians of the Great Plains: Part 2. Platyhelminths of amphibians in Nebraska. Bull Univ Nebraska State Mus 10:65–92

Brooks DR, León-Règagnon V, McLennan DA, Zelmer D (2006) Ecological fitting as a determinant of the community structure of platyhelminth parasites of anurans. Ecology 87:S76–S85

Burch JB (1968) *Erinna newcombi* of Hawaii and *Limnaea onychia* of Japan. Malacol Rev 1:15–30

Chiba S, Davison A (2008) Anatomical and molecular studies reveal several species of the endemic genus *Mandarina* (Pulmonata: Helicoidea) in the Ogasawara Islands. J Moll Stud 74:373–382

Chibwana FD, Nkwengulila G, Locke SA, McLaughlin JD, Marcogliese DJ (2015) Completion of the life cycle of *Tylodelphys mashonense* (Sudarikov, 1971) (Digenea: Diplostomidae) with DNA barcodes and rDNA sequences. Parasitol Res 114:3675–3682

Choudhury A, García-Varela M, Pérez-Ponce de León G (2016) Parasites of freshwater fishes and the Great American Biotic Interchange: a bridge too far? J Helminthol 91:174–196

Collado GA (2017) Unraveling cryptic invasion of a freshwater snail in Chile based on molecular and morphological data. Biodivers Conserv 26:567–578

Collado GA, Valladares MA, Mendez MA (2013) Hidden diversity in spring snails from the Andean Altiplano, the second highest plateau on Earth, and the Atacama Desert, the driest place in the world. Zool Stud 52:50

Collado GA, Valladares MA, Mendez MA (2016) Unravelling cryptic species of freshwater snails (Caenogastropoda, Truncatelloidea) in the Loa River basin, Atacama Desert. Syst Biodivers 14:417–429

Combes C (1968) Biologie, écologie des cycles et biogeography de digénes et monogénes d'amphibiens dans l'est des Pyrénéés. Ann Mus Natl Hist Nat 51:1–195

Cort WW (1912) North American frog bladder flukes. Trans Am Microsc Soc 31:151–168

Cort WW (1915) North American frog lung flukes. Trans Am Microsc Soc 34:203–243

Couceiro L, Lopez L, Sotka EE, Ruiz JM (2012) Molecular data delineate cryptic *Nassarius* species and characterize spatial genetic structure of *N. nitidus*. J Mar Biol Assoc U K 92:1175–1182

Crawford WW (1940) The life history of a gorgoderid trematode, presumably of the genus *Phyllodistomum*. J Parasitol 26:S38

Criscione F, Kohler F (2015) On the land snail *Damochlora* Iredale, 1938 and its cryptic sibling *Nannochlora* n. gen. (Stylommatophora: Camaenidae), each endemic to an island in the Western Australian Kimberley. Molluscan Res 35:275–286

Cutmore SC, Bennett MB, Cribb TH (2010) *Staphylorchis cymatodes* (Gorgoderidae Anaporrhutinae) from carcharhiniform, orectolobiform and myliobatiform elasmobranchs of Australasia: low host specificity, wide distribution and morphological plasticity. Parasitol Int 59:579–586

Dale PJ (1967) The life history of *Gorgodera australiensis* Johnston,1912. Tuatara 15:89–97

De Biasi JB, Tomas ARG, Hilsdorf AWS (2016) Molecular evidence of two cryptic species of *Stramonita* (Mollusca, Muricidae) in the southeastern Atlantic coast of Brazil. Genet Mol Biol 39:392–397

de Lacerda LEM, Richau CS, Amaral CRL, da Silva DA, Carvalho EF, Barbosa dos Santos S (2015) *Ferrissia fragilis* (Tryon, 1863): a freshwater snail cryptic invader in Brazil revealed by morphological and molecular data. Aquat Invasions 10:157–168

Dennis AB, Hellberg ME (2010) Ecological partitioning among parapatric cryptic species. Mol Ecol 19:3206–3225

Depraz A, Hausser J, Pfenninger M (2009) A species delimitation approach in the *Trochulus sericeus/hispidus* complex reveals two cryptic species within a sharp contact zone. BMC Evol Biol 9:171

Detwiler JT, Bos DH, Minchella DJ (2010) Revealing the secret lives of cryptic species: Examining the phylogenetic relationships of echinostome parasites in North America. Mol Phylogenet Evol 55:611–620

Detwiler JT, Zajac AM, Minchella DJ, Belden LK (2012) Revealing cryptic parasite diversity in a definitive host: echinostomes in muskrats. J Parasitol 98:1148–1155

Dillon TD, Frankis RC (2004) High levels of mitochondrial DNA sequence divergence in isolated populations of freshwater snails of the genus *Goniobasis* Lea, 1862. Am Malacol Bull 19:69–77

Dollfus R (1961) Station expérimentale de parasitologie de Richelieu (Indre-et-Loire). Contribution à la faune parasitaire régionale. Ann Parasitol Hum Comp 3:169–451

Donald KM, Kennedy M, Poulin R, Spencer HG (2004) Host specificity and molecular phylogeny of larval Digenea isolated from New Zealand and Australian topshells (Gastropoda: Trochidae). Int J Parasitol 34:557–568

Dronen N (1975) The life cycle of *Haematoloechus coloradensis* Cort, 1915 (Digenea: Plagiorchiidae), with emphasis on host susceptibility to infection. J Parasitol 61:657–660

Dronen NO (2009) Re-evaluation of the species composition of *Bashkirovtrema* Skrjabin, (1944) (Digenea: Echinostomatidae), with the description of two new species of this genus and the proposal of *Kostadinovatrema novaeguiniense* n. g., n. sp. Syst Parasitol 74:169–185

Durand P, Pointier JP, Escoubeyrou K, Arenas JA, Yong M, Amarista M, Bargues MD, Mas-Coma S, Renaud F (2002) Occurrence of a sibling species complex within neotropical lymnaeids, snail intermediate hosts of fascioliasis. Acta Trop 83:233–240

El Ayari T, El Menif NT, Saavedra C, Cordero D, Viard F, Bierne N (2017) Unexpected mosaic distribution of two hybridizing sibling lineages in the teleplanically dispersing snail *Stramonita haemastoma* suggests unusual postglacial redistribution or cryptic invasion. Ecol Evol 7:9016–9026

Elejalde MA, Madeira MJ, Muñoz B, Arrébola JR, Gómez-Moliner BJ (2008) Mitochondrial DNA diversity and taxa delineation in the land snails of the *Iberus gualtieranus* (Pulmonata, Helicidae) complex. Zool J Linn Soc 154:722–737

Emberton KC (1995) Cryptic, genetically extremely divergent, polytypic, convergent, and polymorphic taxa in Madagascan *Tropidophora* (Gastropoda, Pomatiasidae). Biol J Linn Soc 55:183–208

Faltynková A, Našincová V, Kablásková L (2007) Larval trematodes (Digenea) of the great pond snail, *Lymnaea stagnalis* (L.), (Gastropoda, Pulmonata) in Central Europe: a survey of species and key to their identification. Parasite 14:39–51

Faltynková A, Našincová V, Kablásková L (2008) Larval trematodes (Digenea) of planorbid snails (Gastropoda: Pulmonata) in Central Europe: a survey and key to their identification. Syst Parasitol 69:155–178

Fried B, Pane PL, Reddy A (1997) Experimental infection of *Rana pipiens* tadpoles with *Echinostoma trivolvis* cercariae. Parasitol Res 83:666–669

Georgieva S, Selbach C, Faltynková A, Soldánová M, Sures B, Skírnisson K, Kostadinova A (2013) New cryptic species of the 'revolutum' group of Echinostoma (Digenea: Echinostomatidae) revealed by molecular and morphological data. Parasit Vectors 6:64

Gibson DI, Bray RA (1994) The evolutionary expansion and host–parasite relationships of the Digenea. Int J Parasitol 24:1213–1226

Gittenberger A, Gittenberger E (2011) Cryptic, adaptive radiation of endoparasitic snails: sibling species of *Leptoconchus* (Gastropoda: Coralliophilidae) in corals. Organ Divers Evol 11:21–41

Goater TM, Browne CL, Esch GW (1990a) On the life life history and functional morphology of *Halipegus occidualis* (Trematoda: Hemiuridae), with emphasis on the cystophorous cercaria stage. Int J Parasitol 20:923–934

Goater TM, Mulvey M, Esch GW (1990b) Electrophoretic differentiation of two *Halipegus* congeners in a natural amphibian population: comments on genetic diversity in helminth parasites. J Parasitol 76:431–434

Goodchild CG (1943) The life-history of *Phyllodistomum solidum* Rankin, 1937, with observations on the morphology, development and taxonomy of the Gorgoderinae (Trematoda). Biol Bull 84:59–86

Goodchild CG (1948) Additional observations on the life history of *Gorgodera amplicava* Looss, 1899 (Trematoda: Gorgoderidae). J Parasitol 34:407–427

Goodchild CG (1950) Establishment and pathology of gorgoderid infections in anuran kidneys. J Parasitol 36:439–446

Gordy MA, Kish L, Tarrabain M, Hanington PC (2016) A comprehensive survey of larval digenean trematodes and their snail hosts in central Alberta, Canada. Parasitol Res 115:3867–3880

Grabda-Kazubska B (1960) Life cycle of *Haematoloechus similis* (Looss, 1899) (Trematoda: Plagiorchiidae). Acta Parasitol 8:357–367

Gustafson KD, Bolek MG (2016) Effects of trematode parasitism on the shell morphology of snails from flow and nonflow environments. J Morphol 277:316–325

Gustafson KD, Kensigner BJ, Bolek MG, Luttbeg B (2014) Distinct snail (*Physa*) morphotypes from different habitats converge in shell shape and size under common garden conditions. Evol Ecol Res 16:77–89

Haase M, Zielske S (2015) Five new cryptic freshwater gastropod species from New Caledonia (Caenogastropoda, Truncatelloidea, Tateidae). Zookeys 523:63–87

Harwood P (1932) The helminths parasitic in the Amphibia and Reptilia in Houston, Texas, and vicinity. Proc US Natl Mus 81:1–71

Holland MP (2010) Echinostome metacercariae cyst elimination in *Rana clamitans* (Green Frog) tadpoles is age-dependent. J Parasitol 95:281–285

Holland MP, Skelly DK, Kashgarian M, Bolden SR, Harrison LM, Cappello M (2007) Echinostome infection in green frogs (*Rana clamitans*) is stage and age dependent. J Zool 271:455–462

Ingles LG (1932) Four new species of *Haematoloechus* (Trematoda) from Rana aurora draytoni from California. Univ Calif Publ Zool 37:189–202

Ingles LG (1936) Worm parasites of California Amphibia. Trans Am Microsc Soc 55:73–92

Irwin MS (1929) A new lung fluke from *Rana clamitans* Latreille. Trans Am Microsc Soc 48:74–79

Johnson PTJ, Buller ID (2011) Parasite competition hidden by correlated coinfection: using surveys and experiments to understand parasite interactions. Ecology 92:535–541

Johnson PTJ, McKenzie VJ (2008) Effects of environmental change on helminth infections in amphibians: exploring the emergence of *Ribeiroia* and *Echinostoma* infections in North America. In: Fried B, Toledo R (eds) The biology of echinostomes, from the molecule to the community. Springer, Berlin, pp 249–280

Johnson SB, Waren A, Tunnicliffe V, Van Dover C, Wheat CG, Schultz TF, Vrijenhoek RC (2015) Molecular taxonomy and naming of five cryptic species of *Alviniconcha* snails (Gastropoda:

Abyssochrysoidea) from hydrothermal vents. Syst Biodivers 13:278–295

Kanev I, Sorensen R, Sterner M (1998) The identification and characteristics of *Echinoparyphium rubrum* (Cort, 1914) comb. New (Trematoda, Echinostomatidae) based on experimental evidence of the life cycle. Acta Parasitol 43:181–188

Kanev I, Sorensen R, Radev V (2008) Identification problems with species in the *Echinoparyphium recurvatum* complex from physid snails in the USA. Parasitol Res 103:963–965

Kanev I, Fried B, Radev B (2009) Collar spine models in the genus *Echinostoma* (Trematoda: Echinostomatidae). Parasitol Res 105:921–927

Kasl EL, Font WF, Criscione CD (2018) Resolving evolutionary changes in parasite life cycle complexity: molecular phylogeny of the trematode genus *Alloglossidium* indicates more than one origin of precociousness. Mol Phylogenet Evol 126:371–381

Kennedy MJ (1980a) Host-induced variations in *Haematoloechus buttensis* (Trematoda: Haematoloechidae). Can J Zool 58:427–442

Kennedy MJ (1980b) Geographical variation in some representatives of *Haematoloechus* Looss, 1899 (Trematoda: Haematoloechidae) from Canada and the United States. Parasitol Res 58:1151–1167

Kennedy MJ (1981) A revision of the genus *Haematoloechus* Looss, 1899 (Trematoda: Haematoloechidae) from Canada and the United States. Parasitol Res 59:1836–1846

Koehler F, Burghardt I (2016) Cryptic diversity in a widespread land snail: revision of the genus *Xanthomelon* Martens, 1860 from the Australian Monsoon Tropics (Pulmonata, Camaenidae). Zool Scr 45:127–144

Koehler F, Johnson MS (2012) Species limits in molecular phylogenies: a cautionary tale from Australian land snails (Camaenidae: *Amplirhagada* Iredale, 1933). Zool J Linn Soc 165:337–362

Koprivnikar J, Gibson CH, Redfern JC (2002) Infectious personalities: behavioural syndromes and disease risk in larval amphibians. Proc Roy Soc B Biol Sci 279:1544–1550

Koprivnikar J, Marcogliese DJ, Rohr JR, Orlofske SA, Raffel TR, Johnson PTJ (2012) Macroparasite infections of amphibians: what can they tell us? EcoHealth 9:342–360

Kostadinova A (2005) Family Echinostomatidae Looss, 1899. In: Jones A, Bray RA, Gibson DI (eds) Keys to the Trematoda, vol 2. CABI Publishing, Wallingford, pp 9–64

Kraus TJ, Brant SV, Adema CM (2014) Characterization of trematode cercariae from *Physella acuta* in the Middle Rio Grande. Comp Parasitol 81:105–109

Krull WH (1930) The life history of two North American frog lung flukes. J Parasitol 16:207–212

Krull WH (1931) Life history studies on two frog lung flukes, *Pneumonoeces medioplexus* and *Pneumobites parviplexus*. Trans Am Microsc Soc 50:215–277

Krull WH (1932) Studies on the life history of *Haematoloechus longiplexus* (Stafford). Zool Anz 99:231–239

Krull WH (1933) Studies on the life history of a frog lung fluke, *Haematoloechus complexus* (Seely, 1906) Krull, n. comb. Z Parasitenkd 6:192–206

Krull WH (1934) Some additional notes on the life history of a frog lung fluke, *Haematoloechus complexus* (Seely, 1906) Krull. Trans Am Microsc Soc 53:196

Krull HW (1935a) Studies on the life history of a frog bladder fluke, *Gorgodera amplicava* Looss, 1899. Papers of the Michigan Academy of Science, Arts and Letters, vol 20, pp 697–710

Krull HW (1935b) Studies on the life history of *Halipegus occiualis* Stafford, 1905. Am Midl Nat J 16:129–143

Krull HW (1952) Studies on the biology of *Dicrocoelium dendriticum* (Rudolphi, 1819) Looss, 1899 (Trematoda: Dicrocoeliidae), including its relation to the intermediate host, *Cionella lubrica* (Müller). VII. The second intermediate host of *Dicrocoelium dendriticum*. Cornell Vet 42:603–604

Lee T, Kim JJ, Hong HC, Burch JB, Foighil DO (2006) Crossing the Continental Divide: the Columbia drainage species *Juga hemphilli* (Henderson, 1935) is a cryptic member of the eastern North American genus *Elimia* (Cerithioidea: Pleuroceridae). J Moll Stud 72:314–317

León-Règagnon V (2010) Evidence of new species of *Haematoloechus* (Platyhelminthes: Digenea) using partial cox1 sequences. Mitochon DNA 21:S12–S17

León-Règagnon V, Brooks DR (2003) Molecular phylogeny of *Haematoloechus* Looss, 1899 (Digenea: Plagiorchiidae), with emphasis on North American species. J Parasitol 89:1206–1211

León-Règagnon V, Paredes-Calderón EL (2002) *Haematoloechus danbrooksi* n. sp. (Digenea: Plagiorchioidea) from *Rana vaillanti* from Los Tuxtlas, Veracruz, Mexico. J Parasitol 88:1215–1221

León-Règagnon V, Romero-Mayén RA (2013) A new species of *Halipegus* Looss, 1899 (Trematoda: Derogenidae) parasitic of *Rana psilonota* Webb, 2001 of Atenquique, Jalisco, Mexico. J Parasitol 99:1067–1070

León-Règagnon V, Topan J (2018) Taxonomic revision of species of *Haematoloechus* Looss, 1899 (Digenea: Plagiorchioidea), with molecular phylogenetic analysis and the description of three new species from Mexico. Zootaxa 4526:251–302

León-Règagnon V, Brooks DR, Pérez-Ponce de León G (1999) Differentiation of Mexican species of *Haematoloechus* Loss, 1989 (Digenea: Plagiorchiformes): molecular and morphological evidence. J Parasitol 85:935–946

León-Règagnon V, Brooks DR, Zelmer DA (2001) Morphological and molecular description of *Haematoloechus meridionalis* n. sp. (Digenea: Plagiorchioidea: Haematoloechidae) from *Rana vail-*

lanti Brocchi of Guanacaste, Costa Rica. J Parasitol 87:1423–1427

León-Règagnon V, Guillén-Hernández S, Arizmendi-Espinosa MA (2005) Intraspecific variation of *Haematoloechus floedae* Harwood, 1932 (Digenea: Plagiorchiidae), from Rana spp. in North and Central America. J Parasitol 91:915–921

Leung TLF, Donald KM, Keeney DB, Koehler AV, Peoples RC, Poulin R (2009) Trematode parasites of Otago Harbour (New Zealand) soft-sediment intertidal ecosystems: life cycles, ecological roles, and DNA barcodes. N Z J Mar Freshw Res 43:857–865

Locke SA, McLaughlin DJ, Marcogliese DJ (2010) DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. Mol Ecol 19:2813–2827

Looss A (1899) Weitere beiträge zur kenntnis der trematodenfauna Aegyptens, zugleich versuch einer natürlichen gliederung des genus Distomum Retzius. Zool Jahr Abteil Syst Oekol Geogr Tiere 12:521–784

Looss A (1902) Ueber neue und bekannte Trematoden aus Seeschildkriten. Zool Jahrb Syst 16:411–891

Macher JN, Weiss M, Beermann AJ, Leese BJ (2016) Cryptic diversity and population structure at small scales: the freshwater snail *Ancylus* (Planorbidae, Pulmonata) in the Montseny mountain range. Ann Limnol Int J Limnol 52:387–399

Macy RW, Cook WA, Demott WR (1960) Studies on the life cycle of *Halipegus occidualis* Stafford, 1905 (Trematoda: Hemiuridae). Northwest Sci 34:1–17

Madhavi R, Bray R (2018) Digenetic Trematodes of Indian Marine Fishes. Springer, Dordrecht

Manter HW (1938) A collection of trematodes from Florida Amphibia. Trans Am Microsc Soc 57:26–37

Marzouk Z, Aurelle D, Said K, Chenuil A (2017) Cryptic lineages and high population genetic structure in the exploited marine snail *Hexaplex trunculus* (Gastropoda: Muricidae). Biol J Linn Soc 122:411–428

Mata-López R (2006) A new gorgoderid species of the urinary bladder of *Rana zweifeli* from Michoacán, Mexico. Rev Mex Biodivers 77:191–198

Mata-López R, León-Règagnon V (2005) *Gorgoderina festoni* n. sp. (Digenea: Gorgoderidae) in anurans (Amphibia) from México. Syst Parasitol 62:185–190

Mata-López R, León-Règagnon V (2006) Comparative study of the tegumental surface of several species of *Gorgoderina* Looss, 1902 (Digenea: Gorgoderidae), as revealed by scanning electron microscopy. Comp Parasitol 73:24–34

Mata-López R, García-Prieto L, León-Régagnon V (2002) Infracomunidades de helmintos parásitos de *Ambystoma lermaensis* (Caudata: Ambystomatidae) en Lerma, México. Rev Biol Trop 50:303–307

Mata-López R, León-Règagnon V, Brooks DR (2005) Species of *Gorgoderina* (Digenea: Gorgoderidae) in *Rana vaillanti* and *Rana* cf. *forreri* (Anura: Ranidae) from Guanacaste, Costa Rica, including a description of a new species. J Parasitol 91:403–410

McAlpine DF (2006) *Halipegus occidualis* Krull, 1935 and *Halipegus eccentricus* Thomas, 1939 (Digenea, Hemiuridae): proposed conservation of the specific names. Bull Zool Nomencl 63:12–16

McAlpine DF, Burt MD (1998) Taxonomic status of *Halipegus* spp. (Digenea: Derogenidae) parasitic in the mouth and eustachian tubes of North American and Mexican amphibians. J Helmithol Soc Washington 65:10–15

Modica MV, Colangelo P, Hallgass A, Barco A, Oliverio M (2016) Cryptic diversity in a chirally variable land snail. Ital J Zool 83:351–363

Moszczynska A, Locke SA, McLaughlin JD, Marcogliese DJ, Crease TJ (2009) Development of primers for the mitochondrial cytochrome c oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Mol Ecol Resour 1:S75–S82

Nantarat N, Wade CM, Jeratthitikul E, Sutcharit C, Panha S (2014) Molecular evidence for cryptic speciation in the *Cyclophorus fulguratus* (Pfeiffer, 1854) species complex (Caenogastropoda: Cyclophoridae) with description of new species. PLoS One 9:e109785

Nuñez JJ, Vejar-Pardo A, Guzman BE, Barriga EH, Galindo CS (2012) Phylogenetic and mixed Yule-coalescent analyses reveal cryptic lineages within two South American marine snails of the genus *Crepipatella* (Gastropoda: Calyptraeidae). Invert Biol 131:301–311

Odening K (1958) Zur systematik von *Haematoloechus* (Trematoda, Plagiorchiidae). Mitteil Zool Mus Berlin 34:63–108

Olson PD, Tkach VV (2005) Advances and trends in the molecular systematics of the parasitic Platyhelminthes. Adv Parasitol 60:165–243

Patel S, Schell T, Eifert C, Feldmeyer B, Pfenninger M (2015) Characterizing a hybrid zone between a cryptic species pair of freshwater snails. Mol Ecol 24:643–655

Pérez-Ponce de León G, Nadler SA (2010) What we don't recognize can hurt us: a plea for awareness about cryptic species. J Parasitol 96:453–464

Pérez-Ponce de León G, Poulin R (2018) An updated look at the uneven distribution of cryptic diversity among parasitic helminths. J Helminthol 92:197–202

Pérez-Ponce de León G, García-Varela M, Pinacho-Pinacho CD, Sereno-Uribe AL, Poulin R (2016) Species delimitation in trematodes using DNA sequences: middle-American *Clinostomum* as a case study. Parasitology 143:1773–1789

Pienkowska JR, Manganelli G, Giusti F, Hallgass A, Lesicki A (2018) Exploring *Monacha cantiana* (Montagu, 1803) phylogeography: cryptic lineages and new insights into the origin of the English populations (Eupulmonata, Stylommatophora, Hygromiidae). Zookeys 765:1–41

Poulin R (2000) The diversity of parasites. Q Rev Biol 75:277–293

Poulin R (2011) Uneven distribution of cryptic diversity among higher taxa of parasitic worms. Biol Lett 7:241–244

Poulin R, Cribb TH (2002) Trematode life cycles: short is sweet? Trends Parasitol 18:176–183

Poulin R, Leung TLF (2010) Taxonomic resolution in parasite community studies: are things getting worse? Parasitology 137(13):1967–1973

Prasankok P, Tongkerd P, Sutcharit C, Sutcharitb C, Panhab S (2011) Genetic divergence in the snorkel snail, *Rhiostoma housei*, a species complex in Thailand (Caenogastropoda: Cyclophoridae). Biochem Syst Ecol 39:834–840

Pratt HS (1903) Descriptions of four distomes. In: Parker GH (ed) Mark Anniversary Volume to Edward Lawrence Mark. Henry Holt and Company, New York, pp 23–38

Prévot V, Backeljau T, Jordaens K (2015) Morphometric evaluation of DNA-based cryptic taxa in the terrestrial decollate snail genus *Rumina*. J Moll Stud 81:223–232

Prokopič J, Křivanec K (1974) Trematodes of the genus *Haematoloechus* Looss, and their variability. Helminthology 15:779–802

Prudhoe SOBE, Bray RA (1982) Platyhelminth parasites of the amphibia. British Museum (Natural History), Oxford

Puillandre N, Cruaud C, Kantor YI (2010) Cryptic species in *Gemmuloborsonia* (Gastropoda: Conoidea). J Moll Stud 76:11–23

Raffel TR, Hoverman JT, Halstead NT, Michel PJ, Rohr JR (2010) Parasitism in a community context: trait-mediated interactions with competition and predation. Ecology 91:1900–1907

Rama Rao S, Liew TS, Yow YY, Ratnayeke S (2018) Cryptic diversity: two morphologically similar species of invasive apple snail in Peninsular Malaysia. PLoS One 13:e0196582

Rankin JS (1939) The life cycle of the frog bladder fluke, *Gorgoderina attenuata* Stafford, 1902 (Trematoda: Gorgoderidae). Am Midl Nat 21:476–488

Rankin JS (1944) A review of the trematode genus *Halipegus* Loss, 1899, with an account of the life history of *H. amherstensis* n. s. Trans Am Microsc Soc 63:149–164

Razkin O, Sonet G, Breugelmans K, Madeira MJ, Gómez-Moliner BJ, Backeljau T (2016) Species limits, interspecific hybridization and phylogeny in the cryptic land snail complex *Pyramidula*: the power of RADseq data. Mol Phylogenet Evol 101:267–278

Razkin O, Gomez-Moliner BJ, Vardinoyannis K, Martínez-Ortí A, Madeira MJ (2017) Species delimitation for cryptic species complexes: case study of *Pyramidula* (Gastropoda, Pulmonata). Zool Scr 46:55–72

Rhoden HR, Bolek MG (2012) Helminth and leech community structure in tadpoles and caudatan larvae of two amphibian species from western Nebraska. J Parasitol 98:236–244

Rosenberg G (2014) A new critical estimate of named species-level diversity of the recent Mollusca. Am Malacol Bull 32:308–322

Routtu J, Grunberg D, Izhar R, Dagan Y, Guttel Y, Ucko M, Ben-Ami F (2014) Selective and universal primers for trematode barcoding in freshwater snails. Parasitol Res 113:2535–2540

Rudolphi CA (1819) Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi. Berolini, Berlin

Rundell RJ (2008) Cryptic diversity, molecular phylogeny and biogeography of the rock- and leaf litter-dwelling land snails of Belau (Republic of Palau, Oceania). Philos Trans R Soc B 363:3401–3412

Schell SC (1965) The life history of *Haematoloechus breviplexus* Stafford, 1902, (Trematoda: Haplometridae McMullen, 1937), with emphasis on the development of the sporocysts. J Parasitol 51:587–593

Schell SC (1985) Trematodes of North America. University of Idaho Press, Moscow

Schmidt-Hempel P (2011) Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford University Press, Oxford

Scholz T, Besprozvannykh VV, Boutorina TE, Choudhury A, Cribb TH, Ermolenko AV, Faltýnková A, Shedko MB, Shimazu T, Smit NJ (2016) Trematode diversity in freshwater fishes of the Globe I: 'Old World'. Syst Parasitol 93:257–269

Schotthoefer AM, Cole RA, Beasley VR (2003) Relationship of tadpole stage to location of echinostome cercariae encystment and the consequences for tadpole survival. J Parasitol 89:475–482

Seely LA (1906) Two distomes. Biol Bull 10:249–254

Shields JD (1987) Pathology and mortality of the lung fluke *Haematoloechus longiplexus* (Trematoda) in *Rana catesbeiana*. J Parasitol 75:1005–1013

Skrjabin KI, Antipin DN (1962) The superfamily Plagiorchioidea Dollfus, 1930. In: Skrjabin KI (ed) Trematodes of animals and man, vol. 20. Akademy Nauk, CCCR, Moscow, pp 47–163 (in Russian)

Snyder SD (1996) Host specificity among species of *Haematoloechus* (Digenea: Haematoloechidae). PhD thesis, University of Nebraska–Lincoln, Lincoln

Snyder SD, Janovy J Jr (1994) Second intermediate host-specificity of *Haematoloechus complexus* and *Haematoloechus medioplexus* (Digenea: Haematoloechidae). J Parasitol 80:1052–1055

Snyder SD, Janovy J Jr (1996) Behavioral basis of second intermediate host specificity among four species of *Haematoloechus* (Digenea: Haematoloechidae). J Parasitol 82:94–99

Snyder SD, Tkach VV (2001) Phylogenetic and biogeographical relationships among some holarctic frog lung flukes (Digenea: Haematoloechidae). J Parasitol 87:1433–1440

Sorensen RE, Minchella DJ (1998) Parasite influences on host life history: *Echinostoma revolutum* parasitism of *Lymnaea elodes* snails. Oecologia 115:188–195

Stafford J (1902) The American representatives of *Distomum cygnoides*. Zool Jahr Abt Syst Geogr Biol Tiere 17:411–424

Stafford J (1905) Trematodes from Canadian vertebrates. Zool Anz 28:681–694

Standley CJ, Prepelitchi L, Pietrokovsky SM, Issia L, Stothard JR, Wisnivesky-Colli C (2013) Molecular characterization of cryptic and sympatric lymnaeid species from the *Galba*/*Fossaria* group in Mendoza Province, Northern Patagonia, Argentia. Parasit Vectors 6:304

Stigge HA, Bolek MG (2015) The alteration of life history traits and increased success of *Halipegus eccentricus* through the use of a paratenic host: a comparative study. J Parasitol 101:658–665

Stigge HA, Bolek MG (2016a) Anuran host species influence site fidelity of *Halipegus occidualis*. J Parasitol 102:47–53

Stigge HA, Bolek MG (2016b) Evaluating the biological and ecological factors influencing transmission of larval digenetic trematodes: a test of second intermediate host specificity of two North American *Halipegus* species. J Parasitol 102:613–621

Sudarikov VE (1950) The trematodes of vertebrates in the Middle Volga area. Trudy Gelmintol Lab Akad Nauk SSSR 3:131–141

Szuroczki D, Richardson JML (2009) The role of trematode parasites in larval anuran communities: an aquatic ecologist's guide to the major players. Oecologia 161:371–385

Taylor DW (2003) Introduction to Physidae (Gastropoda: Hygrophila); biogeography, classification, morphology. Rev Biol Trop 51:S1–S287

Thomas LJ (1939) Life cycle of a fluke *Halipegus eccentricus* n. sp. found in the ears of frogs. J Parasitol 25:207–221

Tkach VV, Lotz J, Swideerski Z, Esteban JG (2002) On the systematic position of *Ophiosacculus* Macy, 1935 (Digenea: Lecithodendriidae), with the erection of the Ophiosacculinae n. subfam. Syst Parasitol 53:159–167

Toledo R, Carpena I, Espert A, Sotillo J, Munoz-Antoli C, Esteban JG (2006) A quantitative approach to the experimental transmission success of *Echinostoma friedi* (Trematoda: Echinostomatidae) in rats. J Parasitol 92:16–20

Toledo R, Muñoz-Antoli C, Fried B (2007) The use of echinostomes to study host–parasite relationships between larval trematodes and invertebrate and cold-blooded vertebrate hosts. Parasitol Res 100:1177–1185

Ubelaker JE, Olsen OW (1972) Life cycle of *Phyllodistomum bufonis* (Digenea: Gorgoderidae) from the boreal toad, *Bufo boreas*. Proc Helminthol Soc Wash 39:94–100

van Theil PH (1930) Die Entwicklung von *Agamodistomum anophelis* zum *Pneumonoecues variegates* Rud. Zentralbl Bakteriol B 117:103–112

Walther AC, Burch JB, Foighil DO (2010) Molecular phylogenetic revisions of the freshwater limpet genus *Ferrissia* (Planorbidae: Ancylinae) in North America yields two species: *Ferrissia* (*Ferrissia*) *rivularis* and *Ferrissia* (*Kincaidilla*) *fragilis*. Malacologia 53:25–45

Ward HB (1917) On structure and classification of North American parasitic worms. J Parasitol 4:1–11

Weiss M, Weigand H, Weigand AM, Leese F (2018) Genome-wide single-nucleotide polymorphism data reveal cryptic species within cryptic freshwater snail species—the case of the *Ancylus fluviatilis* species complex. Ecol Evol 8:1063–1072

Wetzel EJ, Esch GW (1996) Seasonal population dynamics of *Halipegus occidualis* and *Halipegus eccentricus* (Digenea: Hemiuridae) in their amphibian host, *Rana clamitans*. J Parasitol 82:414–422

Wilke T, Pfenninger M (2002) Separating historic events from recurrent processes in cryptic species: phylogeography of mud snails (*Hydrobia* spp.). Mol Ecol 11:1439–1451

Yamaguti S (1971) Synopsis of digenetic trematodes of vertebrates. Keigaku Publishing, Tokyo

Zamparo D, Ferrao A, Brooks DR, Bettaso J, Mata-Lopez R (2011) New species of *Haematoloechus* (Digenea: Plagiorchidae) in the lung of the foothill yellow-legged frog *Rana boylii* (Anura), from Humboldt County, California, USA. Rev Mexicana Biodivers 82:445–451

Zelmer DA, Brooks DR (2000) *Halipegus eschi* n. sp. (Digenea: Hemiuridae) in *Rana vaillanti* from Guanacaste province, Costa Rica. J Parasitol 86:1114–1117

Zelmer DA, Esch GW (1998a) Bridging the gap: the odonate naiad as a paratenic host for *Halipegus occidualis*. J Parasitol 84:94–96

Zelmer DA, Esch GW (1998b) Interactions between *Halipegus occidualis* and its ostracod second intermediate host: evidence for castration. J Parasitol 84:778–782

Zelmer DA, Esch GW (1999) Reevaluation of the taxonomic status of *Halipegus occidualis* Stafford, 1905 (Digenea: Hemiuridae). J Parasitol 85:157–160

Part IV

Clinical Aspects of Trematode Infections

Epidemiology of Trematode Infections: An Update

12

Jong-Yil Chai and Bong-Kwang Jung

12.1 Introduction

Flukes belonging to the class Trematoda (Phylum Platyhelminthes) are taxonomically diverse and largely consist of the subclasses Monogenea, Digenea, and Aspidogastrea (Beaver et al. 1984). Only those of the subclass Digenea are endoparasites of humans and animals. Species of trematodes important for human infections can be divided into six groups according to their habitat in the definitive host; blood flukes, liver flukes, lung flukes, throat fluke, pancreatic fluke, and intestinal flukes. Blood flukes comprise of more than five species of *Schistosoma* that parasitize the mesenteric venule or vesical or pudendal plexus of the urinary bladder (Beaver et al. 1984). The major liver flukes infecting humans include four species, *Clonorchis sinensis, Opisthorchis viverrini, O. felineus*, and *Fasciola hepatica*. They usually inhabit the bile duct of humans or animals, although *F. hepatica* can also be found in ectopic foci such as the eye, intestinal wall, and muscles. At least eight species of *Paragonimus* are currently acknowledged to be capable of infecting the lungs of human patients; *Paragonimus westermani, P. africanus, P. heterotremus, P. skrjabini, P. miyazakii, P. kellicoti, P. mexicanus*, and *P. uterobilateralis* (Narain et al. 2010). Intestinal flukes are more diverse, including heterophyids (*Metagonimus yokogawai, Heterophyes nocens*, and *Haplorchis taichui*), echinostomes (*Echinostoma revolutum, E. ilocanum, Isthmiophora hortensis, Echinochasmus japonicus, Artyfechinostomum malayanum*, and *Acanthoparyphium tyosenense*), gymnophallids, lecithodendriid-like flukes, microphallids, neodiplostomes, amphistomes, and plagiorchiids (Chai 2007, 2009). Epidemiological characteristics of these trematodes that include the geographical distribution, prevalence and intensity of infection, mode of transmission and infection source, and others related to prevention and control are highly variable according to each trematode species. Trematode infections can be treated using praziquantel (Chai 2013) with the exception of *Fasciola hepatica* infection, which can be treated with triclabendazole. This chapter is an update of the previous version "Chapter 8. Epidemiology of Trematode Infections" in *Digenetic Trematodes* (eds. R Toledo & B Fried), Springer, New York (Chai 2014).

J.-Y. Chai (✉)
Institute of Parasitic Diseases, Korea Association of Health Promotion, Seoul, Republic of Korea

Seoul National University College of Medicine, Seoul, Republic of Korea
e-mail: cjy@snu.ac.kr

B.-K. Jung
Institute of Parasitic Diseases, Korea Association of Health Promotion, Seoul, Republic of Korea

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_12

12.2 Blood Flukes

Among the blood flukes, five species that include *Schistosoma japonicum, S. mekongi, S. mansoni, S. haematobium*, and *S. intercalatum* are known to infect humans (Table 12.1). About 200–300 million people in more than 80 countries are affected by these flukes (Garcia 2007; Chai 2014).

Table 12.1 Human-infecting trematodes with the source of infection and geographical distribution

Species	Source of infection	Geographical distribution
Blood flukes		
Schistosoma japonicum	Contact with water	China, Indonesia, Taiwan, The Philippines
Schistosoma mekongi	Contact with water	Cambodia, Laos
Schistosoma mansoni	Contact with water	Brazil, Burkina Faso, Burundi, Cameroon, Chad, Congo, Cote d'Ivoire, Dutch Guiana, Egypt, Ethiopia, Gambia, Ghana, Guinea, Kenya, Liberia, Madagascar, Malagasy, Malawi, Mali, Mauritania, Mozambique, Namibia, Nigeria, Oman, Puerto Rico, Rwanda, Saudi Arabia, Senegal, Sergipe, Sierra Leone, South Africa, Sudan, Surinam, Tanzania, Togo, Venezuela, Vieques, Uganda, Yemen, Zambia, Zimbabwe
Schistosoma haematobium	Contact with water	Angola, Arabia, Burundi, Burkina Faso, Cameroon, Central Africa, Chad, Congo, Cote d'Ivoire, Egypt, Ethiopia, Gabon, Ghana, Iran, Iraq, Kenya, Lebanon, Madagascar, Malagasy, Mauritius, Morocco, Mozambique, Namibia, Nigeria, Northern Syria, Réunion, Rwanda, Senegal, Sudan, Tanzania, Togo, Uganda, Yemen, Zambia, Zimbabwe
Schistosoma intercalatum	Contact with water	Cameroon, Central African Republic, Chad, D.R. Congo, Egypt, Equatorial Guinea, Gabon, Madagascar, Nigeria, Sao Tome
Schistosoma spp.	Contact with water	Myanmar
Liver flukes		
Clonorchis sinensis	Freshwater fish	China, Korea, North Korea, Russia, Taiwan, Vietnam
Opisthorchis viverrini	Freshwater fish	Cambodia, Laos, Malaysia, Myanmar, Thailand, Vietnam
Opisthorchis felineus	Freshwater fish	Albania, Belarus, France, Germany, Greece, Italy, Kazakhstan, Macedonia, Poland, Romania, Russia, Spain, Switzerland, Turkey, Ukraine
Metorchis conjunctus	Freshwater fish	Canada, USA
Metorchis bilis	Freshwater fish	Central and eastern Europe, Russia
Metorchis orientalis	Freshwater fish	East Asia (human; in China)
Fasciola hepatica	Aquatic vegetation	Bolivia, Chile, China, Cuba, Ecuador, Egypt, France, Iran, Japan, Korea, Peru, Portugal, Spain, Thailand, Vietnam
Fasciola gigantica	Aquatic vegetation	Central African Republic, Chad, China, Ghana, Hawaii, India, Iran, Japan, Kenya, Korea, Malaysia, Niger, Russia, Senegal, Sudan, Tanzania, Thailand, Vietnam
Dicrocoelium dendriticum	Ant	Brazil, Canada, China, Colombia, Cuba, Europe, India, Iran, Japan, Northern coast of Africa, Russia, Syria, The Philippines, Turkey, USA
Dicrocoelium hospes	Ant	Africa
Lung flukes		
Paragonimus westermani	Crab, crayfish	Cambodia, China, India, Japan, Korea, Laos, Malaysia, Nepal, Pakistan, Papua New Guinea, The Philippines, Southeast Siberia, Sri Lanka, Taiwan, Thailand, USA, Vietnam
Paragonimus heterotremus	Crab	China, India, Laos, Thailand, Vietnam
Paragonimus skjrabini	Crab	China, India
Paragonimus miyazakii	Crab	Japan
Paragonimus kellicotti	Crab, crayfish	Canada, USA

(continued)

Table 12.1 (continued)

Species	Source of infection	Geographical distribution
Paragonimus mexicanus	Crab	Costa Rica, Ecuador, Guatemala, Panama, Peru, possibly in Colombia and Brazil
Paragonimus africanus	Crab	Cameroon, Nigeria
Paragonimus uterobilateralis	Crab	Cameroon, Gabon, Liberia, Nigeria
Throat fluke		
Clinostomum complanatum	Freshwater fish	Europe to Far East
Pancreatic fluke		
Eurytrema pancreaticum	Grasshopper	Brazil, China, Europe, Japan, Korea, Malaysia
Intestinal flukes (Table 12.2)		

Table 12.2 Human-infecting intestinal trematodes with the source of infection and geographical distribution

Species	Source of infection	Geographical distribution
Heterophyids		
Metagonimus yokogawai	Freshwater fish	Bulgaria, China, India, Indonesia, Japan, Korea, Russia, Taiwan, Czech Republic, Israel, Serbia, Spain, Ukraine
Metagonimus takahashii	Freshwater fish	Japan, Korea
Metagonimus miyatai	Freshwater fish	Japan, Korea
Heterophyes nocens	Brackish water fish	China, Japan, Korea, Thailand
Heterophyes heterophyes	Brackish water fish	Bangladesh, Egypt, Greece, India, Iran, Italy, Kuwait, Palestine, Russia, Saudi Arabia, Spain, Sri Lanka, Sudan, Thailand, Tunisia, Turkey, United Arab Emirates, Yemen
Heterophyes dispar	Brackish water fish	Eastern Mediterranean, Egypt, Northern Africa, Saudi Arabia, Thailand
Haplorchis taichui	Freshwater fish	Bangladesh, Cambodia, Egypt, Hawaii, India, Iraq, Kuwait, Laos, Malaysia, Palestine, South China, Sri Lanka, Taiwan, Thailand, The Philippines, Vietnam
Haplorchis pumilio	Freshwater fish	Cambodia, Egypt, India, Iraq, Korea, Laos, Malaysia, The Philippines, South China, Sri Lanka, Taiwan, Thailand, Venezuela, Vietnam
Haplorchis yokogawai	Freshwater fish	Australia, Cambodia, Egypt, India, Indonesia, Kuwait, Laos, Malaysia, Southern China, Taiwan, Thailand, The Philippines, Vietnam
Centrocestus formosanus	Freshwater fish	Brazil, China, Colombia, Croatia, Egypt, India, Japan, Lao PDR, Mexico, Taiwan, Thailand, The Philippines, Tunisia, Turkey, USA, Vietnam
Centrocestus armatus	Freshwater fish	Korea, Japan
Centrocestus kurokawai	Freshwater fish	Japan
Procerovum calderoni	Freshwater fish	Africa, China, Egypt, The Philippines
Procerovum varium	Freshwater fish	Australia, Cambodia, China, Egypt, India, Japan, Korea, Laos, The Philippines, Vietnam
Pygidiopsis genata	Freshwater fish	Egypt, Iran, Israel, Kuwait, Palestine, Romania, The Philippines, Tunisia, Ukraine
Pygidiopsis summa	Brackish water fish	Japan, Korea
Stellantchasmus falcatus	Fish	Hawaii, Japan, Korea, Palestine, Taiwan, Thailand, The Philippines, Vietnam
Stictodora fuscata	Brackish water fish	Japan, Korea, Kuwait
Stictodora lari	Brackish water fish	Australia, Japan, Korea, Russia, Vietnam
Acanthotrema felis	Brackish water fish	Korea
Apophallus donicus	Freshwater fish	USA
Ascocotyle longa	Freshwater fish	Europe, Asia, Africa, America
Cryptocotyle lingua	Freshwater fish	Europe, North America, Russia, Denmark, Japan, Korea
Heterophyopsis continua	Brackish water fish	Japan, Korea, the Philippines, Vietnam

(continued)

Table 12.2 (continued)

Species	Source of infection	Geographical distribution
Echinostomes		
Echinostoma revolutum	Freshwater snail	Africa, Asia, Australia, Austria, Bangladesh, Belarus, Brazil, Bulgaria, Cambodia, China, Czech Republic, England, Europe, Finland, France, Germany, India, Indonesia, Iran, Japan, Korea, Laos, Malaysia, New Zealand, North and South America, Poland, Russia, Slovak Republic, Taiwan, Thailand, The Netherlands, Vietnam, Yugoslavia
Echinostoma lindoense	Freshwater snail	Europe, Asia, South America
Echinostoma cinetorchis	Freshwater fish, snail	China, Japan, Korea, Taiwan, Vietnam
Echinostoma ilocanum	Freshwater snail	Bangladesh, Belarus, Cambodia, China, India, Indonesia, Iran, Japan, Korea, Laos, Malaysia, Poland, Russia, Thailand, The Philippines, Vietnam
Echinostoma paraensei	Freshwater snail	Brazil, Australia
Echinostoma angustitestis	Freshwater fish	China
Echinostoma aegyptiacum	Unknown	Egypt, China, Japan, Taiwan, Vietnam, Turkey
Isthmiophora hortensis	Freshwater fish	China, Japan, Korea, Russia
Isthmiophora melis	Freshwater fish, tadpole	Belarus, Bulgaria, Canada, China, Czech Republic, England, France, Germany, Hungary, Lithuania, Poland, Romania, Russia, Taiwan, Ukraine, USA
Echinochasmus japonicus	Freshwater fish	China, Japan, Korea, Kuwait, Laos, Russia, Taiwan, Thailand, Vietnam
Echinochasmus liliputanus	Freshwater fish	China, Egypt, Palestine, Sri Lanka, Syria
Echinochasmus perfoliatus	Freshwater fish	Bulgaria, Croatia, China, Denmark, Egypt, England, Hungary, India, Italy, Japan, Korea, Poland, Romania, Russia, Serbia, Taiwan, Thailand, Ukraine, Vietnam
Echinochasmus fujianensis	Freshwater fish	China
Echinochasmus jiufoensis	Freshwater fish	China
Echinochasmus caninum	Freshwater fish	India, China, Thailand
Artyfechinostomum malayanum	Freshwater snail	China, India, Indonesia, Laos, Malaysia, Singapore, Thailand, The Philippines
Artyfechinostomum sufrartyfex	Freshwater snail	India, Vietnam
Artyfechinostomum oraoni	Freshwater snail	India
Acanthoparyphium tyosenense	Brackish water snail	Korea
Echinoparyphium recurvatum	Freshwater snail	Bulgaria, Canada, Cosmopolitan, Croatia, Czech Republic, Egypt, especially Taiwan, Indonesia, Korea, Japan, Mexico, New Zealand, Poland, Russia, Spain, Thailand, The Philippines, UK, USA
Himasthla muehlensi	Brackish water bivalve	USA
Hypoderaeum conoideum	Freshwater snail	China, Germany, Indonesia, Japan, Mexico, North America, Russia (Siberia), Spain, Taiwan, Thailand
Brachylaimid		
Brachylaima cribbi	Land snail	Australia
Lecithodendriid-like flukes		
Caprimolgorchis molenkampi	Aquatic insect	Indonesia, Laos, Thailand, Cambodia
Phaneropsolus bonnei	Aquatic insect	India, Indonesia, Laos, Malaysia, Thailand, Cambodia
Strigeids		
Cotylurus japonicus	Freshwater snail	China, Japan
Prohemistomum vivax	Brackish and freshwater fish	Egypt, Israel

(continued)

Table 12.2 (continued)

Species	Source of infection	Geographical distribution
Fasciolid		
Fasciolopsis buski	Aquatic plants	Bangladesh, Cambodia, China, India, Indonesia, Japan, Korea, Laos, Malaysia, Myanmar, Nepal, Pakistan, Singapore, Taiwan, Thailand, The Philippines, Vietnam
Amphistomes		
Fischoederius elongatus	Aquatic plants	India, Sri Lanka, China, Japan, Thailand, Vietnam, Taiwan, Russia
Gastrodiscoides hominis	Aquatic plants	India, Pakistan, Myanmar, Vietnam, The Philippines, Indonesia, Malaysia, Thailand, Cambodia, China, Japan, Kazakhstan, Russia, USA, Zambia, Nigeria
Watsonius watsoni	Aquatic plants	Africa, Eastern Asia
Gymnophallid		
Gymnophalloides seoi	Oyster	Korea
Microphallids		
Gynaecotyla squatarolae	Brackish water crab	Japan, Korea
Microphallus brevicaeca	Brackish water crab	The Philippines
Isoparorchiid		
Isoparorchis hypselobagri	Fish	Australia, Bangladesh, China, India, Indonesia, Japan, Pakistan, Russia, Thailand
Nanophyetid		
Nanophyetus salmincola	Fish	Canada, Eastern Siberia, Russia, USA
Neodiplostome		
Neodiplostomum seoulense	Snake, frog	China, Korea
Plagiorchiids		
Plagiorchis muris	Aquatic insect, fish	Cambodia, Central Europe, England, Iran, Ireland, Japan, Korea, Laos, Mexico, The Netherlands, Spain, Taiwan, Thailand, The Philippines, USA, Vietnam
Plagiorchis harinasuta	Unknown	Thailand
Plagiorchis javensis	Insect larva	Indonesia
Plagiorchis philippinensis	Insect larva	The Philippines
Plagiorchis vespertilionis	Insect larva	Afghanistan, Belarus, Canada, China, Denmark, Egypt, France, Hungary, India, Iraq, Italy, Japan, Korea, Madagascar, Mexico, Mongolia, Poland, Romania, Russia, Spain, Taiwan, Turkey, Ukraine, USA

12.2.1 *Schistosoma japonicum* and *Schistosoma mekongi*

Schistosoma japonicum is a species of blood fluke that infects the superior mesenteric venule of humans and animals. It is an oriental type of schistosome. Adult male and female worms copulate and produce about 3000 eggs per worm per day (Garcia 2007). Some of these eggs are excreted in the feces; however, some others become free in the general circulation and can be filtered in the liver, lungs, and even the central nervous system (Garcia 2007). Egg granuloma in the liver parenchyme, fibrosis, portal hypertension, cirrhosis, abdominal distension, and esophageal varices are the main clinical manifestations. Its relationship with colorectal and liver cancer has been documented (Garcia 2007). *Schistosoma mekongi* is biologically similar to *S. japonicum* except in a few different points. *S. mekongi* tends to be more pathogenic than *S. japonicum* (Beaver et al. 1984).

Parasite biology and mode of human infection The snail hosts of *S. japonicum* are several species of *Oncomelania*, namely, *O. nosophora*

(south and southwestern China), *O. hupensis* (Yangtze River basin and south and southeastern China), *O. formosana* (Taiwan), and *O. quadrasi* (the Philippines) (Beaver et al. 1984). They are operculate with conical or turriculate shells, amphibious, freshwater species, and spend some parts of their time out of water, preferring moist soil in marshy habitats, at the edge of slow-flowing streams or irrigation canals (Gryseels and Strickland 2013). The cercariae are discharged only when the snails are at or below the water level (Beaver et al. 1984). Usually, less than 0.5% of the snails are infected with the cercariae at any time point (Gryseels and Strickland 2013). The snail intermediate host of *S. mekongi* is *Neotricula aperta* (syn. *Lithoglyphyopsis aperta* or *Tricula aperta*), which is distributed along the Mekong River, mainly from the Khong Island in Laos to Kratie Province, Cambodia (Ohmae et al. 2004). The cercarial infection rate in snails was 0.3% in Khong Island (Ohmae et al. 2004). The cercariae are furcocercus (fork-tailed) and actively swim in water. They are directly infectious to the skin of humans or animals, and thus contact with water can cause infection. Farmers and canal boatmen are principally infected, but all ages, including children, can be infected while bathing or wading shallow waters (Beaver et al. 1984). Using contaminated water for laundry purposes is also a risk for infection (Beaver et al. 1984). On contact with the skin of the definitive host, the cercariae cast off their tails and penetrate down to the cutaneous capillary beds, and enter the venous circulation (Beaver et al. 1984). The adult flukes, male and female, can live indefinitely in the human body as well as in animal reservoir hosts; survival of worms for 47 years has been documented (Beaver et al. 1984).

Reservoir hosts Humans, dogs, cats, rats, mice, field mice, cattle, water buffaloes, pigs, horses, sheep, and goats have been reported as natural definitive hosts (Beaver et al. 1984). For the purpose of control, attention to domestic cattle and buffaloes in agriculture, and to stray dogs and rats, is essential (Beaver et al. 1984). With regard to *S. mekongi*, the reservoir hosts are wild and domestic mammals that include dogs, cats, and pigs (Ohmae et al. 2004). In the laboratory, mice and hamsters could be successfully infected with *S. mekongi* (Ohmae et al. 2004).

Geographical distribution The current endemic areas of *S. japonicum* include some limited areas of East Asia, particularly Yangtze River basin, Boyang and other big lakes, and mountainous areas of Sichuan and Yunnan Province, China, and Mindanao, Leyte, and other small sites in the Philippines (Fig. 12.1) (Gryseels and Strickland 2013). *S. japonicum* was prevalent in

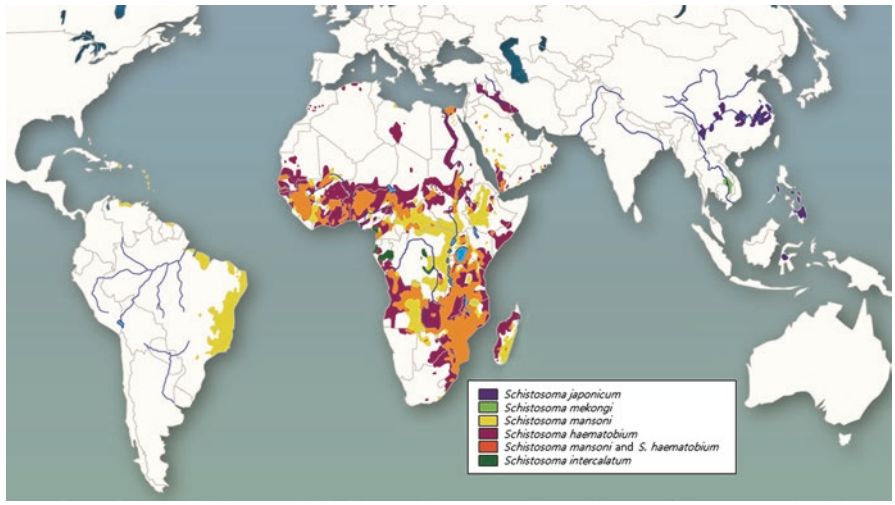

Fig. 12.1 Global distribution of blood flukes (*Schistosoma japonicum, S. mekongi, S. mansoni, S. haematobium*, and *S. intercalatum*). Adapted from Weerakoon et al. (2015)

the 1960s to the 1970s in several parts of Japan (Beaver et al. 1984) but there have been no new infections since 1976 and a declaration was made on the elimination of schistosomiasis in Japan (Ohmae et al. 2004). The Paloe District of Celebes, Indonesia, was endemic with *S. japonicum*; however, it is now unclear whether transmission still occurs in this area (Beaver et al. 1984; Gryseels and Strickland 2013). In Taiwan, enzootic cycle was reported; however, no human schistosomiasis has been documented (Garcia 2007). In 2000, the number of infected people with *S. japonicum* was estimated to be about 1.1 million, exclusively in Asia (Chitsulo et al. 2000). The number of people at risk of *S. mekongi* infection is estimated to be about 60,000 (likely infected cases being 11,000) in Laos and 80,000 in Cambodia (Fig. 12.1) (Chitsulo et al. 2000; Sinuon et al. 2010).

Population epidemiology The greatest exposure to cercariae occurs in boys aged 5–10 years because of recreational activities in water, and thus, the prevalence is highest in children (Garcia 2007). Older children may have less recreational exposure but are likely to be exposed while performing agricultural activities, washing dishes and cloths, and bathing younger siblings in the streams and lakes (Garcia 2007). Most infected individuals have low worm burdens, but a few may have very heavy burdens who probably make the greatest contribution to the dissemination of the infection (Garcia 2007). In the case of *S. mekongi*, the highest rate of infection has also been found in children 7–15 years of age (Beaver et al. 1984).

Environmental factors related to transmission Transmission is seasonal, and the cercarial shedding occurs only in the rainy season when the snails are at or below the water level (Beaver et al. 1984). The vector snails, *Oncomelania* spp., are extremely resistant to a long time dessication, and on contact with water, the snails become active again and shed infective cercariae (Garcia 2007). Heavy rains and flood, construction of dams, and other big environmental changes will affect the transmission of *S. japonicum* infection (Beaver et al. 1984; Garcia 2007). The newly constructed Three Gorges Dam area of China may appear to be an endemic area (Gryseels and Strickland 2013). With regard to *S. mekongi*, transmission occurs in rocky banks of the Mekong River basin, because the natural habitat of the snail host, *N. aperta*, is small crevices in the partially submerged rocks (Urbani et al. 2002). The period of active transmission in *S. mekongi* is during the dry season, from February and April in Cambodia, and March to June in Laos (Urbani et al. 2002).

12.2.2 *Schistosoma mansoni*

Schistosoma mansoni is parasitic in the inferior mesenteric venules and veins (mostly in the large bowel) of humans and rarely some animals like primates (Chai 2014). Adult male and female worms copulate and produce 100–300 eggs per worm per day (Garcia 2007). Some of these eggs are excreted in the feces; however, many others are swept away and become lodged in the microvasculature of the liver and other organs (Garcia 2007; Kaatano et al. 2015a). Eggs infiltrated in the intestinal wall may cause inflammation, thickening, and fibrosis, which lead to intestinal symptoms, including abdominal pain, bloody mucous stools, diarrhea, and dysentery (Beaver et al. 1984). Eggs deposited in the portal triads of the liver stimulate a granulomatous response, leading to continuous fibrosis of the periportal tissue and finally liver cirrhosis (Garcia 2007).

Parasite biology and mode of human infection Freshwater snails, *Biomphalaria glabrata, B. straminea, B. tenagophila* (Western Hemisphere), *B. alexandrina, B. sudanica, B. rüppellii, B. pfeifferi, B. choanomphala. B. smithi*, and *B. stanleyi* (Africa), have been found to be the intermediate host (Beaver et al. 1984; Caldeira et al. 2009). They are non-operculate, without cover or lid on the shell, and characterized by disk- or lens-shaped shells (Gryseels and Strickland 2013). They can survive long protracted droughts, hiding in moist mud until the next rains come and rivers swell again (Gryseels and Strickland 2013). The furcocercus cercariae

swim in water and are infectious to the skin of humans and animals. Direct contact with water containing cercariae can cause infection (Beaver et al. 1984). Farmers are exposed to infection in irrigation ditches, and infection can also occur from bathing and washing dishes and clothes in contaminated water (Beaver et al. 1984; Garcia 2007).

Reservoir hosts The role of reservoir hosts in maintaining the endemicity of *S. mansoni* is negligible, although natural infections were found in monkeys, baboons, and gerbils (Beaver et al. 1984). The source of human infection is almost exclusively derived from humans (Beaver et al. 1984).

Geographical distribution The geographical distribution of *S. mansoni* depends on the distribution of the snail intermediate host and the opportunity to infect humans (Garcia 2007). The endemic areas of *S. mansoni* are in 54 countries, which are scattered in Africa, the Arabian Peninsula, and the Western Hemisphere including South America and the West Indies (Fig. 12.1) (Chitsulo et al. 2000; Gryseels et al. 2006). Egypt, Sudan, Ethiopia, Cameroon, Congo, Uganda, Kenya, Tanzania, Zimbabwe, Zambia, South Africa, Madagascar, Mozambique, Malagasy, Malawi, Gambia, Guinea, Sierra Leone, Liberia, Mali, Mauritania, Senegal, Ghana, Nigeria, Cote d'Ivoire, Togo, Burundi, Burkina Faso, Chad, Rwanda, Namibia, and Oman are the countries in Africa having endemic areas (Beaver et al. 1984; Garcia 2007; Gryseels and Strickland 2013; Siza et al. 2015a, b; Al Abaidani et al. 2016; World Health Organization 2018). Saudi Arabia and Yemen are the Middle East countries where endemic areas of *S. mansoni* were reported (Beaver et al. 1984; Gryseels et al. 2006; Gryseels and Strickland 2013). The endemic countries in the Western Hemisphere include Brazil, Puerto Rico, Surinam, Venezuela, Vieques, Dutch Guiana, and Sergipe (Beaver et al. 1984; Chitsulo et al. 2000; Gryseels and Strickland 2013). However, in Puerto Rico, there was little transmission during the first half of the 1990s and has been disappearing thereafter (Garcia 2007). The estimated number of people infected with *S. mansoni* and/or *S. haematobium* is over 190 million (Chitsulo et al. 2000).

Population epidemiology *S. mansoni* infection is transmitted mainly from infected persons who defecate in or near water where the appropriate snail host resides (Garcia 2007). The overall prevalence in communities living under endemic conditions is usually between 30 and 100% (Gryseels and Strickland 2013). Its prevalence is highest in children, particularly in boys aged 5–10 years (Garcia 2007; Siza et al. 2015a, b). Most infected individuals have low worm burdens, but a few may have very heavy burdens and probably make the greatest contribution to the dissemination of the infection (Garcia 2007).

Environmental factors related to transmission Snail populations, cercarial density, and patterns of human–water contact show strong temporal and spatial variations, resulting in a focal distribution of the infection within countries, regions, and villages (Gryseels et al. 2006). Whereas *S. haematobium* mostly occurs in warm plains, *S. mansoni* can be transmitted in a variety of ecotypes, from savannah to rain forest and highlands of up to 2500 m (Gryseels and Strickland 2013). Transmission of both *S. mansoni* and *S. haematobium* takes place in the great lakes of Central and East Africa (Kaatano et al. 2015b), and also in many other small and large, natural or artificial, lakes (Gryseels and Strickland 2013).

12.2.3 *Schistosoma haematobium* and *Schistosoma intercalatum*

Schistosoma haematobium is a blood fluke that infects the vesical, pelvic, and pudendal plexuses in the urogenital system of humans and a few species of nonhuman primates. Adult females can contain 20–100 eggs in the uterus at one time (Garcia 2007). The eggs are deposited in the venous plexuses of the urinary bladder and excreted in the urine. However, eggs may also be deposited in the rectal venules and excreted in the

feces. In light infections, there may be no clinical symptoms; however, in moderate to severe infections there may be dysuria, frequency, and hematuria due to cystitis (Garcia 2007). In chronic cases, obstructive uropathy, bladder calcification, and even bladder carcinoma may occur (Garcia 2007). *S. intercalatum* is morphologically and biologically similar to *S. haematobium* but clinically similar to *S. mansoni* (Garcia 2007). *S. intercalatum* can cause an intestinal form of schistosomiasis like *S. mansoni*, although characterized by the lesions, mainly situated in the rectum and sigmoid level (Garcia 2007).

Parasite biology and mode of human infection The snail intermediate host of *S. haematobium* is *Bulinus truncatus* in the Mediterranean and Arabian countries, whereas it is *B. truncatus rohlfsi, B. guernei, B. senegalensis, B. globusus*, or *B. africanus* in Africa (Beaver et al. 1984). In India, the snail host is a limpet, *Ferrisia tenuis*, and in Portugal, it is *Planorbis metidjensis* (Beaver et al. 1984). They are aquatic, nonoperculate, without cover or lid on the shell, and characterized by having conic shells with a left-twisted spiral (Gryseels and Strickland 2013). They can survive in the mud when the water dries up, and retain their infectivity and resume cercarial shedding when the rainy season comes again (Garcia 2007; Gryseels and Strickland 2013). The snail hosts of *S. intercalatum*, which morphologically resembles *S. haematobium* but is biologically similar to *S. mansoni* (their habitat is mesenteric veins, and eggs are detected in the feces), are *Bulinus forkskalis* and *B. africanus* (Garcia 2007). The eggs of *S. haematobium* soon hatch on dilution of the urine or stool with ten or more parts of water, and the miracidia become free and swim actively in water (Beaver et al. 1984). The cercariae are infectious to the skin of humans and animals, and direct contact with water containing cercariae can cause infection (Beaver et al. 1984). Farmers, women washing clothes in streams, and children bathing or wading in the water are all subject to exposure (Beaver et al. 1984). In endemic foci of certain countries, religious practices tend to increase pollution of the water and encourage exposure to infections (Beaver et al. 1984). The mode of infection of *S. intercalatum* to humans is similar to that of *S. haematobium* and *S. mansoni*.

Reservoir hosts Humans appear to be the only important reservoir host of *S. haematobium*, although monkeys, baboons, and chimpanzees were found to be naturally infected in endemic areas (Garcia 2007). The source of human infection is almost exclusively derived from humans (Beaver et al. 1984). This is also true for *S. intercalatum*, although natural infection was found also in nonhuman primates, insectivores, marsupials, and rodents (Garcia 2007).

Geographical distribution The geographical distribution of *S. haematobium* depends on the distribution of the snail intermediate host and the opportunity to infect humans (Garcia 2007). The endemic areas, 53 countries (Chitsulo et al. 2000), are scattered in Africa, Asia Minor, Cyprus, the islands off the African east coast, and southern Portugal (Garcia 2007). Sudan, Ethiopia, Uganda, Yemen, east coast from Somaliland to Cape, vast areas in Central Africa, West Africa, and far south (Cote d'Ivoire, Togo, Burkina Faso, Chad, Nigeria, Cameroon, Congo, Angola, Gabon, Namibia), North Africa from Egypt to Morocco, and east coast countries (Madagascar, Malagasy, Mauritius, and Réunion) are well-known endemic areas in Africa (Fig. 12.1) (Beaver et al. 1984; Chitsulo et al. 2000; World Health Organization 2018). Lebanon, northern Syria, Arabia, Iraq, and Iran are west Asian countries having endemic areas (Beaver et al. 1984). In India, an endemic focus was found in an area south of Bombay (Beaver et al. 1984). The estimated number of people infected with *S. mansoni* and/or *S. haematobium* is over 190 million (Chitsulo et al. 2000). *S. intercalatum* has been found in ten countries of Africa (Cameroon, Equatorial Guinea, Gabon, Chad, Nigeria, Central African Republic, Democratic Republic of Congo, and Sao Tome, Egypt, and Madagascar), and a few countries in the Arabian Peninsula (Fig. 12.1) (Chitsulo et al. 2000; Garcia 2007). However, transmission in Chad, Nigeria, Central African Republic, and Democratic

Republic of Congo should be confirmed (Chitsulo et al. 2000). In endemic areas, prevalence of 5–25% has been found (Garcia 2007).

Population epidemiology *S. haematobium* is transmitted mainly from infected persons who urinate in or near water where the appropriate snail host resides (Garcia 2007). The overall prevalence in communities living under endemic conditions is usually between 30 and 100% (Gryseels and Strickland 2013). In most endemic foci, children are more frequently and more heavily exposed and infected than adults (Beaver et al. 1984). Most infected individuals have low worm burdens, but a few may have very heavy burdens and can probably make the greatest contribution to the dissemination of the infection (Garcia 2007). Human *S. intercalatum* infection is all derived from human sources (Garcia 2007).

Environmental factors related to transmission *S. haematobium* mostly occurs in warm plains, whereas *S. mansoni* can be transmitted in a variety of ecotypes (Gryseels and Strickland 2013). Transmission of both *S. mansoni* and *S. haematobium* takes place in the great lakes of Central and East Africa, and also in many other small and large, natural or artificial, lakes in Africa (Gryseels and Strickland 2013). The infected snails can be carried from infected foci into new irrigation projects (Beaver et al. 1984). The environmental factors related to the epidemiology of *S. intercalatum* infection are similar to those of *S. mansoni* (Garcia 2007).

12.3 Liver Flukes

Human-infecting liver flukes are at least ten species (Table 12.1); the major three being *Clonorchis sinensis, Opisthorchis viverrini*, and *O. felineus*. Infections with *Metorchis* species, i.e., *M. conjunctus, M. bilis*, or *M. orientalis*, are emerging zoonotic infections (Chai 2014). About 50 million people are infected with liver flukes with 800 million people at risk of infection, 601 million for *C. sinensis*, 80 million for *O. viverrini* and *O. felineus*, and 91 million for *Fasciola* spp. (Garcia 2007).

12.3.1 *Clonorchis sinensis*

Clonorchis sinensis, the Chinese liver fluke, infects the bile duct of humans and animals in the Far East and Southeast Asia. It can cause inflammation of the bile duct and gall bladder causing cholangitis and cholecystitis, respectively, and obstruction of the biliary tract. The early-stage clinical symptoms include jaundice, low-grade fever, anorexia, easy fatigue, and gastrointestinal disturbances such as epigastric discomfort, indigestion, and diarrhea (Rim 1982a, 1990, 2005). In chronic stages, various complications may occur that include biliary stones, fibrosis and cirrhosis of the liver, and even cholangiocarcinoma (Rim 2005; Chai 2014). It needs first and second intermediate hosts and a definitive host for completion of its life cycle.

Parasite biology and mode of human infection The first intermediate host is the freshwater snail, *Parafossarulus manchouricus* (syn. *P. striatulus*) in China, the Republic of Korea (hereafter Korea), Taiwan, and Russia, and also several other species of the Hydrobiidae (including *P. anomalospiralis*, *Bithynia fuchsiana, B. misella*, and *Alocinma longicornis*), Melaniidae (*Melanoides tuberculata* and *Semisulcospira libertina*), Assimineidae (*Assiminea lutea*), and Thiaridae (*Thiara granifera*) in China and Taiwan (Chen et al. 1994). The second intermediate host, i.e., the source of human and animal infections, is at least 113 species of freshwater fish, with the majority being cyprinoid fish including *Pseudorasbora parva* (Rim 2005). Three species of freshwater shrimps were also reported to be the second intermediate host in China (Rim 2005), The presence of the snail and fish, together with reservoir hosts, is essential to transmission of *C. sinensis* in endemic areas, and this combination must be sustained for the parasite to remain endemic in a region (Chai et al. 2005). The prevalence of infection in the snail host can be as low as 0.08% even in highly endemic areas, but this is sufficient to maintain the life cycle because the infected snails may release an average of 788 cercariae per snail per day, with a maximum of 5,840 cercariae per snail (Rim 1982a). Cercarial shedding can occur from May to October in Korea (Rim 1982a). The

infection rate of freshwater fish with *C. sinensis* metacercariae is up to 100% in endemic areas (Ooi et al. 1997; Cho et al. 2011). The prevalence of *C. sinensis* in endemic areas is, of course, related to the human custom of eating raw fish or shrimps (Chai et al. 2005). In southern China and Hong Kong, the morning congee (rice gruel) with slices of raw freshwater fish is an example of major dietary source of infection (Chen et al. 1994). In Guangdong Province, half-roasted or undercooked fish is another type of food involved, and in Fujian Province, eating raw shrimps is an important mode of human infection (Chen et al. 1994). In Korea, slices of raw freshwater fish with red pepper sauce is the major type of fish dish responsible for *C. sinensis* infection (Chai et al. 2005). In endemic areas of *C. sinensis* infection, small-sized fish, such as *P. parva, Acanthorhodeus* sp., *Rhodeus* sp., and *Hemiculter* sp., are more frequently and more intensively infected with the metacercariae than large fish (Rim 2005). However, large-sized fish such as carps, for example, *Cyprinus carpio, C. carpio nudus*, and *Cyprinus auratus*, are practically more important in inducing human infections in endemic areas (Chai et al. 2005; Rim 2005). The metacercarial burdens of these large carps are generally very low; however, they are preferred as the source of raw fish (Chai et al. 2005; Rim 2005). In contrast, small fish such as *P. parva* generally are much less preferred, particularly in Korea (Rim 1982a; Chai et al. 2005). This contributes to the accumulation of infections from large fish with small numbers of metacercariae over a period of 20–30 years (Rim 1982a).

Reservoir hosts The natural definitive hosts are mammals, including humans, dogs, cats, rats, pigs, badgers, weasels, camels, and buffaloes (Chai et al. 2005). However, the role of the reservoir hosts, especially cats, dogs, and pigs, in maintaining endemicity has not been well established (Chen et al. 1994; Chai et al. 2005). In some areas of China and Vietnam, infection may be high among people and low among domestic animals and the reverse situation is also found; however, in other endemic areas, the prevalence among the reservoir hosts may be the same as that in humans (Chen et al. 1994; De et al. 2003; Chai et al. 2005). This is not a trivial issue because the role of reservoir hosts may have an important bearing on the outcome of mass drug treatment control programs (Chai et al. 2005).

Geographical distribution The major endemic areas of *C. sinensis* include East Asia, particularly China, Taiwan, Korea (South and North), Vietnam, and Russia (Fig. 12.2) (Rim 1990; Chai et al. 2005;

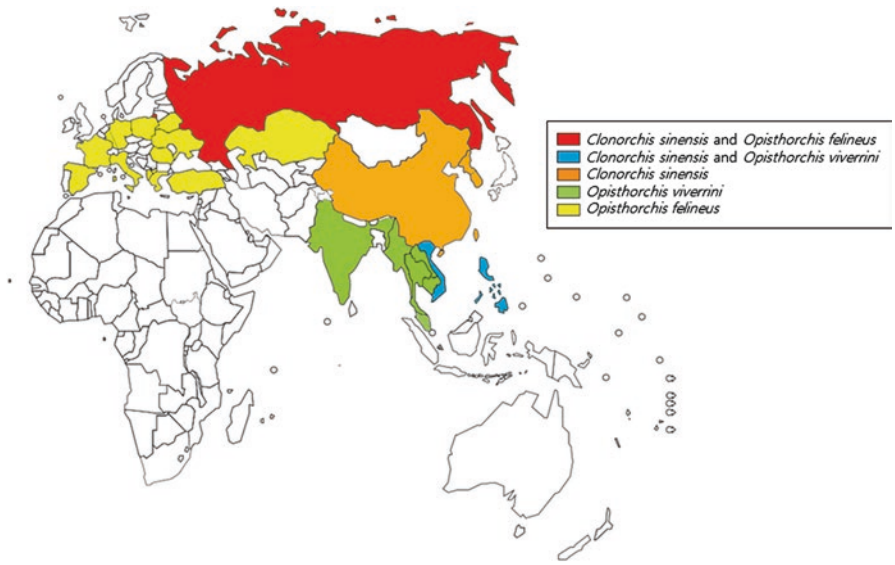

Fig. 12.2 Global distribution of liver flukes (*Clonorchis sinensis, Opisthorchis viverrini*, and *Opisthorchis felineus*)

Hong and Fang 2012; Lee et al. 2018). The number of infected people worldwide is currently estimated at 20 million at the least (Hong and Fang 2012). The geographical distribution is determined predominantly by the distribution of the snail intermediate host (Hong and Fang 2012). It is distributed between E 100° (Hanoi area, Vietnam) and E 140° (Amur River territory, Russia) and N 20° (Hanoi area, Vietnam) and N 50° (Amur River territory, Russia). In Korea, a national survey in 2004 reported 2.9% egg positive rate (Korea Association of Health Promotion 2004), and the number of infected people estimated in Korea was about 1.5 million. Major endemic areas are scattered along the five major rivers, including the Nakdong, Seomjin, Yongsan, Keum, and Han River. One case of clonorchiasis was recently diagnosed in stool examination of 15 refugees from North Korea (Lee et al. 2018). Thus, it seems probable that *C. sinensis* is distributed throughout the Korean Peninsula. In China, a total of 24 endemic localities (provinces, municipalities, and autonomous regions) were reported (Chen et al. 1994). Among them, Guangdong Province (including Hong Kong) and Guangxi Zhuang Autonomous Region, Heilongjiang, Jilin, and Liaoning provinces were the most important regions showing significantly high infection rates of the people (Yu et al. 2003). The number of infected people in nationwide China is estimated at about 12.5 million (Hong and Fang 2012; Fang et al. 2008). In Hong Kong, the prevalence was high before but it has decreased remarkably owing to control measures (Chen et al. 1994). In Taiwan, clonorchiasis was formerly endemic in three localities, Mei-Nung in the south, Sun-Moon Lake in the center, and Miao-Li in the north (Cross 1984). However, the current status is unknown. In Vietnam, clonorchiasis has been endemic mainly in the north, especially along the Red River Delta, including Haiphong and Hanoi (Rim 1982a; De et al. 2003; Chai et al. 2005). The number of population infected in Vietnam was estimated at about one million (Cam et al. 2008). In Russia, human cases infected with *C. sinensis* were reported in the Amur River territory, the far eastern part of the country (Chen et al. 1994). In a southern part of Khabarovsk region, along the Amur River, domestic cats revealed a high (74.6%) infection rate with *C. sinensis* (Chen et al. 1994).

Population epidemiology Characteristic age–sex prevalence patterns are known in endemic areas (Chai et al. 2005). The prevalence is generally higher in men than in women, and higher in adults than in children (Rim 1982a; Chen et al. 1994; Korea Association of Health Promotion 2004). For example, 25–55-year-old men and women over 45 years are the most highly affected groups (Chen et al. 1994). The prevalence begins to increase from the age of 20 years and reaches the peak at the age of 40–50 years, particularly in men (Rim 1990). This reflects most likely the behavior pattern of men, who more often gather together for dinners with raw or pickled fish usually accompanied by alcohol (Chai et al. 2005).

Environmental factors related to transmission Water contamination with human or animal feces containing eggs plays an important role in the transmission of *C. sinensis* (Chen et al. 1994). Pigpens, cowsheds, and even toilets nearby water drainages, streams, and ponds are the major sources of the eggs (Chen et al. 1994). As reported in Hubei Province, the prevalence is higher in plain areas than in mountainous areas because the houses are always near the streams and rivers in plain areas, and water is easily contaminated, resulting in high infection rates of the snails and fish (Chen et al. 1994). With regard to the regulation of snail development as well as parasite development in the snails, temperature and season are the primary determinants, and seasonal variation is evident in the infection prevalence (Rim 1982a; Chen et al. 1994). The cercarial shedding occurs from May to October in Korea and between March and October in Taiwan, which has more southerly latitude (Mas-Coma and Bargues 1997; Chai et al. 2005). Seasonal fluctuation was also recognized in the infection rate of fish hosts (Chen et al. 1994). In Shandong Province, China, the peak prevalence of *C. sinensis* metacercariae in fish occurred in October–November, and the metacercarial density peaked in November (Chen et al. 1994). Thus, it was sug-

gested that, in southern parts of China, the peak risk period for human infection is September to November (Chen et al. 1994). In Korea, seasonal tendency of metacercarial density in fish seems to be a less prominent phenomenon (Chai 2014).

12.3.2 *Opisthorchis viverrini*

Opisthorchis viverrini, the cat liver fluke, infects the bile duct of humans and animals in the Indochina peninsula. The pathogenesis and pathology are similar to those observed in *C. sinensis* infection. Chronic complications may include pyogenic cholangitis, biliary calculi, cholecystitis, cirrhosis of the liver, pancreatitis, and cholangiocarcinoma (Chai 2014). Its potential for inducing cholangiocarcinoma may be higher than that of *C. sinensis* (Chai et al. 2005). It needs first and second intermediate hosts and a definitive host for completion of its life cycle.

Parasite biology and mode of human infection The first intermediate host is the freshwater snail, *Bithynia* (*siamensis*) *goniomphalos, B.* (*siamensis*) *funiculata*, and *B.* (*simensis*) *siamensis* in northeast, north, and central Thailand, respectively (Kaewkes 2003). In Laos, *B.* (*simensis*) *goniomphalos* has been shown to play the role of the first intermediate host (Giboda et al. 1991; Ditrich et al. 1992). In southern parts of Vietnam, cercariae of *O. viverrini* were found from *Melanoides tuberculata* (De et al. 2003). The cercarial prevalence in the snail host may be quite low, 0.08–1.6%, but this level is sufficient to maintain the life cycle (Kaewkes 2003). However, a recent survey reported that the cercarial prevalence was up to 6.9% (average 0.73%) in 25 wetland localities of northeastern Thailand and up to 8.4% (average 1.1%) in 23 wetland areas of Laos (Kiatsopit et al. 2012). Species of freshwater fish reported to be susceptible to *O. viverrini* infection were 18 cyprinoid species including *Cyclocheilichthys siaja, Hampala dispar, Puntius orphoides*, *P. gonionotus, P. proctozysron, P. viehoever, Labiobarbus lineatus, Esomus metallicus*, and *Osteochilus* sp. (Rim 1982b; Kaewkes 2003; Chai et al. 2005). The infection rate of freshwater fish with *O. viverrini* metacercariae is variable according to locality but it can be up to 90–95% in high endemic areas (Sithithaworn and Haswell-Elkins 2003). The presence of the snail and fish hosts, together with the natural definitive host, is essential for transmission of *O. viverrini* in endemic areas (Chai et al. 2005). In northeast Thailand and Laos, it has been well known that "*koi pla*" is an important food source for *O. viverrini* infection, particularly among the Thai of Lao descent and Laotians (Chai et al. 2005). The "*koi pla*" dish consists of raw fish flesh chopped with garlic, lemon juice, fish sauce, chili, roasted ground rice, and local vegetables (Rim 1982b). However, the frequency of "*koi pla*" consumption has declined and generally confined to special social occasions (Sithithaworn and Haswell-Elkins 2003). Instead, the other two types of fish food, called "*pla som*" (moderately fermented fish for a few days to weeks) and "*pla ra*" or "*jaewbhong*" (extensively fermented for at least 2–3 months), have become more important as the infection source of *O. viverrini* (Sithithaworn and Haswell-Elkins 2003). Preserved fish dish like "*pla ra*" or "*jaewbhong*" is an important staple consumed daily by 60–98% of northeastern Thai people and lowland Laotians (Sithithaworn and Haswell-Elkins 2003). However, as the viability of metacercariae depends on the concentration of salt and degree of fermentation, "*koi pla*" is the most highly infective, followed by "*pla som*," and then "*pla ra*" and "*jaewbhong*" in which viable metacercariae are rare (Sithithaworn and Haswell-Elkins 2003). *Opisthorchis viverrini* adult flukes probably survive less than 10 years in the human host (Sithithaworn and Haswell-Elkins 2003).

Reservoir hosts The natural definitive hosts of *O. viverrini* include humans, dogs, cats, rats, and pigs (Sithithaworn and Haswell-Elkins 2003). However, the infection of the animal reservoir hosts is not closely associated with human infections (Sithithaworn and Haswell-Elkins 2003). In areas of high endemicity with *O. viverrini*, humans are the most common definitive host responsible for continuation of the parasite life cycle (Rim 1982b).

Geographical distribution This liver fluke is distributed widely along the Mekong River basin, particularly in Thailand, Laos, Vietnam, Cambodia, and Myanmar (Fig. 12.2) (Chai et al. 2005, 2014a; Sohn et al. 2012; Yong et al. 2012; Aung et al. 2017). The estimated number of infected people in total is 9–10 million (Yossepowitch et al. 2004; Andrews et al. 2008). The geographical distribution of *O. viverrini* is determined in close relationship with the distribution of the snail (*Bithynia* sp.) intermediate host (Chai 2014). The parasite is distributed actually between E 97° (Chiang Mai area, Thailand) and E 107° (Kratie area, Cambodia) and N 10° (Cantho area, Vietnam) and N 20° (Vientiane area, Laos). In Thailand, this liver fluke is distributed mainly in the north (19.3% prevalence; likely to include minute intestinal flukes) and northeast (15.7% prevalence) regions (Sithithaworn and Haswell-Elkins 2003), although the prevalence has shown a substantial decline after the 1990s (Andrews et al. 2008). On the other hand, in Laos, the infection is rather increasingly reported after the 1990s, particularly in the areas along the Mekong River; high prevalences of 53–67% were documented (Rim et al. 2003; Sayasone et al. 2007; Andrews et al. 2008; Chai et al. 2005, 2007, 2009a). Even among schoolchildren, the prevalence was considerably high (10.9%) among 29,846 subjects examined nationwide, with the highest prevalence (25.9%) found in Savannakhet Province (Rim et al. 2003). In Vietnam, there are endemic areas in southern parts, including Phu Yen, Da Nang, Dak Lak, and Binh Dinh (De et al. 2003; Le et al. 2012). The highest prevalence was found among people in Phu Yen (15.2–36.9%) (De et al. 2003). In Cambodia, Takeo and Kratie areas proved to be endemic areas (Sohn et al. 2012; Yong et al. 2012). In Myanmar, the presence of *O. viverrini* in the rural population from lower Myanmar area was confirmed by molecular methods (Aung et al. 2017).

Population epidemiology The population epidemiology of *O. viverrini* infection is closely related to the eating habit of the source food such as "*koi pla*," "*pla som*," and "*pla ra*" in endemic communities. The youngest group (0–5 years) generally shows the lowest prevalence and intensity, while infections in adolescents (15–19 years) often make a plateau followed by a decline thereafter (Rim 1982b; Sithithaworn and Haswell-Elkins 2003). In some areas, the worm burden declines with increase of age, possibly because of a late-developing immune response, lower parasite survival in heavily fibrosed bile ducts, death of parasites in heavily infected persons, or reduced exposure in the elderly (Sithithaworn and Haswell-Elkins 2003). The incidence and worm burdens tend to be greater in males than in females (Sithithaworn and Haswell-Elkins 2003).

Environmental factors related to transmission An important factor responsible for the propagation of *O. viverrini* infection is unsanitary latrine system in rural areas (Rim 1982b). Latrines are often unavailable in remote rural areas, and people have the habit of defecating on the ground in the bush not far from their houses, many of which are situated around lakes, water beds, or on the bank of streams (Rim 1982b). The transmission is often seasonal particularly where changes in rainfall and temperature are marked (Chai et al. 2005). In endemic countries like Thailand, water pollution by feces containing *O. viverrini* eggs takes place mainly in the rainy season, and in the last portion of this season and the first third of dry season the largest number of human infections occur (Rim 1982b). The possible effects of global climate change on *O. viverrini* distribution are debated (Andrews et al. 2008). Increased temperature is likely to reduce the developmental time of immature stages and might potentially reduce cercarial host-searching time (Poulin 2006). An increase in rainfall could lead to the extension of wetlands and, therefore, suitable new habitat, with an increased likelihood of parasitic gene flow between them through the expansion of snail and fish host distributions (Andrews et al. 2008).

12.3.3 *Opisthorchis felineus*

Opisthorchis felineus, the cat liver fluke, infects the bile duct of humans and animals in Eastern and Southern Europe and mid- and western parts of Russia (Chai 2014). The pathogenesis and

pathology are similar to those observed in *C. sinensis* and *O. viverrini* infection (Chai 2014). In chronic infections, anorexia, dyspepsia, dryness of mouth, bitter taste, fatigue, nausea, intolerability for greasy food, and pain in the hypochondrium are the major symptoms (Pozio et al. 2013). In severe cases, acute pancreatitis, bile peritonitis, hepatic abscess, obstruction of bile ducts, jaundice, and recurrent cholangitis may occur (Pozio et al. 2013). Its potential for inducing cholangiocarcinoma has been underestimated (Chai 2014). In Russia, the highest incidence of cholangiocarcinoma in humans was documented in the same area with the highest incidence of *O. felineus* infection (Mordvinov et al. 2012).

Parasite biology and mode of human infection The first intermediate host is *Bithynia leachi* species complex, which includes *B. leachi, B. troscheli*, and *B. inflata* distributed in East Europe (Mordvinov et al. 2012). Various species of freshwater fish take the role of the second intermediate host for *O. felineus*, which include 23 cyprinoid fish species (Mordvinov et al. 2012). The chub (*Idus melanotus*), tench (*Tinca tinca* and *T. vulgaris*), bream (*Abramis brama* and *A. sapa*), barbel (*Barbus barbus*), carp (*Cyprinus carpio*), *Blicca bjorkna, Leuciscus idus, Alburnus lucidus, Aspius aspius*, and *Scardinius erythrophthalmus* are the fish species involved, with the first two being the most commonly infected (Rim 1982b). In the past decade, humans in European Union got infected with *O. felineus* via ingestion of raw marinated fillets of fish (Pozio et al. 2013). However, in Italy, there was an outbreak of *O. felineus* infection in 211 people from 2003 to 2011 through consumption of the raw fillet of the tench (*T. tinca*) fished from two lakes, Bolsena and Bracciano (Pozio et al. 2013). Consumption of raw fish fillets occurs at home, in small restaurants along the shores of lakes, and at gastronomic events, and furthermore, raw fillets of the tench are served at restaurants because of their low cost and great availability during the summer as well as their particular taste (Pozio et al. 2013). Along the tributaries of the Ob River, West Siberia, riparian people eat the freshwater fish *Rutilus rutilus lacustris* (Siberian chebak roach), freshly salted, dried in the sun, or pickled in garlic juice (Rim 1982b).

Reservoir hosts The natural definitive hosts include humans, carnivorous mammals (dogs, cats, and foxes), chipmunks, beavers, Caspian seals, wild pigs, and domestic pigs (Rim 1982b; Mordvinov et al. 2012). Experimental definitive hosts include the hedgehog, rabbit, guinea pig, house mouse, golden hamster, and black-bellied hamster (Mordvinov et al. 2012). Stray cats in central Italy revealed 36.6%, 73.3%, and 40.0% infection rates in the lake coasts of Bolsena, Bracciano, and Trasimeno, respectively (Pozio et al. 2013).

Geographical distribution This fluke is widely distributed from the Iberian Peninsula (Portugal and Spain) to Eastern Europe and West Siberia (north of Kazakhstan) (Fig. 12.2) (Mordvinov et al. 2012). With regard to human infections, patients were recorded previously in Lithuania (before 1901), Poland (before 1937), Romania (before 1957), and Spain (before 1932), but recently no cases seem to occur in these countries (Pozio et al. 2013). However, in the last 50 years, many human cases have been reported in the European Union (Germany, Greece, and Italy), Eastern Europe (Balarus, Russia, and Ukraine), and West Siberia and Kazakhstan (Mordvinov et al. 2012; Pozio et al. 2013) Recently in Italy, there were 8 small outbreaks of human *O. felineus* infection involving a total of 211 people from 2003 to 2011 around two central lakes named Bolsena and Bracciano (Pozio et al. 2013). The estimated number of infected people worldwide totals 1.6 million (Yossepowitch et al. 2004). Infections in snails, fish, or reservoir hosts were reported in Germany, Italy, Poland, Portugal, Spain, Belarus, Russia, Ukraine, and Siberia (Pozio et al. 2013). The parasite is distributed between W 5° (Portugal) and E 95° (Kazakhstan and West Siberia) and N 38–40° (Greece) and N 55–70° (Western Siberia) (Pozio et al. 2013).

Population epidemiology The population epidemiology of *O. felineus* infection is closely

related to the eating habit of the source food such as raw fish fillets of the tench or marinated fish fillets of other kinds of freshwater fish (Pozio et al. 2013). In recent outbreaks in Italy, almost all patients were adults aged 16–84 years and only one was a child aged 9 years; 61.4% were males and 38.6% were females (Pozio et al. 2013). In West Siberia, along the Op River, the greatest incidence was found among housewives, who frequently have a chance to consume pickled, salted, or dried freshwater fish (Rim 1982b).

Environmental factors related to transmission The seasonal dynamics of *Bithynia* snail infection with *O. felineus* cercariae is characterized by a one-peak curve, with the peak observed in July (Rim 1982b). Related with this, the spring season until late May presents a great epidemiological danger for the snails to be infected with miracidia (Rim 1982b). Increased temperature and rainfall may adversely contribute to the spreading of this infection, although no proper documents related to this have been published (Chai 2014).

12.3.4 *Metorchis conjunctus*, *Metorchis bilis*, and *Metorchis orientalis*

Metorchis conjunctus, the Canadian liver fluke, infects carnivorous mammals in Canada and the USA (MacLean et al. 1996). In Canada, only sporadic and asymptomatic human infections have been found since 1946, particularly in aboriginal populations from Quebec to Saskatchewan and the eastern coast of Greenland (MacLean et al. 1996; Behr et al. 1998; Chai et al. 2005). However, an outbreak of acute infection was reported in 1996 among 19 Korean immigrants in Canada; the victims ate wild-caught fish (*Catostomus commersoni*) prepared in insufficiently cooked but traditional dishes (MacLean et al. 1996). Fatigue, upper abdominal tenderness, fever, abdominal pain, headache, weight loss, and anorexia were principal clinical presentations of the patients (MacLean et al. 1996). The first intermediate host is an aquatic snail *Amnicola limosa limosa*, and the second host includes several species of freshwater fish (*C. commersoni, C. catostomus, Salvelinus fontinalis*, and *Perca flavescens*) of which the most important one is the white sucker *C. commersoni* (MacLean et al. 1996). The reservoir hosts include the wolf, fox, coyote, raccoon, muskrat, mink, fisher, dog, and cat (MacLean et al. 1996). Infections have caused death of sled dogs in Central Canada; necropsies indicated that the cause of death was liver damage associated with this fluke infection (MacLean et al. 1996). This parasite is an emerging fish-borne parasitic zoonosis.

Metorchis bilis is an opisthorchiid fluke infecting carnivorous mammals including humans in Central and Eastern Europe and Western Siberia of Russia (Mordvinov et al. 2012). The detailed geographical range of *M. bilis* is thought to considerably overlap with that of *O. felineus* (Mordvinov et al. 2012). The detailed status of human infections and clinical manifestations in human patients are yet unknown. The first intermediate host is freshwater snails *Bithynia tentaculata* in European Territory of Russia and Kazakhstan and *Bithynia inflata, B. troscheli*, and *B. tentaculata* in Western Siberia (Mordvinov et al. 2012). The fish host is the same as those of *O. felineus*; the ide, roach, dace, tench, minnow, gudgeon, verkhavka, and silver carp (Mordvinov et al. 2012). The reservoir hosts include the wolf, white tailed eagle, muskrat, otter, and mink (Mordvinov et al. 2012).

Metorchis orientalis is a species of liver fluke infecting piscivorous birds and mammals including humans in East Asia (Yamaguti 1958; Lin et al. 2001; Cheng et al. 2005). The first report on human infections was made in 2001, which involved 4 (4.2%) of 95 residents examined in Ping Yuan county of Guangdong Province, China; 12 adult flukes were recovered in two purged patients (Lin et al. 2001). However, the detailed status of human infections and potential damage to humans are yet unknown. The geographical range of *M. orientalis* seems to be overlapped with that of *C. sinensis*. The first intermediate host is a freshwater snail *P. manchouricus* (syn. *P. striatulus*), and the fish hosts

are various species of cyprinoid fish that include *P. parva, Pseudogobio esocinus, C. carrassius, C. carpio*, and *Zacco platypus* (Komiya 1965). The reservoir hosts are dogs, cats, ducks, chicken, and geese (Yamaguti 1958; Zhan et al. 2017).

12.3.5 *Fasciola hepatica* and *Fasciola gigantica*

Fasciola hepatica and *F. gigantica*, the sheep liver fluke and the giant cattle liver fluke, respectively, infect the bile duct of domestic animals, including cattle and sheep worldwide (Beaver et al. 1984; Mas-Coma et al. 2005; Liu and Zhu 2013). The parasite life cycle, pathogenesis and pathology, epidemiology, and clinical symptoms are similar between *F. hepatica* and *F. gigantica* (Chai 2014). The juvenile worms migrate through tissues within the abdominal cavity and move to the liver parenchyme and then finally the bile duct or to other ectopic organs (Liu and Zhu 2013). They can cause inflammatory reactions and mechanical damage to the migratory tracks of the flukes. Humans are an accidental host infected through consumption of raw aquatic vegetables or raw liver of infected livestock animals (Liu and Zhu 2013). The acute-stage clinical symptoms include fever, abdominal pain, vomiting, loss of appetite, flatulence, diarrhea, urticaria, jaundice, ascites, and anemia (Liu and Zhu 2013). Chronic symptoms may include biliary colic, abdominal tenderness, hepatomegaly, fatty acid intolerance, pruritus, pancreatitis, cholangitis, cholecystitis, severe anemia, and portal cirrhosis (Liu and Zhu 2013). In ectopic infections, the clinical manifestations vary according to the involved site. The pathogenicity of *F. hepatica* and *F. gigantica* to humans is not recognizably different, although in sheep *F. gigantica* is more pathogenic than *F. hepatica* (Mas-Coma and Bargues 1997).

Parasite biology and mode of human infection The first intermediate host is a wide variety of freshwater snails of the Lymnaeidae (Bargues and Mas-Coma 2005). In Europe, *Galba truncatula* (syn. *Lymnaea truncatula*) is the preferred host snail, and in Africa, *G. truncatula* and *Pseudosuccinea columella* are important ones; in Asia, *G. truncatula* and *Austropeplea ollula* (syn. *A. viridis*) are involved, and in Australia and New Zealand, *Austropeplea tomentosa* (syn. *Lymnaea tomentosa*), *P. columella*, and *G. truncatula* have been recorded (Bargues and Mas-Coma 2005). In Hawaii, Papua New Guinea, the Philippines, and Japan, the snail host involved is *A. ollula*, and in North and Central America, *Fossaria humilis, F. bulimoides, F. cubensis*, and *P. columella* are transmitting *F. hepatica* (Bargues and Mas-Coma 2005). In South America, *Fossara viatrix* (syn. *F. viator*), *F. cubensis, G. truncatula*, and *Lymnaea diaphana* snails are involved (Bargues and Mas-Coma 2005). As for *F. gigantica*, the most important snail species is *Radix auricularia* followed by *Radix rubiginosa, R. natalensis, Lymnaea rufescens, L. acuminata*, and *A. tomentosa* (Liu and Zhu 2013; Phalee et al. 2015). These snails live in deeper water and are close to being true aquatic snails in their behavior (Liu and Zhu 2013). Metacercariae of *F. hepatica* are found encysted on leaves of aquatic vegetables (watercress, alfalfa, and water lettuce) or green vegetation, bark, or other smooth surfaces above or below the water line (Beaver et al. 1984; Mas-Coma and Bargues 1997; Liu and Zhu 2013). They are resistant and remain viable for a long period on the plants when moist, but are killed by excessive heat or dryness (Mas-Coma and Bargues 1997). In Latin America, France, and Algeria, people frequently acquire the disease as a result of eating raw watercress (*Nasturtium officinale*) on which the metacercariae have encysted (Beaver et al. 1984). Raw liver of an infected animal can also cause human infections; in these cases, young worms attach to the pharyngeal mucosa and cause pain, bleeding, edema, and dyspnea, a condition called "halzoun" (Beaver et al. 1984). Possibility of liver infection in humans through consumption of raw animal liver remains to be determined.

Reservoir hosts Mainly, sheep, goats, cattle, and European hares are natural definitive hosts for *F. hepatica* (Chen and Mott 1990; Oyarzún-Ruiz et al. 2018). Whereas most of the flukes in

cattle are eliminated within 9–12 months and they are resistant against challenge infections, sheep and goats are susceptible to reinfection without conferring acquired immunity (Chen and Mott 1990). A wide variety of other domestic and wild animals as well as laboratory animals can be infected with *F. hepatica*, but they are usually not very important for transmission of human infections (Chen and Mott 1990). Reservoir hosts of *F. gigantica* include sheep, goat, cattle, buffalo, camel, pig, horse, and other domestic and wild animals (Mas-Coma et al. 2005).

Geographical distribution The geographical distribution of *F. hepatica* is almost worldwide, especially where there is extensive sheep and cattle raising (Liu and Zhu 2013). Approximately 2.4 million people in more than 60 countries are estimated to be infected and the number of people at risk is more than 180 million globally (Liu and Zhu 2013). Human fascioliasis is a serious public health concern in the Andean countries (Ecuador, Bolivia, Chile, and Peru), the Caribbean area (Cuba), northern Africa (Egypt), Western Europe (Portugal, France, and Spain), and the Caspian areas (Iran, Turkey, and neighboring countries) (Mas-Coma et al. 2005). Sporadic cases have been reported in Korea, Japan, China, Thailand, and Vietnam (Chen and Mott 1990; Mas-Coma et al. 2005; Kang et al. 2014). The highest prevalence and intensity ever reported have been found in the Northern Bolivian Altiplano; in this area, prevalences detected in some communities were up to 72% and 100% by coprological and serological surveys, respectively (Mas-Coma et al. 2005). In this area, intensities reached up to 5000 eggs per gram of feces in children (Esteban et al. 2003). The geographical distribution of *F. gigantica* is a little different from that of *F. hepatica*, and human infections have been reported mainly in Asia (Japan, Korea, China, Russia, Vietnam, Thailand, Malaysia, India, Iran, and many others), Africa (Sudan, Senegal, Chad, Ghana, Niger, Central African Republic, Tanzania, Kenya, and many others), and Hawaii (Mas-Coma and Bargues 1997).

Population epidemiology The prevalence and intensity of *F. hepatica* infection is significantly higher in females than in males in Bolivia and Egypt (Esteban et al. 1999, 2003). This gender role may be related to cultural, hygienic, and behavioral factors, females being occupied in washing household items in large canals where transmitting lymnaeid snails are present, and in agricultural tasks in irrigated plantations such as rice fields (Mas-Coma et al. 2005). Females are also central to meal preparation in houses including management of freshwater plants potentially carrying attached metacercariae (Mas-Coma et al. 2005). However, in Spain, the prevalence was similar in males and females (Chen and Mott 1990). Family clustering of the infected cases is a typical feature because the family shares the same food (Chen and Mott 1990). Fascioliasis is predominantly a rural disease, and sheep- and cattle-herders are more frequently affected than other professions (Chen and Mott 1990). All age groups can be infected; however, those less than 5 years of age had the lowest chance of infection (Chen and Mott 1990).

Environmental factors related to transmission Fascioliasis is unique in its capability of giving rise to human endemic areas from below sea level (on the shores of Caspian Sea) up to very high altitude (in Bolivia, Peru, Ecuador, and Venezuela) (Mas-Coma et al. 2005). In endemic areas, climatic conditions are critical and decisive for the development of both the *Lymnaea* snails and the flukes and transmission (Chen and Mott 1990; Mas-Coma et al. 2005). The snails of *F. hepatica* are more resistant to low temperature compared with high temperature and dessication; they can survive through the winter but become weak at low humidity and temperatures over 25°C (Chen and Mott 1990). In France, human infections were more frequently observed in the years with heavy rain fall (Chen and Mott 1990). Human fascioliasis can occur in any season but more frequently in cooler seasons of the year (Chen and Mott 1990). The metacercariae of *F. gigantica* survive longer at high temperatures and are more susceptible to dessication than those of *F. hepatica* (Mas-Coma et al. 2005).

12.3.6 *Dicrocoelium dendriticum* and *Dicrocoelium hospes*

Although *Dicrocoelium dendriticum* and *D. hospes* are liver flukes of mainly sheep, goat, and cattle, they can accidentally infect humans in rare instances (Traversa et al. 2013). Simple egg positive results in human fecal examinations may represent a spurious infection due to ingestion of infected livers of animals, and genuine infection can be verified through parasite finding in surgical operations, evidence of permanent egg shedding through time, egg recovery in duodenal aspirates, or the existence of related clinical symptoms (Mas-Coma and Bargues 1997). The pathology depends on the number of flukes infected and the duration of infection and in many instances without notable symptoms; however, a prolonged period of constipation or diarrhea, nausea, vomiting, abdominal discomfort, and epigastric pain may occur (Mas-Coma and Bargues 1997). Mechanical and toxic damages to the host are much less than in fascioliasis and opisthorchiasis, because of the small worm size and its smooth and spineless surface (Mas-Coma and Bargues 1997).

Parasite biology and mode of human infection More than 90 land snail species have been found to act as competent vectors of *D. dendriticum*, and several of them, in particular, *Cochlicopa lubrica*, are diffused worldwide (Traversa et al. 2013). *Helicella corderoi* is distributed in Spain, *Zebrina hohenackeri* in the Caucasus, *Helicella obvia* in Germany, and *Cernuella virgata* in Italy (Traversa et al. 2013). The cercariae are extruded from the snails in clusters of thousand, enveloped in a mucilaginous substance, commonly known as "slime ball" and are ingested by various (more than 14) species of ants (*Formica fusca, F. pratensis*, and *F. rufibarbis*), the second intermediate host (Traversa et al. 2013). Humans acquire the infection accidentally by swallowing an infected ant together with the food, such as vegetables, fruits, and others while staying in endemic areas (Mas-Coma and Bargues 1997). Both *D. dendriticum* and *D. hospes* are zoonotic and are able to establish in the bile ducts of humans (Traversa et al. 2013).

Reservoir hosts *D. dendriticum* infection is common in small ruminants in many European and Asian countries, for example, 100% of sheep flocks having individual burdens of 1650–2837 worms (Mas-Coma and Bargues 1997). Cattle are also an important reservoir and the prevalence increases with age (Mas-Coma and Bargues 1997). Goats, deer, elk, rabbits, and pigs are less important reservoir hosts (Mas-Coma and Bargues 1997). *D. dendriticum* also affects species of wild ruminants, such as camelids (lamas and alpacas) in South America and yaks and buffaloes in India (Traversa et al. 2013).

Geographical distribution *D. dendriticum* has a cosmopolitan distribution in herbivorous mammals, mainly ruminants, of the Holartic region (Mas-Coma and Bargues 1997). It is distributed in almost every country in the European continent and adjacent islands and is found along the northern coast of Africa (Mas-Coma and Bargues 1997). In Asia, it is found in Russia, Turkey, Syria, Iran, India, China, the Philippines, and Japan; in the Americas, it is found in the USA, Canada, Cuba, Colombia, and Brazil (Mas-Coma and Bargues 1997). *D. hospes* is found in Africa (Traversa et al. 2013).

Population epidemiology Up to 1982, approximately 300 human cases were reported in the literature based on recovery of eggs in the feces (Mas-Coma and Bargues 1997); however, population epidemiology of human dicrocoeliasis is unknown.

Environmental factors related to transmission Cercariae may be shed from the snails intermittently at short intervals dictated by sudden climatological changes, such as decrease in atmospheric pressure and temperature, and increase in relative humidity notably during thunderstorm (Mas-Coma and Bargues 1997). In sheep pastures in Germany, it was found that the majority of *H. obvia* snails become infected in

the autumn of their second year of life, when their shell diameter is of medium size (Mas-Coma and Bargues 1997). The formation of the slime balls is associated with a drop in the temperature in the snail environment, and the slime ball output can be activated only in May and June (Mas-Coma and Bargues 1997).

12.4 Lung Flukes

Eight species of lung flukes infect humans worldwide, namely, *Paragonimus westermani, P. heterotremus, P. skryabini, P. miyazakii, P. africanus, P. uterobilateralis, P. kellicotti*, and *P. mexicanus* (Table 12.1) (Narain et al. 2010; Sugiyama et al. 2013). Human infection with *P. siamensis*, which was originally described from experimental cats in Thailand, was documented (Wang et al. 2011). However, species identification of the responsible specimen needs further verification (Chai 2014). The estimated number of *Paragonimus*-infected patients globally is 21 million and the number of people at risk is 293 million (Keiser and Utzinger 2009).

12.4.1 *Paragonimus westermani*

Paragonimus westermani (syn. *P. pulmonalis, P. philippinensis*), first discovered in the lungs of a Bengal tiger (Miyazaki 1991), is the representative species of the lung flukes infecting humans and animals. It is distributed mainly in Asia but recently has also been found in Papua New Guinea and North America (Owen 2005; Fried and Abruzzi 2010). Its pulmonary infection can cause fatigue, chest pain, cough, pleural effusion, dyspnea, bronchiectasis, hemoptysis with rusty-colored sputum, pneumothorax, interstitial pneumonitis, and bronchopneumonia (Procop 2009). However, it can also frequently invade other visceral organs, eliciting extrapulmonary paragonimiasis; the brain, spinal cord, and abdominal organs (Miyazaki 1991; Blair et al. 2007). It needs snails as the first intermediate host and crabs and crayfish as the second intermediate host and a wild animal definitive host for completion of its life cycle in nature. A triploid form of *P. westermani*, once called "*P. pulmonalis*" (Miyazaki 1991), was later regarded as a variation within a species, with both being of single origin (Blair et al. 1999).

Parasite biology and mode of human infection The snail intermediate host includes various species of *Semisulcospira* (*S. libertina, S. calculus, S. amurensis, S. extensa, S. multicincta*, and *S. nodiperda*) and *Melanoides tuberculata* (Blair et al. 1999). The reported second hosts are numerous species of freshwater crayfish (*Cambaroides similis, C. schrenki, C. dauricus, Procambarus clarki*, and *Macrobrachium nipponensis*) and crabs (*Eriocheir japonicus, E. sinensis, Parathelphusa maculata, P. malaysiana, Geothelphusa candidiensis, G. miyazakii, Hunanopotamon angulatum, H. obtusum, Potamon hispidum, P. hokuoensis, and Sinopotamon* spp.) (Blair et al. 1999). Human infection is caused by consumption of raw or undercooked freshwater crabs or crayfish. In China, the famous dishes causing human paragonimiasis is "drunken crab" and in Korea, a most common source is "Kejang (= sauced crab)" (Choi 1990; Nakamura-Uchiyama et al. 2002) In Japan, an important infection source is "oborokiro (= crab juice soup)" and in Thailand and the Philippines, "goong ten (= raw crayfish salad)" and "kinuolao (= raw crab)," respectively, are responsible for inducing human paragonimiasis (Kim 1984; Nakamura-Uchiyama et al. 2002). In Korea, the juice of crushed crayfish had been traditionally used as a remedy for measles and other febrile illnesses in children and thus became an important source of infection (Choi 1990); however, this tradition has almost disappeared now. Another important mode of infection in Japan is ingestion of raw wild boar and sika deer meat (venison) containing metacercariae or juvenile worms (Miyazaki and Habe 1976; Shibahara et al. 1992; Nakamura-Uchiyama et al. 2002; Yoshida et al. 2016). Paragonimiasis patients sometimes claim that they had never eaten freshwater crabs or crayfish. Finger contamination with metacercariae might occur while cooking the crabs or crayfish, and the chopping board or

other cooking utensils might also be contaminated (Nakamura-Uchiyama et al. 2002).

Reservoir hosts The definitive hosts for *P. westermani* are humans and wild animals that include monkeys, dogs, foxes, wolves, cats, tigers, lions, leopards, pigs, and rats (Blair et al. 1999). Wild boars and sika deers are paratenic hosts (Nakamura-Uchiyama et al. 2002; Yoshida et al. 2016), in which worms do not mature and stay in muscles and tissues; when these immature worms are eaten by humans, they could develop into adult worms. Experimentally, hamsters, mice, rats, guinea pigs, and rabbits can be infected with *P. westermani*; however, worms seldom mature in the lungs of these hosts and thus these animals are also regarded as paratenic hosts (Blair et al. 1999).

Geographical distribution This fluke has been reported from various parts of Asia, including China, Taiwan, Korea, Japan, Southeast Siberia, the Philippines, Malaysia, Thailand, Cambodia, Laos, Vietnam, Sri Lanka, India, Nepal, and Pakistan (Fig. 12.3) (Blair et al. 1999; Narain et al. 2010; Sugiyama et al. 2013). Recently, the distribution of this species appears to have extended to Papua New Guinea (Owen 2005) and North America, i.e., USA (Fried and Abruzzi 2010; Boland et al. 2011). More than ten million people are estimated to be infected with *P. westermani*.

Population epidemiology Because the diagnosis of *P. westermani* is usually difficult to perform by sputum examination to detect eggs (only less than a half of the patients reveal sputum eggs), the prevalence among people has seldom been studied even in highly endemic areas (Chai 2014). In the past, intradermal tests to detect antibody reactions were popularly used; however, cross-reactions frequently occurred and its specificity was not sufficiently good (Chai 2014). ELISA is currently the most useful tool for the diagnosis of paragonimiasis, and 2.8% in 1993 (out of 3973 cases examined) and 1.1% in 2006 (out of 1869 cases) seroprevalences were reported among hospital-referred general patients in Korea (Lee et al. 2010b). However, not much data are available on the seroepidemiological status of infection using ELISA. The prevalence, as estimated by sputum examination, was generally higher in men than in women and higher in aged people than in young children (Yokogawa et al. 1960; Yokogawa 1965). The higher prevalence in males is probably due to their greater use of crab meat as a stimulant during drinking (Yokogawa et al. 1960; Yokogawa 1965). However, no substantial difference in the male-to-female ratio

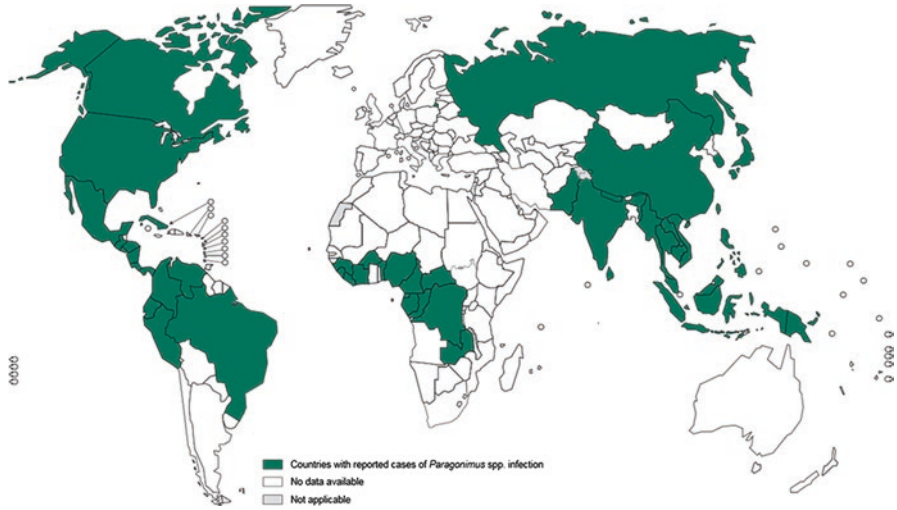

Fig. 12.3 Global distribution of paragonimiasis. Adapted from WHO (2015)

was reported by several workers (Procop 2009). Higher prevalence in children than in adults was also reported in Vietnam (De 2004). Although the incidence has been generally decreasing, sporadic cases are occasionally reported in Korea due to environmental and social changes related to the availability of the crab and crayfish (Koh et al. 2012).

Environmental factors related to transmission The snail intermediate host is most frequently found in swift-moving mountain streams that may be at some distance from human habitations; such streams are also the preferred habitats for the freshwater crabs or crayfish that serve as the second intermediate hosts (Yokogawa et al. 1960, 1965). It seems probable that the miradicia that infect the snails come more commonly from eggs passed by wild or domesticated animals harboring the adult flukes than human patients (Yokogawa et al. 1960). Therefore, mountainous areas rather than plain areas tend more likely to become endemic areas of *P. westermani*. The optimum temperature for the development of *P. westermani* eggs into miracidia was reported to be from 28°C to 32°C; it took 10–16 days (Yokogawa et al. 1960). At temperatures lower than this, 16–25°C, for example, it took longer (25 days) for the eggs to develop into miracidia (Yokogawa et al. 1960).

12.4.2 *Paragonimus heterotremus*

Paragonimus heterotremus (syn. *P. tuanshanensis*) was first found in China in 1964 and is now known to be distributed in southern and western parts of Asia (Blair et al. 1999; Singh et al. 2009; Narain et al. 2010). Clinical manifestations are similar to those seen in *P. westermani* infection (Miyazaki 1991). In a central area of Thailand, there was an outbreak of infection involving at least 33 (sputum egg positive cases) persons out of 503 examined (Miyazaki 1991). In the northern mountainous regions of Vietnam, among 2216 sputum samples examined, 142 (6.4%) revealed eggs of *P. heterotremus* (De 2004). An endemic focus has been found in Nagaland area of India (Singh et al. 2009).

Parasite biology and mode of human infection The snail intermediate host includes *Assiminea* sp., *Neotricular aperta*, *Oncomelania hupensis chiui, O. hupensis formosana, O. hupensis hupensis, O. hupensis nosophora, O. hupensis quadrasi*, *Paludomus* sp., and *Tricula gregoriana* (Blair et al. 1999; Iwagami et al. 2009). The second host is the freshwater crab, *Esanthelphusa dugasti* and *Siamthelphusa paviei* in Thailand, and *Potamon flexum* and *Potamiscus smithianus* in China, *Potamiscus tannanti* in Vietnam, and *Potamiscus manipurensis* in India (Blair et al. 1999; De 2004; Singh et al. 2009). In Vietnam, people like to eat raw crab; 68.1% of people surveyed answered that they had consumed crabs raw (De 2004). In Nagaland area of India, villagers believed that raw crabs or its extract and soup provided them strength and nutrition, and some believed that ingestion of raw crab extract can cure fever and allergy (Singh et al. 2009).

Reservoir hosts *P. heterotremus* has been found to infect humans, dogs, cats, gerbils, and monkeys in nature, and mice, rats, and rabbits can be infected experimentally (Blair et al. 1999). In Vietnam, the infection rate of dogs in northern mountainous areas was 12.1% of 222 dogs examined (De 2004).

Geographical distribution This species has been found in China, Vietnam, Laos, Thailand, and India (Fig. 12.3) (Singh et al. 2009; Narain et al. 2010). Human and/or animal infections have been found in southern parts of China (Guangxi Province), northern mountainous areas of Vietnam, mountainous areas of Laos, central Thailand, and northeastern parts of India (Miyazaki 1991; De 2004; Singh et al. 2009). This lung fluke may potentially be distributed in Cambodia (Miyazaki 1991).

Population epidemiology In an area of northeastern India, the egg positive rate in the sputum was 20.9% in children (55 of 263 examined) and 4.1% in adult population (17 of 412 examined); the egg positivity was not significantly different by sex (Devi et al. 2007). Little information has been available in other countries.

Environmental factors related to transmission Environmental factors, similar to those of *P. westermani*, may also affect the transmission of *P. heterotremus* infection; however, not much information is available.

12.4.3 *Paragonimus skrjabini* and *Paragonimus miyazakii*

The Chinese lung fluke, *Paragonimus skrjabini* (syn. *P. szechuanensis, P. hueitungensis*), was first described from viverrid cats purchased in a market of Guangzhou, China (Chen 1962; Blair et al. 1999) and is now known to be distributed in several Asian countries (Miyazaki 1991; Blair et al. 2005; Singh et al. 2006). Human infections were first found in Sichuan, China (Chen 1962). *P. skrjabini* is a zoonotic parasite, primarily the lung fluke of animals, which is less adapted to humans (Nakamura-Uchiyama et al. 2002). Thus, in *P. skrjabini* infection, subcutaneous, cerebral, and eye involvements are more frequent than pulmonary lesions (Miyazaki 1991; Nakamura-Uchiyama et al. 2002). *P. miyazakii*, which morphologically resembles *P. kellicotti*, was first described from dogs fed the metacercariae from the crab *Geothelphusa dehaani* (syn. *Potamon dehaani*) in Japan (Kamo et al. 1961), and later human infections were found in Kanto District, Japan (Yokogawa et al. 1974). Pleural infections are more common in *P. miyazakii*-infected patients (a less suitable host) compared with *P. westermani*-infected patients in whom lung parenchymatous lesions are more common (Nakamura-Uchiyama et al. 2002).

Parasite biology and mode of human infection The snail intermediate host of *P. skrjabini* is *Akiyoshia orientalis, Assiminea lutea, Tricula* spp. (*T. gushuiensis*), and *Neotricula* spp. (Miyazaki 1991; Blair et al. 1999). The second host is freshwater crabs, *Sinopotamon denticulatum, S. yaanense, Aprapotamon grahami, Isolapotamon* spp., *Tenuilapotamon* spp., and *Potamiscus manipurensis* (Blair et al. 1999; Singh et al. 2006). As for *P. miyazakii*, the snail host is *Bythinella kubotai, Bythinella nipponica*, and *Oncomelania hupensis nosophora*, and the second host is the freshwater crab *Geothelphusa dehaani* (= *Potamon dehaani*) (Blair et al. 1999). In China, consumption of raw, undercooked, or pickled crab is very common (Li et al. 2008), and this may be an important source of infection with *P. skrjabini.* Human infections with *P. miyazakii* occurred by eating raw or improperly cooked potamonid crabs or drinking crab juice, a folk remedy for whooping cough in Japan (Miyazaki 1991). Wild pig or boar meat is a significant alternative source of human infections with *P. miyazakii* in Japan (Nakamura-Uchiyama et al. 2002).

Reservoir hosts Natural definitive hosts for *P. skrjabini* other than humans include monkeys, dogs, cats, and weasels (Blair et al. 1999). Mice, rats, cats, dogs, and monkeys can serve as the experimental definitive hosts (Blair et al. 1999). As for *P. miyazakii*, humans, dogs, cats, martens, weasels, and wild boars are natural definitive hosts (Blair et al. 1999). Experimental hosts include hamsters, mice, guinea pigs, and rabbit (Blair et al. 1999).

Geographical distribution *P. skrjabini* is known to be distributed in 16 provinces of China and some parts of northeastern India (Fig. 12.3) (Miyazaki 1991; Blair et al. 2005; Singh et al. 2006). Miyazaki (Miyazaki 1991) found a worm in Thailand; however, no further reports have been available from Thailand. High prevalences of *P. skrjabini* infection in humans and crabs were recently reported in seven townships surrounding the Three Gorges Reservoir in Yangtze River, China (Zhang et al. 2012). The distribution of *P. miyazakii* has been reported in Japan; Kyushu, Shikoku, and Honshu Prefectures are the main areas (Miyazaki 1991). In Kyushu, Japan, a total of 104 paragonimiasis patients were diagnosed during 1986–1998, among which 6 were caused by *P. miyazakii* (Uchiyama et al. 1999).

Population epidemiology Slightly higher prevalence was noted in *P. skrjabini* infection among the age group 7–17 years than other age groups in Sichuan and Chongqing Province, China (Zhang et al. 2012). On the other hand, all of the ten *P. miyazakii* patients first reported in

Japan were adults whose age ranged 27–50 years; nine were males and one was female (Yokogawa et al. 1974).

Environmental factors related to transmission The construction of the Three Gorges Dam in Yangtze River, China, beginning in 1994 changed the depth and the flow pattern of the Yangtze River, and environmental conditions for *P. skrjabini* infection have been changed to reveal increased prevalences of human and crab infections (Zhang et al. 2012). Abundance of wild animals in the surroundings may also facilitate the transmission of *P. skrjabini* and *P. miyazakii*; however, not much information on this point has been available.

12.4.4 *Paragonimus kellicotti* and *Paragonimus mexicanus*

Paragonimus kellicotti was first found from a cat and a dog in Ohio, USA (Procop 2009). Its autochthonous human infection was first identified in 1986 in Illinois, USA (Mariano et al. 1986) and a total 20 human cases (the majority of cases occurring in Missouri) have been documented (Centers for Disease Control and Prevention 2010; Fried and Abruzzi 2010; Lane et al. 2012). The clinical manifestations are similar to those seen in *P. westermani* infection, and ectopic infections in the brain and the skin were possible (Lane et al. 2009). *P. mexicanus* (syn. *P. peruvianus, P. ecuadoriensis, P. amazonicus, P. caliensis, P. inca*; according to Tongu, 2001) was first collected from two opossums in Mexico (Miyazaki and Ishii 1968) and is now known to be distributed in many Latin American countries. It has been argued that *P. ecuadoriensis* should be resurrected considering its genetic distinctness from *P. mexicanus* (López-Caballero et al. 2013). The clinical manifestations of *P. mexicanus* infection seem not different from those caused by *P. westermani* (Chai 2014).

Parasite biology and mode of human infection The first intermediate hosts of *P. kellicotti* are amphibious snails *Pomatiopsis lapidaria, P. cincinnatiensis*, and *Oncomelania hupensis nosophora* (Blair et al. 1999). The second hosts include various species of crayfish, i.e., *Cambarus bartoni, C. robustus, C. virilis, Orconectes propinquus, O. rusticus, Procambarus blandingi*, and *P. clarkii* (Blair et al. 1999). The snail intermediate hosts for *P. mexicanus* are *Aroapyrgus allei, A. colombiensis*, and *A. costaricensis* (Blair et al. 1999). The second hosts are the freshwater crabs, *Hypolobocera aequadorialis, H. chilensis, H. gracilignathus*, *Pseudothelphusa americana, P. dilatata, P. nayaritae, P. propinqua, P. terrestris*, and *Ptychophallus* spp. (Blair et al. 1999). Some patients of *P. kellicotti* infection ate undercooked crayfish caught from rivers in Missouri, USA (Lane et al. 2009). Mexicans favor ceviche that contains uncooked crustaceans that may contain viable *P. mexicanus* metacercariae (Procop 2009). Peruvians eat raw crab with vegetables and lemon juice (Nakamura-Uchiyama et al. 2002).

Reservoir hosts *P. kellicotti* has been found in mammals, including humans, skunks, red foxes, coyotes, minke, dogs, pigs, cats, and bobcats in central and eastern parts of the USA and adjacent areas of Canada (Procop 2009). The natural definitive hosts for *P. mexicanus* are humans, opossums, dogs, and cats (Blair et al. 1999).

Geographical distribution The geographical distribution of *P. kellicotti* is confined to North America, including mid-west areas and Mississippi Basin of the USA and Atlantic coast, Ontario, and Quebec in Canada (Fig. 12.3) (Blair et al. 1999). *P. mexicanus* is now known to be distributed in Peru, Ecuador, Costa Rica, Panama, Guatemala, and possibly in Colombia and Brazil, although the species occurring in Colombia and Brazil have not been definitely identified (Blair et al. 1999; Velez et al. 2003; Lemos et al. 2007).

Population epidemiology The majority of *P. kellicotti* cases were males and all but one were adults (Lane et al. 2012). Little information is available on the population epidemiology of *P. mexicanus*.

Environmental factors related to transmission Transmission of *P. kellicotti* to natural definitive hosts is closely correlated with the eco-

logical condition and availability of the crayfish host (Chai 2014). In the case of *P. mexicanus*, environmental conditions favorable for survival of the crab hosts can facilitate its transmission to the wild mammalian hosts (Chai 2014).

12.4.5 *Paragonimus africanus* and *Paragonimus uterobilateralis*

Paragonimus africanus was first found in the mongoose from Cameroon in 1965, and now human infections are known in Cameroon and Nigeria (Blair et al. 1999; Aka et al. 2008; Narain et al. 2010). *P. uterobilateralis* was first described from mongooses in Cameroon in 1965, and its human infection have been found in Cameroon, Nigeria, Gabon, Liberia, and Guinea (Blair et al. 1999; Aka et al. 2008; Narain et al. 2010). The clinical features of *P. africanus* and *P. uterobilateralis* are similar to those caused by *P. westermani* (Chai 2014).

Parasite biology and mode of human infection The snail host of *P. africanus* is an aquatic snail *Potadoma freethii* or *Melania* spp., and the second hosts are the freshwater crabs, *Sudanonautes africanus* and *S. granulatus* (syn. *S. pelii*) (Blair et al. 1999; Aka et al. 2008). The snail intermediate host of *P. uterobilateralis* is unknown, and the second hosts are the freshwater crabs *Liberonautes chaperi, L. latidactylus, L. nanoides, L. paludicolis, Sudanonautes africanus, S. granulatus, S. aubryi*, and *S. fowleri* (Blair et al. 1999). Eating uncooked crabs is the principal mode of infection with *P. africanus* and *P. uterobilateralis* (Aka et al. 2008).

Reservoir hosts The natural definitive host for *P. africanus* includes humans, black mongooses, civets, drills, dogs, and Nigerian red-capped mangabeys (Blair et al. 1999; Aka et al. 2008; Friant et al. 2015). The natural definitive hosts for *P. uterobilateralis* include humans, dogs, cats, otters, mongooses, swamps, shrews, and other rodents (Blair et al. 1999).

Geographical distribution *P. africanus* is distributed in Cameroon and Nigeria and human infections were found also in these countries (Fig. 12.3) (Blair et al. 1999). *P. uterobilateralis* is distributed in Liberia, Nigeria, Cameroon, and Gabon, and human infections have been identified in Liberia, Nigeria, Gabon, and possibly in Cameroon (Fig. 12.3) (Sachs et al. 1986; Blair et al. 1999; Aka et al. 2008). Some lung flukes found in Cameroon and some others discovered in Equatorial Guinea, Benin, and Ivory Coast have not been determined at a specific level (Blair et al. 1999; Aka et al. 2008).

Population epidemiology In Cameroon, 454 (4.0%) of 11,236 persons or biological products (intermediate hosts) examined since 1932 have been found infected with *Paragonimus* spp., almost exceptionally *P. africanus* (Aka et al. 2008; Nkouawa et al. 2009). In a peri-urban area of Cameroon, 2.56% of 1482 schoolchildren were sputum/stool egg positive for *P. africanus*; among symptomatic children, 25 (17.0%) of 147 boys and 13 (8.0%) of 162 girls were positive for eggs (Moyou-Somo et al. 2003). In Nigeria, 1778 (25.0%) of 7105 persons or biological products examined since 1939 were found infected with *Paragonimus* spp., particularly, *P. uterobilateralis* (Aka et al. 2008). In an endemic village in Nigeria, the prevalence and intensity was higher in children than in adults and males showed higher prevalence and intensity than females (Ibanga and Eyo 2001).

Environmental factors related to transmission No special environmental factors related to transmission of *P. africanus* and *P. uterobilateralis* have been documented.

12.5 Throat Fluke

12.5.1 *Clinostomum complanatum*

Clinostomum complanatum was first reported by Rudolphi in 1814 from the throat and esophagus of *Ardea cinerea*, an avian species in Germany

(Yamaguti 1958). Various species of freshwater fish have been found to play the role of the second intermediate host, and fish-eating birds and frogs have been found to be the natural definitive host (Yamaguti 1958, 1971; Chung et al. 1995a). *Radix auricularia* and other freshwater snails have been shown to be the first intermediate host (Chung et al. 1998). A human infection with this fluke was first reported in Japan by Yamashita in 1938, and 18 further cases had been added until 2014 in Japan (Hara et al. 2014). The occurrence of human infections is also known in Korea; five cases have been recorded until 2018 (Lee et al. 2017; Song et al. 2018) since the first report of a case by Chung et al. (1995b). The geographical distribution of this parasite extends from Europe to the Far East (Yamaguti 1958, 1971).

12.6 Pancreatic Fluke

12.6.1 *Eurytrema pancreaticum*

Eurytrema pancreaticum was originally described from the pancreatic duct of cattle by Janson in 1899 in Japan (Seo 1978) and has also been found in China, Korea, Malaysia, and also Europe and Brazil (Yamaguti 1971; Seo 1978; Beaver et al. 1984). The snail hosts are land snails such as *Bradybaena similaris* and *Cathaica lavida*, and the second intermediate hosts are grasshoppers *Conocephalus maculatus, Conocephalus gradiatus*, and *Acusta despecta* (Seo 1978). Human infections were found in two cases in China (Hong Kong and Jiangsu Province) and at least six cases in Japan (Beaver et al. 1984).

12.7 Intestinal Flukes

At least 66 different species have been reported as intestinal flukes infecting humans and animals around the world (Table 12.2) (Chai et al. 2009b). Among them, *Metagonimus yokogawai, Heterophyes nocens, Haplorchis taichui, Isthmiophora hortensis, Echinochasmus japonicus, Fasciolopsis buski, Neodiplostomum seoulense, Caprimolgorchis molenkampi, Phaneropsolus bonnei*, and *Gymnophalloides seoi* are important from the public health point of view (Chai et al. 2009b). The estimated number of infected people is at least 40–50 million (Chai et al. 2009b).

12.7.1 *Metagonimus yokogawai, Metagonimus takahashii,* and *Metagonimus miyatai*

Metagonimus yokogawai, M. takahashii, and *M. miyatai* are minute intestinal flukes that belong to the family Heterophyidae (= heterophyids) (Chai et al. 2009b; Chai and Jung 2017). They are distributed mainly in Asia, particularly in the Far East (Yu and Chai 2013; Chai and Jung 2017). *M. yokogawai* was originally described in 1912 in Taiwan and then reported from Asian and European countries (Yu and Chai 2013). *M. takahashii* was originally described in 1930 in Japan and is now known to exist also in Korea (Yu and Chai 2013). *M. miyatai* was originally found in Japan in 1941 but reported as a distinct species in 1993 based on specimens from Japan and Korea (Yu and Chai 2013). These flukes can cause mild to severe gastrointestinal troubles and indigestion, which lead to malabsorption and weight loss. In immunocompromised hosts, the flukes may cause extraintestinal invasion (Chai et al. 2009b).

Parasite biology and mode of human infection The snail hosts for *M. yokogawai* are *Semisulcospira libertina* and *S. coreana*, and those for *M. takahashii* are *S. libertina, S. coreana*, and *Koreanomelania nodifila* (Yu and Chai 2013; Chai 2017). The snail hosts for *M. miyatai* are *S. libertina, S. dolorosa*, and *S. globus* (Yu and Chai 2013). The cercariae are of the ophthalmo-pleuro-lophocercous type (Yu and Chai 2013). After they are shed in water, they freely swim and infect freshwater fish. The fish hosts for *M. yokogawai* include the sweetfish *Plecoglossus altivelis*, dace *Tribolodon hokonensis* or *T. taczanowskii*, and perch *Lateolabrax japonicus* (Yu and Chai 2013). On the other hand, the fish hosts for *M. takahashii* include the

crucian carp *C. carassius*, carp *C. carpio*, dace *T. taczanowskii*, and perch *L. japonicus* (Yu and Chai 2013). The fish hosts of *M. miyatai* include *Z. platypus, Zacco temminckii*, *P. altivelis, T. hakonensis, T. taczanowskii*, *Opsariichthys bidens*, *Morocco steindachneri*, and *Phoxinus lagowskii steindachneri* (Yu and Chai 2013). The metacercariae encyst in the fish muscle but rarely under the scale or in the fin of the fish (Yu and Chai 2013). The metacercariae can live in the fish hosts for at least 2.5 years (Yu and Chai 2013). The usual mode of human infection with *M. yokogawai, M. takahashii*, and *M. miyatai* is ingestion of raw or improperly cooked flesh of the freshwater fish (Chai et al. 2009b; Chai and Jung 2017). Endemic areas are scattered along riparian villages, where local people traditionally eat these raw fish (Yu and Chai 2013; Chai et al. 2015a).

Reservoir hosts The definitive hosts of *Metagonimus* flukes are fish-eating birds and mammals, including humans. In case of *M. yokogawai*, dogs, rats, cats, foxes, and kites (bird) harbor the adult flukes (Yu and Chai 2013). However, the significance of each animal host as the source of human infections (i.e., as reservoir hosts) has not been verified (Yu and Chai 2013). Mice, rats, cats, dogs, gerbils, hamsters, and ducks are experimental definitive hosts for *M. yokogawai* (Yu and Chai 2013). Four (9.8%) of 41 cats purchased from a market in Seoul had *M. yokogawai* infection (Huh et al. 1993), and 78 (17.8%) of 438 feral cats from a market in Busan were found infected with *M. yokogawai* worms (under the name *Metagonimus* sp.) (Sohn and Chai 2005). Pelicans, kites, and other species of birds, and dogs, cats, and other mammals were reported to be the natural and experimental definitive hosts for *M. takahashii* (Yu and Chai 2013). Rats and various strains of mice were used as experimental definitive hosts for *M. takahashii* (Yu and Chai 2013). Natural definitive hosts for *M. miyatai* include the dog, red fox, raccoon dog, and black-eared kite, and mice, rats, hamsters, and dogs are experimental definitive hosts for *M. miyatai* (Yu and Chai 2013).

Geographical distribution The geographical distribution of *M. yokogawai* is wide, from Far Eastern Asia to Middle Europe (Fig. 12.4), although its epidemiological significance in causing human diseases is much less in Europe than in Asia (Yu and Chai 2013). In Korea, almost all large and small rivers and streams in eastern and southern coastal areas are endemic areas of *M. yokogawai* (Chai and Lee 2002; Chai et al. 2009b). The Sumjin, Tamjin, and Boseong Rivers, Geoje Island, and Osip Stream (Samchok-shi, Gangwon-do) are the highest endemic areas with

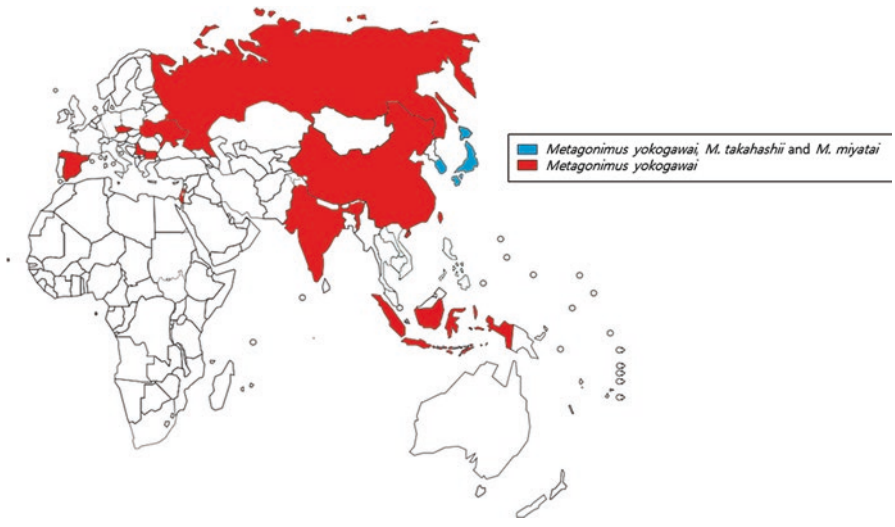

Fig. 12.4 Global distribution of *Metagonimus* spp. (*M. yokogawai, M. takahashii*, and *M. miyatai*)

20–70% egg positive rates of the riparian residents (Chai et al. 2000, 2015a; Yu and Chai 2013). The national average prevalence of heterophyid eggs (mostly *M. yokogawai*) in 2004 was 0.5% and the estimated number of infected people in Korea is about 260,000 (Kim et al. 2009). In Japan, the reported prevalence in humans in Japan had been 0.5–35.1% until the 1960s depending on the locality, which was generally lower than that in Korea. However, in some areas, for example, those along the Takatsu River, Shimane Prefecture, the prevalence among residents in 1965 was high, up to 71.9% among 798 examined (Kagei 1965). In addition, between 1982 and 1988, an epidemiological survey was performed around the Hamamatsu Lake, Shizuoka Prefecture, in which a 13.2% egg positive rate among 4524 lakeside people examined was reported (Ito et al. 1991). In Taiwan, where *M. yokogawai* was originally described, the current status of human infections is unknown, although metacercarial infections in fish have been recently documented (Li 2013). In mainland China, little information is available on human *M. yokogawai* infection, although it was mentioned that human infections exist in Guangdong, Anhui, Hubei, and Zhejiang Province (Yu and Mott 1994). In Russia, *M. yokogawai* infection is highly endemic in the Amur and Ussuri valleys of the Khabarovsk territory, with prevalence among the ethnic minority group of people between 20 and 70% (Yu and Mott 1994). In the north of Sakhalin Island, the infection rate was 1.5% among Russians and 10% among ethnic minorities (Yu and Mott 1994). In Europe, no human infections are known so far. The geographical distribution of *Metagonimus takahashii* is rather narrow, only in Japan and Korea (Yu and Chai 2013). In Japan, this fluke was reported in Okayama, Hiroshima, and around the Lake Biwa (Ito 1964; Urabe 2003). In Korea, the presence of human *M. takahashii* infection was first demonstrated in 1988 from riparian people along the Hongcheon River, Gangwon-do, by recovery of adult flukes (Ahn and Ryang 1988). Subsequently, an endemic focus was later discovered in 1993 from Umsong-gun, Chunchungnam-do, along the upper reaches of the Namhan River (Chai et al. 1993). *M. miyatai* was described as *Metagonimus* Miyata type in 1980 in Korea by detecting eggs in the feces, which was slightly larger in size than those of *M. yokogawai* (Yu and Chai 2013). Later, adult flukes were recovered from 32 people living along the Namhan River in Umsong-gun and Yongwol-gun (Chai et al. 1993). In Japan, epidemiological studies particularly on human infections are scarce. Saito et al. (1997) enlisted dogs, foxes, raccoons, dogs, and black-eared kites for animal definitive hosts in Shimane, Kochi, and Yamagata Prefectures. Cercariae, rediae, and metacercariae were detected in the Hiroi River basin in Nagano Prefecture (Shimazu 2002). Small rivers in Shizuoka Prefecture were found to have *M. miyatai* infection in fish (Kino et al. 2006).

Population epidemiology In a survey performed in Tamjin River basin of Korea in 1977, the egg positive rate of *M. yokogawai* was 26.4% on average and that of males and females was 35.3% and 18.6%, respectively (Chai et al. 1977). The prevalence became higher as the age of people was increased (Chai et al. 1977). This general pattern of high prevalence in male adult population seems to be consistent. In an endemic area of Samchok-shi, Korea, the egg positive rate was remarkably higher in males (46.6%) than in females (16.3%) and the highest in adults aged 61–70 years, followed by 41–50 and 51–60 years (Chai et al. 2000). Among young people under 20 years of age, there were no infected cases (Chai et al. 2000). Age- and sex-prevalences of *M. takahashii* and *M. miyatai* are essentially the same as those of *M. yokogawai* (Chai 2014).

Environmental factors related to transmission Environmental factors related to transmission of *C. sinensis* and *O. viverini* are also applicable to *M. yokogawa, M. takahashii*, and *M. miyatai* (Chai 2014).

12.7.2 *Heterophyes nocens*, *Heterophyes heterophyes*, and *Heterophyes dispar*

Heterophyes nocens was first reported in 1916 from experimental dogs and cats fed mullets (*Mugil cephalus*) in Japan (Chai 2007). Human

infections with this fluke occur in Japan, China, and Korea (Fig. 12.5) (Chai et al. 2009b; Chai and Jung 2017). Cercariae are shed from brackish water snails, *Cerithidea cingulata* (= *Tympanotonus microptera*) (Ito 1964). The second intermediate hosts are brackish water fish, including the mullet or goby *Acanthogobius flavimanus* (Guk et al. 2007a). Humans become infected by eating raw or inadequately cooked fish hosts. Natural definitive hosts other than humans include domestic or feral cats (Chai et al. 2009b, 2013a). Prevalences ranging from 10 to 70% were detected in residents of southwestern coastal areas, including many islands, of Korea (Chai et al. 2004, 2009b). In Japan, human infections were reported from Kochi, Chiba, Yamaguchi, Chugoku, Hiroshima, and Shizuoka Prefectures (Kino et al. 2002; Chai 2007).

Heterophyes heterophyes was first discovered in 1851 at the autopsy of an Egyptian and is now known to cause human infections along the Nile Delta of Egypt and Sudan (Fig. 12.5) (Ransom 1920; Yu and Mott 1994; Chai 2007). It is also present in Greece, Iran, Turkey, Italy, and Tunisia (Fig. 12.5) (Yu and Mott 1994; Chai 2007). The snail host is *Pirenella conica* in Egypt, and the second intermediate hosts are brackish water fish that include *M. cephalus, Tilapia nilotica, Aphanius fasciatus*, and *Acanthogobius* sp. (Chai 2007). The metacercariae of *H. heterophyes* can survive up to 7 days in salted fish (Chai 2007). Humans are contracted by this fluke through eating raw or inadequately cooked brackish water fish. A variety of mammals other than humans take the role of the reservoir host; dogs, foxes, and jackals (Chai 2007). In Egypt, human infections are prevalent on northern parts of the Nile Delta, particularly around the Lakes Manzala, Borollos, and Edco where fishermen and domestic animals frequently consume fish (Yu and Mott 1994). In Khuzestan, Islamic Repubic, the mean prevalence of heterophyid fluke infections was 8% (2–24% in range) (Yu and Mott 1994).

Heterophyes dispar was first discovered in 1902 in the intestines of dogs and cats in Egypt, and then from mammals including the fox and wolf in northern Africa and eastern Mediterranean (Fig. 12.5) (Yu and Mott 1994). Human infections were reported from two Koreans who returned from Saudi Arabia (Chai et al. 1986) and in northeastern Thailand (Yu and Mott 1994). Brackish water fish are the infection source harboring the metacercariae (Chai et al. 2009b).

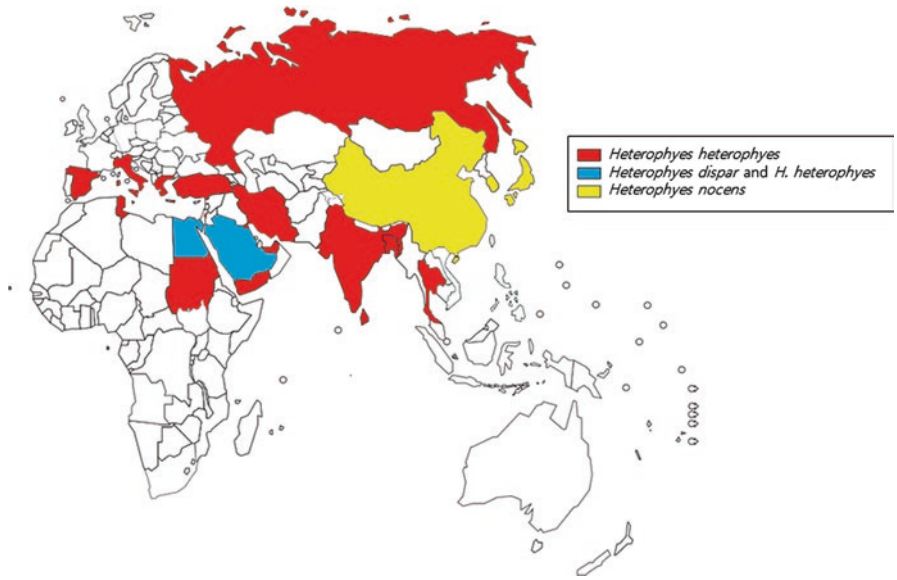

Fig. 12.5 Global distribution of *Heterophyes* spp. (*H. heterophyes, H. nocens*, and *H. dispar*)

12.7.3 *Haplorchis taichui, Haplorchis pumilio*, and *Haplorchis yokogawai*

Haplorchis taichui was described in 1924 from birds and mammals caught in Taiwan (Chai 2007; Chai and Jung 2017). Natural human infections were first reported in the Philippines (Beaver et al. 1984; Mas-Coma and Bargues 1997). This fluke is currently known to be distributed widely in Asia (Taiwan, the Philippines, Malaysia, Thailand, Laos, Vietnam, South China, Bangladesh, India, and Sri Lanka) and the Middle East (Palestine, Iraq, and Egypt) (Fig. 12.6) (Chai et al. 2009b, 2012a; Sohn et al. 2014). Hyperendemic areas of *H. taichui* infections, with average individual worm burdens of 21,565 and 12,079 worms in each patient were recently reported in Saravane and Champasak Province, respectively, in Lao PDR (Chai et al. 2013b). The snail hosts are freshwater dwellers, *Melania obliquegranosa, Melania juncea*, or *Melanoides tuberculata* (Chai et al. 2009b). A wide variety of freshwater fish serve as the second intermediate host; *Cyclocheilichthys repasson, Cyprinus auratus, Cyprinus carpio, Gambusia affinis, Hampala dispar, Labiobarbus leptocheila, Puntius binotatus, Puntius brevis, Puntius gonionotus, Puntius leicanthus, Puntius orphoides, Puntius palata, Pseudorasbora parva, Rhodeus ocellatus*, *Zacco platypus*, *Raiamas guttatus, Mystacoleucus marginatus*, and *Henichoryhnchus siamensis* (Chai et al. 2009b). Dogs, cats, and birds are the natural definitive hosts (Yamaguti 1958).

Haplorchis pumilio was first recorded from birds and mammals in 1886 in Egypt and then subsequently found in Taiwan in 1924 (Chai et al. 2009b). A successful experimental human infection was reported in 1924 (Chai et al. 2009b), and natural human infections (12 cases) were described for the first time in Thailand in 1983 (Radomyos et al. 1983). The geographical distribution of this fluke is essentially the same as that of *H. taichui* and is now known to be distributed in the Philippines, Thailand, Laos, Vietnam, Cambodia, South China, Taiwan, Malaysia, India, Sri Lanka, Iraq, Egypt, and Korea (Fig. 12.6) (Chai et al. 2009b, 2012a, 2014a, 2015b; Chung et al. 2011). The freshwater snail, *Melania reiniana* var. *hitachiens*, is the first intermediate host, and various freshwater fish species, which belong to the Cyprinidae, Siluridae, and Cobitidae, serve as the second intermediate hosts (Chai et al. 2009b). The natural definitive hosts include dogs and cats (Chai et al. 2009b).

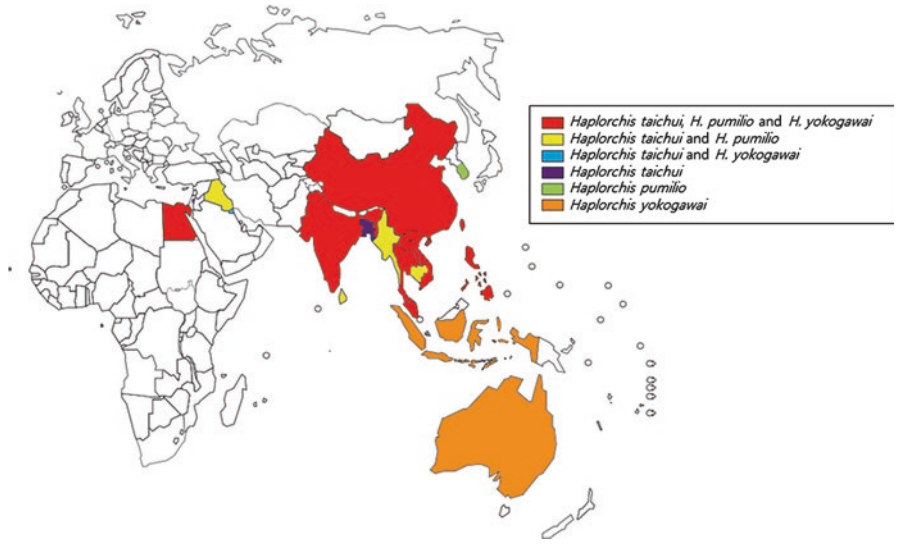

Fig. 12.6 Global distribution of *Haplorchis* spp. (*H. taichui, H. pumilio*, and *H. yokogawai*). In addition to these, *H. taichui* was reported from Hawaii (North America), and *H. pumilio* from Venezuela (South America)

Haplorchis yokogawai was originally described from dogs and cats experimentally fed the mullet *Mugil cephalus* in Taiwan in 1932 (Chai et al. 2009b). An experimental human infection with this fluke was successful in Taiwan; however, natural human infections were first reported in the Philippines (Beaver et al. 1984; Mas-Coma and Bargues 1997). Currently, this fluke is distributed in the Philippines, South China, Malaysia, Indonesia, Thailand, Laos, Vietnam, Cambodia, India, Australia, and Egypt (Fig. 12.6) as human or animal infections (Chai et al. 2009b, 2014a). Cercariae are shed from freshwater snails, *Melanoides tuberculata* or *Stenomelania newcombi* (Chai et al. 2009b). *Cyclocheilichthys armatus, Hampala dispar, Labiobarbus leptocheila, Misgurnus* sp., *Mugil* spp., *Onychostoma elongatum*, *Ophicephalus striatus*, and *Puntius* spp. are the fish intermediate hosts (Chai et al. 2009b). The natural definitive hosts are dogs, cats, cattle, and other mammals (Yamaguti 1958).

12.7.4 *Centrocestus formosanus* and Other *Centrocestus* species

Centrocestus formosanus (syn. *C. caninus*) was originally described from an experimental dog fed freshwater fish infected with the metacercariae in Taiwan by Nishigori in 1924, with a successful experimental infection in a human volunteer and a natural infection in a fox (Chai et al. 2009b; Chai and Jung 2017). Possible occurrence of natural human infections was mentioned in Taiwan and Japan (Chen 1942; Ito 1964) and actual human natural infections were found in China and Vietnam but without detailed description of the worms (Chai et al. 2009b). Two human infections under the name of *C. caninus* were reported in Thailand (Waikagul et al. 1997). However, seven human infections with a detailed worm description were reported recently in Lao PDR (Chai et al. 2013c). These cases were with no exceptions mixed-infected with other trematode species including *O. viverrini* and *H. taichui* (Chai et al. 2013c). The distribution of this fluke is currently almost all over the world, including Asia (Taiwan, China, Japan, Philippines, Thailand, Lao PDR, Vietnam, Cambodia, and India), Europe (Turkey), and North and South Americas (USA, Mexico, and Brazil) (Chai et al. 2009b, 2012a, 2013c, 2014a; Wanlop et al. 2017). The snail, *Stenomelania newcombi*, is the first intermediate host, and the freshwater fish, including *Cyclocheilichthys repasson, Puntius brevis*, and *Osteochilus hasseltii*, play the role of the second intermediate host (Chai et al. 2009b). Natural definitive hosts include dogs, foxes, chickens, and ducks (Chai et al. 2013c).

C. armatus was first reported in Japan from dogs, cats, rabbits, rats, mice, and a human experimentally infected with the metacercariae from cyprinoid fish by Tanabe in 1922 (Chai et al. 2009b; Chai and Jung 2017). Later, a naturally infected human was found in Korea (Hong et al. 1988). The first intermediate host of *C. armatus* is the freshwater snail, *Semisulcospira* sp., and the second hosts are freshwater fish, including *Zacco platypus, Zacco temminckii*, *Rhodeus ocellatus, Gobius similis, Pseudorasbora parva*, and *Pelteobagrus fulvidraco* (Chai et al. 2009b). Its natural definitive hosts are cats and fish-eating birds that include the large egret *Egretta alba modesta* (Chai et al. 2009b, 2013a).

C. cuspidatus was originally reported from a naturally infected dog in Egypt, and then human infections were reported in Egypt, Taiwan, and China (Yu and Mott 1994; Chai and Jung 2017). This fluke was also reported from Kuwait and Tunisia (Chai and Jung 2017).

C. kurokawai was originally reported under the name *C. formosanus* var. *kurokawai* from a naturally infected human; later, the name was changed into *C. kurokawai* (Chai et al. 2013c). There is no information on the life cycle of *C. kurokawai* but it is suggested that the second intermediate host is freshwater fish (Yu and Mott 1994).

12.7.5 *Procerovum calderoni* and *Procerovum varium*

Procerovum calderoni was first reported from five dogs and a native Filipino in the Philippines by Africa and Garcia in 1935 (Chai et al. 2009b).

Later, it was also reported from China and Africa (Chai et al. 2009b). Brackish water snails, *Thiara riquetti*, shed the cercariae (Velasquez 1973a). Freshwater fish species, including *Ophiocephalus striatus*, *Glossogobius giurus*, *Mollienesia latipinna*, *Mugil* sp., and *Creisson validus*, harbor the metacercariae (Velasquez 1973a, 1973b; Yu and Mott 1994).

Procerovum varium was first described from experimentally infected dogs with the metacercariae in the mullet *Mugil cephalus* in Japan by Onji and Nishio in 1916 (Chai et al. 2009b). Experimental human infections were reported successful (Chai et al. 2009b), but there have been no reports on natural human infections. It is now known to be distributed in China, the Philippines, Australia, India, Vietnam, and Korea (Sohn and Chai 2005; Chai et al. 2009b, 2012a). Cats are the natural definitive hosts (Sohn and Chai 2005).

12.7.6 *Pygidiopsis genata* and *Pygidiopsis summa*

Pygidiopsis genata was first discovered by Looss in 1902 in the small intestine of a pelican in Egypt, and then found in pelicans in Romania, dogs and cats (experimental) in the Philippines, a Persian wolf in Berlin Museum, and dogs and cats in Palestine (Witenberg 1929; Africa et al. 1940). In Egypt, this parasite has been found from cats, dogs, foxes, shrews, rats, kites, and ducks (Kuntz and Chandler 1956) and subsequently in humans by recovery of eggs in fecal examinations (Youssef et al. 1987a). This parasite was also recovered from birds in Israel (Dzikowski et al. 2004) and stray cats in Kuwait (El-Azazy et al. 2015). Kuntz and Chandler (1956) treated *P. summa* as a synonym of *P. genata*, without proper explanations. However, both should be treated as distinct species because of various differential points (Sohn et al. 2016). The snail host is *Melanoides tuberculata* (Youssef et al. 1987b) and *Melanopsis costata* (Dzikowski et al. 2004). The second intermediate hosts are brackish water fish, including *Tilapia galilea, Tilapia nilotica, Tilapia simonis, Mugil* sp., and *Barbus canis* (Witenberg 1929; Youssef et al. 1987b). Natural definitive hosts include pelicans, wolves, cats, dogs, foxes, shrews, rats, kites, and ducks (Witenberg 1929; Kuntz and Chandler 1956; El-Dakhly et al. 2017).

Pygidiopsis summa was first found in dogs fed brackish water fish infected with the metacercariae in Japan and thereafter reported from Korea (Chai et al. 2009b; Chai and Jung 2017). Human infections were found by recovery of eggs in the feces in 1929 and of adult flukes in 1965 in Japan (Chai et al. 2009b). In Korea, eight human infections were first discovered from a salt-farm village in the western coastal area of Okku-gun, Chollabuk-do (Seo et al. 1981). It is now known to be distributed also on many western and southern coastal islands in Korea (Chai et al. 2004). The snail intermediate host is a brackish water species, *Cerithidea* sp. or *Tympanotonus* sp., and the second host is brackish water fish, including the mullet *Mugil cephalus* and goby *Acanthogobius flavimanus* (Chai et al. 2009b). Natural definitive hosts other than humans are domestic or feral cats (Sohn and Chai 2005; Chai et al. 2009b).

12.7.7 *Stellantchasmus falcatus*

Stellantchasmus falcatus (syn. *Stellantchasmus pseudocirrata*) was first described from cats experimentally fed the mullet harboring the metacercariae in Japan (Chai et al. 2009b). Human infections were first known in Japan and thereafter in many Asian-Pacific countries, which include the Philippines, Hawaii, Japan, Palestine, Thailand, Cambodia, Vietnam, Taiwan, and Korea (Chai et al. 2009b, 2012a, 2016). The first intermediate host is brackish water snails *Stenomelania newcombi* or *Thiara granifera*, and the second host is the mullet and the half-beaked fish *Dermogenus pusillus* (Chai et al. 2009b). Natural definitive hosts include dogs and cats, and experimental hosts can be rats and mice (Chai et al. 2009b, 2013a).

12.7.8 *Stictodora fuscata* and *Stictodora lari*

Stictodora fuscata was originally described from cats experimentally fed infected mullets in Japan by Onji and Nishio in 1916 (Chai 2007; Chai and Jung 2017). The first human infection was found in a young Korean man who regularly consumed raw mullets and gobies (Chai et al. 1988). Thirteen additional cases were detected in a seashore village of a southwestern coastal area (Chai and Lee 2002; Chai 2007). The fish hosts include the goby *Acanthogobius flavimanus* and the carp *Pseudorasbora parva* (Yamaguti 1958; Chai et al. 2009b). Natural definitive hosts are feral cats and dogs (Yamaguti 1958; Sohn and Chai 2005; Chai et al. 2013a). Experimental definitive hosts include cats (*Felis catus*) (Sohn et al. 1994).

Stictodora lari was first found in the small intestine of the seagull *Larus crassirostris* in Japan (Yamaguti 1939a; Chai et al. 1989, 2009b). The first human cases were reported in six people who resided in two southern coastal villages of Korea (Chai et al. 2002). The snail host reported is a brackish water gastropod *Velacumantus australis* in Australia (Howell 1973). The fish hosts include a number of estuarine fish, including the goby *Acanthogobius flavimanus* (Howell 1973; Chai et al. 2009b). Natural definitive hosts include feral cats (Chai et al. 2013a). Experimental definitive hosts are cats and dogs (Sohn et al. 1994).

12.7.9 Other Heterophyid Flukes (*Acanthotrema*, *Apophallus*, *Ascocotyle*, *Cryptocotyle*, and *Heterophyopsis*)

Acanthotrema felis was first described from the small intestine of stray cats in Korea (Sohn et al. 2003a). Subsequently, an adult specimen was found from a 70-year-old Korean woman residing in a coastal area of Korea (Cho et al. 2010). Thereafter, four more human infections with low worm burdens (total ten worms) were detected in Korea (Chai et al. 2014b). No reports are available on this fluke in other countries. The cercariae and other molluscan larval stages have not yet been found. The metacercariae were discovered from the goby, *Acanthogobius flavimanus* (brackish water fish) (Sohn et al. 2003b; Cho et al. 2012). Only cats were reported to be the natural definitive host (Sohn and Chai 2005; Shin et al. 2009, 2015).

Apophallus donicus was experimentally infected to a human in the USA (Niemi and Macy 1974), and there were other reports of infection with this species in humans where fish are eaten raw (Schell 1985). Heterophyid cercariae shed by the stream snail, *Flumenicola virens*, were found to encyst in hatchery-reared coho salmon, *Oncorhynchus kisutch* (Niemi and Macy 1974). Many other kinds of fish, including blackside dace, suckers, squawfish, redside shiners, rainbow trout, and coho salmon, were found naturally infected with the metacercariae (Niemi and Macy 1974). Reservoir hosts are dogs, cats, rats, foxes, and rabbits (Yamaguti 1958).

Ascocotyle longa (syn. *Phagicola longa*) is an intestinal parasite of fish-eating birds or mammals in Europe, Asia, Africa, and the Americas (Yu and Mott 1994). Human infections presumably with this species (described as a *Phagicola* sp.) were reported in Brazil (Chieffi et al. 1992). A dog was also found infected with this fluke (Chieffi et al. 1992). Freshwater fish are the second intermediate hosts (Chieffi et al. 1992). The taxonomic status of this species in relation to other related species was extensively studied (Scholz 1999).

Cryptocotyle lingua was described from cats, dogs, rats, birds, and wild animals in Europe, North America, Russia, Denmark, and Japan (Yamaguti 1958; Saeed et al. 2006). Human infection with this fluke was reported in Greenland (Yu and Mott 1994). Cercariae develop in littorina snails, *Littorina littoria*, and encyst in the fish *Gobius ruthensparri* and *Labrus bergylta*; adults can be grown in gulls fed metacercarial cysts (Yamaguti 1958).

Heterophyopsis continua was first discovered from experimental cats fed the mullet *Mugil cephalus* that harbored the metacercariae in

Japan (Onji and Nishio 1916). *H. continua* differs from other heterophyid species in its elongate body, genital sucker located separately from the ventral sucker, and two obliquely tandem testes (Chai and Lee 2002). The presence of human infections was first mentioned in Japan (Yamaguti 1958). Subsequently, in the Republic of Korea, two natural human infections were discovered (Seo et al. 1984). Including these two cases, eight human cases in total have been confirmed by the recovery of adult flukes in Korea (Hong et al. 1996a; Chai et al. 1997, 1998). The first intermediate host is unknown. Metacercariae were found in the perch *Lateolabrax japonicus* and the goby *Acanthogobius flavimanus* (Chun 1960). Other fish hosts include shad *Clupanodon punctatus*, conger eel *Conger myriaster*, and sweetfish *Plecoglossus altivelis* (Chai et al. 2009b). Domestic or feral cats, ducks, and seagulls were reported to be natural definitive hosts (Chai et al. 2009b). Experimental definitive hosts include cats, dogs, and domestic chicks (Chai et al. 2009b). The geographical distribution of this species extends from Japan, Korea, the Philippines, and Vietnam (Chai et al. 2009b, 2012a).

12.7.10 *Echinostoma revolutum* Group, *Echinostoma ilocanum*, and Other *Echinostoma* spp.

Echinostoma revolutum, together with *E. lindoense* and *E. cinetorchis*, are the three major so-called 37-collar spined, "revolutum" group, *Echinostoma* spp. infecting humans (Chai 2009, 2015). *E. revolutum* is the oldest species of all echinostomes ever recorded in the literature (Chai 2009, 2015). It was first found in 1798 from a naturally infected wild duck *Anas boschas fereae* in Germany (Kanev 1994) and is now found widely in Asia, Europe, Africa, Australia, New Zealand, and North and South America (Chai et al. 2009b). Its human infection was first reported in 1929 in Taiwan, and the prevalence among people in Taiwan was estimated to be between 2.8 and 6.5% (Yu and Mott 1994). Human infections have also been known in China, Indonesia, Thailand, Russia, Cambodia, and Lao PDR (Chai et al. 2009b, 2012b; Sohn et al. 2011a). In Pursat Province, Cambodia, 7.5–22.4% of schoolchildren were infected with *E. revolutum* (Sohn et al. 2011a). The fluke infects the intestine of humans and animals (birds and mammals) and can cause gastrointestinal troubles, mucosal ulcerations, and mucosal bleeding (Chai 2009, 2015). Its first intermediate host includes freshwater snails, *Lymnaea* sp., *Physa* sp., *Paludina* sp., *Segmentina* sp., and *Heliosoma* sp. (Beaver et al. 1984). The second host includes tadpoles, snails *Physa occidentalis, Lymnaea* sp., and *Filopaludina* sp., and clams *Corbicula producta* (Beaver et al. 1984; Yu and Mott 1994; Chai et al. 2011; Chantima et al. 2013). The source of infection among schoolchildren in Pursat, Cambodia, was undercooked snails and clams of unidentified species sold on the road to their homes after school (Sohn et al. 2011a). Natural definitive hosts are ducks, gooses, rats, muskrats, dogs, and cats (Yamaguti 1958; Chai et al. 2009b, 2013a).

Echinostoma lindoense was originally described from the intestine of mammals in Germany in 1803 (Kanev 1994). High prevalences (24–96%) of human infections were reported in Celebes, Indonesia in 1940, under the name of *E. lindoense* (Chai et al. 2009b). Now this species is known to be distributed widely in Europe, Asia, and South America (Chai et al. 2009b). The snail hosts include *Lymnaea, Planorbarius, Planorbis, Anisus, Gyraulus, Biomphalaria*, and *Viviparus* (Fried et al. 2004). The second hosts are mussels, *Corbicula lindoensis, Corbicula sucplanta*, and *Idiopoma javanica*, and snails, *Biomphalaria glabrata* (Chai et al. 2009b). Natural definitive hosts include birds and mammals (Fried and Graczyk 2004). Rats, mice, ducks, and pigeons can be experimental definitive hosts (Rim 1982c; Beaver et al. 1984).

Echinostoma cinetorchis was first discovered in 1923 from rats in Japan (Chai et al. 2009b). Human infections were first reported in Japan, and then in Korea and China (Chai et al. 2009b, 2015b). The snail hosts are *Hippeutis cantori* and *Segmentina hemisphaerula* (Chai et al. 2009b).

The second hosts include a wide range of freshwater snails (*H. cantori, S. hemisphaerula, Radix auricularia coreana*, *Physa acuta, Cipangopaludina chinensis malleata*, and *Cipangopaludina* sp.), tadpoles (*Rana nigromaculata, R. rugosa*, and *R. japonica*), and freshwater fish (especially the loach *Misgurnus anguillicaudatus*) (Chai et al. 2009b). Natural definitive hosts include rats (Chai et al. 2009b). Experimental definitive hosts include mice and rats (Lee et al. 1988).

Echinostoma ilocanum was first found in 1907 from five prisoners in Manila, Philippines (Chai et al. 2009b). Subsequently, human infections have been found in Celebes, Java, Indonesia, China, Thailand, the Philippines, India, and Cambodia (Sohn et al. 2011b; Chai et al. 2015b, 2018). In northern Luzon, the Philippines, the prevalence among the Ilocano population ranged from 7 to 17% (Chai et al. 2009b), and in Oddar Meanchey Province, Cambodia, the prevalence in students and general population was 0.7% and 1.8%, respectively (Sohn et al. 2011b). The snails host includes *Gyraulus* or *Hippeutis*, and the second hosts are large snails, *Pila conica* (Philippines) and *Viviparus javanicus* (Java); these large snails are the source of human infections (Beaver et al. 1984; Yu and Mott 1994). Natural definitive hosts are rats and dogs (Beaver et al. 1984).

Echinostoma paraensei was first described from experimental rodents (hamsters, rats, and mice) fed the metacercariae in *Biomphalaria* snails in Brazil (Lie and Basch 1967). Eggs of this echinostome were detected in the coprolite of a mummified human body in Brazil; molecular studies suggested them to be eggs of *E. paraensei* (Leles et al. 2014). A molecularly compatible isolate was also detected in Australia (Morgan and Blair 1998).

Echinostoma macrorchis was originally described from field rats in Japan (Ando and Ozaki 1923). Two human infections have been reported in Japan (Majima 1927; Okabe and Okabe 1972). Freshwater snails *Segmentina nitidella* and *Gyraulus chinensis* (syn. *Planorbis compressus japonicus*) serve as the first intermediate host, and the same snails as well as other snail species *Cipangopaludina malleata, Cipangopaludina japonica, Viviparus malleatus, Parafossarulus manchourichus*, and frogs of *Rana* sp. can play the role of second intermediate hosts (Rim 1982c; Yu and Mott 1994). The geographical distribution of this fluke extends from Japan to Taiwan, Korea, and Lao PDR (Lo 1995; Sohn et al. 2013; Sohn and Na 2017).

Echinostoma angustitestis was originally described from experimental dogs fed the metacercariae from freshwater fish in 1977 in China (Yu and Mott 1994). Two human infections were found in Fujian Province, China (Yu and Mott 1994). This echinostome has not been reported from other countries.

Echinostoma aegyptiacum was originally described from a naturally infected rat in Egypt in 1924 (Khalil and Abaza 1924) and then redescribed in Japan (Yamaguti 1939b, 1971). Human infection was first reported in Fujian Province, China (Cheng et al. 2005). Its life cycle and larval development are unknown. The geographical distribution includes Egypt, China, Japan, Taiwan, Vietnam, and Turkey (Yamaguti 1939b, 1971; Fischthal and Kuntz 1975; Cheng et al. 2005; Toan et al. 2008; Gürler and Bakan 2017).

12.7.11 *Isthmiophora hortensis* and *Isthmiophora melis*

Isthmiophora hortensis was originally described in 1926 from rats in Japan, and then from rats in Korea and China (Chai et al. 2009b; Chai 2015). Human infections were first reported in Japan in 1976 and thereafter in Korea and China (Chai and Lee 2002; Chai et al. 2009b). In Liaoning province, China, six patients who had eaten raw loach were found infected, and in Cheongsong-gun, Korea, 22.4% prevalence was reported among the residents living along a small stream where various species of freshwater fish are available (Chai et al. 2009b). Occasionally, clinical cases with significant abdominal symptoms are diagnosed by extracting worms through gastroduodenal endoscopy in Korea (Chai et al. 2009b). The snail host includes *Lymnaea pervia* and *Radix auricularia coreana*, and the second hosts

include loaches, *Misgurnus anguillicaudatus* and *M. mizolepis*, and several other species of freshwater fish, including *Odontobutis obscura interrupta, Moroco oxycephalus, Coreoperca kawamebari*, and *Squalidus coreanus* (Chai et al. 2009b). In Liaoning province, China, 69.7% of the loach *M. anguillicaudatus* from a market was infected with *I. hortensis* (Yu and Mott 1994). Natural definitive hosts include rats, dogs, and cats (Chai et al. 2009b, 2013a). Experimental infection was successful in mice, rats, and humans (Seo et al. 1985).

Isthmiophora melis was first described in 1788 from rodents and carnivores in Europe and North America (Yamaguti 1958; Rim 1982c). Human infection with *I. melis* was first confirmed in 1916 in a diarrheic patient in Romania and then in an autopsy case of a Chinese patient (Beaver et al. 1984). Further human cases were reported in China and Taiwan (Yu and Mott 1994). The snail host is *Stagnicola emarginata angulata* and the second host includes tadpoles and presumably the loach, a kind of freshwater fish (Yu and Mott 1994). Natural definitive hosts include various domestic and wild animals (Chai et al. 2009b).

12.7.12 *Echinochasmus japonicus*, *Echinochasmus lilliputanus*, and Other *Echinochasmus* spp.

Echinochasmus japonicus was first described in 1926 from experimental dogs, cats, rats, mice, and birds fed the metacercariae encysted in freshwater fish in Japan, and is now known to occur in the Far Eastern countries (Chai and Lee 2002; Chai et al. 2009b; Chai 2015). A successful experimental human infection was reported in Japan, and natural human infections were found in China and Korea (Chai et al. 2009b). In Fujian and Guangdong Province, China, *E. japonicus* is prevalent among people (4.9%) and animals (39.7% in dogs and 9.5% in cats) (Yu and Mott 1994). The snail hosts include *Parafossarulus manchouricus*, and the reported second hosts are 18 species of freshwater fish, including *Pseudorasbora parva*, *Hypomesus olidus*, and *Gnathopogon strigatus* (Chai et al. 1985; Choi et al. 2006). Natural definitive hosts are ducks, egrets, cats, and insectivores (Chai et al. 2009b).

Echinochasmus liliputanus was originally described from dogs, cats, and birds in Egypt, Syria, and Palestine (Yamaguti 1958). Human infections were first discovered in Anhui Province, China, in 1991, the prevalence being 13.4% among 2426 people examined (Yu and Mott 1994). Thereafter, more than 2500 human cases have been reported in Anhui Province, China (Xiao et al. 2005). The snail host is *Parafossarulus striatulus*, and the fish host is *Pseudorasbora parva* and goldfish (Xiao et al. 2005). Humans may be infected with this parasite through consumption of raw or improperly cooked fish or drinking untreated water containing the cercariae (Chai et al. 2009b). Natural definitive hosts include badgers, foxes, raccoons, dogs, and cats (Chai et al. 2009b).

Echinochasmus perfoliatus was first described from dogs in Romania in 1902, and then found from dogs and cats in Hungary in 1908 (Chai et al. 2009b). It is now a common intestinal fluke of dogs and cats in Hungary, Italy, Romania, Russia, Japan, China, and Taiwan, and of red foxes in Denmark (Chai et al. 2009b). An experimental and a natural human infection were reported in Japan (Chai et al. 2009b). Thereafter, 1.8% prevalence was reported among people in Guangdong, Fujian, Anhui, and Hubei Provinces of China (Yu and Mott 1994). The snail host includes *Parafossarulus manchouricus, Bithynia leachi*, and *Lymnaea stagnalis* (Yamaguti 1958). The fish hosts are *Carassius* sp., *Zacco platypus, Zacco teminckii*, and *Pseudorasbora parva*, and the metacercariae are encysted only on the gills (Rim 1982c; Yu and Mott 1994). Natural definitive hosts are rats, cats, dogs, foxes, fowls, and wild boars (Chai et al. 2009b).

Echinochasmus fujianensis was first described in 1992 from humans, dogs, cats, pigs, and rats in Fujian Province, China (Yu and Mott 1994). In five areas of Fujian Province, China, the prevalence among residents ranged 1.6–7.8%, and about two-thirds of the infected people were children of 3–15 years (Yu and Mott 1994). The snail host is

Bellamya aeruginosa and the fish hosts are *Pseudorasbora parva* and *Cyprinus carpio* (Yu and Mott 1994). Natural definitive hosts include dogs, cats, pigs, and rats (Yu and Mott 1994).

Echinochasmus jiufoensis was described as a new species in 1988 found at an autopsy of a 6-month-old girl who died from pneumonia and dehydration in Guangzhou, China (Liang and Ke 1988). Its life cycle is unknown.

Echinochasmus caninum (syn. *Episthochasmus caninum, Episthmium caninum*) was originally reported from dogs by Verma (1935) in India. Kobayasi (1942) found its metacercariae in fish *Macropodus opercularis* and adult flukes in dogs in Hainan Island, China. Three human infections were reported from northeastern Thailand (Radomyos et al. 1985, 1991). The snail host is yet unknown. The geographical distribution of this flukes includes India, Thailand, and China (Verma 1935; Kobayasi 1942; Radomyos et al. 1985, 1991).

12.7.13 *Artyfechinostomum malayanum*, *Artyfechinostomum sufrartyfex*, and *Artyfechinostomum oraoni*

Artyfechinostomum malayanum (syn. *Echinostoma malayanum*) was first described from a human in Malaysia under the name of *Echinostoma malayanum* (Beaver et al. 1984), and then found in Malaysia, Singapore, Thailand, Cambodia, Indonesia, India, the Philippines, and Lao PDR (Chai et al. 2009b, 2012b; Sohn et al. 2017). The first intermediate host is a freshwater snail, *Indoplanorbis exustus* and *Gyraulus convexiusculus*, and the cercariae encyst in various species of snails, i.e., *Indoplanorbis exustus, Gyraulus convexiusculus, Pila scutata, Digoniostoma pulchella*, and *Lymnaea (Bullastra) cumingiana* (Yu and Mott 1994; Belizario et al. 2007). Natural definitive hosts include humans, pigs, rats, cats, dogs, mice, hamsters, and house-shrews (Beaver et al. 1984; Chai et al. 2009b). Human infections have been reported in Malaysia, Thailand, Indonesia, India, the Philippines, and Lao PDR (Chai et al. 2009b, 2012b).

Artyfechinostomum sufrartyfex (syn. *Paryphostomum sufrartyfex, Artyfechinostomum mehrai*) was first found from an Assamese girl in India in 1915, and then found in pigs in India in 1962 (Yu and Mott 1994). Dogs and rats are also natural definitive hosts (Yu and Mott 1994). The first intermediate host (for *A. mehrai*) is known to be freshwater snails *Indoplanorbis exustus* and *Lymnaea luteola* (Raghunathan and Srinivasan 1962), and metacercariae occur in freshwater snails (*Indoplanorbis exustus, Lymnaea luteola*, and *Digoniostoma pulchella*), fish (*Bourbus stigma*), or frogs (Raghunathan and Srinivasan 1962; Nath 1969; Yu and Mott 1994). Further, human infections were reported under the name *Paryphostomum sufrartyfex* by Reddy and Varmah (1950) and the name *Artyfechinostomum mehrai* by Raghunathan and Srinivasan (1962), Reddy et al. (1964), and Kaul et al. (1974). The geographical distribution includes India and Vietnam (Tran et al. 2016).

Artyfechinostomum oraoni was first reported in 1989 from 20 human infections in a tribal community near Calcutta, India (Bandyopadhyay et al. 1989, 1995). The snail host is *Lymnaea* sp. (Maji et al. 1995), and the second host is unknown. *A. oraoni* may provoke fatal diarrhea in pigs (Bandyopadhyay et al. 1989).

12.7.14 Other Echinostome Flukes (*Acanthoparyphium*, *Echinoparyphium*, *Himasthla*, and *Hypoderaeum*)

Acanthoparyphium tyosenense was first described in 1939 based on worms recovered from the small intestines of the duck *Melanitta fusca stejnegeri* and *M. nigra americana* caught in Korea (Chai 2009). It is now known to be distributed in Korea and Japan (Kim et al. 2004). Human infections were first discovered in two coastal villages of Chollabuk-do (Province), Korea (Chai et al. 2001). The snail host is marine megagastropods *Lunatia fortuni* and *Glassaulax*

didyma (Kim et al. 2004) or marine gastropods *Tympanotonus microptera, Cerithidea cingulata*, and *Cerithidea largillierti* (Chai et al. 2009b). The second hosts include brackish water bivalves, i.e., *Mactra veneriformis, Solen grandis, Solen strictus*, and *Ruditapes philippinarum*, and brackish water gastropod, *Neverita bicolor* (Chai et al. 2001; Kim et al. 2004). The patients in Korea used to eat improperly cooked marine bivalves and gastropods (Chai et al. 2001). Natural definitive hosts include ducks, and experimental infection of chicks and seagulls *Larus crassiostris* was successful (Chai et al. 2009b).

Echinoparyphium recurvatum was first found in 1873 from various avian species, and now recognized as a parasite of also mammals (Chai et al. 2009b). The first human infection was identified in Taiwan in 1929 and then in Indonesia and Egypt (Beaver et al. 1984; Yu and Mott 1994). The snail hosts include *Physa alexandrina, P. fontinalis, Planorbis planorbis, Lymnaea pervia, L. peregra*, *Valvata piscinalis*, and *Radix auricularia coreana* (Sohn 1998; Chai et al. 2009b). The second hosts are tadpoles and frogs of *Rana temporaria* and snails, *P. planorbis, Lymnaea* sp., *R. auricularia coreana*, and *Lymnaea stagnalis* (Chai et al. 2009b). Natural definitive hosts are house rats, wild rats (*Arvicanthis niloticus*), and various species of birds (Chai et al. 2009b).

Himasthla muehlensi was originally described by Vogel in 1933 (Chai 2007). Five adult flukes were found from a German patient who lived in Colombia and traveled to New York City where he had eaten raw clams *Venus mercenaria* (Beaver et al. 1984). A species of marine operculate snail *Littorina littoria* serves as the first intermediate host and the bivalve mollusks *Mytilus* and *Mya* spp. as the host for metacercariae (Beaver et al. 1984). Birds are natural definitive hosts (Beaver et al. 1984).

Hypoderaeum conoideum was discovered in 1872 from various species of birds in Europe and is now known to be an intestinal fluke of the duck, goose, and fowl in Europe, Japan, and Siberia (Yamaguti 1958; Rim 1982c; Beaver et al. 1984). Human infections were first reported from northeastern Thailand, where the prevalence of *H. conoideum* was 55% among the 254 residents examined (Yokogawa et al. 1965). The snail host includes *Planorbis corneus, Indoplanorbis exustus, Lymnaea stagnalis, L. limosa, L. ovata, L. tumida, L. pegrera, L. corvus*, and *L. rubiginosa*, and the second hosts are snails and tadpoles (Chai et al. 2009b).

12.7.15 *Brachylaima cribbi*

Brachylaima cribbi was originally reported by Butcher and Grove (2001) in Australia. The first human infection with this fluke was reported in South Australia (Butcher et al. 1998) and subsequently ten adults and children in South Australia (Butcher et al. 2003) were reported as infected. Birds, reptiles, and mammals were found to be infected with this fluke (Butcher et al. 1998; Butcher and Grove 2001, 2005). The first intermediate host is a helicid land snail, *Theba pisana*, and cercariae begin to emerge 8 weeks after exposure to the eggs (Butcher and Grove 2001). Cercariae encyst in other species of helicid land snails, such as, *Cernuella virgata*, which serve as the source of human infections (Butcher and Grove 2005). Symptoms due to this fluke infection vary depending on the worm burden and include diarrhea, abdominal pain, low-grade fever, and fatigue (Butcher et al. 2003).

12.7.16 *Caprimolgorchis molenkampi* and *Phaneropsolus bonnei*

Caprimolgorchis molenkampi (syn. *Paralecithodendrium molenkampi, Prosthodendrium molenkampi*, and *Fontius molenkampi*) was first described in 1951 from two human autopsies in Indonesia (Manning and Lertprasert 1973). Subsequently, this fluke was found in 14 human autopsies in northeastern Thailand (Manning et al. 1970; Manning and Lertprasert 1973). Since then, high prevalences were reported in different areas of northeast Thailand and Lao PDR

(Chai et al. 2009b). The snail host is presumed to be *Bithynia goniomphalos* but needs confirmation (Manning and Lertprasert 1973). The second host is the naiads and adults of dragon- and damselflies (Manning and Lertprasert 1973). People in northeastern Thailand and Lao PDR occasionally eat naiads of these insects (Chai et al. 2009b). Natural definitive hosts are humans, rats, and bats (Manning and Lertprasert 1973).

Phaneropsolus bonnei was first described by Lie in 1951 from a human autopsy in Indonesia, and then found in monkeys in Malaysia and India by Lie in 1962 (Manning et al. 1970). Subsequently, this fluke was reported from 15 human autopsies in northeastern Thailand (Manning et al. 1970). Thereafter, high prevalences were found in various localities of northeastern Thailand and Lao PDR (Chai et al. 2009b). The snail host is presumed to be *Bithynia goniomphalos* but needs confirmation (Manning and Lertprasert 1973). The second host is insects, particularly naiads and adults of dragon- and damselflies (Manning and Lertprasert 1973). Local people in northeastern Thailand and Lao PDR occasionally eat naiads of these insects (Chai et al. 2009b). Natural definitive hosts other than humans include humans and monkeys (Manning and Lertprasert 1973).

12.7.17 *Cotylurus japonicus* and *Prohemistomum vivax*

Cotylurus japonicus was first described by Ishii in 1932 in Japan (Chai 2007). The first human infection with this fluke was reported from a 13-year-old girl in Hunan Province, China (Yu and Mott 1994). Ducks were found to be infected with this fluke (Yu and Mott 1994). The first intermediate hosts are freshwater snails belonging to the genera *Stagnicola, Lymnaea, Physa*, and *Heligsoma*, and cercariae encyst in the same snail hosts to become specialized metacercariae known as tetracotyles (Fried et al. 2004). Infection may occur when birds or mammals ingest tetracotyles in infected snails (Fried et al. 2004).

Prohemistomum vivax (syn. *Prohemistomum spinulosum*) was originally reported as a cercaria (*Cercaria vivax*) shed from the snail *Cleopatra bulimoides* by Sonsino 1892 in Egypt (Azim 1933). Azim (1933) experimentally obtained its metacercariae in *Gambusia affinis* and *Tilapia nilotica* fish and also adults from cats and dogs fed the fish; the adult worm was assigned as *Prohemistomum vivax*. Its first human infection was found in Cairo, Egypt (Nasr 1941). The geographical distribution of this parasite is in Egypt and Israel (Bowman 2014).

12.7.18 *Fasciolopsis buski*

Fasciolopsis buski, the largest fluke among those parasitizing the human host, was first discovered in 1843 in the duodenum of an Indian sailor (Beaver et al. 1984; Mas-Coma et al. 2005; Tandon et al. 2013). This fluke is now known to be a common intestinal parasite of humans and pigs in China, Taiwan, Thailand, Vietnam, Laos, Cambodia, Bangladesh, India, Indonesia, Myanmar, the Philippines, Singapore, and Malaysia (Chai et al. 2009b; Tandon et al. 2013). The number of population infected with *F. buski* is estimated to be at least 10 million in southern Asia (Tandon et al. 2013). The prevalence among human population ranged from 0.04% in Cambodia to 8.6–50% in Bangladesh, 25–61% in Taiwan, and up to 85% in some areas of China (Tandon et al. 2013). The snail host is *Segmentina* sp., *Hippeutis* sp., and *Gyraulus* sp. (Mas-Coma et al. 2005). Metacercariae may float in the water or they can attach to the body surface of aquatic plants, such as water chestnut *Eliocharis tuberose*, water caltrop *Trapa natans*, water hyacinth *Eichhornia* sp., roots of the lotus, water bamboo *Zizania* sp., and other aquatic vegetations such as *Salvinia, Valisneria*, and *Eichornia* spp. (Beaver et al. 1984; Yu and Mott 1994; Tandon et al. 2013). Consumption of raw or improperly cooked aquatic plants, or peeling off the hull or skin of the plants by mouth before eating the raw nut is the principal mode of human infection (Yu and Mott 1994). Natural definitive hosts include pigs, dogs, and rabbits (Tandon et al. 2013).

12.7.19 *Fischoederius elongatus*, *Gastrodiscoides hominis*, and *Watsonius watsoni*

Fischoederius elongatus is an intestinal parasite of ruminants originally reported in 1883 (Chai et al. 2009b). It is infected by ingesting aquatic plants having metacercariae (Yu and Mott 1994). Only one human infection, a 35-year-old woman, was reported from Guandong, China, in 1992 (Yu and Mott 1994). The patient complained of epigastric pain for several months (Chai et al. 2009b).

Gastrodiscoides hominis was originally described from an Indian patient in 1876 (Beaver et al. 1984). Now it is known to be a common parasite of humans and pigs in India, Pakistan, Myanmar, Vietnam, the Philippines, Thailand, China, Kazakhstan, Indian immigrants in Guyana, and the Volga Delta in Russia (Beaver et al. 1984; Mas-Coma et al. 2005). In human infections, worms attach to the cecum and ascending colon and may produce mucous diarrhea (Beaver et al. 1984). The planorbid freshwater snails, *Helicorbis coenosus* shed the cercariae (Beaver et al. 1984), and the cercariae encyst on aquatic plants, or in tadpoles, frogs, and crayfish (Yu and Mott 1994). Pigs, mouse deers, field rats, and rhesus monkeys are reservoir hosts (Beaver et al. 1984; Yu and Mott 1994).

Watsonius watsoni, an aquatic plant-borne trematode first reported in 1904 was discovered only once at the autopsy of a West African Negro who died of severe diarrhea (Beaver et al. 1984). Many worms were recovered from the intestine, some attached to the duodenal and jejunal wall, and others free in the lumen of the colon (Beaver et al. 1984). Various species of primates are natural hosts of this parasite in eastern Asia and Africa (Beaver et al. 1984).

12.7.20 *Gymnophalloides seoi*

Gymnophallloides seoi was first discovered in 1988 from a Korean woman suffering from acute pancreatitis and gastrointestinal discomfort (Lee et al. 1993; Chai et al. 2003). The home village of the patient, a southwestern coastal island (Aphaedo Island, Shinan-gun, Korea), was subsequently found to be a highly endemic area with 49% prevalence and heavy worm loads of 106–26,373 worms per person (Chai et al. 2003). Now *G. seoi* is known to be distributed on 25 seashore villages of western and southern coastal islands and 3 coastal villages (land) of Korea (Chai et al. 2009b). The first intermediate host has not been determined. The second host is the oyster *Crassostrea gigas* (Chai et al. 2003). People in endemic areas are infected through consumption of raw oysters. Aged people tend to show higher infection rate than children but there is no sex difference in the prevalence. Natural definitive hosts other than humans are the Palearctic oystercatchers *Haematopus ostralegus* and feral cats (Ryang et al. 2000; Shin et al. 2009). Experimental definitive hosts are wading birds, such as, the Kentish plover *Charadrius alexandrinus*, Mongolian plover *Charadrius mongolus*, grey plover *Pluvialis squatarola* and mammals including gerbils, hamsters, cats, and several strains of mice (Lee et al. 1997; Ryang et al. 2001).

12.7.21 *Gynaecotyla squatarolae* and *Microphallus brevicaecae*

Gynaecotyla squatarolae was originally reported in 1934 from birds in Japan under the name *Levinseniella squatarolae* (Yamaguti 1971). Human infection was first documented in 2011 from a Korean female who habitually consumed brackish water crabs in soy sauce (Chung et al. 2011). The clinical significance of this fluke is unknown. The snail host is not yet determined. The second hosts are brackish water crabs *Macrophthalmus dilatatus, M. japonicus*, and *Helice depressa* (Yamaguti 1971; Lee et al. 2010a). Natural definitive hosts are avian species that include *Squatarola hypomelaena, Erolia alpina sakhalina*, and *Arenaria interpres interpres* (Yamaguti 1971; Seo et al. 2007).

Microphallus brevicaecae (syn. *Spelotrema brevicaecae*) was originally reported in 1935 from birds and accidentally also from a human autopsy case and subsequently from 11 additional

cases in the Philippines (Africa et al. 1940). Eggs of this fluke caused acute cardiac dilatation and egg granuloma in the heart, brain, and spinal cord (Africa et al. 1940). The snail host is unknown. The second hosts are a brackish water crab *Carcinus maenas* and shrimp *Macrobrachium* sp. (Beaver et al. 1984). Natural definitive hosts include birds and mammals including monkeys (Yamaguti 1971).

12.7.22 *Isoparorchis hypselobagri*

Isoparorchis hypselobagri was originally reported from the swim bladder of siluroid fishes, including catfish, in 1898 by Billet (Cribb 1988). Two human infections have been reported in the literature; once in India and once in China (Seo 1978). The first intermediate host is yet undetermined but the second intermediate host has been shown to be various species of fish (Cribb 1988). The geographical distribution of this parasite extends from India to Pakistan, Bangladesh, Thailand, China, Australia, Japan, Russia, and Indonesia (Cribb 1988).

12.7.23 Nanophyetus salmincola

Nanophyetus salmincola (syn. *Troglotrema salmincola*) was originally reported from dogs with a fatal disease following the ingestion of uncooked salmon in the Pacific Coast of North America in 1925–1926 (Witenberg 1932). The parasite involved was named *Nanophyes salmincola* by Chapin in 1926 and then revised as *Nanophyetus salmincola* in 1927 by Chapin (Witenberg 1932). Human infections were first reported by Skrjabin and Podjapolskaja in 1931 among aborigines of eastern Siberia (under the name *N. schikhobalowi*, which is now regarded as a subspecies of *N. salmincola*) (Witenberg 1932). Thereafter, at least 20 cases of *N. salmincola* infection were found in the USA since 1974 (Witenberg 1932; Eastburn et al. 1987). Nanophyetiasis is now endemic in the far-eastern part of Russia including the Amur and Ussuri valleys of Khabarovsk territory and north Sakhalin, where the average prevalence is 5% (Yu and Mott 1994). The natural definitive hosts are mammals, including dogs, cats, raccoons, and foxes, and birds in North America, Canada, and Eastern Siberia (Beaver et al. 1984; Chai et al. 2005; Chai 2007). Its snail host is *Oxytrema silicula*, and the second intermediate hosts are a wide variety of fish, including salmon, trout, and non-salmonid fish (Yu and Mott 1994). This fluke has been proven to be the vector of a rickettsia, *Neorickettsia helmintheca*, which causes a serious and often fatal systemic infection known as salmon poisoning in animals such as dogs, foxes, and coyotes. Salmon poisoning has not been reported in humans.

12.7.24 *Neodiplostomum seoulense*

Neodiplostomum seoulense was originally described in 1964 from house rats in Korea (Seo 1990). This parasite is now known to be distributed countrywide among rodents in Korea, predominantly in mountainous areas (Seo 1990) and also in a northeastern part of China (Chai et al. 2009b). Human infection was first reported in 1982 from a young man who suffered from acute abdominal pain and fever and had a history of consuming improperly cooked snakes 7 days prior to hospital admission (Seo 1990). In experimental mice and rats, severe mucosal damage with frequent host death was observed (Lee et al. 1985; Chai et al. 2009b), and fecundity reduction after survival from lethal infection was reported in BALB/c mice (Shin et al. 2016). Twenty-six additional human cases were found from soldiers who had eaten raw snakes during their survival training (Hong et al. 1984; Chai et al. 2009b). The snail hosts are *Hippeutis cantori* and *Segmentina (Polypylis) hemisphaerula* (Chai et al. 2009b). The second hosts include tadpoles and frogs of *Rana* sp.; the snake *Rhabdophis tigrina* is regarded a paratenic host (Seo 1990). Natural definitive hosts are rats and mice (Seo 1990). Experimental definitive hosts include mice, rats, and guinea pigs (Seo 1990).

12.7.25 *Plagiorchis muris* and Other *Plagiorchis* spp.

Plagiorchis muris was originally described in 1922 based on worms recovered from mice experimentally infected with the metacercariae in Japan (Hong et al. 1996b). An experimental human infection was reported in the USA, and natural human infections have been documented in Japan and Korea (Hong et al. 1996b; Chai et al. 2009b). The snail host is *Lymnea pervia* in Japan and *Stagnicola emarginata angulata* in the USA (Chai et al. 2009b). The second hosts include mosquito larvae, insect naiads, freshwater snails, and freshwater fish (Hong et al. 1996b). Natural definitive hosts are rats, mice, and cats (Chai et al. 2009b). Rats can be used as an experimental definitive host (Hong et al. 1996b).

Plagiorchis harinasutai was reported as a new species in 1989, based on specimens recovered from four human cases in Thailand (Radomyos et al. 1989). The life cycle, pathology, clinical manifestations, and public health importance are not known (Radomyos et al. 1989).

Plagiorchis javensis was originally described in 1940 from a human case in Indonesia, and two additional cases were reported in Indonesia (Yu and Mott 1994). The second hosts are presumed to be larval insects, and reservoir hosts are birds and bats (Yu and Mott 1994).

Plagiorchis philippinensis was first discovered in 1937 at the autopsy of a resident in Manila, Philippines (Yu and Mott 1994). The second host is insect larvae, and reservoir hosts include birds and rats (Yu and Mott 1994).

Plagiorchis vespertilionis was originally described in 1780 from the brown long-eared bat in Europe, and then found in many countries, including Korea (Guk et al. 2007b). Human infection was reported in a Korean patient who habitually consumed raw flesh of snakehead mullet and gobies (Guk et al. 2007b); however, whether these fish took the role for a source of infection is unclear. Its clinical significance is unknown. The snail host is *Lymnaea stagnalis*, and the second hosts include mosquito larvae, caddis-fly larvae, mayfly larvae, and dragonfly nymphs (Macy 1960).

References

Africa CM, de Leon W, Garcia EY (1940) Visceral complications in intestinal heterophyidiasis of man. Acta Medica Philippina (Monographic Series) 1:1–132

Ahn YK, Ryang YS (1988) Epidemiological studies on *Metagonimus* infection along the Hongcheon river, Kangwon Province. Korean J Parasitol 26:207–213. (in Korean)

Aka NA, Adoubryn K, Rondelaud D, Dreyguss G (2008) Human paragonimiasis in Africa. Ann Afr Med 7:153–162

Al Abaidani I, Al-Abri S, Shaban M, Ghugey SL, Al Kathery S, Al-Mashikhi K, Garba A, Gabrielli AF (2016) Decline in transmission of schistosomiasis mansoni in Oman. Infect Dis Poverty 5:112

Ando R, Ozaki Y (1923) On four new species of trematodes of the family Echinostomatidae. Dobutsugaku Zasshi 35:108–119. (in Japanese)

Andrews RH, Sithithaworn P, Petney TN (2008) *Opisthorchis viverrini*: an underestimated parasite in world health. Trends Parasitol 24:497–501

Aung WPP, Htoon TT, Tin HH, Thinn KK, Sanpool O, Jongthawin J, Sadaow L, Phosuk I, Rodpai R, Intapan PM, Maleewong W (2017) First report and molecular identification of *Opisthorchis viverrini* infection in human communities from Lower Myanmar. PLoS One 12:e0177130

Azim MA (1933) On *Prohemistomum vivax* (Sonsino, 1892) and its development from *Cercaria vivax* Sonsino, 1892. Z Parasitenkd 5:432–436

Bandyopadhyay AK, Manna B, Nandy A (1989) Human infection of *Artyfechinostomum oraoni* n. sp. (Paryphostominae: Echinostomatidae) in a tribal community, "Oraons" in West Bengal, India. Indian J Parasitol 13:191–196

Bandyopadhyay AK, Maji AK, Manna B, Bera DK, Addy M, Nandy A (1995) Pathogenicity of *Artyfechinostomum oraoni* in naturally infected pigs. Trop Med Parasitol 46:138–139

Bargues MD, Mas-Coma S (2005) Reviewing lymnaeid vectors of fascioliasis by robosomal DNA sequence analyses. J Helminthol 79:257–267

Beaver PC, Jung RC, Cupp EW (1984) Clinical parasitology, 9th edn. Lea and Febiger, Philadelphia

Behr MA, Gyorkos TW, Kokoskin E, Ward BJ, MacLean JD (1998) North American liver fluke (*Metorchis conjunctus*) in a Canadian aboriginal population: a submerging human pathogen? Can J Public Health 89:258–259

Belizario VY, Geronilla GG, Anastacio MBM, de Leon WU, Suba-an AP, Sebastian AC, Bangs MJ (2007) *Echinostoma malayanum* infection, the Philippines. Emerg Infect Dis 13:1130–1131

Blair D, Xu ZB, Agatsuma T (1999) Paragonimiasis and the genus *Paragonimus*. Adv Parasitol 42:113–222

Blair D, Chang Z, Chen M, Cui A, Wu B, Agatsuma T, Iwagami M, Corlis D, Fu C, Zhan X (2005) *Paragonimus skrjabini* Chen, 1959 (Digenea:

Paragonimidae) and related species in eastern Asia: a combined molecular and morphological approach to identification and taxonomy. Syst Parasitol 60:1–21

Blair D, Agatsuma T, Wang W (2007) Paragonimiasis. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites. Springer, New York, pp 117–150

Boland JM, Vaszar LT, Jones JL, Mathison BA, Rovzar MA, Colby TV, Leslie KO, Tazelaar HD (2011) Pleuropulmonary infection by *Paragonimus westermani* in the United States: a rare cause of eosinophilic pneumonia after ingestion of live crabs. Am J Surg Pathol 35:707–713

Bowman A (2014) *Prohemistomum vivax* (Sonsino, 1892) Azim, 1933. Website of American Association of Veterinary Parasitologists (AAVP). pp 1–2

Butcher AR, Grove DI (2001) Description of life-cycle stages of *Brachylaima cribbi* n. sp. (Digenea: Brachylaimidae) derived from eggs recovered from human feces in Australia. Syst Parasitol 49:211–221

Butcher AR, Grove DI (2005) Second intermediate host land snails and definitive host animals of *Brachylaima cribbi* in southern Australia. Parasite 12:31–37

Butcher AR, Parasuramar P, Thompson CS, Grove DI (1998) First report of the isolation of an adult worm of the genus *Brachylaima* (Digenea: Brachylaimidae), from the gastrointestinal tract of a human. Int J Parasitol 28:607–610

Butcher AR, Palethope HM, Grove DI (2003) Response to re-infection with *Brachylaima cribbi* in immunocompetent and immunodeficient mice. Parasitol Int 52:219–228

Caldeira RL, Janootti-Passos LK, Carvalho DS (2009) Molecular epidemiology of Brazilian *Biomphalaria*: a review of the identification of species and the detection of infected snails. Acta Trop 111:1–6

Cam CDT, Yajima A, Viet KN, Montresor A (2008) Prevalence, intensity and risk factors for clonorchiasis and possible use of questionnaires to detect individuals at risk in northern Vietnam. Trans R Soc Trop Med Hyg 102:1263–1268

Centers for Disease Control and Prevention (2010) Human paragonimiasis after eating raw or undercooked crayfish-Missouri, July 2006-September 2010. Morb Mortal Wkly Rep 59:1573–1576

Chai JY (2007) Intestinal flukes. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites, vol 11. Springer, New York, pp 53–115

Chai JY (2009) Echinostomes in humans. In: Fried B, Toledo R (eds) The biology of echinostomes. Springer, New York, pp 147–183

Chai JY (2013) Praziquantel treatment in trematode and cestode infections: an update. Infect Chemother 45:32–43

Chai JY (2014) Epidemiology of trematode infections. Adv Exp Med Biol 766:241–292

Chai JY (2015) Echinostomes. In: Xiao L, Ryan U, Feng Y (eds) Biology of foodborne parasites. CRE Press, Taylor & Francis Group, Boca Raton, pp 351–369

Chai JY (2017) Metagonimus. In: Liu D (ed) Laboratory models for foodborne infections. CRC Press Taylor & Francis Group, Boca Raton, pp 743–763

Chai JY, Jung BK (2017) Fishborne zoonotic heterophyid infections: an update. Food Waterborne Parasitol 8–9:33–63

Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 51:129–154

Chai JY, Cho SY, Seo BS (1977) Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea. IV. An epidemiological investigation along Tamjin River Basin, South Cholla Do, Korea. Korean J Parasitol 15:115–120

Chai JY, Hong SJ, Son DW, Lee SH, Seo BS (1985) Metacercariae of the *Echinochasmus japonicus* encysted in a fresh water fish, *Pseudorasbora parva*, and their development in experimental mice. Korean J Parasitol 23:221–229

Chai JY, Seo BS, Lee SH, Hong SJ, Sohn WM (1986) Human infections by *Heterophyes heterophyes* and *H. dispar* imported from Saudi Arabia. Korean J Parasitol 24:82–88

Chai JY, Hong SJ, Lee SH, Seo BS (1988) *Stictodora* sp. (Trematoda: Heterophyidae) recovered from a man in Korea. Korean J Parasitol 26:127–132

Chai JY, Park SK, Hong SJ, Choi MH, Lee SH (1989) Identification of *Stictodora lari* (Heterophyidae) metacercariae encysted in the brackish water fish, *Acanthogobius flavimanus*. Korean J Parasitol 27:253–259

Chai JY, Huh S, Yu JR, Kook J, Jung KC, Park EC, Sohn WM, Hong ST, Lee SH (1993) An epidemiological study of metagonimiasis along the upper reaches of the Namhan River. Korean J Parasitol 31:99–108

Chai JY, Kim IM, Seo M, Guk SM, Kim JL, Sohn WM, Lee SH (1997) A new focus of *Heterophyes nocens*, *Pygidiopsis summa*, and other intestinal flukes in a coastal area of Muan-gun, Chollanam-do. Korean J Parasitol 35:233–238

Chai JY, Song TE, Han ET, Guk SM, Park YK, Choi MH, Lee SH (1998) Two endemic foci of heterophyids and other intestinal fluke infections in southern and western coastal areas in Korea. Korean J Parasitol 36:155–161

Chai JY, Han ET, Park YK, Guk SM, Kim JL, Lee SH (2000) High endemicity of *Metagonimus yokogawai* infection among residents of Samchok-shi, Kangwon-do. Korean J Parasitol 38:33–36

Chai JY, Han ET, Park YK, Guk SM, Lee SH (2001) *Acanthoparyphium tyosenense*: the discovery of human infection and identification of its source. J Parasitol 87:794–800

Chai JY, Han ET, Park YK, Guk SM, Park JH, Lee SH (2002) *Stictodora lari* (Digenea: Heterophyidae): the discovery of the first human infections. J Parasitol 88:627–629

Chai JY, Choi MH, Yu JR, Lee SH (2003) *Gymnophalloides seoi*: a new human intestinal trematode. Trends Parasitol 19:109–112

Chai JY, Park JH, Han ET, Shin EH, Kim JL, Guk SM, Hong KS, Lee SH, Rim HJ (2004) Prevalence of *Heterophyes nocens* and *Pygidiopsis summa* infections among residents of the western and southern coastal islands of the Republic of Korea. Am J Trop Med Hyg 71:617–622

Chai JY, Murrell KD, Lymbery AJ (2005) Fish-borne parasitic zoonoses: status and issues. Int J Parasitol 35:1233–1254

Chai JY, Han ET, Guk SM, Shin EH, Sohn WM, Yong TS, Eom KS, Lee KH, Jeong HG, Ryang YS, Hoang EH, Phommasack B, Insisiengmay B, Lee SH, Rim HJ (2007) High prevalence of liver and intestinal fluke infections among residents of Savannakhet Province, Laos. Korean J Parasitol 45:213–218

Chai JY, Han ET, Shin EH, Sohn WM, Yong TS, Eom KS, Min DY, Um JY, Park MS, Hoang EH, Phommasack B, Insisiengmay B, Lee SH, Rim HJ (2009a) High prevalence of *Haplorchis taichui, Prosthodendrium molenkampi*, and other helminth infections among people in Khammouane province, Lao PDR. Korean J Parasitol 47:243–247

Chai JY, Shin EH, Lee SH, Rim HJ (2009b) Foodborne intestinal flukes in Southeast Asia. Korean J Parasitol 47:S69–S102

Chai JY, Sohn WM, Na BK, Nguyen VD (2011) *Echinostoma revolutum*: metacercariae in *Filopaludina* snails from Nam Dihn Province, Vietnam, and adults from experimental hamsters. Korean J Parasitol 49:449–455

Chai JY, De NV, Sohn WM (2012a) Foodborne trematode metacercariae in fish from northern Vietnam and their adults recovered from experimental hamsters. Korean J Parasitol 50:317–325

Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Hoang EH, Phammasack B, Insisiengmay B, Rim HJ (2012b) Echinostome flukes recovered from humans in Khammouane Province, Lao PDR. Korean J Parasitol 50:269–272

Chai JY, Na BK, Sohn WM (2013a) Trematodes recovered in the small intestine of stray cats in the Republic of Korea. Korean J Parasitol 51:99–106

Chai JY, Yong TS, Eom KS, Min DY, Jeon HK, Kim TY, Jung BK, Sisabath L, Insisiengmay B, Phommasack B, Rim HJ (2013b) Hyperendemicity of *Haplorchis taichui* infection among riparian people in Saravane and Champasak Province, Lao PDR. Korean J Parasitol 51:305–311

Chai JY, Sohn WM, Yong TS, Eom KS, Min DY, Lee MY, Lim H, Insisiengmay B, Phommasack B, Rim HJ (2013c) *Centrocestus formosanus* (Heterophyidae): human infections and the infection source in Lao PDR. J Parasitol 99:531–536

Chai JY, Sohn WM, Na BK, Yong TS, Eom KS, Yoon CH, Hoang EH, Jeoung HG, Socheat D (2014a) Zoonotic trematode metacercariae in fish from Phnom Penh and Pursat, Cambodia. Korean J Parasitol 52:35–40

Chai JY, Kim JL, Seo M (2014b) Four human cases of *Acanthotrema felis* (Digenea: Heterophyidae) infection in Korea. Korean J Parasitol 52:291–294

Chai JY, Jung BK, Kim DG, Kim JL, Lim H, Shin EH, Lee KH, Kim MR, Han SJ, Yeom JH, Park SM, Hwang JS (2015a) Heterophyid trematodes recovered from people residing along the Boseong River, South Korea. Acta Trop 148:142–146

Chai JY, Sohn WM, Jung BK, Yong TS, Eom KS, Min DY, Insisiengmay B, Insisiengmay S, Phommasack B, Rim HJ (2015b) Intestinal helminths recovered from humans in Xieng Khouang Province, Lao PDR with a particular note on *Haplorchis pumilio* infection. Korean J Parasitol 53:439–445

Chai JY, Sohn WM, Na BK, Jeoung HG, Sinuon M, Socheat D (2016) *Stellantchasmus falcatus* (Digenea: heterophyidae) in Cambodia: discovery of metacercariae in mullets and recovery of adult flukes in an experimental hamster. Korean J Parasitol 54:537–541

Chai JY, Sohn WM, Cho J, Eom KS, Yong TS, Min DY, Hoang EH, Phommasack B, Insisiengmay B, Rim HJ (2018) *Echinostoma ilocanum* infection in two residents of Savannakhet Province, Lao PDR. Korean J Parasitol 56:77–81

Chantima K, Chai JY, Wongsawad C (2013) *Echinostoma revolutum*: freshwater snails as the second intermediate hosts in Chiang Mai, Thailand. Korean J Parasitol 51:183–189

Chen HT (1942) The metacercaria and adult of *Centrocestus formosanus* (Nishigori, 1924), with notes on the natural infection of rats and cats with *C. armatus* (Tanabe, 1922). J Parasitol 28:285–298

Chen HT (1962) The etiologic agent of human paragonimiasis in China. Chin Med J 81:345–353

Chen M, Mott KE (1990) Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. Trop Dis Bull 87:R1–R38

Chen M, Lu Y, Hua X, Mott KE, World Health Organization (1994) Progress in assessment of morbidity due to *Clonorchis sinensis* infection: a review of recent literature. Trop Dis Bull 91:R7–R65

Cheng Y, Xu L, Chen B, Li LS, Zhang RY, Lin CX, Lin JX, Li YS, Li YR, Fang YY, Lin KQ, Zheng GB (2005) Survey on the current status of important human parasitic infections in Fujian Province. Chin J Parasitol Parasit Dis 23:283–287. (in Chinese)

Chieffi PP, Gorla MCO, Torres DM, Dias RM, Mangini AC, Monteiro AV, Woiciechovski E (1992) Human infection by *Phagicola* sp. (Trematoda, Heterophyidae) in the municipality of Registro, São Paulo State, Brazil. J Trop Med Hyg 95:346–348

Chitsulo L, Engels D, Montresor A, Savioli L (2000) The global status of schistosomiasis and its control. Acta Trop 77:41–51

Cho SH, Cho PY, Lee DM, Kim TS, Kim IS, Hwang EJ, Na BK, Sohn WM (2010) Epidemiological survey on the infection of intestinal flukes in residents of Muangun, Jeollanam-do, the Republic of Korea. Korean J Parasitol 48:133–138

Cho SH, Sohn WM, Na BK, Kim TS, Kong Y, Eom K, Seok WS, Lee T (2011) Prevalence of *Clonorchis sinensis* metacercariae in freshwater fish from three

latitudinal regions in the Korean Peninsula. Korean J Parasitol 49:385–398

Cho SH, Kim IS, Hwang EJ, Kim TS, Na BK, Sohn WM (2012) Infection status of estuarine fish and oysters with intestinal fluke metacercariae in Muan-gun, Jeollanam-do, Korea. Korean J Parasitol 50:215–220

Choi DW (1990) *Paragonimus* and paragonimiasis in Korea. Korean J Parasitol 28:S79–S102

Choi MH, Kim SH, Chung JH, Jang HJ, Eom JH, Chung BS, Sohn WM, Chai JY, Hong ST (2006) Morphological observations of *Echinochasmus japonicus* cercariae and the in vitro maintenance of its life cycle from cercariae to adults. J Parasitol 92:236–241

Chun SK (1960) A study on some trematodes whose intermediate hosts are brackish water fish (I) The life history of *Heterophyes continus*, the intermediate host of which is *Lateolabrax japonicus*. Bull Pusan Fish Coll 3:40–44

Chung DI, Kong HH, Moon CH (1995a) Demonstration of the second intermediate hosts of *Clinostomum complanatum* in Korea. Korean J Parasitol 33:305–312

Chung DI, Moon CH, Kong HH, Choi DW, Lim DK (1995b) The first human case of *Clinostomum complanatum* (Trematoda: Clinostomidae) infection in Korea. Korean J Parasitol 33:219–213

Chung DI, Kong HH, Joo CY (1998) *Radix auricularia coreana*: natural snail host of *Clinostomum complanatum* in Korea. Korean J Parasitol 36:1–6

Chung OS, Lee HJ, Kim YM, Sohn WM, Kwak SJ, Seo M (2011) First report of human infection with *Gynaecotyla squatarolae* and first Korean record of *Haplorchis pumilio* in a patient. Parasitol Int 60:227–229

Cribb TH (1988) Two new digenetic trematodes from Australian freshwater fishes with notes on previously described species. J Nat Hist 22:27–43

Cross JH (1984) Changing patterns of some trematode infections in Asia. Arzneimittelforschung (Drug Research) 34:1224–1226

De NV (2004) Epidemiology, pathology and treatment of paragonimiasis in Vietnam. Southeast Asian J Trop Med Public Health 35:331–336

De NV, Murrell KD, Cong LD, Cam PD, Chau le V, Toan ND, Dalsgaard A (2003) The food-borne trematode zoonoses of Vietnam. Southeast Asian J Trop Med Public Health 34:12–34

Devi KR, Narain K, Bhattacharya S, Negmu K, Agatsuma T, Blair D, Wickramashinghe S, Mahanta J (2007) Pleuropulmonary paragonimiasis due to *Paragonimus heterotremus*: molecular diagnosis, prevalence of infection and clinicoradiological features in an endemic area of northeastern India. Trans R Soc Trop Med Hyg 101:786–792

Ditrich O, Nasincova V, Scholz T, Giboda M (1992) Larval stages of medically important flukes (Trematoda) from Vientiane Province, Laos. Part II. Cercariae. Ann Parasitol Hum Comp 67:75–81

Dzikowski R, Levy MG, Poore MF, Flowers JR, Paperna I (2004) Use of rDNA polymorphism for identification of Heterophyidae infecting freshwater fishes. Dis Aquat Org 59:35–41

Eastburn RL, Tritsche TR, Terhune CA Jr (1987) Human intestinal infection with *Nanophyetus salmincola* from salmonid fishes. Am J Trop Med Hyg 36:586–591

El-Azazy OMS, Abdou NEM, Khalil AI, Al-Batel MK, Majeed QAH, Henedi AAR, Tahrani LMA (2015) Potential zoonotic trematodes recovered in stray dogs from Kuwait Municipality, Kuwait. Korean J Parasitol 53:279–287

El-Dakhly MM, Aboshinaf ASM, El-Nahass E, Gharib ALF (2017) A preliminary study on the helminth fauna in necropsied stray cats (*Felis catus*) in Beni-Suef, Egypt. J Adv Vet Res 7:87–92

Esteban JG, Flores A, Angles R, Mas-Coma S (1999) High endemicity of human fascioliasis between Lake Titicaca and La Paz valley, Bolivia. Trans R Soc Trop Med Hyg 93:151–156

Esteban JG, Gonzalez C, Curtale F, Muñoz-Antoli C, Valero MA, Bargues MD, el-Sayed M, el-Wakeel AA, Abdel-Wahab Y, Montresor A, Engels D, Savioli L, Mas-Coma S (2003) Hyperendemic fascioliasis associated with schistosomiasis in villages of the Nile Delta, Egypt. Am J Trop Med Hyg 69:429–437

Fang YY, Chen YD, Li XM, Wu J, Zhang QM, Ruan CW (2008) Current prevalence of *Clonorchis sinensis* infection in endemic areas of China. Chin J Parasitol Parasit Dis 26(2):99–103. (in Chinese)

Fischthal JH, Kuntz RE (1975) Some digenetic trematodes of mammals from Taiwan. Proc Helminthol Soc Wash 42:149–157

Friant S, Brown K, Saari MT, Segel NH, Slezak J, Goldberg TL (2015) Lung fluke (*Paragonimus africanus*) infects Nigerian red-capped mangabeys and causes respiratory disease. Int J Parasitol Parasites Wildlife 4:329–332

Fried B, Abruzzi A (2010) Food-borne trematode infections of humans in the United States of America. Parasitol Res 106:1263–1280

Fried B, Graczyk TK (2004) Recent advances in the biology of *Echinostoma* species in the '*revolutum*' group. Adv Parasitol 58:139–195

Fried B, Graczyk TK, Tamang L (2004) Food-borne intestinal trematodiases in humans. Parasitol Res 93:159–170

Garcia LS (2007) Diagnostic medical parasitology, 5th edn. ASM Press, Washington, DC

Giboda M, Ditrich O, Scholz T, Viengsay T, Bouaphanh S (1991) Current status of food-borne parasitic zoonoses in Laos. Southeast Asian J Trop Med Public Health 22:S56–S61

Gryseels B, Strickland GT (2013) Schistosomiasis. In: Magill AJ, Ryan ET, Hill DR, Solomon T (eds) Hunter's tropical medicine and emerging infectious diseases, 9th edn. Elsevier Saunders, London, New York, Oxford, St. Louis, Sydney, Toronto, pp 868–883

Gryseels B, Polman K, Clerinx J, Kestens L (2006) Human schistosomiasis. Lancet 368:1106–1118

Guk SM, Shin EH, Kim JL, Sohn WM, Hong KS, Yoon CH, Lee SH, Rim HJ, Chai JY (2007a) A survey of *Heterophyes nocens* and *Pygidiopsis summa* metacercariae in mullets and gobies along the coastal areas of the Republic of Korea. Korean J Parasitol 45:205–211

Guk SM, Kim JL, Park JH, Chai JY (2007b) A human case of *Plagiorchis vespertilionis* (Digenea: Plagiorchiidae) infection in the Republic of Korea. J Parasitol 93:1225–1227

Gürler AT, Bakan N (2017) Helminths of laboratory animals in Turkey: review. J Lab Anim 1:41–48

Hara H, Miyauchi Y, Tahara S, Yamashita H (2014) Human laryngitis caused by *Clinostomum complanatum*. Nagoya J Med Sci 76:181–185

Hong ST, Fang Y (2012) *Clonorchis sinensis* and clonorchiasis, an update. Parasitol Int 61:17–24

Hong ST, Cho TK, Hong SJ, Chai JY, Lee SH, Seo BS (1984) Fifteen human cases of *Fibricola seoulensis* infection in Korea. Korean J Parasitol 22:61–65

Hong SJ, Seo BS, Lee SH, Chai JY (1988) A human case of *Centrocestus armatus* infection in Korea. Korean J Parasitol 26:55–60

Hong SJ, Chang CK, Lee DH, Woo HC (1996a) One human case of natural infection by *Heterophyopsis continua* and three other species of intestinal trematodes. Korean J Parasitol 34:87–90

Hong SJ, Woo HC, Chai JY (1996b) A human case of *Plagiorchis muris* (Tanabe, 1922: Digenea) infection in the Republic of Korea: freshwater fish as a possible source of infection. J Parasitol 82:647–649

Howell MJ (1973) The resistance of cysts of *Stictodora lari* (Trematoda: Heterophyidae) to encapsulation by cells of the fish host. Int J Parasitol 3:653–659

Huh S, Sohn WM, Chai JY (1993) Intestinal parasites of cats purchased in Seoul. Korean J Parasitol 31:371–373

Ibanga ES, Eyo VM (2001) Pulmonary paragonimiasis in Oben community in Akamkpa local government area, Cross River State, Nigeria: prevalence and intensity of infection. Trans R Soc Trop Med Hyg 95:159–160

Ito J (1964) *Metagonimus* and other human heterophyid trematodes. Prog Med Parasitol Jpn 1:314–393

Ito J, Mochizuki H, Ohno Y, Ishiguro M (1991) On the prevalence of *Metagonimus* sp. among the inhabitants at Hamamatsu basin in Shizuoka Prefecture, Japan. Jpn J Parasitol 40:274–278

Iwagami M, Rajapakse RPV, Yatawara L, Kano S, Agatsuma T (2009) The first intermediate host of *Paragonimus westermani* in Sri Lanka. Acta Trop 109:27–29

Kaatano GM, Min DY, Siza JE, Yong TS, Chai JY, Ko Y, Chang SY, Changalucha JM, Eom KS, Rim HJ (2015a) *Schistosoma mansoni*-related hepatosplenic morbidity in adult population on Kome Island, Sengerema District, Tanzania. Korean J Parasitol 53:545–551

Kaatano GM, Siza JE, Mwanga JR, Min DY, Yong TS, Chai JY, Ko Y, Chang SY, Kullaya CM, Rim HJ, Changalucha JM, Eom KS (2015b) Integrated schistosomiasis and soil-transmitted helminthiasis control over five years on Kome Island, Tanzania. Korean J Parasitol 53:535–543

Kaewkes S (2003) Taxonomy and biology of liver flukes. Acta Trop 88:177–186

Kagei N (1965) Epidemiological studies of metagonimiasis in Japan. I. Epidemiological survey of metagonimiasis among the Takatsu River, Shimane Prefecture. Bull Inst Public Health Jpn 14:213–227. (in Japanese)

Kamo H, Nishida H, Hatsushika R, Tomimura T (1961) On the occurrence of a new lung fluke, *Paragonimus miyazakii* n. sp. in Japan (Trematoda: Troglotrematidae). Yonago Acta Med 5:43–52

Kanev I (1994) Life-cycle, delimitation and redescription of *Echinostoma revolutum* (Froelich, 1982) (Trematoda: Echinostomatidae). Syst Parasitol 28:125–144

Kang BK, Jung BK, Lee YS, Hwang IK, Lim H, Cho J, Hwang JH, Chai JY (2014) A case of Fasciola hepatica infection mimicking cholangiocarcinoma and ITS-1 sequencing of the worm. Korean J Parasitol 52:193–196

Kaul BK, Singhal CD, Pillai PN (1974) *Artyfechinostomum mehrai* infestation with bowel perforation. J Indian Med Assoc 63:263–265

Keiser J, Utzinger J (2009) Food-borne trematodiases. Clin Microbiol Rev 22:466–483

Khalil M, Abaza MS (1924) A new trematode parasite of the rat, *Echinostoma aegyptiaca* nov. sp. Report and Notes of the Public Health Laboratories 6:187–189

Kiatsopit N, Sithithaworn P, Saijuntha W, Boonmars T, Tesana S, Sithithaworn J, Petney TN, Andrews RH (2012) Exceptionally high prevalence of infection of *Bithynia siamensis goniomphalos* with *Opisthorchis viverrini* cercariae in different wetlands in Thailand and Lao PDR. Am J Trop Med Hyg 86:464–469

Kim DC (1984) *Paragonimus westermani*: life cycle, intermediate hosts, transmission to man and geographical distribution in Korea. Arzneimittelforschung (Drug Research) 34:1180–1183

Kim YK, Yu JE, Chung EY, Chung PR (2004) *Acanthoparyphium tyosenense* (Digenea: Echinostomatidae): experimental confirmation of the cercaria and its complete life history in Korea. J Parasitol 90:97–102

Kim TS, Cho SH, Huh S, Kong Y, Sohn WM, Hwang SS, Chai JY, Lee SH, Park YK, Oh DK, Lee JK, Korea Association of Health Promotion (2009) A nationwide survey on the prevalence of intestinal parasitic infections in the Republic of Korea. Korean J Parasitol 47:37–47

Kino H, Oishi H, Ohno Y, Mitsuru I (2002) An endemic human infection with *Heterophyes nocens* Onji et Nishio, 1916 at Mikkabi-cho, Shizuoka, Japan. Jpn J Trop Med Hyg 30:301–304

Kino H, Suzuki T, Oishi H, Suzuki S, Yamagiwa S, Ishiguro M (2006) Geographical distribution of *Metagonimus yokogawai* and *M. miyatai* in Shizuoka Prefecture, Japan, and their site preferences in the sweetfish, *Plecoglossus altivelis*, and hamsters. Parasitol Int 55:201–206

Kobayasi H (1942) Studies on the flukes in Hainan Island. III. On the flukes belonging to the Family Echinostomatidae Odhner, 1910, *Episthochasmus caninum* Verma, 1935. Acta Nippon Med Trop 4:97–105

Koh EJ, Kim SK, Wang KC, Chai JY, Chong S, Park SH, Cheon JE, Phi JH (2012) The return of an old worm: cerebral paragonimiasis presenting with intracerebral hemorrhage. J Korean Med Sci 27:1428–1432

Komiya Y (1965) Metacercaria in Japan and adjacent territories. In: Progress of medical parasitology in Japan, vol 2. Meguro Parasitological Museum, Tokyo, pp 1–328

Korea Association of Health Promotion (2004) Prevalence of intestinal parasitic infections in Korea—the 7th Report (Monographic series in Korean). Seoul

Kuntz RE, Chandler AC (1956) Studies on Egyptian trematodes with special reference to the heterophyid of mammals. I. Adult flukes, with descriptions of *Phagicola longicollis* n. sp., *Cynodiplostomum namrui* n. sp., and a *Stephanoprora* from cats. J Parasitol 42:445–459

Lane MA, Barsanti MC, Santos CA, Yeung M, Lubner SJ, Weil GJ (2009) Human paragonimiasis in North America following ingestion of raw crayfish. Clin Infect Dis 49:e55–e61

Lane MA, Macros LA, Onen NF, Demertzis LM, Hayes EV, Davila SZ, Nurutdinova DR, Bailey TC, Weil GJ (2012) *Paragonimus kellicotti* fluke infections in Missouri, USA. Emerg Infect Dis 18:1263–1267

Le TH, Nguyen NTB, Truong NH, De NV (2012) Development of mitochondrial loop-mediated isothermal application for detection of the small liver fluke *Opisthorchis viverrini* (Opisthorchiidae; Trematoda; Platyhelminthes). J Clin Microbiol 50:1178–1184

Lee SH, Yoo BH, Hong ST, Chai JY, Seo BS, Chi JG (1985) A histopathological study on the intestine of mice and rats experimentally infected by *Fibricola seoulensis*. Korean J Parasitol 23:58–72

Lee SH, Lee JK, Sohn WM, Hong ST, Hong SJ, Chai JY (1988) Metacercariae of *Echinostoma cinetorchis* encysted in the fresh water snail, *Hippeutis (Helicorbis) cantori*, and their development in rats and mice. Korean J Parasitol 26:189–197

Lee SH, Chai JY, Hong ST (1993) *Gymnophalloides seoi* n. sp. (Digenea: Gymnophallidae), the first report of human infection by a gymnophallid. J Parasitol 79:677–680

Lee SH, Park SK, Seo M, Guk SM, Choi MH, Chai JY (1997) Susceptibility of various animals and strains of mice to *Gymnophalloides seoi* infection and the effetcs of immunosuppression in C3H/HeN mice. J Parasitol 83:883–886

Lee HJ, Chai JY, Lee JW, Jin H, Min KH, Cho YJ, Seo M (2010a) Surveys of *Gynaecotyla squatarolae* and *Microphallus koreana* (Digenea: Microphallidae) metacercariae in two species of estuarine crabs in western coastal areas, Korea. Korean J Parasitol 48:81–83

Lee MK, Hong SJ, Kim HR (2010b) Seroprevalence of tissue invading parasitic infections diagnosed by ELISA in Korea. J Korean Med Sci 25:1272–1276

Lee GS, Park SW, Kim J, Seo KS, You JH, Moon HC, Hong GY (2017) A case of endoscopically treated laryngopharyngitis resulting from *Clinostomum complanatum* infection. Korean J Gastroenterol 69:177–180. (in Korean)

Lee YI, Seo M, Kim SB (2018) Infections of soil-transmitted helminth in refugees from North Korea. Korean J Parasitol 56:291–294

Leles D, Cascardo P, Freire AS, Maldonado A Jr, Sianto L, Araújo A (2014) Insights about echinostomiasis by paleomolecular diagnosis. Parasitol Int 63:646–649

Lemos ACM, Coelho JC, Matos ED, Montal G, Aguiar F, Badaró R (2007) Paragonimiasis: first case report in Brazil. Braz J Infect Dis 11:1–5

Li MH (2013) *Metagonimus yokogawai*: metacercariae survey in fishes and its development to adult worms in various rodents. Parasitol Res 112:1647–1653

Li Q, Wei F, Liu W, Yang S, Zhang X (2008) Paragonimiasis: an important food-borne zoonosis in China. Trends Parasitol 24:318–323

Liang C, Ke XL (1988) *Echinochasmus jiufoensis* sp. nov., a human parasite from Guangzhou (Trematoda: Echinostomatidae). Acta Zootaxon Sin 13:4–8. (in Chinese)

Lie KJ, Basch PF (1967) The life history of *Echinostoma paraensei* sp. n (Trematoda: Echinostomatidae). J Parasitol 53:1192–1199

Lin J, Chen Y, Li Y (2001) The discovery of natural infection of human with *Metorchis orientalis* and the investigation of its focus. Chin J Zoonoses 17:38–53. (in Chinese)

Liu D, Zhu XQ (2013) Fasciola. In: Liu D (ed) Molecular detection of human parasitic pathogens. CRC Press, Taylor & Francis Group, Boca Raton, pp 343–351

Lo CT (1995) *Echinostoma macrorchis*: life history, population dynamics of intramolluscan stages, and the first and second intermediate hosts. J Parasitol 81:569–576

López-Caballero J, Oceguera-Figueroa A, León-Règagnon V (2013) Detection of multiple species of human *Paragonimus* from mexico using morphological data and molecular barcodes. Mol Ecol Resour 13:1125–1136

MacLean JD, Arthur JR, Ward BJ, Gyorkos TW, Curtis MA, Kokoskin E (1996) Common-source outbreak of acute infection due to the North American liver fluke *Metorchis conjunctus*. Lancet 347:154–158

Macy RW (1960) The life cycle of *Plagiorchis vespertilionis parorchis* n. ssp., (Trematoda: Plagiorchidae), and observations on the effects of light on the emergence of the cercaria. J Parasitol 46:337–345

Maji AK, Manna B, Bandyopadhyay AK, Bera DK, Addy M, Nandy A (1995) Studies on the life cycle of *Artyfechinostomum oraoni* Bandyopadhyay, Manna & Nandy, 1989: embryogenesis and development in the intermediate host. Indian J Med Res 102:124–128

Majima M (1927) On *Echinostoma macrorchis* found in man. Kumamoto Igakkai Zasshi No. 2252, 2260–2263 (in Japanese)

Manning GS, Lertprasert P (1973) Studies on the life cycle of *Phaneropsolus bonnei* and *Prosthodendrium molenkampi* in Thailand. Ann Trop Med Parasitol 67:361–365

Manning GS, Diggs CL, Viyanant V, Lertprasert P, Watanasirmkit K (1970) Preliminary report on *Phaneropsolus bonnei* Lie Kian Joe, 1951. A newly discovered human intestinal fluke from northeastern Thailand. J Med Assoc Thail 53:173–178

Mariano EG, Borja SB, Vruno MJ (1986) A human infection with *Paragonimus kellicotti* (lung fluke) in the Unites States. Am J Clin Pathol 86:685–687

Mas-Coma S, Bargues MD (1997) Human liver flukes: a review. Res Rev Parasitol 57:145–218

Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278

Miyazaki I (1991) An illustrated book of helminthic zoonoses. International Medical Foundation of Japan, Tokyo

Miyazaki I, Habe S (1976) A newly recognized mode of infection with the lung fluke, *Paragonimus westermani* (Kerbert 1878). J Parasitol 62:646–648

Miyazaki I, Ishii Y (1968) Studies on the Mexican lung flukes, with special reference to a description of *Paragonimus mexicanus* sp. nov. (Trematoda: Troglotrematidae). Jpn J Parasitol 17:445–453

Mordvinov VA, Yurlova NI, Ogorodova LM, Katokhin AV (2012) *Opisthorchis felineus* and *Metorchis bilis* are the main agents of liver fluke infection of humans in Russia. Parasitol Int 61:25–31

Morgan JA, Blair D (1998) Mitochondrial ND1 gene sequences used to identify echinostome isolates from Australia and New Zealand. Int J Parasitol 28:493–502

Moyou-Somo R, Kefie-Arrey C, Dreyfuss G, Dumas M (2003) An epidemiological study of pleuropulmonary paragonimiasis among pupils in the peri-urban zone of Kumba town, Meme Division, Cameroon. BMC Public Health 3:40

Nakamura-Uchiyama F, Mukae H, Nawa Y (2002) Paragonimiasis: a Japanese perspective. Clin Chest Med 23:409–420

Narain K, Agatsuma T, Blair D (2010) Paragonimus. In: Liu D (ed) Molecular detection of foodborne pathogens. CRC Press, Taylor & Francis Group, Boca Raton, pp 827–837

Nasr M (1941) The occurrence of *Prohemistomum vivax* (Sonsino, 1892) Azim, 1933 infection in man, with a description of the parasite. Lab Med Prog 2:135–149

Nath D (1969) *Rana cyanophlyctis* as the second intermediary of *Artyfechinostomum sufrartyfex*. Curr Sci 14:342–343

Niemi DR, Macy RW (1974) The life cycle and infectivity to man of *Apophallus donicus* (Skrjabin and Lindtrop, 1919) (Trematoda: Heterophyidae) in Oregon. Proc Helminthol Soc Wash 41:223–229

Nkouawa A, Okamoto M, Mabou AK, Edinga E, Yamasaki H, Sako Y, Nakao M, Nakaya K, Blair D, Agatsuma T, Enyong P, Shibahara T, Moyou-Somo R, Ito A (2009) Paragonimiasis in Cameroon: molecular identification, serodiagnosis and clinical manifestations. Trans R Soc Trop Med Hyg 103:255–261

Ohmae H, Sinuon M, Kirinoki M, Matsumoto J, Chigusa Y, Socheat D, Matsuda H (2004) Schistosomiasis mekongi: from discovery to control. Parasitol Int 53:135–142

Okabe N, Okabe K (1972) On a human case infected by three species of trematodes. Nippon Iji Shimpo 2531:46–48. (in Japanese)

Onji Y, Nishio T (1916) On the trematodes whose intermediate host is brackish water fishes. Chiba Igaku Semmon Gakko Zasshi 81/82:229–249. (in Japanese)

Ooi HK, Chen CI, Lin SC, Tung KC, Wang JS, Kamiya M (1997) Metacercariae in fishes of Sun Moon Lake which is an endemic area for *Clonorchis sinensis* in Taiwan. Southeast Asian J Trop Med Public Health 28:S222–S223

Owen IL (2005) Parasitic zoonoses in Papua New Guinea. J Helminthol 79:1–14

Oyarzún-Ruiz P, Alvelo C, Vera F, Moroni M (2018) Histopathological findings of *Fasciola hepatica* infection in non-native European hare (*Lepus europaeus*) in Southern Chile. Braz J Vet Parasitol 14. pii: S1984-29612018005013102

Phalee A, Wongsawad C, Rojanapaibul A, Chai JY (2015) Experimental life history and biological characteristics of *Fasciola gigantica* (Digenea: Fasciolidae). Korean J Parasitol 53:59–64

Poulin R (2006) Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. Parasitology 132:143–151

Pozio E, Armigbacco O, Ferri F, Gomez Morales MA (2013) *Opisthorchis felineus*, an emerging infection in Italy and its implication for the European Union. Acta Trop 126:54–62

Procop GW (2009) North American paragonimiasis (caused by *Paragonimus kellicoti*) in the context of global paragonimiasis. Clin Microbiol Rev 22:415–446

Radomyos P, Bunnag D, Harinasuta T (1983) *Haplorchis pumilio* (Looss) infection in man in northeastern Thailand. Southeast Asian J Trop Med Public Health 14:223–227

Radomyos P, Bunnag D, Harinasuta T (1985) Report of *Episthmium caninum* (Verma, 1935) Yamagui, 1958 (Digenea: Echinostomatidae) in man. Southeast Asian J Trop Med Public Health 16:508–511

Radomyos P, Bunnag D, Harinasuta C (1989) A new intestinal fluke, *Plagiorchis harinasutai*, n. sp. Southeast Asian J Trop Med Public Health 20:101–107

Radomyos P, Charoenlarp P, Radomyos B (1991) Human *Episthmium caninum* (Digenea: Echinostomatidae) infection: report of two more cases. J Trop Med Parasitol 14:48–50

Raghunathan VS, Srinivasan T (1962) *Artyfechinostomum mehrai* infestation. J Indian Med Assoc 38:485–487

Ransom BH (1920) Synopsis of the trematode family Heterophyidae with descriptions of a new genus and five new species. Proc U S Natl Mus 57(2322):527–573

Reddy G, Varmah K (1950) *Paryphostomum sufrartyfex* (intestinal fluke) infection in man. Ind Med Gaz 85:546–547

Reddy DB, Ranganaykamma I, Venkataratnam D (1964) *Artyfechinostomum mehrai* infestation in man. J Trop Med Hyg 67:58–59

Rim HJ (1982a) Clonorchiasis. In: Steele JH, Hillyer GV, Hopla CE (eds) CRC handbook series in zoonoses, Section C: parasitic zoonoses, vol III. Trematode zoonoses. CRC Press Inc., Boca Raton, pp 17–32

Rim HJ (1982b) Opisthorchiasis. In: Steele JH, Hillyer GV, Hopla CE (eds) CRC handbook series in zoonoses, Section C: parasitic zoonoses, vol III. Trematode zoonoses. CRC Press Inc., Boca Raton, pp 109–121

Rim HJ (1982c) Echinostomiasis. In: Steele JH, Hillyer GV, Hopla CE (eds) CRC handbook series in zoonoses, Section C: parasitic zoonoses, vol III. Trematode zoonoses. CRC Press Inc., Boca Raton, pp 53–69

Rim HJ (1990) Clonorchiasis in Korea. Korean J Parasitol 28:S63–S78

Rim HJ (2005) Clonorchiasis: an update. J Helminthol 79:269–281

Rim HJ, Chai JY, Min DY, Cho SY, Eom KS, Hong SJ, Sohn WM, Yong TS, Deodato G, Standgaard H, Phommasack B, Yun CH, Hoang EH (2003) Prevalence of intestinal parasite infections on a national scale among primary schoolchildren in Laos. Parasitol Res 91:267–272

Ryang YS, Yoo JC, Lee SH, Chai JY (2000) The Palearctic oystercatcher *Haematopus ostralegus*, a natural definitive host for *Gymnophalloides seoi*. J Parasitol 86:418–419

Ryang YS, Yoo JC, Lee SH, Chai JY (2001) Susceptibility of avian hosts to experimental *Gymnophalloides seoi* infection. J Parasitol 87:454–456

Sachs R, Albiez EJ, Voelke J (1986) Prevalence of *Paragonimus uterobilaterialis* infection in children in a Liberian village. Trans R Soc Trop Med Hyg 80:800–801

Saeed I, Maddox-Hyttel C, Monrad J, Kapel CMO (2006) Helminths of red foxes (*Vulpes vulpes*) in Denmark. Vet Parasitol 139:168–179

Saito S, Chai JY, Kim KH, Lee SH, Rim HJ (1997) *Metagonimus miyatai* sp. nov. (Digenea: Heterophyidae), a new intestinal trematode transmitted by freshwater fishes in Japan and Korea. Korean J Parasitol 35:223–232

Sayasone S, Odermatt P, Phoumindr N, Vongsaravane X, Sensombath V, Phetsouvanh R, Choulamany X, Strobel M (2007) Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. Trans R Soc Trop Med Hyg 101:40–47

Schell SC (1985) Handbook of trematodes of North America North of Mexico. University Press of Idaho, Moscow

Scholz T (1999) Taxonomic study of *Ascocotyle* (*Phagicola*) *longa* Ransom, 1920 (Digenea: Heterophyidae) and related taxa. Syst Parasitol 43:147–158

Seo BS (1978) Clinical parasitology. Il-Cho-Kak, Seoul (in Korean)

Seo BS (1990) *Fibricola seoulensis* Seo, Rim and Lee, 1964 (Trematoda) and fibricoliasis in man. Seoul J Med 31:61–96

Seo BS, Hong ST, Chai JY (1981) Studies on intestinal trematodes in Korea III. Natural human infection of *Pygidiopsis summa* and *Heterophyes heterophyes nocens*. Seoul J Med 22:228–235

Seo BS, Lee SH, Chai JY, Hong SJ (1984) Studies on intestinal trematodes in Korea. XIII. Two cases of natural human infection by *Heterophyopsis continua* and the status of metacercarial infection in brackish water fishes. Korean J Parasitol 22:51–60

Seo BS, Chun KS, Chai JY, Hong SJ, Lee SH (1985) Studies on intestinal trematodes in Korea XVII. Development and egg laying capacity of *Echinostoma hortense* in albino rats and human experimental infection. Korean J Parasitol 23:24–32

Seo M, Guk SM, Lee SH, Chai JY (2007) *Gynaecotyla squatarolae* (Digenea: Microphallidae) from rats experimentally infected with metacercariae from the shore crab, *Macrophthalmus dilatatus*. Korean J Parasitol 45:199–204

Shibahara T, Nishida H, Torii M (1992) Experimental infection of wild boars with metacercariae of *Paragonimus miyazakii* (Trematoda: Troglotrematidae). Jpn J Parasitol 41:274–278

Shimazu T (2002) Life cycle and morphology of *Metagonimus miyatai* (Digenea: Heterophyidae) from Nagano, Japan. Parasitol Int 51:271–280

Shin EH, Park JH, Guk SM, Kim JL, Chai JY (2009) Intestinal helminth infections in feral cats and a raccoon dog on Aphaedo Island, Shinan-gun, with a special note on *Gymnophalloides seoi* infection in cats. Korean J Parasitol 47:189–191

Shin SS, Oh DS, Ahn KS, Cho SH, Lee WJ, Na BK, Sohn WM (2015) Zoonotic intestinal trematodes in stray cats (*Felis catus*) from riverside areas of the Republic of Korea. Korean J Parasitol 53:209–213

Shin EH, Im TK, Park YK, Cho J, Kim JL, Chai JY (2016) Fecundity reduction of BALB/c mice after survival from lethal *Neodiplostomum seoulense* infection. Parasitol Res 115:2051–2059

Singh ST, Singh DL, Sugiyama H (2006) Possible discovery of Chinese lung fluke, *Paragonimus skrjabini* in Manipur, India. Southeast Asian J Trop Med Public Health 37:S53–S56

Singh TS, Sugiyama H, Unchara A, Hiese S, Khalo K (2009) *Paragonimus heterotremus* infection in Nagaland: a new focus of paragonimiasis in India. Indian J Med Microbiol 27:123–127

Sinuon M, Sayasone S, Odermatt-Biays S, Phompida S, Duong S, Odermatt P (2010) *Schistosoma mekongi* in Cambodia and Lao People's Democratic Republic. Adv Parasitol 72:179–203

Sithithaworn P, Haswell-Elkins M (2003) Epidemiology of *Opisthorchis viverrini*. Acta Trop 88:187–194

Siza JE, Kaatano GM, Chai JY, Eom KS, Rim HJ, Yong TS, Min DY, Chang SY, Ko Y, Changalucha JM (2015a) Prevalence of schistosomes and soil-transmitted helminths among schoolchildren in Lake Victoria basin, Tanzania. Korean J Parasitol 53:515–524

Siza JE, Kaatano GM, Chai JY, Eom KS, Rim HJ, Yong TS, Min DY, Chang SY, Ko Y, Changalucha JM (2015b) Prevalence of schistosomes and soil-transmitted helminths and morbidity associated with schistosomiasis among adult population in Lake Victoria basin, Tanzania. Korean J Parasitol 53:525–533

Sohn WM (1998) Life history of *Echinoparyphium recurvatum* (Trematoda: Echinostomatidae) in Korea. Korean J Parasitol 36:91–98

Sohn WM, Chai JY (2005) Infection status with helminthes in feral cats purchased from a market in Busan, Republic of Korea. Korean J Parasitol 43:93–100

Sohn WM, Na BK (2017) *Echinostoma macrorchis* (Digenea: Echinostomatidae): metacercariae in *Cipangopaludina chinensis malleata* snails and adults from experimental rats in Korea. Korean J Parasitol 55:541–548

Sohn WM, Han GG, Kho WG, Chai JY, Lee SH (1994) Infection status with the metacercariae of heterophyid flukes in the brackish water fish from Haenam-gun, Chollanam-do, Korea. Korean J Parasitol 32:163–169

Sohn WM, Han ET, Chai JY (2003a) *Acanthotrema felis* n. sp. (Digenea: Heterophyidae) from the small intestine of stray cats in the Republic of Korea. J Parasitol 89:154–158

Sohn WM, Han ET, Seo M, Chai JY (2003b) Identification of *Acanthotrema felis* (Digenea: Heterophyidae) metacercariae encysted in the brackish water fish *Acanthogobius flavimanus*. Korean J Parasitol 41: 101–105

Sohn WM, Chai JY, Yong TS, Eom KS, Yoon CH, Sinuon M, Socheat D, Lee SH (2011a) *Echinostoma revolutum* infection in children, Pursat Province, Cambodia. Emerg Infect Dis 17:117–119

Sohn WM, Kim HJ, Yong TS, Eom KS, Jeong HG, Kim JK, Kang AR, Kim MR, Park JM, Ji SH, Sinuon M, Socheat D, Chai JY (2011b) *Echinostoma ilocanum* infection in Oddar Meanchey Province, Cambodia. Korean J Parasitol 49:187–190

Sohn WM, Yong TS, Eom KS, Pyo KH, Lee MY, Lim H, Choe S, Jeong HG, Sinuon M, Socheat D, Chai JY (2012) Prevalence of *Opisthorchis viverrini* infection in humans and fish in Kratie Province, Cambodia. Acta Trop 124:215–220

Sohn WM, Chai JY, Na BK, Yong TS, Eom KS, Park H, Min DY, Rim HJ (2013) *Echinostoma macrorchis* in Lao PDR: metacercariae in *Cipangopaludina* snails and adults from experimentally infected animals. Korean J Parasitol 51:191–196

Sohn WM, Yong TS, Eom KS, Min DY, Lee D, Jung BK, Banouvong V, Insisiengmay B, Phammasack B, Rim HJ, Chai JY (2014) Prevalence of *Haplorchis taichui* among humans and fish in Luang Prabang Province, Lao PDR. Acta Trop 136:74–80

Sohn WM, Kim DG, Jung BK, Cho J, Chai JY (2016) *Pygidiopsis cambodiensis* n. sp. (Digenea: Heterophyidae) from experimental hamsters infected with metacercariae in mullets from Cambodia. Parasitol Res 115:123–130

Sohn WM, Yong TS, Eom KS, Sinuon M, Jeoung WG, Chai JY (2017) *Artyfechinostomum malayanum*: metacercariae encysted in *Pila* sp. snails purchased from Phnom Penh, Cambodia. Korean J Parasitol 55:341–345

Song HB, Choi MH, Chung EJ (2018) Human laryngeal infection by *Clinostomum complanatum*. Am J Trop Med Hyg 98:7–8

Sugiyama H, Sing TS, Rangsiruji A (2013) Paragonimus. In: Liu D (ed) Molecular detection of human parasitic pathogens. CRC Press, Taylor & Francis Group, Boca Raton, pp 423–435

Tandon V, Roy B, Prasad PK (2013) Fasciolopsis. In: Liu D (ed) Molecular detection of human parasitic pathogens. CRC Press, Taylor & Francis Group, Boca Raton, pp 353–364

Toan PQ, Luc PV, Ha PV (2008) Some supplementary data on parasitic trematode of house rat (*R. flavipectus*) Nghe An, Ha Tinh Provinces and describe to *Centrocestus formosanus* (Heterophyidae) which species the first time found from the rat in Vietnam. Tap chi SINH HOC 31:9–13. (in Vietnamese)

Tongu Y (2001) The species of *Paragonimus* in Latin America. Bulletin of Faculty of Health Sciences, Okayama University Medical School 12:1–5

Tran BT, Nguyen ST, Nguyen TT, Luc PV, Mafie E, Rupa FH, Sato H (2016) Endoparasites of Vietnamese lizards recorded in the last 50 years (1966-2015). Jpn J Vet Parasitol 15:34–58

Traversa D, Lorruso V, Otranto D (2013) Dicrocoelium. In: Liu D (ed) Molecular detection of human parasitic pathogens. CRC Press, Taylor & Francis Group, Boca Raton, pp 323–331

Uchiyama F, Morimoto Y, Nawa Y (1999) Re-emergence of paragonimiasis in Kyushu, Japan. Southeast Asian J Trop Med Public Health 30:686–691

Urabe M (2003) Trematode fauna of prosobranch snails of the genus *Semisulcospira* in Lake Biwa and the connected drainage syste. Parasitol Int 52:21–34

Urbani C, Sinuon M, Socheat D, Pholsena K, Strandgaard H, Odermatt P, Hatz C (2002) Epidemiology and control of mekongi schistosomiasis. Acta Trop 82:157–168

Velasquez CC (1973a) Life cycle of *Procerovum calderoni* (Africa and Garcia, 1935), Price, 1940 (Trematoda: Digenea: Heterophyidae). J Parasitol 59:813–816

Velasquez CC (1973b) Observations on some Heterophyidae (Trematoda: Digenea) encysted in Philippine fishes. J Parasitol 59:77–84

Velez I, Velasquez LE, Velez ID (2003) Morphological description and life cycle of *Paragonimus* sp. (Trematoda: Troglotrematidae): causal agent of human paragonimiasis in Colombia. J Parasitol 89:749–755

Verma SC (1935) Studies on the Indian species of the genus *Echinochasmus*, Part I. and on an allied

new genus *Episthochasmus*. Proc Indian Acad Sci 1:837–856

Waikagul J, Wongsaroj T, Radomyos P, Meesomboon V, Praewanich R, Jongsuksuntikul P (1997) Human infection of *Centrocestus caninus* in Thailand. Southeast Asian J Trop Med Public Health 28:831–835

Wang W, Blair D, Min T, Li F, Wang D (2011) *Paragonimus* worm from a New Guinea native in 1926. Asian Pac J Trop Med 4:76–78

Wanlop A, Wongsawad C, Prattapong P, Wongsawad P, Chontananarth T, Chai JY (2017) Prevalence of *Centrocestus formosanus* metacercariae in ornamental fish from Chiang Mai, Thailand, with molecular approach using ITS2. Korean J Parasitol 55:445–449

Weerakoon KG, Gobert GN, Cai P, McManus DP (2015) Advances in the diagnosis of human Schistosomiasis. Clin Microbiol Rev 28:939–967

WHO (2015) Investing to overcome the global impact of neglected tropical diseases. Third WHO report on neglected tropical diseases. WHO/Department of Control of Neglected Tropical Diseases, Geneva

WHO (2018) Schistosomiasis and soil-transmitted helminthiases: numbers of people treated in 2017. Weekly Epidemiol Rec 93:681–692. Geneva, Switzerland

Witenberg G (1929) Studies on the trematode-family Heterophyidae. Ann Trop Med Parasitol 23:131–268

Witenberg G (1932) On the anatomy and systematic position of the causative agent of so-called salmon poisoning. J Parasitol 18:258–263

Xiao X, Wang T, Zheng X, Shen G, Tian Z (2005) In vivo and in vitro encystment of *Echinochasmus liliputanus* cercariae and biological activity of the metacercariae. J Parasitol 91:492–498

Yamaguti S (1939a) Studies on the helminth fauna of Japan. Part 25. Trematodes of birds, IV. Jpn J Zool 8:129–210

Yamaguti S (1939b) Studies on the helminth founa of Japan. Part 27. Trematodes of mammals, II. Jpn J Med Sci VI Bacteriol Parasitol 1:131–153

Yamaguti S (1958) Part I. The digenetic trematodes of vertebrates. In: Systema Helminthum, vol I. Interscience Publishers Inc., New York

Yamaguti S (1971) Synopsis of digenetic trematodes of vertebrates, vol I & II. Keigaku Pub Co., Tokyo

Yokogawa M (1965) *Paragonimus* and paragonimiasis. Adv Parasitol 3:99–158

Yokogawa S, Cort WW, Yokogawa M (1960) *Paragonimus* and paragonimiasis. Exp Parasitol 10:81–205

Yokogawa M, Harinasuta C, Charoenlarp P (1965) *Hypoderaeum conoideum* (Block, 1782) Dietz, 1909, a common intestinal fluke of man in the north-east Thailand. Jpn J Parasitol 14:148–153

Yokogawa M, Araki K, Saito K, Momose T, Kimura M, Suzuki S, Chiba N, Kutsumi H, Minai M (1974) *Paragonimus miyazakii* infections in man first found in Kanto District, Japan especially, on the methods of immunosero-diagnosis for paragonimiasis. Jpn J Parasitol 23:167–179. (in Japanese)

Yong TS, Shin EH, Chai JY, Sohn WM, Eom KS, Lee DM, Park K, Jeoung HG, Hoang EH, Lee YH, Woo HJ, Lee JH, Kang SI, Cha JK, Lee KH, Yoon CH, Sinuon M, Socheat D (2012) High prevalence of *Opisthorchis viverrini* infection in a riparian population in Takeo Province, Cambodia. Korean J Parasitol 50:173–176

Yoshida A, Matsuo K, Moribe J, Tanaka R, Kikuchi T, Nagayasu E, Misawa N, Maruyama H (2016) Venison, another source of *Paragonimus westermani* infection. Parasitol Int 65:607–612

Yossepowitch O, Gotesman T, Assous M, Marva E, Zimlichman R, Dan M (2004) Opisthorchiasis from imported raw fish. Emerg Infect Dis 10:2122–2126

Youssef MM, Mansour NS, Awadalla HN, Hammouda NA, Khalifa R, Boulos LM (1987a) Heterophyid parasites of man from Idku, Maryut and Manzala Lakes areas in Egypt. J Egypt Soc Parasitol 17: 475–479

Youssef MM, Mansour NS, Hammouda NA, Awadalla HN, Khalifa R, Boulos LM (1987b) Studies on some developmental stages in the life cycle of *Pygidiopsis genata* Looss, 1907 (Trematoda: Heterophyidae) from Egypt. J Egypt Soc Parasitol 17:463–474

Yu JR, Chai JY (2013) Metagonimus. In: Liu D (ed) Molecular detection of human parasitic pathogens. CRC Press, Taylor & Francis Group, Boca Raton, pp 389–398

Yu SH, Mott KE (1994) Epidemiology and morbidity of food-borne intestinal trematode infections. Trop Dis Bull 91:R125–R152

Yu SH, Mansanori K, Li XM, Xu LQ, Lan CG, Rui L (2003) Epidemiological investigation on *Clonorchis sinensis* in human population in an area of south China. Jpn J Infect Dis 56:168–171

Zhan X, Li C, Wu H, Sun E, Zhu Y (2017) Investigation on the endemic characteristics of *Metorchis orientalis* in Huainan area, China. Nutr Hosp 34:675–679

Zhang XL, Wang Y, Wang GX, Chen WB, He X, Niu H, Li ZL, Chen L, Wang LF (2012) Distribution and clinical features of *Paragonimus skrjabini* in Three Gorges Reservoir region. Parasitol Int 61:645–649

Trematode Genomics and Proteomics

13

Javier Sotillo, Mark S. Pearson, and Alex Loukas

13.1 Introduction

Trematodes have an enormous impact on the health and economy of communities, particularly on the poorest populations from tropical and subtropical regions of the world. Indeed, trematode infections make a vast contribution to morbidity in the form of disability-adjusted life years (DALYs) lost (Fürst et al. 2012), and, in addition, infection with some trematodes has been strongly associated with cancer (Brindley and Loukas 2017). Despite the 56.2 million people infected with food-borne trematodiases (Fürst et al. 2012) and 239 million schistosome-infected people (Vos et al. 2012), these are still considered neglected tropical diseases and are receiving disproportionately low levels of attention, particularly when compared to other infectious diseases such as malaria and tuberculosis (Henry J Kaiser Family Foundation 2018; Reed and McKerrow 2018).

This lack of responsiveness from funding bodies is reflected by the relative paucity of genomic and proteomic studies and other "omic" resources currently available for trematodes. For example, despite the genome of a malaria parasite (*Plasmodium falciparum*) being first published in the early 2000s (Gardner et al. 2002), the first genome of a trematode (*Schistosoma japonicum*) was not released until 2009 (Berriman et al. 2009; The *Schistosoma japonicum* Genome and Functional Analysis 2009). Furthermore, most of the published genomes from trematodes are simply draft assemblies (Young et al. 2014; Coghlan et al. 2018) which, although providing useful information that can be used as a starting point in helminth research, are unfinished assemblages and usually contain errors that might lead to a limited or misinformed understanding of the parasite's biology. There have been further limitations in the study of trematodes and helminths in general, mainly the availability and accessibility of samples, which leads to a lack of physical material; however, newer sequencing technologies and sample preparation techniques have to some degree addressed these difficulties. Furthermore, gene prediction and post-assembly processes have also been challenging when applied to the study of helminths in general and trematodes in particular due to the lack of model organisms. Gene prediction software can now be trained with manually annotated and validated sets of genes, efficiently producing more accurate results in subsequent genome analyses (Holroyd and Sanchez-Flores 2012).

Despite these limitations, a significant effort from organisations such as the Wellcome Trust Sanger Institute (WTSI) and the Washington

J. Sotillo (✉) · M. S. Pearson · A. Loukas
Centre for Molecular Therapeutics, Australian Institute for Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia
e-mail: javier.sotillo@jcu.edu.au; mark.pearson@jcu.edu.au; alex.loukas@jcu.edu.au

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_13

University's Genome Institute (WUGI) has been made in recent years to provide the research community with more datasets, web-based resources and other tools for the study of trematodes (Howe et al. 2017; Martin et al. 2018). Recently, the International Helminth Genomes Consortium, involving researchers from WTSI, WUGI and other institutions, has published the most comprehensive comparative genomics analysis of 81 of the major parasitic helminths, including the genomes of 11 trematodes (5 schistosomatids, 2 liver flukes and 1 intestinal fluke) (Coghlan et al. 2018), providing a significant advance in the generation of necessary resources for research into parasitic worms.

The field of trematode proteomics has not advanced at the same rate as the genomics field. However, in the very recent years, the development of highly sensitive mass spectrometers, as well as the availability of novel databases, is enabling high-throughput analyses of trematode proteomes. The analysis of these proteomes will allow us to characterise the host–parasite molecular interface and understand the parasite's biology and its relationship with the host (Sotillo et al. 2017). New advances in technology and data processing such as proteogenomics are helping in data annotation of genes and genomes and provide a new approach to identify novel peptides and proteins involved in host–parasite interactions (Sotillo et al. 2017).

Herein, we summarise the recent advances in genomics and proteomics of trematodes, with an emphasis on the discovery of the genes and proteins involved in parasite establishment and survival that can be exploited for diagnostic and vaccine purposes. We also provide information on the different techniques, tools and resources currently available for the study of trematodes and their biology.

13.2 A Brief History of Trematode Genomic Sequencing

The chain termination sequencing method introduced by Sanger et al. in 1977 (Sanger et al. 1977) marked a major turning point in the history of DNA sequencing. Despite being widely used for prokaryotes and, even, parasitic protozoans (Gardner et al. 2002; Loman and Pallen 2015), it was not until 2009 that this technology was used to first sequence the genome of a trematode (Berriman et al. 2009) (Fig. 13.1). During the late 1990s, expressed sequence tag (EST) technology significantly contributed to the identification of transcripts from different parasites. For instance, in 1994, only 105 genes had been discovered for all *Schistosoma* spp.; however, the generation and analysis of ESTs allowed researchers to identify 607 ESTs from *S. mansoni* adult worms in 1995 (Franco et al. 1995) (Fig. 13.1). This cost-effective technique provided the scientific community with 163,000 ESTs from six developmental stages of *S. mansoni* less than a decade later (Verjovski-Almeida et al. 2003), and, in 2009, this number significantly increased for different platyhelminthes (~450,000 platyhelminth ESTs in GenBank) (Brindley et al. 2009).

The technology continued to develop, and other platforms such as Roche 454 and Illumina, which offered 1000-fold increases in throughput over the Sanger sequencing technology, became commercially available and started to be used by the parasitology community (The *Schistosoma japonicum* Genome and Functional Analysis 2009; Brindley et al. 2009). The increased read length and reduced cost have recently allowed researchers to sequence the transcriptomes and genomes of several trematodes of human importance (Huang et al. 2013; Young et al. 2014; McNulty et al. 2017) (Fig. 13.1).

Most of this work has been the result of a collaboration between several groups and the establishment of different consortia aimed at sequencing the transcriptomes and genomes of trematodes of human and agricultural importance (Holroyd and Sanchez-Flores 2012; Martin et al. 2015). The World Health Organization (WHO) established the Schistosoma Genome Network in 1995 with the aims of identifying new targets for vaccine and drug development by developing resources and providing genomic and proteomic datasets (Johnston 1997). The network generated a near-complete description of the genomic complement of two medically important trematodes

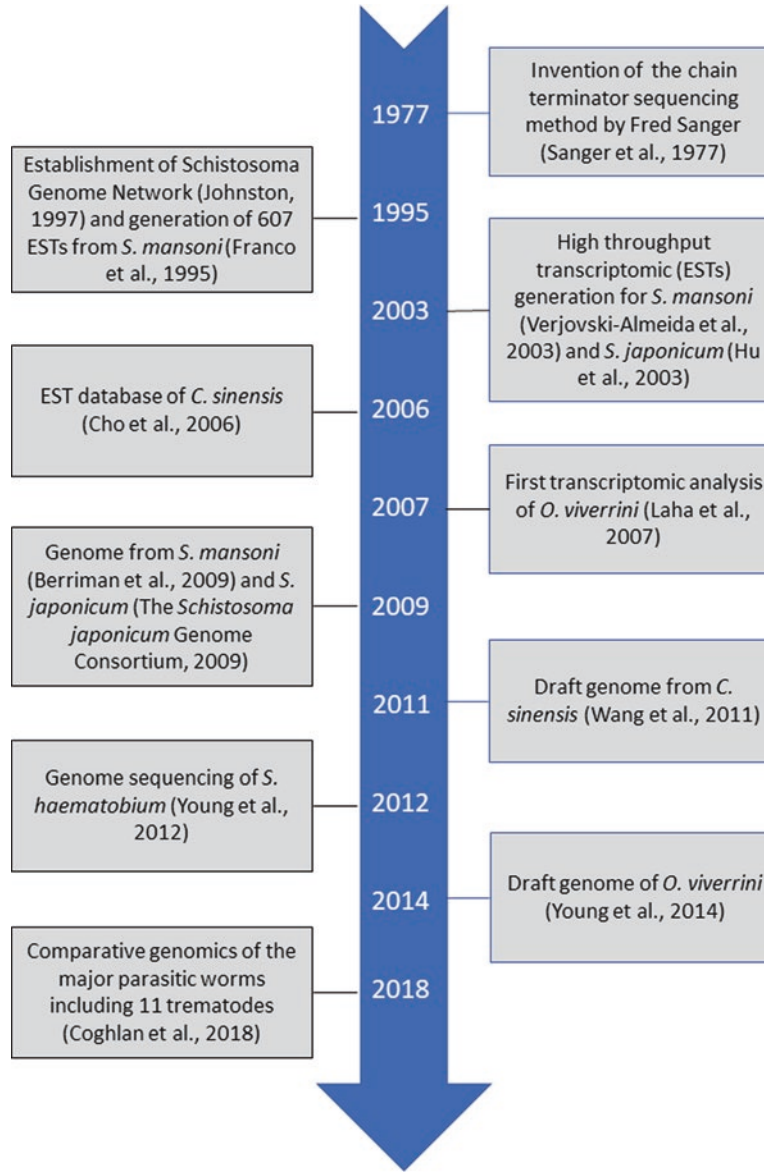

Fig. 13.1 Schematic of the major transcriptomic and genomic advances of trematodes detailed over time

by performing two different EST sequencing projects for *S. japonicum* and *S. mansoni* that were published in 2003 (Hu et al. 2003; Verjovski-Almeida et al. 2003) (Fig. 13.1). Together, these projects added 16,347 new sequences to GenBank and spurred a new era in the transcriptomics of not only *Schistosoma* but also helminth parasites in general (Sotillo et al. 2017).

More recently, researchers from WUGI and WTSI have been working together to sequence the genomes of trematode species of human, animal and agricultural importance (50 Helminth Genome Initiative (Wellcome Trust Sanger Institute 2014)). This initiative has resulted in the sequencing of 81 genomes from helminths, including 11 from trematodes, aimed at providing the scientific community with invaluable draft genome data focusing on the major differences between parasite lineages and providing information on key genes relevant to parasitism, the modulation of the immune response and parasite migration and feeding (Coghlan et al. 2018).

These fruitful collaborations have resulted not only in a flood of new genomic datasets but also in the development of tools and resources aiming to serve helminth parasitologists such as WormBase ParaSite (Howe et al. 2017), SchistoDB (Zerlotini et al. 2013), Trematode.net (Martin et al. 2015) and the resources provided by NIH-NIAID Schistosomiasis Resource Center at the Biomedical Research Institute (Cody et al. 2016). These resources will be explored in more detail in Sect. 13.5 of this chapter.

13.3 Trematode Genomics and Proteomics

13.3.1 Introduction

Trematodes can be classified according to numerous factors, including transmission route (i.e. plant-borne, food-borne), type of definitive host (i.e. trematodes of veterinary or human importance) and anatomic location of adult worms within the definitive host (i.e. liver flukes, intestinal flukes, blood flukes). For the purpose of comparing the genomic and proteomic features of these trematodes, the classification based on the site where the adult worm resides can provide more insights into the biology of the different parasites, including differences in the genes and proteins involved in the intra-organ migration and establishment of juvenile/adult worms, feeding and defence mechanisms. For instance, *Echinostoma caproni* does not undergo any intra-organ migration and settles in the intestine of the definitive host after ingestion, while *Opisthorchis viverrini* migrates from the stomach to the bile ducts after a short transit through the duodenum and ampulla of Vater (Nithikathkul et al. 2007), and *Fasciola hepatica* traverses the intestinal wall into the peritoneal cavity before migrating to the liver capsule and parenchyma (Cwiklinski et al. 2016). This difference in the biology of the parasites is reflected in, for example, the types of proteases and peptidases secreted by the worms. Similarly, molecules implicated in feeding and protection from oxidative stress and damage provoked by the host are different between the flukes residing in the blood (i.e. schistosomes) and the bile (*O. viverrini*, *Clonorchis sinensis* and *F. hepatica*) of the definitive hosts.

13.3.2 Liver Flukes

13.3.2.1 Background

The liver flukes are members of a polyphyletic group of platyhelminthes of veterinary and human importance (Lotfy et al. 2008). This group is comprised mainly of digeneans from the family Fasciolidae (such as *F. hepatica*, *Fasciola gigantica* and *Fasciola magna*) and Opisthorchiidae (*C sinensis*, *O. viverrini* and *Opisthorchis felineus*), which are distributed worldwide (Mas-Coma et al. 2009; Sripa et al. 2012). Liver flukes are probably the most pathogenic human-infecting trematodes in terms of parasite-associated morbidity. Indeed, both *O. viverrini* and *C. sinensis* are classified as Group 1 human biological agents (carcinogens) by the International Agency for Research on Cancer (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012). The infection routes for both families are completely different. While infection with members of the Opisthorchiidae occurs when contaminated fish is ingested raw or undercooked (Sripa et al. 2012), species belonging to the Fasciolidae family are plant-borne trematodes, and infection only occurs when watercress containing the infective stage metacercariae are eaten (Mas-Coma et al. 2009). The socioeconomic and medical importance of all liver flukes is indisputable, and, in addition, *F. hepatica* causes huge losses in the cattle industry (Torgerson and Claxton 1999; Arias et al. 2011; Jaja et al. 2017; Mazeri et al. 2017).

13.3.2.2 *Fasciola* spp.: Genomics and Proteomics

Infection by members of the genus *Fasciola* is one of the most important food-borne trematodiases in terms of both zoonotic and human impact (Torgerson et al. 2015). All *Fasciola* species follow a two-host life cycle, involving freshwater snails of the family Lymnaeidae (Bargues et al. 2001). While *F. hepatica* is distributed worldwide due to the extensive range of snail intermediate

hosts, *F. gigantica* has been detected in western, sub-Saharan and eastern Africa, as well as in countries from the Near East, such as Iran as a result of the presence of its specific lymnaeid vector, snails of the genus *Radix*. Due to the medical and socioeconomic importance as well as its wide distribution, *F. hepatica* has received generous attention from researchers and funding bodies which has resulted in a substantial amount of "omics" data.

Early studies focused on characterising the secretome of *F. hepatica* (*Fh*ES) using radiometabolic labelling to differentiate between the proteins of the various lifecycle stages, highlighting that proteins from immature worms were more immunoreactive than those from mature adult worms and that many of the immunodominant molecules were glycoproteins (Dalton et al. 1985; Irving and Howell 1982). However, it wasn't until 1995 when the first proteomic identification of *Fh*ES was performed (Tkalcevic et al. 1995), with a total of seven proteins, most of which were proteases including cathepsin B, cathepsin L and asparaginyl endopeptidase, being detected (Tkalcevic et al. 1995). In an attempt to characterise more *Fh*ES proteins, Jefferies et al. developed a 2D-gel method to separate and characterise the proteomes from *F. hepatica*, including somatic and *Fh*ES proteins (Jefferies et al. 2000); however, they did not identify any proteins using mass spectrometry.

Despite the advances in mass spectrometry technology and protein separation in the early 2000s, researchers still experienced difficulties in identifying proteins from *F. hepatica* and other trematodes due mainly to the lack of appropriate databases, which consequently bolstered efforts to address the genomic aspects of these parasites. Indeed, in 2001, the mitochondrial genome from *F. hepatica* was published, providing significant information for the study of parasite populations and for the development of diagnostic markers (Le et al. 2001). Furthermore, a significant advance in the study of *F. hepatica* was provided by the WTSI, who generated 6819 EST sequences and made them available for download in 2006 (ftp://ftp.sanger.ac.uk/pub/pathogens/*Fasciola/hepatica*/ESTs/). Despite the fact that these were unannotated sequences, this database was useful in subsequent proteomic studies (Chemale et al. 2006, 2010; Robinson et al. 2009). Several proteins of interest in host–parasite interactions such as glutathione S-transferases (GSTs), calcium-binding proteins, mucin-like proteins and the helminth-defence molecule were identified in the proteomic studies that made use of this resource (Chemale et al. 2006; Robinson et al. 2011; Banford et al. 2013).

Despite the high number of ESTs deposited by WTSI since 2006, these were still unannotated, and only a few hundred sequences available in GenBank were fully characterised and annotated by 2009. Furthermore, these sequences were highly redundant. Robinson et al. performed the first integrated transcriptomic and proteomic analysis of *F. hepatica*, making use of the large EST database from WTSI to characterise *Fh*ES, with particular emphasis on proteases as virulence and tissue-damaging factors (Robinson et al. 2009). Over 40% of the 160 identified cDNAs encoding secreted proteins were cathepsin L cysteine proteases, which were hypothesised to have a role in the digestion of blood macromolecules (Robinson et al. 2009). Furthermore, the second most abundant protein present in the analysis was a saposin-like molecule, also implicated in digestion (cell lysis) mechanisms, and a representative of a protein family whose members have been tested as vaccine candidates against *F. hepatica*, *F. gigantica* and other trematodes (Hillyer 2005; Kueakhai et al. 2013). In 2010, however, only 22 mRNA sequences had been deposited from juvenile adult worms in GenBank; thus, there was an increasing need to improve gene annotation and characterise all the ESTs from different life stages. Cancela et al. characterised 1684 ESTs from the juvenile stage, most of them stage-specific, showing that the parasite can produce molecules (i.e. proteases and other enzymes) in response to stage-specific needs such as migration or feeding (Cancela et al. 2010).

These latest studies led the way for Young and co-workers to provide the first high-throughput transcriptomic analysis of the adult stage of *F. hepatica* in 2010 (Young et al. 2010b) and *F. gigantica* in 2011 (Young et al. 2011). A total

of 590,927 high-quality *F. hepatica* sequence reads were generated, resulting in 15,423 supercontigs averaging 745 bp (±517 bp) which were enriched for open reading frames (ORFs). In addition, more than 40% of the ORF-enriched sequences were annotated using different bioinformatic approaches, which was a significant advance in the field (Young et al. 2010b). Similarly, the same group published the transcriptome of *F. gigantica* 1 year later, generating >20 million sequence reads before assembly that resulted in 30,513 ORF-enriched sequences (Young et al. 2011). Unsurprisingly, both datasets presented a high degree of similarity in gene composition, sharing, in particular, antioxidants, heat shock proteins and cysteine proteases (Young et al. 2011). Furthermore, other groups also generated significant amounts of high-quality contigs from *F. hepatica* adults using 454 sequencing that contributed significantly to the datasets interrogated in proteomic studies. For instance, Wilson and collaborators generated ~20,000 contigs that were used in the proteomic analysis performed on the tegument of *F. hepatica* adult worms, where 229 tegumental proteins were identified (Wilson et al. 2011).

The first genome assembly from *F. hepatica* (UK strain) was published as a draft in 2015 (Cwiklinski et al. 2015), and, only 1 year later, a reference genome was published for *F. hepatica* (Oregon strain) (McNulty et al. 2017) which was 1.14 Gb in size (N50 = 2036 and N50 length = 161 kb) and encoded for 14,642 predicted proteins (McNulty et al. 2017). The majority of gene sequence data was supported by RNA-Seq data (McNulty et al. 2017). An interesting observation from this study (and highlighting the utility of "omic" data to make inferences regarding parasite biological function) was that, despite the presence of anaerobic fermentation from carbohydrates to lactate as a metabolic pathway, energy metabolism is driven through the more energy-efficient malate dismutation pathway, as evidenced by identification of all the cognate enzymes (McNulty et al. 2017). Additionally, enzymatic pathways for fatty acid elongation by reversal of beta-oxidation and fatty acid catabolism are also present in the *F. hepatica* genome (as with other liver flukes and as opposed to blood flukes) which may be due to the high content of fatty acids in the bile ducts (McNulty et al. 2017).

Most interestingly, the sequencing *of F. hepatica* (Oregon and Uruguay isolates) led to the discovery of *Neorickettsia* as an endobacteria of this species, which was confirmed and validated using PCR and 16S rRNA sequencing. *Neorickettsia* were detected in varying numbers in the reproductive tissues (ovary, ootype, Mehlis' gland, vitelline glands) and in intrauterine eggs as well as in mature eggs isolated from liver tissue, but not in the somatic tissue, suggesting vertical transmission of this endobacteria (McNulty et al. 2017).

13.3.2.3 *Opisthorchis* spp.: Genomics and Proteomics

Two species from the genus *Opisthorchis* are medically important in humans: *O. viverrini* and *Opisthorchis felineus*. While the former is present in Southeast Asia in countries such as Thailand, Cambodia and Laos (Sripa et al. 2010a), the latter has been found mainly in Russia and Central-Eastern Europe (Pozio et al. 2013; Pakharukova and Mordvinov 2016). Infection with *O. viverrini* is a major public health problem not only because of the infection-associated morbidity but also because infection with this parasite leads to cholangiocarcinoma (CCA), a fatal bile duct cancer (Sripa et al. 2012). *O. felineus* has been less studied, but the importance of this parasite in some territories of the Russian Federation and some European countries is indisputable, with almost two million people infected (Pakharukova and Mordvinov 2016). Furthermore, there has been recent reports suggesting that this species should also be categorised as a class I carcinogen by IARC since multiple precancerous lesions have been observed in experimental models (Gouveia et al. 2017).

In the late 2000s, a combined effort between laboratories from Australia and Thailand led to the characterisation of 5000 cDNAs accounting for 1932 contigs, representing ~14% of the entire transcriptome (Laha et al. 2007). Using 454 sequencing technology, the transcriptome of *O. viverrini* was analysed and made publicly avail-

able in 2010 (Young et al. 2010a). From the cDNA libraries obtained and analysed in this study, over 60,000 nucleotide sequences contained a predicted ORF, from which 4144 were full-length transcripts and more than 24,000 were partial transcripts (start codon or stop codon only) (Young et al. 2010a). Despite this transcriptomic data being of great importance, there was a need for the generation and assembly of a genome that could give deeper insights into the biology of *O. viverrini*. In 2014, a draft genome for this carcinogenic trematode was published which was 634.5 Mb in size (N50 = 1,323,951 bp) and inferred to have 16,379 protein-encoding genes using RNA-Seq data (Young et al. 2014). The current version of this draft genome is available at www.parasite.wormbase.org (620 Mb in size, 16,356 coding genes and N50 = 1.3 Mb).

Deciphering the *O. viverrini* genome provided key information on the molecular biology of the parasite and how it has adapted to live in the hostile environment of the bile ducts. For instance, the parasite secretes a plethora of antioxidants such as GST to protect against free radicals present in the bile (Young et al. 2014). Indeed, GST is a good vaccine candidate as described later in this chapter. Furthermore, the parasite has adapted to a low-oxygen environment by (1) developing high oxygen affinity haemoglobin (Kiger et al. 1998) and (2) transcribing genes involved in anaerobic glycolysis, such as phosphoenolpyruvate carboxykinase (PEPCK), allowing the liver fluke to be a facultative anaerobe (Young et al. 2014). In addition, the bile ducts are enriched in lipidic nutrients, and the parasite has developed pathways involved in the absorption and uptake of these nutrients, including pathways required for the degradation and uptake of very-low (VLDL), low (LDL), intermediate (IDL) and high-density lipoproteins (HDL) (Young et al. 2014). Among these proteins, Young et al. identified different phospholipases like phospholipase A2, cysteine, serine and aspartic peptidases as well as proteins involved in amino acid transportation (Young et al. 2014). The inability of *O. viverrini* to synthesise cholesterol de novo has necessitated the evolution of proteins for the transport and absorption of this molecule, such as a homologue of the scavenger-like receptor (SR-B1), a LDLR-related protein 1 receptor, CD36-like receptors, an acid lipase, Niemann-Pick-like proteins and adenosine triphosphate-binding cassette transporter 1 complex4 (Young et al. 2014).

As with all the members from the class Trematoda, and most helminths in general, *O. viverrini* secretes different molecules that play a key factor in host–parasite interactions. When studying the secretome of helminths, most efforts have been focused on the study of the molecules aimed at suppressing the host immune response and facilitating parasite survival, including proteins involved in penetration and digestion; however, in the case of *O. viverrini*, secreted molecules that drive pathogenesis have received most attention in recent years (Suttiprapa et al. 2018). Furthermore, the ES products and tegument of the trematodes constitute the major interface between the parasite and its host, and its composition determines the success or failure of infection. The first high-throughput proteomic study aimed at characterising the molecules present in these two compartments was performed by Mulvenna et al. (2010b). To analyse the proteins present in the tegument of *O. viverrini*, Mulvenna et al. employed two different approaches: a sequential extraction of the tegument and a surface biotinylation approach. The sequential extraction of the tegument identified 160 proteins, mostly cytosolic and cytoskeletal and of mitochondrial origin (Mulvenna et al. 2010b). A total of 25 of these proteins were also identified using the biotinylation approach, where proteins from the external surface of the flukes were biotinylated and then isolated using streptavidin, a method which identifies proteins present only on the outer surface of live worms and minimises potential contamination with proteins located in the inner tegument. Recently, three members from the tetraspanin family of integral membrane proteins have been shown to be highly abundant in the fluke's tegument and extracellular vesicles (EVs) and have been shown to play a key role in tegument formation (Piratae et al. 2012; Chaiyadet et al. 2017). Knocking down the tetraspanin-encoding genes *Ov-tsp-1*, *Ov- tsp-2*

and *Ov-tsp-3* resulted in a highly vacuolated tegument (in the case of *Ov-tsp-1* and *Ov-tsp-2*), thinner tegument (in the case of *Ov-tsp-1*) or thicker but less electron-dense tegument (in the case of *Ov-tsp-3*) (Piratae et al. 2012; Chaiyadet et al. 2017). These results suggest that *O. viverrini* tetraspanins could be tested as potential vaccine candidates against opisthorchiasis, as they have been (effectively) in the case of other trematodiases (Merrifield et al. 2016).

The proteins secreted by *O. viverrini* (*Ov*ES) have also been shown to interact with the bile duct epithelium (Sripa and Kaewkes 2000; Chaiyadet et al. 2015), highlighting the importance of these molecules in host–parasite communications. Furthermore, proteins present in *Ov*ES have been shown to induce IL-6 secretion by human cholangiocytes (Chaiyadet et al. 2015) and promote cell proliferation (Smout et al. 2009, 2015). Mulvenna et al. characterised 43 proteins in *Ov*ES after culturing adult worms for 7 days (Mulvenna et al. 2010b) by performing OFFGEL fractionation coupled to LC-MS/MS and multiple reaction monitoring (MRM). Among the identified proteins, two molecules have received further attention due to their roles in carcinogenesis and pathogenesis: thioredoxin and granulin. In most eukaryotic systems, thioredoxin reductase (TrxR) and Trx peroxidase (TPx) form the thioredoxin (Trx) system, which plays an important role in protecting against oxidative damage. However, in *O. viverrini* and other trematodes, TrxR is replaced by thioredoxin glutathione reductase, which can reduce both Trx and glutathione (Tripathi et al. 2017; Suttiprapa et al. 2018). It has been shown that Trx can downregulate apoptotic genes and upregulate anti-apoptosis-related genes including apoptosis signalling kinase 1, caspase 9, caspase 8, caspase 3 and survivin (Matchimakul et al. 2015). Trx has been hypothesised to not only contribute to prevention of oxidative stress damage but also to inhibit apoptosis (Matchimakul et al. 2015; Suttiprapa et al. 2018).

A member of the granulin family, *Ov*-GRN-1, initially identified in *Ov*ES by Mulvenna et al. (2010b), has been shown to be a potent growth factor and have a role in tumour promotion (Laha et al. 2007; Smout et al. 2009, 2015). This protein is secreted by the adult worm, and transcriptomic data suggests that *Ov-grn-1* is highly expressed also in juvenile worms (Young et al. 2014). In addition to *Ov*-GRN-1, a second granulin (*Ov*-GRN-2) was also found in the *O. viverrini* genome as well as the progranulin, *Ov*-PRGN, which contains eight granulin domains (Young et al. 2014). However, only *Ov*-GRN-1 has been found in *Ov*ES. The cell-growth potential of *Ov*-GRN-1 was confirmed using antibodies against this protein, which blocked *Ov*ES-induced cell proliferation (Smout et al. 2009), and, furthermore, its effect on wound healing and angiogenesis has been recently validated using the full-length protein as well as some of its constituent peptides (Smout et al. 2015; Bansal et al. 2017).

Most likely as a result of adaptation to the harsh environment where *O. viverrini* adult worms live, the parasite can express a plethora of proteases. Indeed, there are 386 genes encoding for predicted proteases in the genome of this liver fluke, particularly metalloproteases and serine proteases, although other families such as aspartic and cysteine proteases are also expanded (Young et al. 2014). *O. viverrini* secretes a large amount of cathepsin F enzymes and far fewer cathepsin L proteases (Pinlaor et al. 2009; Kaewpitoon et al. 2008), whereas other liver flukes such as *F. hepatica* secrete predominantly cathepsin L, but not cathepsin F proteases (Robinson et al. 2008), which has been hypothesised to be related to the different migration patterns followed by both liver flukes during maturation (Suttiprapa et al. 2018). Different cathepsins (cathepsin B1 and cathepsin F1) have been shown to degrade fibronectin and the extracellular matrix at a different optimal pH, which could be a strategy of the parasite to digest large numbers of extracellular matrix proteins (Sripa et al. 2010b). Aspartic proteases such as *Ov*-APR-1 have been identified in *Ov*ES, as well as in the gut of the parasite, confirming a role for this protein in the digestion of erythrocyte and serum proteins (Suttiprapa et al. 2009, 2018). In this sense, it has been suggested that the parasite requires a multienzyme network (including *Ov*-CF-1, *Ov*-CB-1 and *Ov*-APR-1) to accomplish

complete digestion of haemoglobin as occurs in other trematodes, including *Schistosoma mansoni* (Skelly et al. 2014), and nematodes, such as *Ancylostoma caninum* (Williamson et al. 2004; Delcroix et al. 2006).

13.3.2.4 *Clonorchis sinensis*: Genomics and Proteomics

The liver fluke *C. sinensis* is also classified as a Group 1 carcinogen by the IARC (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012) and, as with *O. viverrini*, is linked to the development of CCA in humans (Brindley et al. 2015). This parasite is also a fish-borne trematode, and different species of snails and fish can act as first and second intermediate hosts, respectively, depending on the area and country (Tang et al. 2016). It is endemic in Vietnam, China, South Korea, North Korea and far-eastern Russia, and over 200 million people are at risk, with more than 15 million people infected worldwide (Tang et al. 2016).

During the mid-2000s, a total of ~8000 ESTs were identified from *C. sinensis* (Lee et al. 2003; Cho et al. 2006), and the complete transcriptome (>50,000 sequences assembled) was published in 2010 (Young et al. 2010a), laying the foundation for the following genomic and proteomic studies. Only 1 year later, another transcriptomic study and a draft genome were published for the oriental liver fluke (Wang et al. 2011; Yoo et al. 2011) which had a total size of 516 Mb with ~16,000 predicted protein-coding genes (N50 length = 42 kb) (Wang et al. 2011). As with *O. viverrini*, *C. sinensis* can obtain energy through aerobic or anaerobic pathways, which is a result of the diverse hosts and niches colonised by the different life stages (i.e. intermediate hosts—aerobic vs. bile ducts—anaerobic). In addition, genes related to proteases, energy metabolism, motility, antioxidants and reproduction are significantly expressed in adults when compared to other life stages (Yoo et al. 2011). A new reannotation of the genome (Table 13.1) has shown that, despite some pathways like glycolysis, the TCA cycle and oxidative phosphorylation being quite conserved with other helminths, the expression pattern of molecules involved in other metabolic pathways, such as the fatty acid related genes, was different, probably due, again, to the different needs of the adult worms as a result of the colonisation niche (Huang et al. 2013). In this sense, as with *O. viverrini* and in contrast to blood flukes and tapeworms, both juvenile and adult stages of *C. sinensis* transcribe genes involved in processing lipoproteins present in the bile (Young et al. 2014).

An analysis of the genome identified 749 proteins to be putatively secreted and 2275 to have a transmembrane domain (Wang et al. 2011). These two proteomes have been well characterised using different proteomic approaches.

Table 13.1 Main statistics of the genomes from trematodes discussed in this book chapter

	Genome size (Gb)	Scaffold length (Mb)	N50 length (kb)	Number predicted proteins	References
Clonorchis sinensis	0.547	547	417.5	13,634	Huang et al. (2013)
Echinostoma caproni	0.834	835	27	18,607	Coghlan et al. (2018)
Fasciola hepatica (isolate Oregon)	1.4	2036	161	14,642	McNulty et al. (2017)
Fasciola hepatica (isolate UK)	1.275	1000	204	22,676	Cwiklinski et al. (2015)
Opisthorchis viverrini	0.634	620	1323	16,356	Young et al. (2014)
Schistosoma haematobium	0.375	376	317.5	11,140	Young et al. (2012)
Schistosoma japonicum	0.402	403	176.9	12,738	The *Schistosoma japonicum* Genome and Functional Analysis (2009)
Schistosoma mansoni	0.409	410	50.5	14,519	Protasio et al. (2012)

When possible, the reannotation features were provided instead of the original assembly

For instance, in one of the first studies analysing the proteomes of *C. sinensis*, the secreted products (*Cs*ES) obtained after 4 h culture of adult worms were separated using a 2D-gel approach, and 340 protein spots were visualised (Ju et al. 2009). The authors built a custom-made database containing 28,762 ESTs and were able to identify 62 of the spots, including some detoxification enzymes (i.e. GST and TPx), and a number of cysteine proteases (Ju et al. 2009). Furthermore, using an immunoproteomic approach, they identified asparaginyl endopeptidases and cysteine proteases as proteins with the highest antigenicity. Further studies characterised a total of 110 proteins and identified fructose-1,6-bisphosphatase as a major antigen present in *Cs*ES (Zheng et al. 2011). The composition of *Cs*ES has also been studied over time after comparing samples at 0–3 h, 3–6 h, 6–12 h, 12–24 h, 24–36 h and 36–48 h post incubation of worms in DMEM (Zheng et al. 2013). A total of 187, 80, 103, 58, 248 and 383 proteins were found, respectively, and the authors selected methionine aminopeptidase 2 and the acid phosphatase for further exploration and molecular characterisation (Zheng et al. 2013). Despite the absence of a high-throughput analysis of the *C. sinensis* tegumental proteome, different individual proteins from the tegument have been well characterised, and some of them have been hypothesised to be druggable targets or effective vaccine candidates (Huang et al. 2007; Chung et al. 2018; Kim et al. 2017).

13.3.3 Blood Flukes

13.3.3.1 Background

Schistosomiasis is a debilitating disease caused by species from the genus *Schistosoma*. Three main species affect humans: *S. mansoni*, *S. japonicum* (both of them causing intestinal schistosomiasis) and *S. haematobium*, the agent of urogenital schistosomiasis (McManus et al. 2018). As with most trematodes, these parasites require a snail for an intermediate host: freshwater snails for *S. mansoni* and *S. haematobium* and amphibious snails for *S. japonicum*. Infection occurs when cercariae released by these snails penetrate the human host skin (Tucker et al. 2013), losing their tail and transforming into a schistosomula that will migrate through the body until reaching the mesenteric (*S. mansoni*, *S. japonicum*) or the perivesicular veins (*S. haematobium*) (Tucker et al. 2013).

While *S. haematobium* and *S. mansoni* are present in Africa and the Middle East, only the latter is found also in the Americas (Colley et al. 2014). *S. japonicum* is located in China and other countries of Asia (Colley et al. 2014). Pathogenesis is strongly related to deposition of eggs (Burke et al. 2009), which induce granulomas when trapped in the tissues leading to local inflammation and liver fibrosis (in the case of intestinal schistosomiasis) and a variety of urogenital pathogenesis and bladder disfunctions that can lead to squamous cell carcinoma (Burke et al. 2009; Barsoum 2013; Brindley and Loukas 2017) as well as in genital malignancy in women (Michelle-North et al. 2003; Chenault and Hoang 2006). Indeed, *S. haematobium* is one of the three helminths classified as a class I carcinogen by the IARC (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2012).

13.3.3.2 Genomics and Proteomics

Due to the socioeconomic and medical importance of *Schistosoma*, these parasites have received more attention than liver flukes or intestinal flukes. Indeed, supported by the World Health Organization (WHO), the Schistosoma Genome Network was initiated in 1995 to develop datasets and resources aimed at identifying new targets for vaccine and drug development (Johnston 1997). This network generated data every year that complemented the genomic information available in the mid-1990s (only 105 genes for all *Schistosoma* species available in GenBank in 1995) and that culminated in the sequencing of 168,347 EST sequences from *S. mansoni* and *S. japonicum* in 2003 (Hu et al. 2003; Verjovski-Almeida et al. 2003).

The first proteomic studies were performed in the early 1980s and focused on the investigation of the tegument of *S. mansoni*, which was thought to play a key role in the evasion of host immune responses among other functions (Roberts et al.

1983). Using alkaline phosphatase as an intrinsic marker, Roberts and co-workers developed the widely used method of freeze-thaw-vortex to remove the tegument (Roberts et al. 1983); unfortunately, due to the limitations on mass spectrometry at that time, very little information was obtained from the constituents of this tissue (Roberts et al. 1983). Two decades later, thanks to advances in mass spectrometry and the characterisation of a significant amount of genes that could be interrogated for proteomic studies, several proteomic analyses have been performed (Curwen et al. 2004; Cheng et al. 2005; Van Balkom et al. 2005; Braschi et al. 2006a, b; Braschi and Wilson 2006). One of these studies characterised the soluble proteins across four lifecycle stages from *S. mansoni* (Curwen et al. 2004), and another compared the male and female proteome of *S. japonicum* (Cheng et al. 2005), but both used the peptide mass fingerprinting identification approach after a 2D-gel separation, and only 32 and 27 spots were identified, respectively.

A seminal study identified 43 and 207 proteins in the tegument and stripped *S. mansoni* worms, respectively (while 179 were common to both fractions), although the limitations in protein annotations were evident at that time and almost 30% of the proteins presented no homology against any other species (Van Balkom et al. 2005). Braschi and co-workers also provided a comprehensive analysis of the tegumental proteins from *S. mansoni* by performing a differential extraction of the tegument using reagents of increasing solubilising power (Braschi et al. 2006b) as well as a tegument biotinylation approach of the surface-exposed proteins which were recovered using streptavidin beads (Braschi and Wilson 2006), identifying a significant amount of proteins compared to other studies.

The sequencing of the nuclear genome from *S. mansoni* provided new avenues for the study of the worm's biology (Berriman et al. 2009). This first assembly delivered a 363 Mb genome encoding at least 11,809 genes and provided more information on the deficit of the worm in lipid metabolism, as well as on the kinome and the proteases encoded by the parasite (Berriman et al. 2009). This assembly was further improved 3 years later by Protasio et al. (2012) by using Sanger capillary and deep-coverage Illumina sequencing from clonal worms. Furthermore, by using transcriptomic data, the number of encoded genes was updated to 10,852 and provided information on the temporal changes in the expression of 9535 of these genes (Table 13.1) (Protasio et al. 2012). The genome of the other two schistosomes of human importance—*S. japonicum* and *S. haematobium*—have also been published (The *Schistosoma japonicum* Genome and Functional Analysis 2009; Young et al. 2012), although these genomes have not been updated using new sequencing technologies or new transcriptomic or proteomic data.

Recent proteomic studies have made use of the available genomic information to identify novel tegumental and secreted proteins from different life stages of schistosomes. The *S. japonicum* proteins from different life stages have been well characterised (Mulvenna et al. 2010a; Hong et al. 2011, 2012; Zhang et al. 2013; Liu et al. 2015; Zhai et al. 2018). For instance, the proteins from the tegument of *S. japonicum* adult worms were revealed by biotinylation and tandem mass spectrometry (Mulvenna et al. 2010a). Proteins from *S. japonicum* schistosomula collected from hosts with different susceptibility to the parasite have been characterised, and the main differences were observed in proteins related with metabolism, stress responses and cellular movement (Hong et al. 2011). In general, fewer proteomic studies have been performed on *S. mansoni* extracts since the publication of the genome. A quantitative analysis of the proteins expressed on the tegument from *S. mansoni* schistosomula has been performed, providing information not only on the nature of proteins present on the parasite's tegument but also their changes during maturation of the schistosomula (Sotillo et al. 2015). Mathieson and Wilson characterised the *S. mansoni* egg secretions in order to understand the egg's interaction with the host and the formation of granulomas (Mathieson and Wilson 2010). Furthermore, effort has been devoted to understanding the relationships between the parasites and their intermediate hosts, and a proteomic

analysis of the *S. mansoni* miracidium provided information on proteases, venom allergen-like proteins and other proteins that might be involved in snail-parasite interplay.

13.3.4 Intestinal Flukes

13.3.4.1 Diplostomiasis, Gymnophalloidiasis and Heterophyidiasis

Not many trematodes are known to parasitise the intestines of humans. Parasites from the family Diplostomatidae, in particular members from the genera *Alaria*, *Neodiplostomum* and *Fibricola*, can parasitise humans, although only *Neodiplostomum seoulense* and *Fibricola cratera* colonise the intestine (Toledo et al. 2011). Other families, such as the Gymnophallidae and the Heterophyidae include some important human pathogens (i.e. *Gymnophallidae seoi*, *Haplorchis* spp. and *Metagonimus* spp.) (Toledo et al. 2011; Waikagul and Thaenkham 2014). Most of these trematodes can cause gastrointestinal discomfort, abdominal pain, fever, diarrhoea and even severe enteritis (Chai and Lee 2002; Chai et al. 2003). Furthermore, infection with *G. seoi* is associated with a variety of pancreatic diseases and may require medical attention (Chai et al. 2003).

Despite their medical importance in some countries, genomic and proteomic information on these species is scarce. Some immunological methods have been developed for the diagnosis of *Metagonimus yokogawai* and *Heterophyes taichui* and other metagonimiases, mainly using crude extracts from metacercariae and adult worms (Yull et al. 1987; Lee et al. 1993; Ditrich et al. 1991). More recently, a 100 kDa somatic antigen from *M. yokogawai* has been characterised. Monoclonal antibodies against this antigen also react with other trematodes including *G. seoi*, *Paragonimus westermani*, *C. sinensis* and *F. hepatica* (Han et al. 2014), which compromises its ability to be a suitable and specific diagnostic candidate against *M. yokogawai*.

Since heterophyidiasis is becoming a public health threat in several countries, some efforts have been made to characterise these parasites at a genomic level. For instance, the near-complete ribosomal transcription units of *Haplorchis pumilio* and *H. taichui* have been determined and annotated, providing new information that can be used for the diagnosis of heterophyid species of human importance (Le et al. 2017). Furthermore, a multiplex qPCR developed recently can detect and differentiate up to eight intestinal parasites including *M. yokogawai* and *G. seoi*, with high sensitivity and specificity (Won et al. 2016).

13.3.4.2 The Family Echinostomatidae: Genomics and Proteomics

The family Echinostomatidae comprises several hermaphroditic trematodes with an ubiquitous distribution (Toledo et al. 2009). They can parasitise a wide variety of definitive hosts, including birds, mammals, reptiles or fishes, and usually reside in the intestine of their definitive hosts, although other sites of infections have been observed (Toledo 2009). Infection with echinostomes in humans is rare and is usually limited to East and Southeast Asia (Chai 2009). In some parts of Cambodia, the prevalence ranges from 7.5 to 22.4% (Sohn et al. 2011).

Several echinostomatids, such as *E. caproni* and *Echinostoma friedi*, have been widely used as models to study intestinal trematode infections (Toledo 2009), which has supported the interest of researchers to understand the genomic and proteomic composition of these parasites. Several proteomic studies, including analysis of the secretome and the immunomodulatory proteins, from *E. caproni* and *E. friedi* have been performed, although the lack of a parasite-specific genomic database precluded researchers from performing a deep and high-throughput analysis (Sotillo et al. 2008, 2010; Cortés et al. 2016; Bernal et al. 2006; Guillou et al. 2007). Individual proteins from several echinostomatids, including *E. caproni* and *Echinostoma trivolvis*, have also been characterised, helping to decipher the biology of these worms (Pisciotta et al. 2005; Marcilla et al. 2007; Higón et al. 2008).

The gold standard in diagnosis of echinostomatids is the detection of eggs in the faeces using microscopy (Toledo et al. 2011). The molecular identification and diagnosis of echinostomatids

remain very challenging mainly because of the lack of genomic and proteomic data supporting these methods, and these approaches have only been assessed in experimental infections (Toledo et al. 2003, 2004).

Recently, effort has been devoted to deciphering the mitochondrial genome of some echinostomatids, in particular *Echinostoma hortense* (Liu et al. 2016) and *E. caproni* (unpublished, GenBank: AP017706.1). Mitochondrial genomes provide important information regarding population genetics and evolutionary studies and can offer valuable genetic markers for diagnostics. In the case of echinostomatids, this would be of great value since there is a significant controversy on the use of morphological features (i.e. size, shape, and arrangement of collar spines, the development of circumoral disc and also the testicular shape) for the classification of echinostomes (Kostadinova et al. 2003; Saijuntha et al. 2011).

The generation of a transcriptomic database using 454 sequencing technologies allowed the identification of 180 proteins present in the excretory/secretory (ES) products of *E. caproni* (Garg et al. 2013), which contrasts with the 39 proteins identified using the NCBInr database (Sotillo et al. 2010). Indeed, in the latter study, only 6.7% of the total identified peptides had homology and could be assigned to proteins despite using multiple search engines such as Mascot® (http://www.matrixscience.com/) and ProteinPilot® (https://sciex.com/products/software/proteinpilot-software) (Sotillo et al. 2010). This was hypothesised to be related to a lack of homology between the proteins secreted by *E. caproni* and the ones deposited in the NCBInr database at the time of the study, which is supported by the fact that mainly highly conserved metabolic enzymes and structural proteins, such as enolase, actin, glyceraldehyde 3P dehydrogenase (GAPDH) and aldolase (Sotillo et al. 2010), were identified. Indeed, these were also the types of proteins found when the secretions of *E. friedi* were compared from adult worms obtained from hosts with different degrees of permissiveness (Bernal et al. 2006).

Currently, the genome from *E. caproni* has been sequenced as part of the 50 Helminth Genome Initiative by the Parasite Genomic group at the WTSI, in collaboration with Rafael Toledo (Universidad de Valencia) (Coghlan et al. 2018), and is deposited in WormBase ParaSite (https://parasite.wormbase.org/Echinostoma_caproni_prjeb1207/Info/Index/). Using Illumina paired-end sequencing, they have generated a 835 Mb genome in length with a scaffold N50 size of 27 kb. This genome contains 18,607 genes; however, this is still a draft and the number might change.

13.3.5 Lung Flukes

13.3.5.1 Paragonimiasis

Paragonimiasis is a disease caused by trematodes from the genus *Paragonimus*. Different species affect humans and other mammalian hosts (mainly carnivores) which become infected when eating partially cooked or poorly processed crustaceans that contain the infective metacercariae (Blair et al. 1999). After ingestion, the metacercariae excyst in the small intestine and juvenile worms penetrate through the intestinal wall into the abdominal cavity, migrating through the diaphragm and the pleural cavity to finally establish in the lungs, where they start depositing eggs (Liu et al. 2008).

There are several species of lung flukes of human importance, including *Paragonimus westermani* and *Paragonimus heterotremus*, which are found predominately in Asia; *Paragonimus uterobilateralis* and *Paragonimus africanus*, which are endemic to Africa; and *Paragonimus kellicotti* and *Paragonimus mexicanus,* which are found in the Americas (Chai 2013).

Clinical symptoms of pulmonary paragonimiasis range from cough and blood-tinged sputum to even necrosis of lung parenchyma, fibrotic encapsulation and dyspnea (Liu et al. 2008; Sripa et al. 2010a). Furthermore, some ectopic cases in aberrant sites such as the brain have been reported (Chai 2013). The wide distribution of species of the family Paragonomidae and the estimation that almost 293 million people are at risk (Keiser and Utzinger 2009) make this disease a trematodiasis of medical and economic importance.

13.3.5.2 Genomics and Proteomics

Despite the significance of this disease, very little information on the genomic and proteomic aspects of *Paragonimus* spp. can be found. As an example, until 2015 only 456 protein sequences from the genus *Paragonimus* were available in NCBI's non-redundant protein database (NR) (Li et al. 2016). Early efforts from different groups aimed at characterising immunodominant antigens released by the adult parasite or the eggs. Wongkham and co-workers developed an immunoblotting test for the detection of IgG4 antibodies to a 31.5 kDa protein present in the ES products of *P. heterotremus* that was highly sensitive and specific (100% and 96.9%, respectively), although they didn't identify the antigen (Wongkham et al. 2005). A subsequent study identified a heat shock protein as one of the most immunodominant antigens from the eggs of *P. westermani* and determined its suitability as a serodiagnostic antigen (90.2% sensitivity and 100% specificity) (Lee et al. 2007). Furthermore, three novel cysteine proteases have been identified as immunodominant antigens from the ES products of adult *P. westermani* using 2D gels and MALDI-TOF/TOF, and their potential use in serodiagnosis has been suggested (Lee et al. 2006).

Recently, most efforts have focused on sequencing the mitochondrial genome (Biswal et al. 2014) and the transcriptome and nuclear genome from different species of the genus *Paragonimus* (McNulty et al. 2014; Blair et al. 2016; Li et al. 2016). Indeed, draft genomes of *P. westermani*, *P. skrjabini miyazakii*, *P. heterotremus* and *P. kellicotti* are available from Trematode.net (http://trematode.net/TN_frontpage.cgi) (Martin et al. 2018), although these results haven't been published yet. This rapid expansion in the genomic data available from species of the Troglotrematidae family is allowing for the development of markers for population studies (Biswal et al. 2014) and novel antigens to be used in serodiagnosis (McNulty et al. 2014; Li et al. 2016) of lung fluke infections.

13.4 Other Technologies

13.4.1 Proteome Microarrays

The upsurge in availability of "omics" information for the trematodes has set the stage for the development of new technology platforms that exploit this goldmine of bioinformatic and proteomic data to further our understanding of trematode biology. One such platform is the proteome microarray, which uses a cell-free protein expression system to produce hundreds or thousands of recombinant proteins which are then printed on nitrocellulose arrays coated on microscope slides. Originally developed to study the host humoral immune response to viral, bacterial and malaria infections (Doolan et al. 2003; Davies et al. 2005), the technology made use of available genomic information to array the predicted immunome (the protein subset of an organism that is capable of inducing a host immune response upon infection) of these pathogens, which was then probed with antisera from infected hosts to aid in vaccine and diagnostic antigen discovery.

Due to the availability of comprehensively annotated genomes for *S. mansoni* and *S. japonicum* (Berriman et al. 2009; The *Schistosoma japonicum* Genome and Functional Analysis 2009) and the publication of adult tegumental proteomes from these parasites (Van Balkom et al. 2005; Cheng et al. 2005; Braschi et al. 2006a, b; Mulvenna et al. 2010a), the first schistosome (in fact, the first multicellular pathogen) protein microarray was developed in 2010 (Driguez et al. 2010). This "proof of concept" array comprised a subset of 180 and 42 proteins from the *S. japonicum* and *S. mansoni* proteomes, respectively, which were predicted to be (1) immunogenic and potentially protective tegument antigens from the adult and schistosomula stages and (2) not cross-reactive with mammalian host homologues (Driguez et al. 2010). Further, this platform offered an advantage over other immunoproteomic approaches in that there is no inherent bias resulting from the differences in protein abundance of parasite preparations as there is no need for MS identification of antigens

(Driguez et al. 2010). Studies utilising this protein array have been oriented towards the identification of vaccine antigens and have identified numerous candidates, both previously documented (validating protein array technology as a vaccine antigen discovery tool) and novel, through probing with sera from animal models and human subjects with varying susceptibility to schistosomiasis (Gaze et al. 2014; McWilliam et al. 2014; Pearson et al. 2015; Driguez et al. 2015). An additional study probing the same array with sera from *S. japonicum*-infected subjects with different pathologies found that antibody signatures could discriminate between individuals with varying disease states (Driguez et al. 2016), implying that array technology such as this could also be used to identify antigens of prognostic value.

In a similar approach to identify *S. japonicum* antigens of diagnostic utility, 204 predicted signal peptide-containing proteins were expressed with GST fusion tags and attached to glutathione immobilised microplates, and this proteomic platform screened with sera from individuals positive and negative for *S. japonicum* infection. The most sero-reactive protein identified from the array screen, SjSP13—a saposin-like molecule, was then validated by ELISA against sera from 1371 individuals, confirming the significant diagnostic potential of this antigen identified by protein array technology (Xu et al. 2014).

Building on the success of the "proof of concept" schistosome array, a second-generation proteome microarray was constructed that comprised 992 proteins from *S. mansoni*, representing approximately 10% of the predicted proteome of 10,852 proteins (De Assis et al. 2016). Arrayed proteins were selected from various proteomic analyses of the juvenile and adult tegument (219 proteins) (Braschi and Wilson 2006; Castro-Borges et al. 2011a, b; Sotillo et al. 2015), an immunoproteomic screen of adult worm extracts with *S. mansoni*-infected human sera (59 proteins) and bioinformatic predictions of signal peptide (SP)-containing proteins (714 proteins). While a pilot array containing a randomly selected subset (92) of these proteins has been validated by probing with *S. mansoni*-infected human sera (De Assis et al. 2016), no subsequent study utilising the larger array has been published. In an effort to comprehensively apply protein array technology to the third medically important species of schistosome, *S. haematobium*, a proteome microarray for this parasite has been created which contains 993 proteins (8% of the predicted proteome of 13,073 proteins) selected from analyses of the tegument and ES proteomes (approximately 70% of selected proteins) and bioinformatic predictions of SP-containing proteins (approximately 30% of selected proteins) (Pearson et al. unpublished).

13.4.2 CRISPR/Cas9 Technology

Since the first application of RNAi to trematodes (silencing of the RNA encoding cathepsin B in *S. mansoni*) in 2005 (Correnti et al. 2005), our knowledge of gene function of these parasites has expanded rapidly, offering valuable insights into trematode biology and revealing genes encoding proteins essential for parasite survival which can be targeted for chemotherapeutic and immunotherapeutic intervention. RNAi studies in trematodes have undoubtedly benefited from the wealth of genomic data available for these parasites, but it is the gene-editing CRISPR/Cas9 technology which will be most enabled in this post-genomic era of the trematodes as careful and comprehensive gene annotations (e.g. the delineation of intron/exon boundaries, identification of protospacer adjacent motifs required for Cas9 nuclease function) are required for the successful design of the small guide RNAs which are the facilitators of the technique (Wang et al. 2016). While still in its relative infancy in the field of parasitology, the first applications of CRISPR/Cas9 technology to trematodes have been reported in *S. mansoni* (Ittiprasert et al. 2018) and *O. viverrini* (Arunsan et al. 2018). For the *S. mansoni* study, targeted editing of the egg ribonuclease, omega-1, resulted in an 80% reduction in omega-1 transcripts (despite only 4.5% of the sequenced amplicons spanning the target site

harbouring a mutation), reduced ribonuclease and Th2-polarising ability in egg lysates and a diminished volume of pulmonary granulomas surrounding mutated eggs after injection of the ova into the tail vein of mice (Ittiprasert et al. 2018). The carcinogenic growth factor, granulin, was the target of CRISPR/Cas9 gene editing in *O. viverrini*. Gene-edited newly excysted juvenile flukes, despite exhibiting many of the hallmarks of an active infection, induced attenuated biliary hyperplasia as long as 60 days postinfection—highlighting the stability of the gene-editing process and thus its potential to transform trematode functional genomics (Arunsan et al. 2018).

13.4.3 Metabolomics

Metabolomics, the study of the repertoire of endogenously synthesised small (non-proteinaceous) molecules of an organism (Lima et al. 2017), has been referred to as the "apogee of the omic trilogy" (Patti et al. 2012) because the molecules which are studied represent the endpoints of protein expression and function (characterised by proteomics) and which have been driven by gene expression and regulation (characterised by genomics). The development of increasingly sensitive spectrophotometric methods has enabled the identification of the entire metabolic repertoires, and thus metabolomics can be integrated with equally sensitive proteomics and genomics to facilitate systems-level understanding of biological processes.

This integrated approach has the capacity to transform our knowledge of trematode biology and parasitism; for example, the metabolic profile of specific disease states can be linked to proteomic make-up to help understand, predict and prevent infection and pathogenesis, and elucidation of novel biochemical pathways can identify parasite-specific enzymes which can serve as candidates for chemotherapeutic intervention. The literature on metabolic profiling of schistosomes and schistosome infection is relatively extensive (Lima et al. 2017), and similar studies are emerging for *Opisthorchis* (Kokova et al. 2017) and *Fasciola* (Saric et al. 2012). The ability to complement comprehensive metabolomic studies with proteomic and genomic data will undoubtedly facilitate more in-depth investigations into these parasites and the debilitating diseases they cause.

13.5 Tools and Resources

Since the early 2000s, different databases such as Nematode.net have provided useful tools for studying parasitic worms (Martin et al. 2015); however, this resource was mainly focused on nematodes. Recently, the group from WUGI introduced Trematode.net, a site dedicated to provide resources focused on the phylum Platyhelminthes. It currently includes data for 17 species, including intestinal trematodes like *E. caproni*, liver flukes such as *O. viverrini*, *C. sinensis*, *F. hepatica* and *F. gigantica*, blood flukes such as several species from the family Schistosomatidae and lung trematodes from the genus *Paragonimus*. WUGI is making a considerable effort to provide the community with a collection of databases hosting resources for helminths, all under the name of Helminth.net (www.helminth.net), providing comprehensive functional gene/protein annotation as well as tissue-specific expression information for nematodes (www.nematode.net), trematodes (www.trematodes.net) and cestodes in the near future (Cestodes.net).

In addition to serving as a repository for different "omics" data from trematodes, Trematode.net also provides additional services such as gene annotations (TremaBrowse), pathway visualisation (TremaPath), blast capability (TremaBlast) and microbiome interaction. Trematode.net hosts a central repository of data (TremaGene), which contains over one million RNA-Seq and almost eight million genomic reads as well as different mass spectrometry data such as immunoreactive proteins from different trematodes (www.trematodes.net). Using TremaGene, researchers can define a specific set of data (i.e. gene ontology, Kegg identifier or InterproScan annotation) for a particular species and download the protein and/or

nucleotide sequence (as available). Scientists can also search custom sequence(s) against deduced protein sets using TremaBlast and can visualise gene predictions and trematode genomes using TremaBrowse, both integrated in Trematode.net. Furthermore, TremaPath provides information about the enzymatic pathways present in given organisms based on actual transcript data (Martin et al. 2018).

Contemporaneous to the publication of the *S. mansoni* and *S. japonicum* genomes, an integrated genomic and functional genomic database for species from the genus *Schistosoma* (http://schistodb.net) was developed (Zerlotini et al. 2009). Initially, SchistoDB was developed to provide scientists with a visualisation tool of the *S. mansoni* genome and integrated other data obtained previously (such as ESTs) (Zerlotini et al. 2009); however, since its creation, it has been further developed, and it now integrates whole genome sequencing and annotation along with experimental data (Zerlotini et al. 2013). Using the same database structural framework as EuPathDB (Aurrecoechea et al. 2017), SchistoDB includes supplemental bioinformatic analyses and a web interface for data mining. As is the case with Trematode.net, the database also serves as a tool for sequence retrieval, blast and genome browser as well as for the visualisation of metabolic pathways.

Taking advantage of the infrastructure and expertise from the well-developed WormBase project, researchers from WTSI and the European Molecular Biology Laboratory (EMBL) have developed WormBase ParaSite (http://parasite.wormbase.org). This new resource provides consistent functional annotation information as well as gene expression and comparative analysis data by curating the data from 12 species of trematodes as well as cestodes, free-living flatworms, monogeneans and nematodes for a total of 148 species (Howe et al. 2017). Initiatives started by WTSI, such as the 50 Helminth Genome Initiative, have resulted in a flood of new draft genomes that are the foundation of WormBase ParaSite (Howe et al. 2017; Coghlan et al. 2018).

As with other resources, WormBase ParaSite's main mission is to make data publicly available and provide the tools for individual or bulk download (Howe et al. 2017). In addition, they have incorporated a set of tools and resources that facilitate genome annotation and visualisation. When available, gene annotations can be retrieved from the original source; however, they have also developed a pipeline that has been used to annotate most of the genomes sequenced as part of the 50 Helminth Genome Initiative as well as to provide comparative genomic data (Howe et al. 2017). A genome browser, blast and advanced querying tools are also available at WormBase Parasite, with results fully exportable in a variety of formats. Additional features are planned for the near future, such as the identification of putative targets for anti-helminthic drugs by linking the data from WormBase ParaSite with the ChEMBL database (Bento et al. 2014).

In addition to the tools aimed at providing data at the genomic and transcriptomic level, other platforms have been established to provide information and reagents from trematodes and other helminths. The Schistosome Related Reagent Repository (SR3), also known as the Schistosomiasis Resource Center at the Biomedical Research Institute (BRI) and funded by the National Institute of Allergy and Infectious Disease of the National Institutes of Health, has provided training, information and reagents to the scientific community for over 40 years (Cody et al. 2016; Lewis et al. 2008). Currently, the SR3 distributes reagents for research in schistosomiasis all over the world, including countries such as the USA, Canada, the UK and Australia (Lewis et al. 2008). Among these reagents they provide scientists with infected and uninfected intermediate snail hosts for all three human-infecting schistosomes (*Biomphalaria glabrata*, *Oncomelania hupensis* and *Bulinus truncatus*), as well as with nucleic acid (DNA and RNA) material from these snails and parasites. SR3 also provides with *Schistosoma* spp.-infected mice or hamsters as well as nucleic acids from *S. mansoni*, *S. japonicum* and *S. haematobium* (Cody et al. 2016). In addition to supplying material, the Schistosomiasis Resource Center provides educational courses on schistosomiasis aimed at training students and technicians on how to maintain the life cycle, as well as other

courses aimed at providing students with the necessary skills to study the molecular biology of schistosomes (Cody et al. 2016).

13.6 Conclusion

The last decade has seen an exponential growth in genomic and proteomic information from trematodes and helminths in general, from the first genome sequencing to the latest high-throughput proteomic analyses. Most of these advances arrived with the development of technologies such as second- (and third-)generation DNA sequencers and the high-sensitivity time of flight and ion trap mass spectrometers together with the growth in open-source software able to handle big data files.

Currently, we have transcriptomic and genomic data for over ten trematode species, including blood, liver and intestinal flukes; however, most of this data is still in an incomplete (draft) state. The third generation of DNA sequencers has the advantage of producing longer reads, which results in a less challenging genome assembly and transcript reconstruction. Indeed, this technology is providing reference-quality genome sequences for prokaryotes (Loman and Pallen 2015), and it will soon be applied to the field of helminthology. This technology together with proteomic data (proteogenomics) will advance the field significantly, providing high-quality information on the genes and proteins from trematodes that will form the basis for a deeper understanding of parasitism (Sotillo et al. 2017).

Once the problem of precise assemblies has been tackled, the challenge will reside in providing the right annotation for all genes, particularly for those lacking homologues in other species and conserved protein domains (Palevich et al. 2018). There is a substantial amount of "hypothetical" genes and proteins identified in the different datasets, and the availability of high-quality "omics" information will allow a lot of these genes to be updated with the right annotation. However, the unique parasitic lifestyles of helminths has resulted in evolution of novel and uncharacterised genes that allow them to adapt to the different niches they colonise, and these genes and proteins will need to be studied cooperatively by synergising efforts from different groups and combining different "omic" techniques with appropriate molecular technologies if we are to ever completely unravel the mysteries of parasite biology.

Acknowledgements This work was supported by a programme grant from the National Health and Medical Research Council (NHMRC) [programme grant number 1037304] and a Senior Principal Research fellowship from NHMRC to AL (1117504). The funders had no role in study design, decision to publish or preparation of the manuscript.

References

Arias M, Lomba C, Dacal V, Vázquez L, Pedreira J, Francisco I, Piñeiro P, Cazapal-Monteiro C, Suárez JL, Díez-Baños P, Morrondo P, Sánchez-Andrade R, Paz-Silva A (2011) Prevalence of mixed trematode infections in an abattoir receiving cattle from northern Portugal and north-west Spain. Vet Rec 168:408

Arunsan P, Ittiprasert W, Smout MJ, Cochran CJ, Mann VH, Chaiyadet S, Karinshak SE, Sripa B, Young ND, Sotillo J, Loukas A, Brindley PJ, Laha T (2018) Programmed mutation of liver fluke granulin using CRISPR/Cas9 attenuates virulence of infection-induced hepatobiliary morbidity. BioRxiv. https://doi.org/10.7554/eLife.41463

Aurrecoechea C, Barreto A, Basenko EY, Brestelli J, Brunk BP, Cade S, Crouch K, Doherty R, Falke D, Fischer S, Gajria B, Harb OS, Heiges M, Hertz-Fowler C, Hu S, Iodice J, Kissinger JC, Lawrence C, Li W, Pinney DF, Pulman JA, Roos DS, Shanmugasundram A, Silva-Franco F, Steinbiss S, Stoeckert JCJ, Spruill D, Wang H, Warrenfeltz S, Zheng J (2017) EuPathDB: the eukaryotic pathogen genomics database resource. Nucleic Acids Res 45:D581–D591

Banford S, Drysdale O, Hoey EM, Trudgett A, Timson DJ (2013) FhCaBP3: a *Fasciola hepatica* calcium binding protein with EF-hand and dynein light chain domains. Biochimie 95:751–758

Bansal PS, Smout MJ, Wilson D, Cobos-Caceres C, Dastpeyman M, Sotillo J, Seifert J, Brindley PJ, Loukas A, Daly N (2017) Development of a potent wound healing agent based on the liver fluke granulin structural fold. J Med Chem 60:4258–4266

Bargues MD, Vigo M, Horak P, Dvorak J, Patzner RA, Pointier JP, Jackiewicz M, Meier-Brook C, Mas-Coma S (2001) European Lymnaeidae (Mollusca: Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA ITS-2 sequences. Infect Genet Evol 1:85–107

Barsoum RS (2013) Urinary schistosomiasis: review. J Adv Res 4:453–459

Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, Krüger FA, Light Y, Mak L, Mcglinchey S, Nowotka M, Papadatos G, Santos R, Overington JP (2014) The ChEMBL bioactivity database: an update. Nucleic Acids Res 42:D1083–D1090

Bernal D, Carpena I, Espert AM, De La Rubia JE, Esteban JG, Toledo R, Marcilla A (2006) Identification of proteins in excretory/secretory extracts of *Echinostoma friedi* (Trematoda) from chronic and acute infections. Proteomics 6:2835–2843

Berriman M, Haas BJ, Loverde PT, Wilson RA, Dillon GP, Cerqueira GC, Mashiyama ST, Al-Lazikani B, Andrade LF, Ashton PD, Aslett MA, Bartholomeu DC, Blandin G, Caffrey CR, Coghlan A, Coulson R, Day TA, Delcher A, Demarco R, Djikeng A, Eyre T, Gamble JA, Ghedin E, Gu Y, Hertz-Fowler C, Hirai H, Hirai Y, Houston R, Ivens A, Johnston DA, Lacerda D, Macedo CD, McVeigh P, Ning Z, Oliveira G, Overington JP, Parkhill J, Pertea M, Pierce RJ, Protasio AV, Quail MA, Rajandream MA, Rogers J, Sajid M, Salzberg SL, Stanke M, Tivey AR, White O, Williams DL, Wortman J, Wu W, Zamanian M, Zerlotini A, Fraser-Liggett CM, Barrell BG, El-Sayed NM (2009) The genome of the blood fluke *Schistosoma mansoni*. Nature 460:352

Biswal DK, Chatterjee A, Bhattacharya A, Tandon V (2014) The mitochondrial genome of *Paragonimus westermani* (Kerbert, 1878), the Indian isolate of the lung fluke representative of the family Paragonimidae (Trematoda). PeerJ 2:e484

Blair D, Xu ZB, Agatsuma T (1999) Paragonimiasis and the genus *Paragonimus*. Adv Parasitol 42:113–222

Blair D, Nawa Y, Mitreva M, Doanh PN (2016) Gene diversity and genetic variation in lung flukes (genus *Paragonimus*). Trans R Soc Trop Med Hyg 110:6–12

Braschi S, Wilson RA (2006) Proteins exposed at the adult schistosome surface revealed by biotinylation. Mol Cell Proteomics 5:347–356

Braschi S, Borges WC, Wilson RA (2006a) Proteomic analysis of the schistosome tegument and its surface membranes. Mem Inst Oswaldo Cruz 101:205–212

Braschi S, Curwen RS, Ashton PD, Verjovski-Almeida S, Wilson A (2006b) The tegument surface membranes of the human blood parasite *Schistosoma mansoni*: a proteomic analysis after differential extraction. Proteomics 6:1471–1482

Brindley PJ, Loukas A (2017) Helminth infection-induced malignancy. PLoS Pathog 13:e1006393

Brindley PJ, Mitreva M, Ghedin E, Lustigman S (2009) Helminth genomics: the implications for human health. PLoS Negl Trop Dis 3:e538

Brindley PJ, Costa JMCD, Sripa B (2015) Why does infection with some helminths cause cancer? Trends Cancer 1:174–182

Burke ML, Jones MK, Gobert GN, Li YS, Ellis MK, McManus DP (2009) Immunopathogenesis of human schistosomiasis. Parasite Immunol 31:163–176

Cancela M, Ruétalo N, Dell'oca N, Da Silva E, Smircich P, Rinaldi G, Roche L, Carmona C, Alvarez-Valín F, Zaha A (2010) Survey of transcripts expressed by the invasive juvenile stage of the liver fluke *Fasciola hepatica*. BMC Genomics 11:227

Castro-Borges W, Dowle A, Curwen RS, Thomas-Oates J, Wilson RA (2011a) Enzymatic shaving of the tegument surface of live schistosomes for proteomic analysis: a rational approach to select vaccine candidates. PLoS Negl Trop Dis 5:e993

Castro-Borges W, Simpson DM, Dowle A, Curwen RS, Thomas-Oates J, Beynon RJ, Wilson RA (2011b) Abundance of tegument surface proteins in the human blood fluke *Schistosoma mansoni* determined by QconCAT proteomics. J Proteome 74:1519–1533

Chai JY (2009) Echinostomes in humans. In: Toledo R, Fried B (eds) The biology of echinostomes: from the molecule to the community. Springer, New York, pp 147–183

Chai JY (2013) Paragonimiasis. Handb Clin Neurol 114:283–296

Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 51:129–154

Chai JY, Choi MH, Yu JR, Lee SH (2003) *Gymnophalloides seoi*: a new human intestinal trematode. Trends Parasitol 19:109–112

Chaiyadet S, Smout M, Johnson M, Whitchurch C, Turnbull L, Kaewkes S, Sotillo J, Loukas A, Sripa B (2015) Excretory/secretory products of the carcinogenic liver fluke are endocytosed by human cholangiocytes and drive cell proliferation and IL6 production. Int J Parasitol 45:773–781

Chaiyadet S, Krueajampa W, Hipkaeo W, Plosan Y, Piratae S, Sotillo J, Smout M, Sripa B, Brindley PJ, Loukas A, Laha T (2017) Suppression of mRNAs encoding CD63 family tetraspanins from the carcinogenic liver fluke *Opisthorchis viverrini* results in distinct tegument phenotypes. Sci Rep 7:14342–14342

Chemale G, Morphew R, Moxon JV, Morassuti AL, Lacourse EJ, Barrett J, Johnston DA, Brophy PM (2006) Proteomic analysis of glutathione transferases from the liver fluke parasite, *Fasciola hepatica*. Proteomics 6:6263–6273

Chemale G, Perally S, Lacourse EJ, Prescott MC, Jones LM, Ward D, Meaney M, Hoey E, Brennan GP, Fairweather I, Trudgett A, Brophy PM (2010) Comparative proteomic analysis of triclabendazole response in the liver fluke *Fasciola hepatica*. J Proteome Res 9:4940–4951

Chenault C, Hoang MP (2006) An unusual cervical finding. Arch Pathol Lab Med 130:e37–e38

Cheng GF, Lin JJ, Feng XG, Fu ZQ, Jin YM, Yuan CX, Zhou YC, Cai YM (2005) Proteomic analysis of differentially expressed proteins between the male and female worm of *Schistosoma japonicum* after pairing. Proteomics 5:511–521

Cho PY, Lee MJ, Kim TI, Kang SY, Hong SJ (2006) Expressed sequence tag analysis of adult *Clonorchis sinensis*, the Chinese liver fluke. Parasitol Res 99:602

Chung E, Kim YJ, Lee MR, Cho SH, Ju JW (2018) A 21.6 kDa tegumental protein of *Clonorchis sinensis* induces a Th1/Th2 mixed immune response in mice. Immun Inflamm Dis 6(4):435–447

Cody JJ, Ittiprasert W, Miller AN, Henein L, Mentink-Kane MM, Hsieh MH (2016) The NIH-NIAID Schistosomiasis Resource Center at the Biomedical Research Institute: molecular redux. PLoS Negl Trop Dis 10:e0005022

Coghlan A, Tyagi R, Cotton JA, Holroyd N, Rosa BA, Tsai IJ, Laetsch DR, Beech RN, Day TA, Hallsworth-Pepin K, Ke HM, Kuo TH, Lee TJ, Martin J, Maizels RM, Mutowo P, Ozersky P, Parkinson J, Reid AJ, Rawlings ND, Ribeiro DM, Swapna LS, Stanley E, Taylor DW, Wheeler NJ, Zamanian M, Zhang X, Allan F, Allen JE, Asano K, Babayan SA, Bah G, Beasley H, Bennett HM, Bisset SA, Castillo E, Cook J, Cooper PJ, Cruz-Bustos T, Cuéllar C, Devaney E, Doyle SR, Eberhard ML, Emery A, Eom KS, Gilleard JS, Gordon D, Harcus Y, Harsha B, Hawdon JM, Hill DE, Hodgkinson J, Horák P, Howe KL, Huckvale T, Kalbe M, Kaur G, Kikuchi T, Koutsovoulos G, Kumar S, Leach AR, Lomax J, Makepeace B, Matthews JB, Muro A, O'Boyle NM, Olson PD, Osuna A, Partono F, Pfarr K, Rinaldi G, Foronda P, Rollinson D, Samblas MG, Sato H, Schnyder M, Scholz T, Shafie M, Tanya VN, Toledo R, Tracey A, Urban JF, Wang LC, Zarlenga D, Blaxter ML, Mitreva M, Berriman M, International Helminth Genomes (2018) Comparative genomics of the major parasitic worms. Nat Genet 51:163–174

Colley DG, Bustinduy AL, Secor WE, King CH (2014) Human schistosomiasis. Lancet 383:2253–2264

Correnti JM, Brindley PJ, Pearce EJ (2005) Long-term suppression of cathepsin B levels by RNA interference retards schistosome growth. Mol Biochem Parasitol 143:209–215

Cortés A, Sotillo J, Muñoz-Antolí C, Trelis M, Esteban JG, Toledo R (2016) Definitive host influences the proteomic profile of excretory/secretory products of the trematode *Echinostoma caproni*. Parasit Vectors 9:185

Curwen RS, Ashton PD, Johnston DA, Wilson RA (2004) The *Schistosoma mansoni* soluble proteome: a comparison across four life-cycle stages. Mol Biochem Parasitol 138:57–66

Cwiklinski K, Dalton JP, Dufresne PJ, La Course J, Williams DJ, Hodgkinson J, Paterson S (2015) The *Fasciola hepatica* genome: gene duplication and polymorphism reveals adaptation to the host environment and the capacity for rapid evolution. Genome Biol 16:71–71

Cwiklinski K, O'neill SM, Donnelly S, Dalton JP (2016) A prospective view of animal and human Fasciolosis. Parasite Immunol 38:558–568

Dalton JP, Tom TD, Strand M (1985) *Fasciola hepatica*: comparison of immature and mature immunoreactive glycoproteins. Parasite Immunol 7:643–657

Davies DH, Liang X, Hernandez JE, Randall A, Hirst S, Mu Y, Romero KM, Nguyen TT, Kalantari-Dehaghi M, Crotty S, Baldi P, Villarreal LP, Felgner PL (2005) Profiling the humoral immune response to infection by using proteome microarrays: high-throughput vaccine and diagnostic antigen discovery. Proc Natl Acad Sci U S A 102:547

De Assis RR, Ludolf F, Nakajima R, Jasinskas A, Oliveira GC, Felgner PL, Gaze ST, Loukas A, Loverde PT, Bethony JM, Correa-Oliveira R, Calzavara-Silva CE (2016) A next-generation proteome array for *Schistosoma mansoni*. Int J Parasitol 46:411–415

Delcroix M, Sajid M, Caffrey CR, Lim KC, Dvořák J, Hsieh I, Bahgat M, Dissous C, McKerrow JH (2006) A multienzyme network functions in intestinal protein digestion by a platyhelminth parasite. J Biol Chem 281:39316–39329

Ditrich O, Kopacek P, Giboda M, Gutvirth J, Scholz T (1991) Serological differentiation of human small fluke infections using *Opisthorchis viverrini* and *Haplorchis taichui* antigens. Southeast Asian J Trop Med Public Health 22:S174–S178

Doolan DL, Aguiar JC, Weiss WR, Sette A, Felgner PL, Regis DP, Quinones-Casas P, Yates JR, Blair PL, Richie TL, Hoffman SL, Carucci DJ (2003) Utilization of genomic sequence information to develop malaria vaccines. J Exp Biol 206:3789

Driguez P, Doolan DL, Loukas A, Felgner PL, McManus DP (2010) Schistosomiasis vaccine discovery using immunomics. Parasit Vectors 3:4

Driguez P, Mcwilliam HE, Gaze S, Piedrafita D, Pearson MS, Nakajima R, Duke M, Trieu A, Doolan DL, Cardoso FC, Jasinskas A, Gobert GN, Felgner PL, Loukas A, Meeusen E, Mcmanus DP (2015) Specific humoral response of hosts with variable schistosomiasis susceptibility. Immunol Cell Biol 94:52–65

Driguez P, Li Y, Gaze S, Pearson MS, Nakajima R, Trieu A, Doolan DL, Felgner PL, Hou X, Cardoso FC, Jasinskas A, Gobert GN, Loukas A, McManus DP (2016) Antibody signatures reflect different disease pathologies in patients with schistosomiasis due to *Schistosoma japonicum*. J Infect Dis 213:122–130

Franco GR, Adams MD, Soares MB, Simpson AJG, Venter JC, Pena SDJ (1995) Identification of new *Schistosoma mansoni* genes by the EST strategy using a directional cDNA library. Gene 152:141–147

Fürst T, Keiser J, Utzinger J (2012) Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect Dis 12:210–221

Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan MS, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DMA, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419:498

Garg G, Bernal D, Trelis M, Forment J, Ortiz J, Valero ML, Pedrola L, Martinez-Blanch J, Esteban JG, Ranganathan S, Toledo R, Marcilla A (2013) The transcriptome of *Echinostoma caproni* adults: further characterization of the secretome and identification of new potential drug targets. J Proteome 89:202–214

Gaze S, Driguez P, Pearson MS, Mendes T, Doolan DL, Trieu A, McManus DP, Gobert GN, Periago MV, Correa-Oliveira R, Cardoso FC, Oliveira G, Nakajima R, Jasinskas A, Hung C, Liang L, Pablo J, Bethony JM, Felgner PL, Loukas A (2014) An immunomics approach to schistosome antigen discovery: antibody signatures of naturally resistant and chronically infected individuals from endemic areas. PLoS Pathog 10:e1004033

Gouveia MJ, Pakharukova MY, Laha T, Sripa B, Maksimova GA, Rinaldi G, Brindley PJ, Mordvinov VA, Amaro T, Santos LL, Costa JMCD, Vale N (2017) Infection with *Opisthorchis felineus* induces intraepithelial neoplasia of the biliary tract in a rodent model. Carcinogenesis 38:929–937

Guillou F, Roger E, Moné Y, Rognon A, Grunau C, Théron A, Mitta G, Coustau C, Gourbal BEF (2007) Excretory–secretory proteome of larval *Schistosoma mansoni* and *Echinostoma caproni*, two parasites of *Biomphalaria glabrata*. Mol Biochem Parasitol 155:45–56

Han ET, Yang HJ, Park YJ, Park JH, Chai JY (2014) *Metagonimus yokogawai*: a 100-kDa somatic antigen commonly reacting with other trematodes. Korean J Parasitol 52:201–204

Henry J Kaiser Family Foundation (2018) The U.S government and global neglected tropical disease efforts. https://www.kff.org/global-health-policy/fact-sheet/the-u-s-government-and-global-neglected-tropical-diseases/. Accessed 22 Oct 2018

Higón M, Monteagudo C, Fried B, Esteban JG, Toledo R, Marcilla A (2008) Molecular cloning and characterization of *Echinostoma caproni* heat shock protein-70 and differential expression in the parasite derived from low- and high-compatible hosts. Parasitology 135:1469–1477

Hillyer GV (2005) Fasciola antigens as vaccines against fascioliasis and schistosomiasis. J Helminthol 79:241–247

Holroyd N, Sanchez-Flores A (2012) Producing parasitic helminth reference and draft genomes at the Wellcome Trust Sanger Institute. Parasite Immunol 34:100–107

Hong Y, Peng J, Jiang W, Fu Z, Liu J, Shi Y, Li X, Lin J (2011) Proteomic analysis of *Schistosoma japonicum* schistosomulum proteins that are differentially expressed among hosts differing in their susceptibility to the infection. Mol Cell Proteomics 10:M110.006098

Hong Y, Sun A, Zhang M, Gao F, Han Y, Fu Z, Shi Y, Lin J (2012) Proteomics analysis of differentially expressed proteins in schistosomula and adult worms of *Schistosoma japonicum*. Acta Trop 126:1–10

Howe KL, Bolt BJ, Shafie M, Kersey P, Berriman M (2017) WormBase ParaSite—a comprehensive resource for helminth genomics. Mol Biochem Parasitol 215: 2–10

Hu W, Yan Q, Shen DK, Liu F, Zhu ZD, Song HD, Xu XR, Wang ZJ, Rong YP, Zeng LC, Wu J, Zhang X, Wang JJ, Xu XN, Wang SY, Fu G, Zhang XL, Wang ZQ, Brindley PJ, McManus DP, Xue CL, Feng Z, Chen Z, Han ZG (2003) Evolutionary and biomedical implications of a *Schistosoma japonicum* complementary DNA resource. Nat Genet 35:139

Huang Y, Zhou Z, Hu X, Wei Q, Xu J, Wu Z, Yu X (2007) A novel tegumental protein 31.8 kDa of *Clonorchis sinensis*: sequence analysis, expression, and immunolocalization. Parasitol Res 102:77–81

Huang Y, Chen W, Wang X, Liu H, Chen Y, Guo L, Luo F, Sun J, Mao Q, Liang P, Xie Z, Zhou C, Tian Y, Lv X, Huang L, Zhou J, Hu Y, Li R, Zhang F, Lei H, Li W, Hu X, Liang C, Xu J, Li X, Yu X (2013) The Carcinogenic liver fluke, *Clonorchis sinensis*: new assembly, reannotation and analysis of the genome and characterization of tissue transcriptomes. PLoS One 8:e54732

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2012) Biological agents. Volume 100 B. A review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum 100:1–441

Irving DO, Howell MJ (1982) Characterization of excretory-secretory antigens of *Fasciola hepatica*. Parasitology 85:179–188

Ittiprasert W, Mann VH, Karinshak SE, Coghlan A, Rinaldi G, Sankaranarayanan G, Chaidee A, Tanno T, Kumkhaek C, Prangtaworn P, Mentink-Kane MJ, Cochran CJ, Driguez P, Holroyd N, Tracey A, Rodpai RH, Everts B, Hokke CH, Hoffmann KF, Berriman M, Brindley PJ (2018) Programmed genome editing of the omega-1 ribonuclease of the blood fluke, *Schistosoma mansoni*. bioRxiv. https://doi.org/10.7554/eLife.41337

Jaja IF, Mushonga B, Green E, Muchenje V (2017) Financial loss estimation of bovine fasciolosis in slaughtered cattle in South Africa. Parasite Epidemiol Control 2:27–34

Jefferies JR, Brophy PM, Barrett J (2000) Investigation of *Fasciola hepatica* sample preparation for two-dimensional electrophoresis. Electrophoresis 21: 3724–3729

Johnston DA (1997) The WHO/UNDP/World Bank Schistosoma genome initiative: current status. Parasitol Today 13:45–46

Ju JW, Joo HN, Lee MR, Cho SH, Cheun HI, Kim JY, Lee YH, Lee KJ, Sohn WM, Kim DM, Kim IC, Park BC, Kim TS (2009) Identification of a serodiagnostic antigen, legumain, by immunoproteomic analysis of excretory-secretory products of *Clonorchis sinensis* adult worms. Proteomics 9:3066–3078

Kaewpitoon N, Laha T, Kaewkes S, Yongvanit P, Brindley PJ, Loukas A, Sripa B (2008) Characterization of cysteine proteases from the carcinogenic liver fluke, *Opisthorchis viverrini*. Parasitol Res 102:757–764

Keiser J, Utzinger J (2009) Food-borne trematodiases. Clin Microbiol Rev 22:466–483

Kiger L, Rashid AK, Griffon N, Haque M, Moens L, Gibson QH, Poyart C, Marden MC (1998) Trematode hemoglobins show exceptionally high oxygen affinity. Biophys J 75:990–998

Kim YJ, Yoo WG, Lee MR, Kang JM, Na BK, Cho SH, Park MY, Ju JW (2017) Molecular and structural characterization of the tegumental 20.6-kda protein in *Clonorchis sinensis* as a potential druggable target. Int J Mol Sci 18:557

Kokova DA, Kostidis S, Morello J, Dementeva N, Perina EA, Ivanov VV, Ogorodova LM, Sazonov AE, Saltykova IV, Mayboroda OA (2017) Exploratory metabolomics study of the experimental opisthorchiasis in a laboratory animal model (golden hamster, *Mesocricetus auratu*s). PLoS Negl Trop Dis 11:e0006044

Kostadinova A, Hernious EA, Barret J, Littlewood DTJ (2003) Phylogenetic relationships of *Echinostoma rudolphi*, 1809 (Digenea: Echinostomatidae) and related genera re-assessed via DNA and morphological analyses. Syst Parasitol 54:18

Kueakhai P, Changklungmoa N, Riengrojpitak S, Chaichanasak P, Meemon K, Chaithirayanon K, Chantree P, Sansri V, Itagaki T, Sobhon P (2013) Vaccine potential of recombinant saposin-like protein 2 against Fasciolosis gigantica in mice. Vaccine 31:5518–5523

Laha T, Pinlaor P, Mulvenna J, Sripa B, Sripa M, Smout MJ, Gasser RB, Brindley PJ, Loukas A (2007) Gene discovery for the carcinogenic human liver fluke, *Opisthorchis viverrini*. BMC Genomics 8:189

Le TH, Blair D, McManus DP (2001) Complete DNA sequence and gene organization of the mitochondrial genome of the liver fluke, *Fasciola hepatica* L (Platyhelminthes; Trematoda). Parasitology 123:609–621

Le TH, Nguyen KT, Nguyen NTB, Doan HTT, Dung DT, Blair D (2017) The ribosomal transcription units of *Haplorchis pumilio* and *H. taichui* and the use of 28S rDNA sequences for phylogenetic identification of common heterophyids in Vietnam. Parasit Vectors 10:17

Lee SC, Chung YB, Kong Y, Kang SY, Cho SY (1993) Antigenic protein fractions of *Metagonimus yokogawai* reacting with patient sera. Korean J Parasitol 31:43–48

Lee JS, Lee J, Park SJ, Yong TS (2003) Analysis of the genes expressed in *Clonorchis sinensis* adults using the expressed sequence tag approach. Parasitol Res 91:283–289

Lee EG, Na BK, Bae YA, Kim SH, Je EY, Ju JW, Cho SH, Kim TS, Kang SY, Cho SY, Kong Y (2006) Identification of immunodominant excretory–secretory cysteine proteases of adult *Paragonimus westermani* by proteome analysis. Proteomics 6:1290–1300

Lee JS, Lee J, Kim SH, Yong TS (2007) Molecular cloning and characterization of a major egg antigen in *Paragonimus westermani* and its use in ELISA for the immunodiagnosis of paragonimiasis. Parasitol Res 100:677–681

Lewis FA, Liang Y-S, Raghavan N, Knight M (2008) The NIH-NIAID schistosomiasis resource center. PLoS Negl Trop Dis 2:e267

Li BW, McNulty SN, Rosa BA, Tyagi R, Zeng QR, Gu KZ, Weil GJ, Mitreva M (2016) Conservation and diversification of the transcriptomes of adult *Paragonimus westermani* and *P. skrjabini*. Parasit Vectors 9:497

Lima OE, Esteves CZ, Oliveira DN, Guerreiro TM, Melo CFOR, Catharino RR (2017) Mass spectrometry and metabolomics—a new approaches for helminth biochemical studies. In: Rodrigo L (ed) Human Helminthiasis. IntechOpen, London, pp 19–37

Liu Q, Wei F, Liu W, Yang S, Zhang X (2008) Paragonimiasis: an important food-borne zoonosis in China. Trends Parasitol 24:318–323

Liu M, Ju C, Du XF, Shen HM, Wang JP, Li J, Zhang XM, Feng Z, Hu W (2015) Proteomic analysis on cercariae and schistosomula in reference to potential proteases involved in host invasion of *Schistosoma japonicum* larvae. J Proteome Res 14:4623–4634

Liu ZX, Zhang Y, Liu YT, Chang QC, Su X, Fu X, Yue DM, Gao Y, Wang CR (2016) Complete mitochondrial genome of *Echinostoma hortense* (Digenea: Echinostomatidae). Korean J Parasitol 54:173–179

Loman NJ, Pallen MJ (2015) Twenty years of bacterial genome sequencing. Nat Rev Microbiol 13:787

Lotfy WM, Brant SV, Dejong RJ, Le TH, Demiaszkiewicz A, Rajapakse RPVJ, Perera VBVP, Laursen JR, Loker ES (2008) Evolutionary origins, diversification, and biogeography of liver flukes (Digenea, Fasciolidae). Am J Trop Med Hyg 79:248–255

Marcilla A, Pérez-García A, Espert A, Bernal D, Muñoz-Antolí C, Esteban JG, Toledo R (2007) *Echinostoma caproni*: identification of enolase in excretory/secretory products, molecular cloning, and functional expression. Exp Parasitol 117:57–64

Martin J, Rosa BA, Ozersky P, Hallsworth-Pepin K, Zhang X, Bhonagiri-Palsikar V, Tyagi R, Wang Q, Choi YJ, Gao X, McNulty SN, Brindley PJ, Mitreva M (2015) Helminth.net: expansions to Nematode.net and an introduction to Trematode.net. Nucleic Acids Res 43:D698–D706

Martin J, Tyagi R, Rosa BA, Mitreva M (2018) A multi-omics database for parasitic nematodes and trematodes. In: Kollmar M (ed) Eukaryotic genomic databases: methods and protocols. Springer, New York, pp 371–397

Mas-Coma S, Valero MA, Bargues MD (2009) *Fasciola*, Lymnaeids and Human Fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv Parasitol 69:41–146

Matchimakul P, Rinaldi G, Suttiprapa S, Mann VH, Popratiloff A, Laha T, Pimenta RN, Cochran CJ, Kaewkes S, Sripa B, Brindley PJ (2015) Apoptosis of cholangiocytes modulated by thioredoxin of carcinogenic liver fluke. Int J Biochem Cell Biol 65:72–80

Mathieson W, Wilson RA (2010) A comparative proteomic study of the undeveloped and developed *Schistosoma mansoni* egg and its contents: the miracidium, hatch fluid and secretions. Int J Parasitol 40:617–628

Mazeri S, Rydevik G, Handel I, Bronsvoort BMD, Sargison N (2017) Estimation of the impact of F*asciola hepatica* infection on time taken for UK beef cattle to reach slaughter weight. Sci Rep 7:7319

McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ, Zhou XN (2018) Schistosomiasis. Nat Rev Dis Primers 4:13

McNulty SN, Fischer PU, Townsend RR, Curtis KC, Weil GJ, Mitreva M (2014) Systems biology studies of adult paragonimus lung flukes facilitate the identification of immunodominant parasite antigens. PLoS Negl Trop Dis 8:e3242

McNulty SN, Tort JF, Rinaldi G, Fischer K, Rosa BA, Smircich P, Fontenla S, Choi YJ, Tyagi R, Hallsworth-Pepin K, Mann VH, Kammili L, Latham PS, Dell'oca N, Dominguez F, Carmona C, Fischer PU, Brindley PJ, Mitreva M (2017) Genomes of *Fasciola hepatica* from the Americas reveal colonization with *Neorickettsia* endobacteria related to the agents of Potomac horse and human Sennetsu fevers. PLoS Genet 13:e1006537

McWilliam HEG, Driguez P, Piedrafita D, McManus DP, Meeusen ENT (2014) Discovery of novel *Schistosoma japonicum* antigens using a targeted protein microarray approach. Parasit Vectors 7:290

Merrifield M, Hotez PJ, Beaumier CM, Gillespie P, Strych U, Hayward T, Bottazzi ME (2016) Advancing a vaccine to prevent human schistosomiasis. Vaccine 34:2988–2991

Michelle-North MD, Irin-Dubinchik MD, Adel-Hamid MD, Salah-Elderiny MD, Raja-Sayegh MD (2003) Association between cervical schistosomiasis and cervical cancer: a report of 2 cases. J Reprod Med 48:4

Mulvenna J, Moertel L, Jones MK, Nawaratna S, Lovas EM, Gobert GN, Colgrave M, Jones A, Loukas A, McManus DP (2010a) Exposed proteins of the *Schistosoma japonicum* tegument. Int J Parasitol 40:543–554

Mulvenna J, Sripa B, Brindley PJ, Gorman J, Jones MK, Colgrave ML, Jones A, Nawaratna S, Laha T, Suttiprapa S, Smout MJ, Loukas A (2010b) The secreted and surface proteomes of the adult stage of the carcinogenic human liver fluke *Opisthorchis viverrini*. Proteomics 10:1063–1078

Nithikathkul C, Tesana S, Sithithaworn P, Balakanich S (2007) Early stage biliary and intrahepatic migration of *Opisthorchis viverrini* in the golden hamster. J Helminthol 81:39–41

Pakharukova MY, Mordvinov VA (2016) The liver fluke *Opisthorchis felineus*: biology, epidemiology and carcinogenic potential. Trans R Soc Trop Med Hyg 110:28–36

Palevich N, Britton C, Kamenetzky L, Mitreva M, De Moraes Mourão M, Bennuru S, Quack T, Scholte LLS, Tyagi R, Slatko BE (2018) Tackling hypotheticals in helminth genomes. Trends Parasitol 34:179–183

Patti GJ, Yanes O, Siuzdak G (2012) Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 13:263

Pearson MS, Becker L, Driguez P, Young ND, Gaze S, Mendes T, Li XH, Doolan DL, Midzi N, Mduluza T, McManus DP, Wilson RA, Bethony JM, Nausch N, Mutapi F, Felgner PL, Loukas A (2015) Of monkeys and men: immunomic profiling of sera from humans and non-human primates resistant to schistosomiasis reveals novel potential vaccine candidates. Front Immunol 6:213

Pinlaor P, Kaewpitoon N, Laha T, Sripa B, Kaewkes S, Morales ME, Mann VH, Parriott SK, Suttiprapa S, Robinson MW, To J, Dalton JP, Loukas A, Brindley PJ (2009) Cathepsin F cysteine protease of the human liver fluke, *Opisthorchis viverrini*. PLoS Negl Trop Dis 3:e398

Piratae S, Tesana S, Jones MK, Brindley PJ, Loukas A, Lovas E, Eursitthichai V, Sripa B, Thanasuwan S, Laha T (2012) Molecular Characterization of a tetraspanin from the human liver fluke, *Opisthorchis viverrini*. PLoS Negl Trop Dis 6:e1939

Pisciotta JM, Ponder EL, Fried B, Sullivan D (2005) Hemozoin formation in *Echinostoma trivolvis* rediae. Int J Parasitol 35:1037–1042

Pozio E, Armignacco O, Ferri F, Gomez-Morales MA (2013) *Opisthorchis felineus*, an emerging infection in Italy and its implication for the European Union. Acta Trop 126:54–62

Protasio AV, Tsai IJ, Babbage A, Nichol S, Hunt M, Aslett MA, De Silva N, Velarde GS, Anderson TJC, Clark RC, Davidson C, Dillon GP, Holroyd NE, Loverde PT, Lloyd C, Mcquillan J, Oliveira G, Otto TD, Parker-Manuel SJ, Quail MA, Wilson RA, Zerlotini A, Dunne DW, Berriman M (2012) A systematically improved high quality genome and transcriptome of the human blood fluke *Schistosoma mansoni*. PLoS Negl Trop Dis 6:e1455

Reed SL, McKerrow JH (2018) Why funding for neglected tropical diseases should be a global priority. Clin Infect Dis 67:323–326

Roberts SM, MacGregor AN, Vojvodic M, Wells E, Crabtree JE, Wilson RA (1983) Tegument surface membranes of adult *Schistosoma mansoni*: development of a method for their isolation. Mol Biochem Parasitol 9:105–127

Robinson MW, Dalton JP, Donnelly S (2008) Helminth pathogen cathepsin proteases: it's a family affair. Trends Biochem Sci 33:601–608

Robinson MW, Menon R, Donnelly SM, Dalton JP, Ranganathan S (2009) An integrated transcriptomics and proteomics analysis of the secretome of the helminth pathogen *Fasciola hepatica*. Mol Cell Proteomics 8:1891

Robinson MW, Donnelly S, Hutchinson AT, To J, Taylor NL, Norton RS, Perugini MA, Dalton JP (2011) A family of helminth molecules that modulate innate cell responses via molecular mimicry of host antimicrobial peptides. PLoS Pathog 7:e1002042

Saijuntha W, Sithithaworn P, Duenngai K, Kiatsopit N, Andrews RH, Petney TN (2011) Genetic variation and relationships of four species of medically important echinostomes (Trematoda: Echinostomatidae) in South-East Asia. Infect Genet Evol 11:375–381

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci 74:5463

Saric J, Want EJ, Duthaler U, Lewis M, Keiser J, Shockcor JP, Ross GA, Nicholson JK, Holmes E, Tavares MFM (2012) Systematic evaluation of extraction methods for multiplatform-based metabotyping: application to the *Fasciola hepatica* metabolome. Anal Chem 84:6963–6972

Skelly PJ, Da'Dara AA, Li XH, Castro-Borges W, Wilson RA (2014) Schistosome feeding and regurgitation. PLoS Pathog 10:e1004246

Smout MJ, Laha T, Mulvenna J, Sripa B, Suttiprapa S, Jones A, Brindley PJ, Loukas A (2009) A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini,* promotes proliferation of host cells. PLoS Pathog 5:e1000611

Smout MJ, Sotillo J, Laha T, Papatpremsiri A, Rinaldi G, Pimenta RN, Chan LY, Johnson MS, Turnbull L, Whitchurch CB, Giacomin PR, Moran CS, Golledge J, Daly N, Sripa B, Mulvenna JP, Brindley PJ, Loukas A (2015) Carcinogenic Parasite secretes growth factor that accelerates wound healing and potentially promotes neoplasia. PLoS Pathog 11:e1005209

Sohn WM, Chai JY, Yong TS, Eom KS, Yoon CH, Sinuon M, Socheat D, Lee SH (2011) *Echinostoma revolutum* infection in children, Pursat Province, Cambodia. Emerg Infect Dis 17:117–119

Sotillo J, Valero L, Sánchez Del Pino MM, Fried B, Esteban JG, Marcilla A, Toledo R (2008) Identification of antigenic proteins from *Echinostoma caproni* (Trematoda) recognized by mouse immunoglobulins M, A and G using an immunoproteomic approach. Parasite Immunol 30:271–279

Sotillo J, Valero ML, Sánchez Del Pino MM, Fried B, Esteban JG, Marcilla A, Toledo R (2010) Excretory/secretory proteome of the adult stage of *Echinostoma caproni*. Parasitol Res 107:691–697

Sotillo J, Pearson M, Becker L, Mulvenna J, Loukas A (2015) A quantitative proteomic analysis of the tegumental proteins from *Schistosoma mansoni* schistosomula reveals novel potential therapeutic targets. Int J Parasitol 45:505–516

Sotillo J, Toledo R, Mulvenna J, Loukas A (2017) Exploiting helminth–host interactomes through big data. Trends Parasitol 33:875–888

Sripa B, Kaewkes S (2000) Localisation of parasite antigens and inflammatory responses in experimental opisthorchiasis. Int J Parasitol 30:735–740

Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ (2010a) Food-borne trematodiases in Southeast Asia: epidemiology, pathology, clinical manifestation and control. Adv Parasitol 72:305–350

Sripa J, Laha T, To J, Brindley PJ, Sripa B, Kaewkes S, Dalton JP, Robinson MW (2010b) Secreted cysteine proteases of the carcinogenic liver fluke, *Opisthorchis viverrini*: regulation of cathepsin F activation by autocatalysis and trans-processing by cathepsin B. Cell Microbiol 12:781–795

Sripa B, Brindley PJ, Mulvenna J, Laha T, Smout MJ, Mairiang E, Bethony JM, Loukas A (2012) The tumorigenic liver fluke *Opisthorchis viverrini*—multiple pathways to cancer. Trends Parasitol 28:395–407

Suttiprapa S, Mulvenna J, Huong NT, Pearson MS, Brindley PJ, Laha T, Wongkham S, Kaewkes S, Sripa B, Loukas A (2009) Ov-APR-1, an aspartic protease from the carcinogenic liver fluke, *Opisthorchis viverrini*: functional expression, immunolocalization and subsite specificity. Int J Biochem Cell Biol 41:1148–1156

Suttiprapa S, Sotillo J, Smout M, Suyapoh W, Chaiyadet S, Tripathi T, Laha T, Loukas A (2018) *Opisthorchis viverrini* proteome and host–parasite interactions. Adv Parasitol 102:45–72

Tang ZL, Huang Y, Yu XB (2016) Current status and perspectives of *Clonorchis sinensis* and clonorchiasis: epidemiology, pathogenesis, omics, prevention and control. Infect Dis Poverty 5:71–71

The *Schistosoma japonicum* Genome and Functional Analysis (2009) The *Schistosoma japonicum* genome reveals features of host-parasite interplay. Nature 460:345–351

Tkalcevic J, Ashman K, Meeusen E (1995) *Fasciola hepatica*—rapid identification of newly excysted juvenile proteins. Biochem Biophys Res Commun 213:169–174

Toledo R (2009) Echinostomes in the definitive host: a model for the study of host-parasite relationships. In: Toledo R, Fried B (eds) The biology of echinostomes: from the molecule to the community. Springer, New York, pp 185–206

Toledo R, Espert AM, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2003) Development of an antibody-based capture enzyme-linked immunosorbent assay for detecting *Echinostoma caproni* (trematoda) in experimentally infected rats: kinetics of coproantigen excretion. J Parasitol 89:1227–1231

Toledo R, Espert A, Muñoz-Antoli C, Marcilla A, Fried B, Esteban JG (2004) Kinetics of *Echinostoma caproni* (Trematoda: Echinostomatidae) antigens in feces and serum of experimentally infected hamsters and rats. J Parasitol 90:752–758

Toledo R, Esteban JG, Fried B (2009) Recent advances in the biology of Echinostomes. Adv Parasitol 69:147–204

Toledo R, Bernal MD, Marcilla A (2011) Proteomics of foodborne trematodes. J Proteome 74:1485–1503

Torgerson P, Claxton J (1999) Epidemiology and control. In: Dalton JP (ed) *Fasciolosis*. CABI International Publishing, Wallingford, pp 113–149

Torgerson PR, Devleesschauwer B, Praet N, Speybroeck N, Willingham AL, Kasuga F, Rokni MB, Zhou XN, Fèvre EM, Sripa B, Gargouri N, Fürst T, Budke CM, Carabin H, Kirk MD, Angulo FJ, Havelaar A, De Silva N (2015) World Health Organization estimates of the

global and regional disease burden of 11 foodborne parasitic diseases, 2010: a data synthesis. PLoS Med 12:e1001920

Tripathi T, Suttiprapa S, Sripa B (2017) Unusual thiol-based redox metabolism of parasitic flukes. Parasitol Int 66:390–395

Tucker MS, Karunaratne LB, Lewis FA, Freitas TC, Liang YS (2013) Schistosomiasis. Curr Protoc Immunol 103:19.1.1–19.1.58

Van Balkom BW, Van Gestel RA, Brouwers JF, Krijgsveld J, Tielens AG, Heck AJ, Van Hellemond JJ (2005) Mass spectrometric analysis of the *Schistosoma mansoni* tegumental sub-proteome. J Proteome Res 4:958–966

Verjovski-Almeida S, Demarco R, Martins EAL, Guimarães PEM, Ojopi EPB, Paquola ACM, Piazza JP, Nishiyama MY Jr, Kitajima JP, Adamson RE, Ashton PD, Bonaldo MF, Coulson PS, Dillon GP, Farias LP, Gregorio SP, Ho PL, Leite RA, Malaquias LCC, Marques RCP, Miyasato PA, Nascimento ALTO, Ohlweiler FP, Reis EM, Ribeiro MA, Sá RG, Stukart GC, Soares MB, Gargioni C, Kawano T, Rodrigues V, Madeira AMBN, Wilson RA, Menck CFM, Setubal JC, Leite LCC, Dias-Neto E (2003) Transcriptome analysis of the acoelomate human parasite *Schistosoma mansoni*. Nat Genet 35:148

Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Almazroa MA, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basáñez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabé E, Bhalla K, Bhandari B, Bikbov B, Abdulhak AB, Birbeck G, Black JA, Blencowe H, Blore D, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng ATA, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, De Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD (2012) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2163–2196

Waikagul J, Thaenkham U (2014) Approaches to research on the systematics of fish-borne Trematodes. Academic, Amsterdam

Wang X, Chen W, Huang Y, Sun J, Men J, Liu H, Luo F, Guo L, Lv X, Deng C, Zhou C, Fan Y, Li X, Huang L, Hu Y, Liang C, Hu X, Xu J, Yu X (2011) The draft genome of the carcinogenic human liver fluke *Clonorchis sinensis*. Genome Biol 12:R107

Wang H, La Russa M, Qi LS (2016) CRISPR/Cas9 in genome editing and beyond. Annu Rev Biochem 85:227–264

Wellcome Trust Sanger Institute (2014). http://www.sanger.ac.uk/science/collaboration/50hgp. Accessed 22 Oct 2018

Williamson AL, Lecchi P, Turk BE, Choe Y, Hotez PJ, Mckerrow JH, Cantley LC, Sajid M, Craik CS, Loukas A (2004) A multi-enzyme cascade of hemoglobin proteolysis in the intestine of blood-feeding hookworms. J Biol Chem 279:35950–35957

Wilson RA, Wright JM, De Castro-Borges W, Parker-Manuel SJ, Dowle AA, Ashton PD, Young ND, Gasser RB, Spithill TW (2011) Exploring the *Fasciola hepatica* tegument proteome. Int J Parasitol 41:1347–1359

Won EJ, Kim SH, Kee SJ, Shin JH, Suh SP, Chai JY, Ryang DW, Shin MG (2016) Multiplex real-time pcr assay targeting eight parasites customized to the Korean population: potential use for detection in diarrheal stool samples from gastroenteritis patients. PLoS One 11:e0166957

Wongkham C, Intapan PM, Maleewong W, Miwa M (2005) Evaluation of human IgG subclass antibodies in the serodiagnosis of paragonimiasis heterotremus. Asian Pac J Allergy Immunol 23:205–211

Xu X, Zhang Y, Lin D, Zhang J, Xu J, Liu YM, Hu F, Qing X, Xia C, Pan W (2014) Serodiagnosis of *Schistosoma japonicum* infection: genome-wide identification of a protein marker, and assessment of its diagnostic validity in a field study in China. Lancet Infect Dis 14:489–497

Yoo WG, Kim DW, Ju JW, Cho PY, Kim TI, Cho SH, Choi SH, Park HS, Kim TS, Hong SJ (2011) Developmental transcriptomic features of the carcinogenic liver fluke, *Clonorchis sinensis*. PLoS Negl Trop Dis 5:e1208

Young ND, Campbell BE, Hall RS, Jex AR, Cantacessi C, Laha T, Sohn WM, Sripa B, Loukas A, Brindley PJ, Gasser RB (2010a) Unlocking the transcriptomes of two carcinogenic parasites, *Clonorchis sinensis* and *Opisthorchis viverrini*. PLoS Negl Trop Dis 4:e719

Young ND, Hall RS, Jex AR, Cantacessi C, Gasser RB (2010b) Elucidating the transcriptome of *Fasciola hepatica*—a key to fundamental and biotechnological discoveries for a neglected parasite. Biotechnol Adv 28:222–231

Young ND, Jex AR, Cantacessi C, Hall RS, Campbell BE, Spithill TW, Tangkawattana S, Tangkawattana P, Laha T, Gasser RB (2011) A portrait of the transcriptome of the neglected trematode, *Fasciola gigantica*—biological and biotechnological implications. PLoS Negl Trop Dis 5:e1004

Young ND, Jex AR, Li B, Liu S, Yang L, Xiong Z, Li Y, Cantacessi C, Hall RS, Xu X, Chen F, Wu X, Zerlotini A, Oliveira G, Hofmann A, Zhang G, Fang X, Kang Y, Campbell BE, Loukas A, Ranganathan S, Rollinson D, Rinaldi G, Brindley PJ, Yang H, Wang J, Wang J, Gasser RB (2012) Whole-genome sequence of *Schistosoma haematobium*. Nat Genet 44:221

Young ND, Nagarajan N, Lin SJ, Korhonen PK, Jex AR, Hall RS, Safavi-Hemami H, Kaewkong W, Bertrand D, Gao S, Seet Q, Wongkham S, Teh BT, Wongkham C, Intapan PM, Maleewong W, Yang X, Hu M, Wang Z, Hofmann A, Sternberg PW, Tan P, Wang J, Gasser RB (2014) The *Opisthorchis viverrini* genome provides insights into life in the bile duct. Nat Commun 5:4378

Yull CS, Il KS, Yong KS (1987) Specific IgG antibody responses in experimental cat metagonimiasis. Korean J Parasitol 25:149–153

Zerlotini A, Heiges M, Wang H, Moraes RLV, Dominitini AJ, Ruiz JC, Kissinger JC, Oliveira G (2009) SchistoDB: a *Schistosoma mansoni* genome resource. Nucleic Acids Res 37:D579–D582

Zerlotini A, Aguiar ERGR, Yu F, Xu H, Li Y, Young ND, Gasser RB, Protasio AV, Berriman M, Roos DS, Kissinger JC, Oliveira G (2013) SchistoDB: an updated genome resource for the three key schistosomes of humans. Nucleic Acids Res 41:D728–D731

Zhai Q, Fu Z, Hong Y, Yu X, Han Q, Lu K, Li H, Dou X, Zhu C, Liu J, Lin J, Li G (2018) iTRAQ-based comparative proteomic analysis of Adult *Schistosoma japonicum* from water buffalo and yellow cattle. Front Microbiol 9:99

Zhang M, Hong Y, Han Y, Han H, Peng J, Qiu C, Yang J, Lu K, Fu Z, Lin J (2013) Proteomic analysis of tegument-exposed proteins of female and male *Schistosoma japonicum* worms. J Proteome Res 12:5260–5270

Zheng M, Hu K, Liu W, Hu X, Hu F, Huang L, Wang P, Hu Y, Huang Y, Li W, Liang C, Yin X, He Q, Yu X (2011) Proteomic analysis of excretory secretory products from *Clonorchis sinensis* adult worms: molecular characterization and serological reactivity of a excretory–secretory antigen-fructose-1,6-bisphosphatase. Parasitol Res 109:737–744

Zheng M, Hu K, Liu W, Li H, Chen J, Yu X (2013) Proteomic analysis of different period excretory secretory products from *Clonorchis sinensis* adult worms: molecular characterization, immunolocalization, and serological reactivity of two excretory secretory antigens—methionine aminopeptidase 2 and acid phosphatase. Parasitol Res 112:1287–1297

14 Diagnosis of Human Trematode Infections

J. Guillermo Esteban, Carla Muñoz-Antoli, Rafael Toledo, and Lawrence R. Ash

14.1 Introduction

Digenetic trematodes, or digeneans or flukes, are a major group of human parasites included within the phylum platyhelminthes, commonly called "flatworms." Although the number of fluke species parasitizing humans is large, some of them are only sporadically reported in humans or these may be isolated cases. However, this chapter focuses on the diagnosis of species of great medical importance as a large number of individuals are infected.

The human trematode species being the subject of this chapter are grouped according to the typical microhabitat in which the adult parasite usually resides. In this sense, four different groups will be considered: liver, lung, intestinal, and blood flukes. However, among the various parasite stages involved in the life cycle of each species, the egg, and the adult specimen can be found in the definitive host and can be used for the diagnosis of the trematode infection. Nevertheless, this chapter focuses mainly on the parasitological diagnosis based on the finding and recognition of eggs as they can be obtained more easily. Thus, in basic diagnosis, the clinical sample usually required for finding eggs of the trematode species included in each of these groups are: feces for liver, lung, intestinal, and blood flukes; urine for blood fluke species only; and sputum for lung flukes. But, the possibility that parasites may occur in sites other than their usual locations must always be considered.

In the first part of this chapter, the current status of knowledge on diagnostic techniques used when examining feces, urine, and sputum, which are the clinical samples used for direct or parasitological diagnosis based on the search, finding, and identification of trematode eggs is reviewed. Moreover, some general comments will be made on other diagnostic techniques.

The second part of the chapter is dedicated to the most significant characteristics used to identify eggs and also to other aspects concerning the patient's anamnestic data, as well as the analysis of the different morphological characteristics or particular features that have to be considered in the establishment of a precise diagnosis of the species included within each genus, for each of the trematode groups. Nevertheless, diagnosis at species level based on eggs might be complicated or even impossible given the morphological

J. G. Esteban (✉) · C. Muñoz-Antoli · R. Toledo
Área de Parasitología, Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain
e-mail: Jguillermo.Esteban@uv.es; Carla.Munoz@uv.es; Rafael.Toledo@uv.es

L. R. Ash
Infectious and Tropical Diseases, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA, USA
e-mail: larryash@ucla.edu

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6_14

convergence and similarity of some trematode eggs. Thus, it might be convenient to carry out a diagnosis at genus, or even family, level. Furthermore, some complementary information on other diagnostic techniques used, not exclusively based on the finding and recognition of eggs, will be given.

14.2 Diagnostic Techniques for Human Trematodiases

14.2.1 Parasitological Techniques

Microscopic demonstration of trematode eggs in feces, urine, and sputum remains the most widespread tool in fluke diagnosis. For each sample, the main techniques that can be used in parasitological diagnosis are compiled, which are habitually applied in laboratories of clinical parasitology as they are fundamental, well known, and can easily be reproduced. Nevertheless, it must be highlighted that there is no technique that may be considered definitive and unique and may fail when used in a single sample.

For fecal samples Flukes produce eggs, mostly passed in feces. As a consequence, fecal samples can be used for their diagnosis and the analysis of more than one sample per patient always increases the likelihood of a finding, particularly when keeping in mind the natural day-to-day fluctuations of fecal egg excretion.

Diagnosis can be carried out by wet mount, which is the simplest and most widely used technique for the examination of feces, and this method should be performed in all laboratories, especially in cases of heavy infections. However, if the number of eggs is low, as in cases of light infections, the examination of a small amount of feces used for a direct wet mount may be insufficient to reveal their presence. As the burden or intensity of parasitation of a patient cannot be known beforehand, the use of concentration techniques is always required.

Concentration techniques through the flotation of parasites in aqueous zinc sulfate, a saturated salt solution, or a magnesium sulfate solution are probably less efficient and, therefore, appear to be inappropriate for the detection of some flukes, basically those producing large eggs (i.e., *Fasciola, Fasciolopsis, Schistosoma*). Consequently, sedimentation procedures, achieved by simple gravity or through centrifugation, are probably most frequently used in diagnostic laboratories as the sediment will generally contain the eggs present in the stool sample. Among the different sedimentation techniques, the spontaneous sedimentation in tube technique, as well as the rapid sedimentation technique modified by Lumbreras, used for intestinal parasites, and, in particular, for *Fasciola hepatica* human infection diagnosis (Lumbreras et al. 1962; Marco-Flores et al. 2002; Terashima et al. 2009), stand out. The same sedimentation techniques, but using centrifugation, also called "diphasic sedimentation," i.e., the formalin-ether (or formalin-ethyl acetate; or formalin-gasoline) sedimentation and the MIF concentration techniques, depending upon the type of preservative used for fixed fecal samples (SAF or Formalin 10% and MIF, respectively), or some modifications of them, are the most highly effective ones (Ritchie 1948; Blagg et al. 1955; Allen and Ridley 1970; Knight et al. 1976; Ash and Orihel 1991). Nevertheless, the "diphasic sedimentation" by means of the formol-ethyl acetate sedimentation concentration technique has shown low sensitivity in the diagnosis of low intensity of some trematodiasis infections, at least when only a single stool sample is analyzed (Lier et al. 2009a).

A new technique for diagnosis has been proposed based on the differential sedimentation of eggs of *Schistosoma mansoni* when subjected to a slow continuous flux of 3% saline solution through a porous plaque (Coelho et al. 2009).

Quantitative coprological data analysis in epidemiological surveys is also possible using different quantitative methods. Stoll's dilution egg counting technique was one of the first techniques used (Stoll 1923).

Although not being habitually applied in hospital laboratories, the Kato-Katz technique is most widely used for diagnosing flukes in field surveys in numerous countries as it is simple, quantitative,

inexpensive, and provides reproducible results (Katz et al. 1972; WHO 1991; Ash et al. 1994) although sensitivity and negative predictive values are highly dependent on the prevalence and intensity of infection among the population. In this sense, when multiple samples (two or three repeated stool examinations on three consecutive days) are microscopically examined, a significant increase in the sensitivity of this technique is observed. However, among the various drawbacks of this technique, it should be cited that egg recognition between semipermeable cellophane and the microscope slide might often be difficult due to the deficient observation of some morphological details between slide and cover.

The more recent FLOTAC technique (Cringoli 2006; Cringoli et al. 2010) is a multivalent fecal egg count technique originally developed in veterinary medicine which has shown to be highly efficient in the diagnosis of soil-transmitted helminth infections (Utzinger et al. 2008; Knopp et al. 2009a, b; Habtamu et al. 2011). Although studies proving its wide applicability for the diagnosis of trematodiases are still scarce, this technique has shown to be useful, at least until now, for the diagnosis of schistosomiasis caused by *S. mansoni* (Glinz et al. 2010; Barda et al. 2013), human dicrocoeliasis (Jeandron et al. 2010, 2011; Gualdieri et al. 2011), and even experimental fascioliasis in rats (Duthaler et al. 2010). However, it is a more complicated technique and, consequently, may require improvements in terms of cost and accessibility for basic laboratories (Habtamu et al. 2011), as well as adequate training of laboratory workers. The Percoll separation technique for the detection of intestinal schistosomiasis is used in hospitals and laboratories dedicated to research and clinical investigations (Eberl et al. 2002).

For embryonated eggs containing a miracidium when passed in feces, i.e., schistosome eggs, the miracidial hatching technique (Cheng 1989), using a side-arm distillation flask, can be applied to help in the detection of miracidia that have hatched from the eggs. This long-established, yet tedious technique is a traditional approach for assessing trematode infection and has been used for more than five decades, and continues to be widely used, in some countries, e.g., China (Zhou et al. 2011). This method is simple, and its potential for high sensitivity has been recognized but not standardized for quantitative measurement (Ross et al. 2001). More recently, a similar technique has been proposed for diagnosing schistosomiasis in individual or large-scale investigations (Jurberg et al. 2008).

Finally, a new diagnostic technique, named by the authors "Helmintex," has been developed for the detection of schistosome eggs through their interaction with paramagnetic beads in a magnetic field (Teixera et al. 2007). Its high sensitivity may improve diagnosis in cases of light infections, e.g., in infected travellers with low burdens, although its cost-effectiveness must be assessed in relation to other techniques.

For urine samples Urine samples are primarily examined for *S. haematobium* eggs although it should also be noted that subjects with *S. mansoni* infection may pass eggs of this parasite in their urine. As the number of eggs in urine varies throughout the day, a single collection of terminal urine of at least 10 mL should be collected between 10 am and 2 pm, with prior physical exercise combined with the intake of liquids. Alternatively, a 24-h collection of terminal urine can be made. The entire sample must be examined as eggs may be very scarce. If urine is kept for an hour or longer, 1 mL of undiluted formalin (37% formaldehyde solution) should be added to each 100 mL of urine. This will preserve any eggs that might be present, but if not available, 2 mL of ordinary household bleach can be added to each 100 mL of urine (Ash and Orihel 1991; WHO 1991).

The methods used for the detection of eggs are based on sedimentation and filtration. The sedimentation method for a 24-h terminal urine collection is less sensitive but cheaper and simpler to perform. The syringe filtration method, using a polycarbonate or nylon filter (pore size 12–20 μm), or alternatively a paper filter, placed in a filter holder (diameter 13 or 16 mm), is used in public health care mainly when quantitative information is required (WHO 1991).

The miracidium hatching test, described above in fecal samples, may also be used for examining large quantities of urine.

For sputum samples Human paragonimiasis, the only lung fluke infection, may be diagnosed by finding characteristic eggs in sputum. In this infection, the sputum is often viscous and may be tinged with blood and brownish material (the so-called iron filings). When examined on a direct smear, it will show clusters of typical *Paragonimus* eggs. To ensure a good sample, the sputum must be collected in the early morning and the patient's mouth has to be rinsed with hydrogen peroxide beforehand. The sputum may be directly centrifuged and the sediment examined as a direct wet mount; if it is viscous, an equal volume of 3% sodium hydroxide must be added, centrifuged, and the sediment examined; or a formalin-ether concentration procedure may be performed on the sample before examination (Ash and Orihel 1991).

A recent study documents the usefulness and validity of the Ziehl-Neelsen staining technique for the detection of *Paragonimus* eggs in sputum slides, which appears to have superior sensitivity to the standard wet film direct sputum examination, the best cost-effectiveness, and, moreover, eliminates the risk of tuberculosis transmission (Slesak et al. 2011).

Due to the difficulties in obtaining a good clinical sample containing material from the lower respiratory passages, rather than a superficial sample consisting primarily of saliva, it is more common to look for *Paragonimus* eggs in feces using any of the aforementioned techniques for fecal samples.

14.2.2 Other Diagnostic Techniques

When patients are in the invasive or acute phase of the disease (consequently without egg production), or with a low parasite burden (therefore, with low shedding or even the absence of egg shedding), in chronic, ectopic, or even spurious infections, or when flukes are unable to reach maturity, parasitological techniques are inappropriate, and consequently other diagnostic techniques have to be applied for human fluke diagnosis. In this context, noninvasive (based on imaging diagnosis) and invasive (based of obtaining different aspirates and body fluids) techniques should be mentioned.

In the course of their normal life cycles, the adult specimens of trematodes settle in organs and tissues in such a manner that if these are examined along that time, the adult flukes or even the eggs may be found. Some trematodes, however, may migrate to organs or tissue other than typical, the so-called ectopic sites. Consequently, biopsy material or tissue removed during surgery or at necropsy can be examined directly or can be fixed and examined as stained histological sections for diagnostic purposes. It is a sensitive and specific clinical diagnostic method in routinary clinical practice, being neither simple nor convenient for population-based surveys. The recognition and proper identification of parasites, even their broad classification, in microscopic sections of human tissue may be impossible unless the microscopist has considerable experience. In this sense, the specimens should be submitted to parasitological experts. In addition, several texts and reviews that provide detailed information on the pathology of trematode infections and the morphology of parasites in tissue can also be consulted (Fried and Haseeb 1991; Orihel and Ash 1995; Meyers et al. 2000).

Immunodiagnostic tests provide the advantage of being applicable during all stages of the disease and, consequently, they are commonly used in the diagnosis of trematode infections in field situations. However, routine diagnosis in a laboratory of clinical parasitology is a very different matter.

Among the different immunodiagnostic techniques used, the Enzyme-Linked Immunosorbent Assays (ELISA) is the most sensitive and most widely used routine method for diagnosing many trematode infections. Nevertheless, all these immunological methods present problems related to the methodology used in the process of antigen obtention as well as false-positive results as a consequence of antigen and antibody detection

even after parasitological cure. Moreover, they often cannot distinguish between current and past infection and may even present different levels of cross-reactivity. For these reasons, molecular tools should be considered despite their higher cost and the requirement for special laboratory equipment (Bergquist et al. 2009).

In the last years, attention has focused on teleparasitology as a diagnostic tool as it allows the rapid web-transfer of parasitological microscopic images of specimen preparations in the field or laboratory to a local o central server where an expert in the field in question is able to establish a fast and qualified diagnosis (Johansen et al. 2010). An automated technique for counting parasite eggs in feces using fluorescence labelling, smartphone image capture, and computational image analysis has been developed (Slusarewicz et al. 2016), based on the optics and computational power of smartphones to produce convenient analytical instruments and diagnostic devices for use outside the parasitology laboratory (Ephraim et al. 2015; Sowerby et al. 2016).

Recently, Alva et al. (2017) developed a software based on pattern recognition analysis from microscopy digital images of fecal smears, capable of automatically recognizing and diagnosing common human parasites, among them *F. hepatica*. The mathematical algorithm processes the image by first converting it to grayscale, then applies a 14-step filtering process, and produces askeletonized and tricolored images. This algorithm has shown sensitivities of 99.1% to 100% and specificities of 98.1% to 98.3%, respectively.

14.3 Parasitological Features

14.3.1 General Features

Each trematode species produces eggs that exhibit a natural biological variation in size, but are, for the most part, highly uniform in shape, color, and developmental stage. When an egg or an egg-like object is found, some features should be carefully observed in order to make a specific identification. According to Ash and Orihel (2007), the most significant characteristics used to identify eggs are as follows:

- ***Size:*** Length and width constitute suitable criteria of great relevance in the case of many parasite species, as size variability can be considerable, from 18 μm to above 150 μm in length, and of a diameter between 12 and 14 μm or even above 90 μm.
- ***Shape:*** Egg shape also varies greatly, typically spheric to elongate or ovoid, being common in most species. Nonetheless, subtle differences in shape may be particularly relevant when separating species even within the same genus.
- ***Developmental stage after shedding:*** In trematode eggs, the developmental stage of the ovum within freshly passed eggs is characteristic for each parasite species. Concretely, they are unembryonated eggs containing vitelline cells (usually corresponding to eggs of a larger size) and others that are embryonated containing a miracidium (schisotosomes and eggs of a smaller size) when passed in feces.
- ***Thickness of eggshell:*** Usually, trematode eggs have a smooth shell that may vary considerably in thickness, depending on the species, although it is an important feature in separating eggs from vegetable cells or other plant material that may have irregular, limiting membranes. In some cases, modifications of the shell structure, i.e., operculum (present in all trematode eggs other than schistosome eggs), spines (only in schistosome eggs), and knobs (only in some eggs), may be important in the identification.

Besides the listed criteria, other aspects concerning the patient's anamnestic data have to be considered:

- ***Geographical distribution:*** The trematode group includes species that are typically cosmopolitan, while others have a concrete and specific distribution. Thus, a trematodiasis may be endemic in one country, area, or zone or may be present through isolated or sporadic human cases. Moreover, it has to be taken into account that several human fluke species may

occasionally appear very far away from the original endemic area. These are either imported cases resulting from international travel, refugees, seasonal workers, expatriates of NGOs or soldiers on international missions, children adopted abroad, immigrants, and visiting friends and relatives, or they may be related to the consumption of certain foodstuffs, above all imported fish, as fish exporters tend to send shipments by air freight and without freezing in order to reach a competitive edge in the market with the ensuing increase in the risk of different fish-borne trematodiasis for the consumer.

- The geographical distribution is related to the biological cycle of each species, which, from a general point of view, involves two or three different hosts: a vertebrate definitive host; an invertebrate first intermediate host (usually a gastropod mollusk), either terrestrial or aquatic according to the nature of the life cycle; and frequently, a second intermediate host (carrying an encysted metacercarial stage). Thus, it is the first intermediate host that, as a consequence of the marked specificity of the trematode species for their respective first intermediate snail host species, acts as a limiting factor of the geographical distribution of each trematode.
- ***Sociocultural, environmental, and hygienic factors:*** The assessment of these factors is crucial as they are related to the infection route. Among trematode species, two different routes are known: the great majority of species show an indirect or passive infection mechanism related to food habits (Fried et al. 2004; Chai et al. 2005; Keiser and Utzinger 2005, 2009; Mas-Coma et al. 2005, 2007, 2018; Graczyk and Fried 2007; Sithithaworn et al. 2007; Keiser et al. 2010; Sripa et al. 2010; Fürst et al. 2012a, b; Toledo et al. 2012; Hung et al. 2013). The definitive host becomes infected when the encysted metacercarial stage is ingested, either with the intermediate host (numerous invertebrates and poikilothermal vertebrates—amphibians, annelids, arthropods, fish, mollusks) being raw or undercooked, on vegetation or with water. Other species show a direct or active infection route related to contact with freshwater leading to the active penetration of the infective form (furcocercaria) through the skin.
- ***Clinical manifestations***: In certain trematodiases, the symptoms presented by the patients, basically in chronic phases of trematode diseases, may be useful in the establishment of the etiological diagnosis of the distomatosis. Nevertheless, patients often develop subclinical or only mild symptoms when the number of parasites is low, which may be the situation in numerous cases, so that infected subjects do not attend specialists. In other cases, no pathognomonic symptoms are involved and consequently may be confused with infections of a different etiology. Consequently, in these cases, symptomatology is of little importance when establishing the correct specific diagnosis.
- ***Other general features***: It is worth mentioning that atypical or distorted eggs may occasionally be seen, necessitating in these cases the search for more typical forms in order to make a reliable diagnosis. Also, the availability of trained and experienced personnel being able to visualize and recognize trematode eggs in different clinical samples is of utmost importance. In this context, along recent years, web-based virtual microscopy for parasitology is being adapted for education and quality assurance (Linder et al. 2008).

14.3.2 Particular Features

Liver Flukes Within this group, 11 species have been reported in humans (Mas-Coma and Bargues 1997; Mas-Coma et al. 2000), and what the most relevant of them have in common is their hepatic location (bile ducts and gall bladder) of the adult stage and eggs expelled through the host's feces. According to medical importance, owing to the number of people infected, six species of four genera (*Clonorchis, Opisthorchis*, *Dicrocoelium*, and *Fasciola*) belonging to three trematode families (Opisthorchiidae, Dicrocoeliidae, and Fasciolidae) are the subject of the present analysis.

Genus *Clonorchis* The eggs of the so-called Chinese or Oriental liver fluke, *C. sinensis*, are broadly ovoid, moderately thick-shelled, light yellowish-brown, with a large convex seated operculum, which fits into a rimmed extension of the eggshell, giving a "shoulder-like" appearance. At the abopercular end, there is often a small protuberance or small knob (Fig. 14.1a). Mature eggs are embryonated when laid, containing a miracidium, and measure 26–35 × 12–19 µm (average 29 × 16 µm), which is the typical morphology of eggs of this species in feces or duodenal fluid using light microscopy (Ash and Orihel 2007). Nevertheless, in patients with biliary obstruction, eggs are not passed in stools but may be found in bile sediments, appearing aggregated and adhered to or wrapped with bilirubinate particles or mucoid matter, as well as in gallstones, where they appear deformed with eggshell thickening, in which deposits of bilirubinate or mucoid matter may be observed, having lost the operculum, or without a miracidium (Quiao et al. 2012).

As a consequence of the small size of eggs, and the need to differentiate them from eggs of the other two major opisthorchiids (mainly *O. viverrini*) and heterophyid (i.e., *Heterophyes, Metagonimus, Haplorchis*) flukes, which may be

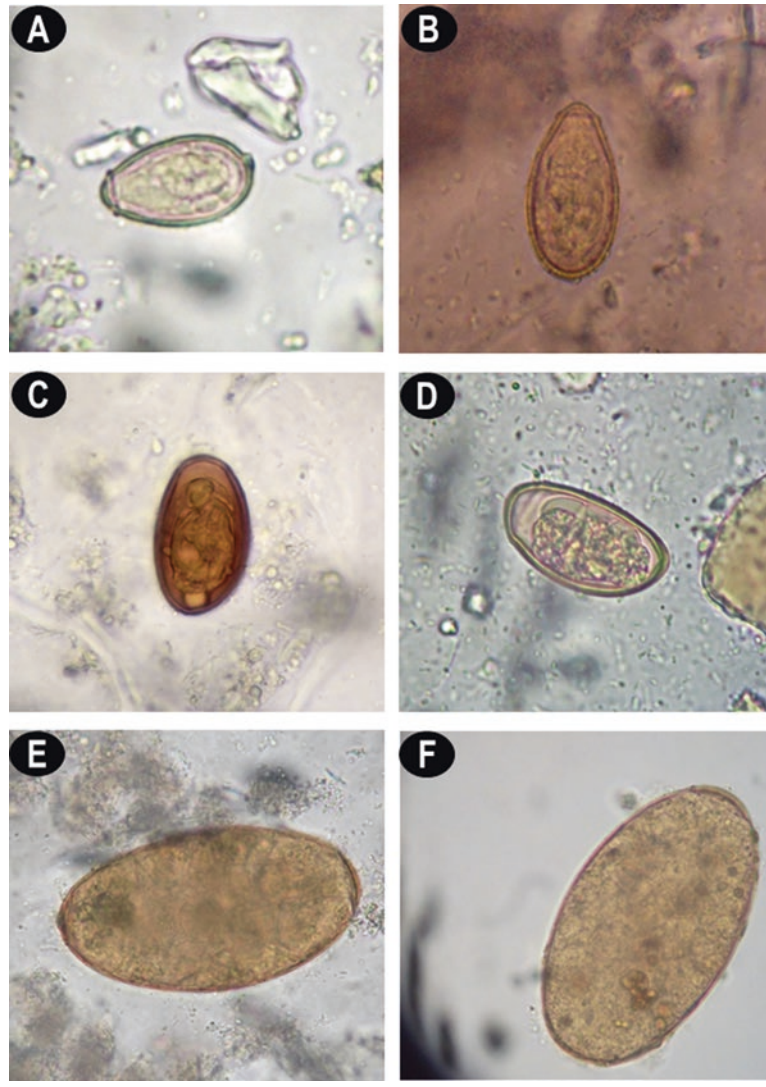

Fig. 14.1 Photomicrographs of various eggs of liver flukes detected in formalin-preserved human fecal material: (**a**) *Clonorchis sinensis* (size: 27.5 × 17.5 µm); (**b**) *Opisthorchis* sp. (30.0 × 15.0 µm); (**c**, **d**) *Dicrocoelium dendriticum* (42.5 × 22.5 µm and 41.3 × 22.5 µm, respectively); and (**e**, **f**) *Fasciola hepatica* (127.5 × 72.5 µm and 130.0 × 77.5 µm, respectively)

present in the same area, parasitological diagnosis must be carried out with caution (Rim 2005; Doanh and Nawa 2016). In fact, in a comparative study carried out between eggs of *C. sinensis* and Heterophyidae in Korea, the results revealed that differential diagnosis of human infection by fecal examination is inconclusive, and thus isolation of adult worms is required to determine the exact species (Lee et al. 1984). Nevertheless, the "shoulders" of eggs have been used for differentiation, as they are usually not as prominent in *O. viverrini*, and heterophyid eggs usually have an inconspicuous operculum flush with its shell surface. The surface ultrastructure of eggs by scanning electron microscopy in Korea revealed that *C. sinensis* eggs are covered with prominent and elevated ridges called "muskmelon-like structures," which were not observed in some heterophyid (i.e., *Metagonimus, Heterophyes, Stellantchasmus, Stictodora, Pygidiopsis*) and gymnophallid (*Gymnophalloides*) eggs (Lee et al. 2012a).

Different types of cholangiography, mainly retrograde endoscopic cholangiopancreatography, as well as ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and tissue harmonic imaging are techniques which permit obtention of radiological images used for the clinical diagnosis of pathological effects and complications derived from clonorchiasis infection, particularly in subjects with moderate or heavy infection (Choi et al. 2004a, b; Rim 2005; Choi and Hong 2007; Tang et al. 2016). However, these methods exhibit relatively poor sensitivity and are nonspecific, and it can be difficult for inexperienced staff to use these methods (Tang et al. 2016).

Various antigen proteins of *C. sinensis* have been identified in the crude extract and excretory-secretory products of adult worms or purified from soluble extracts of worm lysates, and also some recombinant *C. sinensis* proteins have been obtained (Kim et al. 2009; Hong and Fang 2012; Huang et al. 2012b; Lee et al. 2012b; Li et al. 2012; Johansen et al. 2015; Tang et al. 2016). They have either been used unblended or in cocktails for diagnostic purposes in various immunological tests, i.e., an intradermal test (IDT), an indirect hemagglutination test (IHA), counter-immunoelectrophoresis (CIEP), a complement fixation test (CFT), an indirect fluorescent antibody test (IFAT), and an ELISA (Chen et al. 1994; Sithithaworn et al. 2007; Hong and Fang 2012). Among them, ELISA is the most commonly used technique for antibody and circulating antigen detection in serological tests, as well as for urinary antibody and stool antigen detections (Chen et al. 1994; Mazidur Rahman et al. 2012; Tang et al. 2016). However, the results obtained with this particular technique present different levels of sensitivity and specificity depending upon of high and low-risk patient groups (Kim et al. 2010; Hong and Fang 2012).

In recent years, various PCR-based techniques have been developed for detecting different stages of *C. sinensis* in humans and animals (fishes/snails) in different clinical samples (fecal samples; stones from bile ducts; liver tissue) (Petney et al. 2013; Johansen et al. 2015; Tang et al. 2016). Conventional PCR and real-time PCR have been applied to diagnose *C. sinensis* (Jang et al. 2007; Traub et al. 2009; Huang et al. 2012a, b; Quiao et al. 2012; Sanpool et al. 2012) in endemic areas where this species is found by itself (Cai et al. 2012). In addition, molecular tools have also been developed to discriminate this species from the other two *Opisthorchis* spp., using PCR-restriction fragment length polymorphism (PCR-RFLP), multiplex PCR, loop-mediated isothermal amplification (LAMP), and multiplex ligation-dependent probe amplification (MLPA) (Le et al. 2006a; Sato et al. 2009; Cai et al. 2010, 2012; Sun et al. 2011; Kaewkong et al. 2013), and also to discriminate this species from some heterophyid species (Sato et al. 2009). A duplex real-time fluorescence resonance energy transfer PCR followed by melting curve analysis has been developed for the detection and differentiation of eggs of *C. sinensis* and *O. viverrini* in human fecal samples in single or mixed infections (Sanpool et al. 2012). Tantrawatpan et al. (2014) established a DNA pyrosequencing-based identification as a valuable tool for differentiating *C. sinensis, O. viverrini*, and some heterophyid species. More recently, Yang et al. (2018) developed a duplex PCR able to detect and differentiate simultaneously *C. sinensis* and *F. hepatica.*

Genus *Opisthorchis* The species *O. viverrini* and *O. felineus* are included in this genus. The egg of either species is elongate ovoid, light yellowish-brown, 22–36 × 11–22 μm (mean 28 × 16 μm) in size, with an operculum that fits into the thickened rim of the shell proper ("shoulder"-like), a minute tubercular terminal thickening (not always visible), and contains a miracidium when passed in feces (Fig. 14.1b). Duodenal aspirates and bile examinations are suitable alternatives to stool samples for the parasitological confirmation of eggs of these species (Ash and Orihel 2007).

The dimensions of the eggs are 22–32 × 11–22 μm in *O. viverrini* and 21–36 × 10–17 μm in *O. felineus* though the distinction between them is very difficult when based on the morphological characteristics owing to their overlapping intraspecific variability. However, worth mentioning are the differences found by different authors with regard to the ratio of the mean length over the mean breadth of the eggs (1.75 in *O. viverrini* vs 2.75 in *O. felineus*) (Rim 1982; Harinasuta et al. 1993). Although both *Opisthorchis* species are distributed very far away from each other (Thailand, Laos, Vietnam and Cambodia in *O. viverrini* vs Russia, Ukraine, Belarus, Kazakhstan, and the Baltic countries in *O. felineus*) (Sripa et al. 2007; Mordvinov et al. 2012), the export and import of fish and fish products worldwide may cause the presence of *Opisthorchis* in countries where it is a priori not expected (Yossepowitch et al. 2004; Armignacco et al. 2008, 2013; Pozio et al. 2013).

In the case of *C. sinensis*, eggs closely resemble those of *O. viverrini* in size and shape and the geographical distribution overlaps with that of *O. viverrini* in several countries of Southeast Asia (Sripa et al. 2007; Doanh and Nawa 2016). In this case, special attention should be paid to "the shoulder" which in the case of *O. viverrini* is usually not as prominent.

The distinction of *O. viverrini* eggs from various intestinal flukes such as heterophyids (*Haplorchis* and *Stellantchasmus*) and lecithodendriids (*Prosthodendrium* and *Phaneropsolus*) seems to be most problematic point. Nevertheless, some facts such as the presence of muskmelon-like structures on *O. viverrini* is very characteristic and distinctly different from small intestinal fluke eggs (Kaewkes et al. 1991; Tesana et al. 1991; Ditrich et al. 1992), or the absence of iodophilic bodies in *O. viverrini* eggs and its presence in *Prosthodendrium* and *Phaneropsolus* when stained with iodine, may be of diagnostic usefulness (Kaewkes et al. 1991; Tesana et al. 1991). The use of potassium permanganate solution (1% w/v) in fecal examination can also be helpful for the differentiation of *O. viverrini* eggs (the muskmelon-like ridge was clearly observed on the egg surface) from *Haplorchis* (a light striae pattern is revealed) and *Phaneropsolus* (a smooth egg shell is observed, while the small knob and the shoulder are not as prominent as in those other genera) eggs (Tesana et al. 1991; Sukontason et al. 1999).

In the case of *O. felineus* diagnostic difficulties mainly occur with a minor opisthorchiid species, *Metorchis bilis*, since both species overlap in a considerable territory of Central and Eastern Europe and Western Siberia (Mordvinov et al. 2012) though the first is mainly recorded in Eurasia. The difficulties of discovering and differentiating eggs of both species in feces (average of 26 × 10 μm in *O. felineus* vs 30 × 20 μm in *M. bilis*) does not make a specific diagnosis with the help of only light microscopy possible.

X-ray diagnosis (cholecystocholangiography), US, CT, and MRI are methods commonly employed for a preliminary human opisthorchiasis diagnosis, even in field surveys (Upatham and Viyanant 2003; Lim et al. 2008; Mairiang et al. 2012).

As in the case of *C. sinensis*, several immunological techniques have been developed either through antibody or circulating antigen detection, or through coproantigen detection, the majority being developed as ELISAs (Wongratanacheewin et al. 2003; Levy et al. 2005; Sripa et al. 2011, 2012; Sawangsoda et al. 2012; Duenngai et al. 2013; Gomez-Morales et al. 2013; Watwiengkam et al. 2013; Teimoori et al. 2015).

Some tumor markers (CA 125 and CA 19-9), measured by radioimmunoassay in serum samples, have been used in the early detection of

O. viverrini-associated cholangiocarcinoma (Mulvenna et al. 2012), which has more recently been the target of studies aiming at the discovery and verification of new biomarkers based on strategic samples collection (Wongratanacheewin et al. 2002).

In recent years, DNA hybridization and PCR-based techniques have been used for the identification of opisthorchiasis agents (Wongratanacheewin et al. 2003; Sripa et al. 2012; Arimatsu et al. 2015). Concretely, PCR methods for the detection of *Opisthorchis* eggs in human stool samples in areas where they are the only species (Stensvold et al. 2006; Duenngai et al. 2008, 2013; Johansen et al. 2010; Sripa et al. 2011), or coexist with another opisthorchiid—*M. bilis* (Pauly et al. 2003; Kang et al. 2008), and even in areas where they coexist with other liver (*C. sinensis*) and intestinal (*Haplorchis taichui*) flukes have been developed (Lovis et al. 2009; Sato et al. 2010; Sun et al. 2011; Arimatsu et al. 2012; Sanpool et al. 2012; Wongsawad et al. 2012). Recently, PCR and PCR-RFLP methods have been used for discriminating eggs of *O. viverrini*, *C. sinensis*, and small intestinal flukes (Buathong et al. 2017). PCR and real-time PCR techniques for *O. viverrini* and *H. taichui* are used in areas where both species coexist (Lamaningao et al. 2017).

Highly specific and sensitive modalities of LAMP techniques have been used to differentiate DNA of *O. viverrini* (Arimatsu et al. 2012, 2015), as well as to differentiate it from other liver (*Clonorchis, Fasciola*) and intestinal (*Haplorchis*) flukes (Cai et al. 2010; Le et al. 2012a).

Genus *Dicrocoelium* The most important species of commonly named "lancet flukes" or "small liver flukes" is *D. dendriticum*, whose eggs have an asymmetrical oval shape, a thick, dark brown shell, and an inconspicuous operculum (Fig. 14.1c). They are embryonated containing a miracidium when passed in feces, and measure 35–45 × 22–30 µm. As the result of the ingestion of infected livers from herbivorous animals, eggs may be found in human feces. These cases are "spurious infections" or "pseudo-parasitisms," which have to be distinguished from genuine infections (Wolfe 2007; Cabeza-Barrera et al. 2011; Moure et al. 2017). Therefore, it might be interesting to study the egg content in detail, as different phases of embryonation in the egg, and a variable shell color (yellow, orange, or light brown) can be observed (Fig. 14.1d) although in cases of genuine dicrocoeliasis all the eggs must be well embryonated (containing a ciliated embryo inside) and most would even have a deep golden-brown color (Moure et al. 2017). Nevertheless, either informing the patient about not ingesting animal liver for some time, together with the microscopic analysis of three fecal samples collected on consecutive or even alternating days, or the examination of duodenal or biliary aspirates should be the most reliable protocols to establish the distinction between false and genuine infections. In any case, having well trained and experienced personnel available able to visualize and recognize this trematode egg is of utmost importance.

Finally, molecular techniques based on mitochondrial and nuclear ribosomal DNA sequences may also be applied (Sandoval et al. 2013; Liu et al. 2014).

Genus *Fasciola* The eggs of the two species included in this genus, *F. hepatica* and *F. gigantica*, are large, operculated—with a small and indistinct operculum—light yellowish-brown, broadly elipsoidal, and unembryonated when excreted in feces (Fig. 14.1e, f).

In guides for clinicians and diagnostic analysts, as well as in books of medical parasitology and/or tropical medicine it is common to find the size of *Fasciola* eggs within the specific range of 130–150 × 63–90 µm for *F. hepatica* and 160–196 × 70–90 µm for *F. gigantica*, being the most frequent criterion used for the differentiation between both species (Ash and Orihel 2007). Moreover, the usual presence of a roughened or irregular area at the abopercular end of the *Fasciola* egg shell is used to distinguish them from *Fasciolopsis buski* eggs, an intestinal fluke with very similar eggs in size and morphology.

The results obtained in a study carried out to validate the identification of *Fasciola* species based on the shape and size of eggs shed by humans have made it necessary to reconsider the knowledge of parasitological diagnosis available so far, as the application of the classic egg size range and even the irregularity in the egg shell may lead to erroneous conclusions (Valero et al. 2009a; Mas-Coma et al. 2014b). Thus, for areas where *F. gigantica* is absent (as in the Americas and Europe), the values for *F. hepatica* eggs in human stools should be 100–162 × 66–105 μm, while for areas where both fasciolid species are present (as in many parts of Africa and Asia), the egg size values should be 107–172 × 64–95 μm for *F. hepatica* and 151–182 × 85–106 μm for *F. gigantica* (Valero et al. 2009a). Moreover, the results of that study showed that the roughened or irregular area present, according to the traditional criterion, at the abopercular end of the *Fasciola* egg shell is population-dependent (frequent or infrequent according to the geographic origin of the human samples analyzed) (Fig. 14.1e, f) and consequently should not be used as a pathognomonic criterion to separate the eggs of both genus, *Fasciola* and *Fasciolopsis* (Valero et al. 2009a; Mas-Coma et al. 2014b). In such instances, clinical evaluation and the geographic zone of origin of the patient may be an important aspect in diagnosis, especially when considering that range values now overlap with the size of eggs belonging to other trematode species also able to infect humans and presenting a similar morphology (i.e., species of the genera *Gastrodiscoides* and *Echinostoma*).

Adult flukes and eggs may also be found by means of invasive techniques applied to obtain duodenal fluid, duodenal and biliary aspirate. Also, surgery or a histological exam of the liver and/or biopsy of other organs may be required (Mas-Coma et al. 1999).

The possibility of spurious fascioliasis cases due to the ingestion of infected liver from ruminants and the resulting presence of eggs in feces cannot be ruled out. Like in *D. dendriticum* infection, repetition of stool examination after a few days of a liver-free diet is most reliable when establishing the distinction between spurious and genuine infections.

Noninvasive diagnostic techniques such as radiology, radioisotope scanning, US, CT, and MRI are helpful in human fascioliasis diagnosis (Esteban et al. 2008; Dusak et al. 2012; Patel et al. 2016). But, US and CT may sometimes confuse malignancy or stones. The most useful diagnostic test for bile duct examination is cholangiography by endoscopic retrograde and magnetic resonance cholangiopancreatographies (Ashrafi et al. 2014).

In the last decade, significant contributions to human fascioliasis diagnosis focusing on direct parasitological techniques and indirect immunological tests have been made (Esteban et al. 2008; Mas-Coma et al. 2014a; Sarkari and Khabisi 2017; Webb and Cabada 2018).

Mas-Coma et al. (2014a) carried out a revision of human fascioliasis diagnosis by stool and blood techniques, focusing on advantages and weaknesses, sample management, egg differentiation, qualitative and quantitative diagnosis, antibody and antigen detection, post-treatment monitoring, and post-control surveillance. Their main conclusions refer to the pronounced difficulties of diagnosing fascioliasis in humans given the different infection phases and parasite migration capacities, clinical heterogeneity, immunological complexity, different epidemiological situations and transmission patterns, the lack of a diagnostic technique covering all needs and situations, and the advisability for a combined use of different techniques, at least including a stool technique and a blood technique.

Different immunological techniques based on the determination of serum anti-*Fasciola* antibodies, circulating secretory antigens or testing of coproantigens have been reported (Sarkari and Khabisi 2017; Webb and Cabada 2018). Several antigenic fractions of *Fasciola*, as well as purified and recombinant antigens, have been successfully used for the serodiagnosis of fascioliasis. Among the different proteins (cathepsin proteases, saposin like-proteins, fatty acid binding proteins, glutathione *S*-transferase, etc.) documented, cathepsins, and recently glutathione *S*-transferase proteins, are most frequently used for detecting anti-*Fasciola* antibodies employing a large number of enzyme-linked immunosorbent assay tech-

niques, from in-house assays—with sensitivity, respectively, specificity reaching almost 100% (Figueroa-Santiago et al. 2011; Martínez-Sernández et al. 2011; Rahimi et al. 2011; Ali 2012; Allam et al. 2012; Valero et al. 2012a; Gonzales Santana et al. 2013; Gottstein et al. 2014; Shafiei et al. 2015; Shalaby et al. 2015; Mokhtarian et al. 2016a, b; Shin et al. 2016; Kazantseva et al. 2017; Khan et al. 2017; Mirzadeh et al. 2017; Sarkari and Khabisi 2017; Aguayo et al. 2018) to a commercial test currently available.

Indeed, the DRG *F. hepatica* IgG ELISA test is a qualitative microtiter strip-based enzyme-linked immunosorbent assay for the detection of IgG class antibodies in human serum against excretion/secretion antigens of *F. hepatica* predominantly containing fluke cysteine proteases, with sensitivity and specificity values of 95.3% and 95.7%, respectively (Valero et al. 2012a). This technique can be applied as an individual serodiagnostic test when backed up by a compatible clinical history in combination with a second diagnostic method for other cross-reactive helminth infections as well as in future epidemiological studies (Valero et al. 2012a).

The SeroFluke is a lateral flow immunoassay or immunochromatographic test, based on the detection of recombinant cathepsin L1 from *F. hepatica* that can be used with serum or whole blood samples and can be employed in major hospitals in hypoendemic countries, as well as in endemic/hyperendemic regions where point-of-care testing is required (Martínez-Sernández et al. 2011).

The MM3-COPRO ELISA test has been applied for the detection of the *Fasciola* coproantigen in hospital patients, as well as in human surveys carried out in hyperendemic areas of Andean countries (Ubeira et al. 2009; Valero et al. 2012b). The high sensitivity and specificity shown by this technique allows fast large mass screening, detection in the chronic phase, early detection of treatment failure or reinfection in post-treatment subjects, and is even useful in surveillance programs (Valero et al. 2012b). However, this technique falls short when evaluating the fluke burden on its own. The use of a new preservative/diluent CoproGuard™, developed for preservation of *Fasciola* coproantigens, has proved to enhance coproantigen extraction and the antigenicity throughout the complete observation period (Ubeira et al. 2009). Moreover, taking into account that this technique can detect animal infections by both *Fasciola* species may be of great value to ensure human diagnosis, above all, in areas where both fasciolids coexist (Valero et al. 2009b; Mas-Coma et al. 2009).

Gottstein et al. (2014) used two recombinant antigens of *F. hepatica*, saposin-like protein-2 and cathepsin L-1, in conjunction with ELISAs for serodiagnosis of human fascioliasis, and the results obtained with 99% specificity, 87% sensitivity, and 97% overall accuracy, make this approach recommendable for the routine serodiagnosis of chronic fascioliasis in clinical laboratories.

IgM Fas2 ELISA has been evaluated as a method to distinguish the different phases of fascioliasis, reaching a sensitivity of 43.4%, respectively, 100% specificity for *Fasciola* infection in individuals presenting with positive total *F. hepatica* Fas2 antibodies and infections of varying durations (Kazantseva et al. 2017).

An immunoblot directed at IgG antibodies to recombinant FhSAP2 protein is offered by the US Center for Disease Control and Prevention for serologic testing of fascioliasis (Shin et al. 2016).

Recently, Aguayo et al. (2018) have demonstrated that glutathione *S*-transferase protein is a good antigen for serodiagnosis of chronic human fascioliasis by indirect ELISA, presenting a high sensitivity (94.3%) but a poor discrimination between true positive and true negative sera (80.3% specificity).

Finally, and for differential diagnosis among *Fasciola* species, particularly in areas of distributional and zonal overlap, several PCR-based approaches, including PCR-linked restriction fragment length polymorphism, PCR-linked single-strand conformation polymorphism and specific PCR assays, have been developed (Rokni et al. 2010; Ai et al. 2011; Alasaad et al. 2011; Prasad et al. 2011; Le et al. 2012b). In order to detect *Fasciola* DNA in human stool specimens, an isothermal PCR method (recombinase poly-

merase amplification) has been developed able to detect DNA in 50% of samples from subjects with fascioliasis and negative microscopy testing (Cabada et al. 2017).

Recently, a new molecular diagnostic workflow being highly sensitive for the detection and quantification of *Fasciola* in fecal samples has been developed, involving sedimenting and pelleting the samples before DNA isolation in order to concentrate the eggs, followed by disruption by bead-beating in a benchtop homogenizer to ensure access to DNA (Calvani et al. 2017).

Lung flukes Various species belonging to the genus *Paragonimus* are included in this group, whose adult stage parasitizes the lungs of humans, among other definitive hosts; paragonimiasis being the disease caused by these flukes. These adult trematodes discharge operculated and unembryonated eggs, being subsequently found in sputum or swallowed and excreted in feces. Nevertheless, the sensitivity of a sputum sample is greater than that of a stool sample, although in the latter case, sensitivity reaches 25% when three stool samples are examined (Procop 2009). It has been suggested that the examination of a stool sample may be superior to a sputum sample in young and the very old subjects (Cabrera 1984). In this sense, Procop (2009) suggested that if paragonimiasis is suspected on a clinical or radiologic basis, a stool examination for parasite eggs should be performed in conjunction with an examination of respiratory secretions, even though the diagnosis yield of a stool examination may be low.

Genus *Paragonimus* The eggs are moderately large, thick-shelled, golden-brown, and broadly ovoid. At the abopercular end of the egg, the shell is somewhat thickened (Ash and Orihel 2007). Although different species of *Paragonimus* have been described (Blair et al. 1999; Doanh et al. 2007; López-Caballero et al. 2013), the most relevant morphological characteristics, together with the geographic distribution, used to establish the diagnosis of the species most frequently found in humans (Cabaret et al. 1999; Ash and Orihel 2007; Blair et al. 2007; Procop 2009) are:

P. westermani: the eggs are large (80–120 × 45–70 μm), have a moderately thick, golden-brown shell that is thickened at the abopercular end, have a flattened, seated, and rimmed operculum, and it is broadest near the operculum (Fig. 14.2a, b); it is the most common species in Asia, but a number of other species, mainly *P. skrjabini* and *P. heterotremus*, have also been reported from China, Japan, Thailand, and countries along the Mekong River.

P. africanus: the eggs are similar (70–125 × 42–60 μm, mean: 91 × 49 μm) to *P. westermani* in size although they tend to be narrower and the operculum is frequently more prominent; the thickening of its shell at the abopercular end is not as pronounced as in other species (Fig. 14.2c); it is an exclusively African species (West Africa: Equatorial Guinea, Cameroon, Nigeria, and possibly Ivory Coast).

P. uterobilateralis: the eggs are smaller (50–95 × 35–55 μm; mean: 69 × 42 μm) than the eggs of *P. westermani* and *P. africanus*, and its operculum is often not as prominent as in other species, and the thickening of its shell at the abopercular end is not as obvious; it is the other African species (West Africa: Gabon, Cameroon, Nigeria, and Liberia).

P. mexicanus: the eggs are smaller in size (65–79 × 39–47 μm) when compared to *P. westermani*, and have a slightly thinner shell; its operculum and the thickening of its shell at the abopercular end (0.64–1.34 μm) are readily seen, being broadest at its equatorial part (Fig. 14.2d); and it is the most frequently species found in Latin America although other related species (*P. caliensis*, *P. ecuadosiensis*, *P. peruvianus*, etc.) have also been described.

P. kellicotti: the eggs are similar (83–100 × 55–65 μm) to *P. westermani*, but smaller than *P. mexicanus* in size; broadest at its median part, its operculum is prominent and the thickening of its shell at the abopercular end (1.68–2.68 μm) is more pronounced than in *P. mexicanus*; it is a species rarely reported in humans in North America.

The differentiation of *Paragonimus* species on the basis of egg morphology is difficult. Thus, microscopic studies of many eggs in the samples

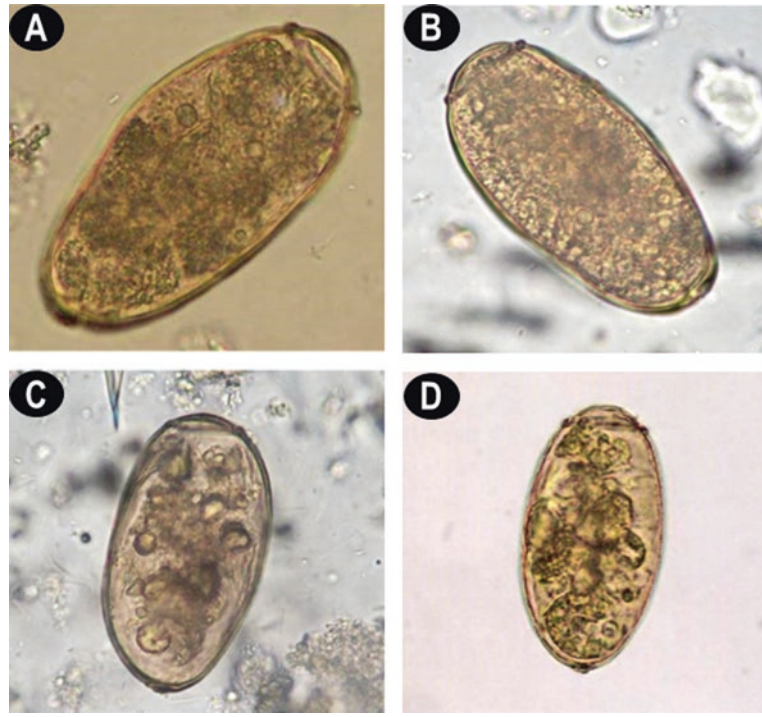

Fig. 14.2 Photomicrographs of various eggs of lung flukes of the genus *Paragonimus* detected (**a**–**c**) in formalin-preserved human fecal material: (**a**, **b**) *P. westermani* (size: 102.5 × 60.0 μm and 95.0 × 45.0 μm, respectively); (**c**) *P. africanus* (92.5 × 47.5 μm); and (**d**) *P. mexicanus* [from Ash and Orihel (2007) with permission]

to appreciate variations in size and shape, as well as in the appearance of the operculum, are convenient combined with adequate parasitological training. In any case, the knowledge of other epidemiological features, mainly about how and where this trematodiasis has been contracted, may be helpful.

Eggs are also sometimes found in pleural effusion or pleural lesions removed surgically (Castilla et al. 2003), and occasionally the cytological examination of fluid obtained by thoracentesis, paracentesis, or fine-needle aspiration reveals eggs (Marty and Neafie 2000; Zarrin-Khameh et al. 2008). Adult specimens have occasionally been coughed up and expectorated (Vanijanonta et al. 1981).

Biopsy of lung and other organs or tissues where the fluke may be ectopically located (Marty and Neafie 2000; Blair et al. 2007; Procop 2009; Liang et al. 2018) may demonstrate the presence of eggs, whose size, shell thickness, and the presence of the operculum usually allow at least an identification at genus level. However, adult or immature worms are very rarely found (Orihel and Ash 1995).

Diagnosis of this trematodiasis often has to be made using medical imaging methods (chest X-rays, CT, 18F-fluorodeoxyglucose positron emission tomography, US, and MRI), above all, when the fluke is found at pulmonary and ectopic locations (mainly cerebral), or atypical manifestations of the disease are presented, being especially relevant in the fields of neurosurgery and neuroradiology (Procop 2009; Henry et al. 2012; Koh et al. 2012; Shim et al. 2012; Xia et al. 2016; Gong et al. 2017). In geographic areas where paragonimiasis pulmonar overlaps with pulmonary tuberculosis, special attention must be paid to imaging findings in the diagnosis, as they are often clinically similar. The presence of a linear track to the pleura is a good criterion to separate paragonimiasis from other infectious or non-infectious lung diseases (Kim et al. 2011; Henry et al. 2012; Lall et al. 2013).

Immunodiagnostic techniques are an alternative in lung fluke diagnosis. Major revisions (Maleewong 1997; Blair et al. 1999, 2007; Procop 2009; Sripa et al. 2010; Chai 2013) of the most commonly used immunodiagnostic techniques, applied with varying success, have been

compiled, including: an IDT, CFT, immunodiffusion methods (double immunodiffusion or Ouchterlony method, IEP and CIEP), IHA, IFAT, and, above all, different variants of tests based on ELISA, multiple-dot assay, immunoblotting, and the dot-immunogold filtration assay.

Crude adult worm extracts and partially purified antigens derived from trematode tissues or excretory-secretory products have been used in the diagnosis of this lung trematodiasis. Some components from adult worms such as 24-, 27-kDa P. westermani; 31.5-, 35-kDa P. *heterotremus*; and 34-, 21/23-kDa P. *kellicotti* have received special attention as antigenic targets (Yang et al. 2004; Wongkham et al. 2005; Fischer et al. 2013). Some proteins have been identified as cysteine proteases (Lee et al. 2006), and one of them (PwCP2) has been cloned and tested in sera from patients with paragonimiasis westermani, showing high sensitivity and specificity (Yang et al. 2004). A recombinant major protein of a *P. westermani* egg antigen developed offers a highly sensitive and specific ELISA assay for the diagnosis of paragonimiasis (Lee et al. 2007). All these antigens have been used for the detection in blood, urine, pleural effusion, or even in cerebrospinal fluid, of parasite-specific antibodies, mainly IgG, although IgE and Ig M have also been suggested, through ELISA or Western blot techniques, which are now most widely used for serological diagnosis of paragonimiasis (Wongkham et al. 2005; Nkouawa et al. 2009; Fischer et al. 2011, 2013; Lane et al. 2012; Ahn et al. 2015; Qiu et al. 2016; Yoonuan et al. 2016; Gong et al. 2017; Yu et al. 2017). A dot-immunogold filtration assay kit was also developed in China for the detection of antibody anti-*P. westermani*, using a *P. westermani* antigen and reporting a sensitivity and specificity of 99 and 92%, respectively (Gan et al. 2005).

In the last decade, molecular tools have been commonly used as the genetic marker of species identification and phylogenetic studies in the genus *Paragonimus* (Doanh et al. 2008; Iwagami et al. 2008; Procop 2009). Conventional PCR has been used to detect *P. kellicotti* DNA in clinical samples (lung biopsy and sputum) although its possible application to other clinical samples, such as bronchoalveolar lavage fluid, pleural fluid or feces may be assumed (Fischer et al. 2011). Amplification of the nuclear ribosomal second internal transcribed spacer region (ITS2) and/or partial mitochondrial cytochrome oxidase subunit 1 gene (cox1) from *Paragonimus* DNA eggs collected from sputum or feces of patients infected with different species (*P. westermani, P. heterotremus, P. pseudoheterotremus, P. proliferus*) have been used for species identification (Chang et al. 2000; Le et al. 2006b; Devi et al. 2007; Yahiro et al. 2008; Zhou et al. 2008; Doanh et al. 2011; Intapan et al. 2012). A copro-DNA test for detection of *P. africanus* DNA from feces has been evaluated and it has been found to be more sensitive than microscopic search for eggs in feces (Nkouawa et al. 2009).

A LAMP assay for the detection *of P. westermani* DNA in humans has also been developed, being highly specific, sensitive, rapid, simple, and cost-effective, as well as being approximately 100 times more sensitive than conventional specific PCR (Chen et al. 2011). The recent application of pyrosequencing technology to discriminate between different *Paragonimus* species co-existing in endemic areas is also worth mentioning, which is a real-time DNA sequencing technique whose applicability has been confirmed in field samples (Tantrawatpan et al. 2013). The combination of a real-time fluorescence resonance energy transfer PCR and melting curve analysis using one set of primers and fluorophore-labelled hybridization probes specific for the 28S rDNA region was developed for the molecular detection of *Paragonimus* and distinguishing it from *Echinostoma* and *Fasciola* eggs (Tantrawatpan et al. 2016).

Intestinal flukes About 70 species belonging to different families are reported in this group infecting humans around the world (Toledo et al. 2006, 2014; Chai 2007, 2009). Among them, echinostomes and heterophyids are the two major groups in terms of the number of species involved, the number of people infected, and the distribution of endemic areas. Fasciolid, gastrodiscid, and lecithodendriid species are also relevant, and there are numerous reports of other small fluke

species of animals accidentally found in humans in Southeast Asia. All the species of this group are food-borne flukes transmitted by freshwater fish and snails, amphibians, terrestrial snakes, crustaceans, and aquatic insects as well as plants (Chai 2007; Chai et al. 2009b; Toledo et al. 2014), and consequently the endemicity of these flukes is associated with cultural and eating habits.

From an exclusively diagnostic point of view, species presenting large eggs (>50 μm) and others, the vast majority, presenting small eggs (<50 μm) are included in this group. Although accurate species identification is often difficult as the eggs of most of these flukes are similar in size and morphology, the most problematic distinction occurs within the group producing small eggs, called "minor intestinal flukes" (MIF) by some authors (Lee et al. 1984, 2012a; Kaewkes et al. 1991; Chai 2007, 2009); an abbreviation apparently rather inadequate as it coincides with a fecal preservative (merthiolate iodine formaldehyde—MIF) used in stool examinations. The term "tiny intestinal flukes" (TIF) is occasionally used as well (Ash and Orihel 2007). In this context, it might seem more convenient to use the denomination "small intestinal flukes" (SIF), reserving the term "small trematode eggs" (STE) for the eggs of small liver and intestinal flukes (Chai et al. 2007, 2009a).

Echinostomatids About 15 species of various genera have been reported causing human echinostomiasis in Asia and the western Pacific and probably also in Africa (Yu and Mott 1994; Toledo et al. 2006, 2014; Chai 2007, 2009; Toledo and Esteban 2016). The diagnosis of these intestinal flukes is based on the recovery of eggs in feces (Fig. 14.3a), usually being oval in shape, variable in size (but more than 50 μm), yellow, dark brown, or silver-white in color, with a thin and refractory shell, unembryonated when laid, and with a small, inconspicuous operculum and roughening or slight thickening of their shell at the abopercular end (Ash and Orihel 2007; Esteban and Muñoz-Antolí 2009; Toledo and Esteban 2016). Although specific diagnosis can be made through careful observations and measurements of the eggs, the recovery and identification of the adult fluke is strongly required for a definitive diagnosis, especially in areas where different species may cause human infection (Chai 2009). Nevertheless, the genus *Echinostoma* comprises the largest number of species producing eggs between 77–82 × 52–55 μm for *E. angustitestis* and 115–130 × 68–80 μm for *E. hortense* (Chai 2009).

Echinostome eggs are similar those of fasciolids (*Fasciola* and *Fasciolopsis*), and even to those of gastrodiscids (*Gastrodiscoides*), making an adequate distinction rather difficult. However, the presence of wrinkles or a thickening at the abopercular end of the shell of echinostome eggs may facilitate their differentiation (Toledo and Esteban 2016).

Occasionally, human echinostomiasis has been revealed by gastroduodenal endoscopy performed in relation to severe gastrointestinal symptoms or colonoscopy (Toledo and Esteban 2016).

Immunological and molecular methods for the diagnosis of human echinostomiasis have not been developed (Toledo and Esteban 2016) although a singleplex real-time fluorescence resonance energy transfer PCR with melting curve analysis has been developed to differentiate eggs in feces of *E. malayanum* from *P. heterotremus* and *F. gigantica* (Tantrawatpan et al. 2016).

Fasciolids The largest fluke parasitizing humans, *Fasciolopsis buski*, which is largely confined to the oriental countries of the Far East and Southeast Asia is included in this group (Yu and Mott 1994; Graczyk et al. 2001; Mas-Coma et al. 2005; Sripa et al. 2010; Toledo et al. 2014). Diagnosis is made by examining fecal specimens for the operculate, unembryonated, ellipsoidal and yellow eggs, measuring 130–140 × 80–85 μm (Fig. 14.3b). The similarity in size and morphology of the eggs of this species and *F. hepatica* may lead to difficulties in establishing a correct diagnosis in areas of Southeast Asia where these species overlap in geographic distribution. In this case, certain facts—such as the difficult visualization of the operculum in *Fasciolopsis* eggs and that the shell at the abopercular end is not blem-

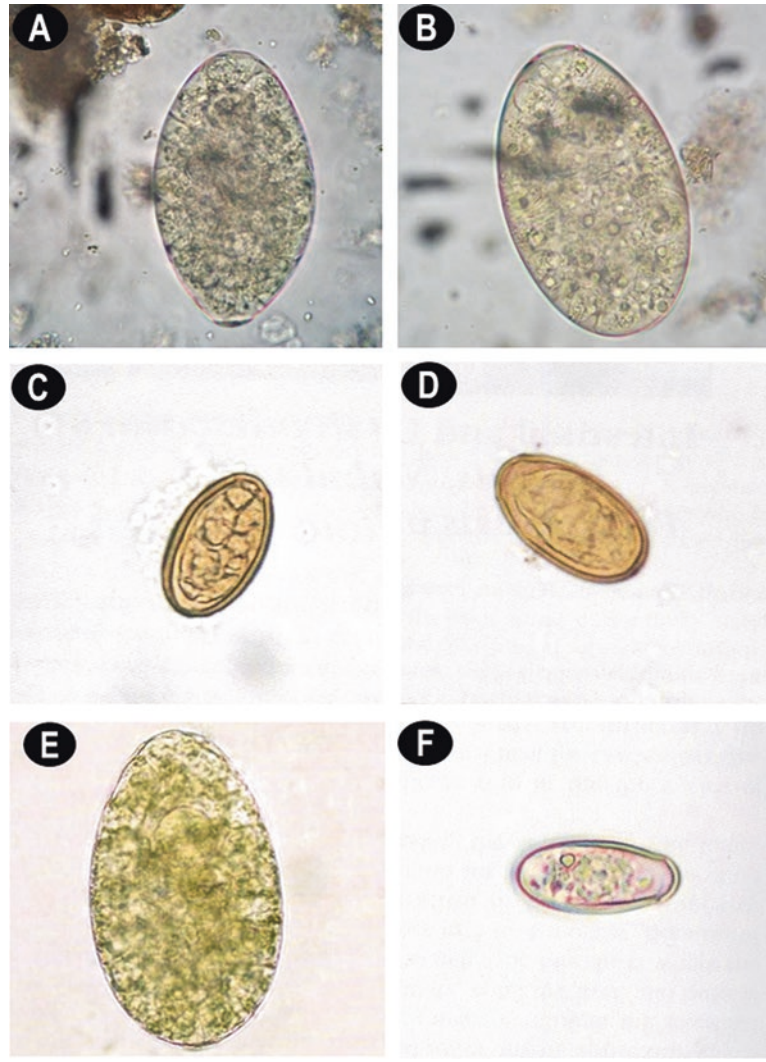

Fig. 14.3 Photomicrographs of various eggs of intestinal flukes detected (**a**, **b**) in formalin-preserved human fecal material: (**a**) *Echinostoma* sp. (size: 127.5 × 72.5 µm); (**b**) *Fasciolopsis buski* (140.0 × 80.0 µm); and *Heterophyes heterophyes* (**c**), *Metagonimus yokogawai* (**d**), *Gastrodiscoides hominis* (**e**) and *Prosthodendrium molenkampi* (**f**) [from Ash and Orihel (2007) with permission]

ished as is often the case in *F. hepatica* eggs—should be mentioned although the clinical evaluation and the geographic zone of origin of the patient may be decisive in the diagnosis (Valero et al. 2009a). Echinostome eggs have a slight thickening of their shell at the abopercular end that may facilitate their distinction from *Fasciolopsis* eggs (Ash and Orihel 2007). However, the troublesome diagnosis concerning the group of large trematodes eggs can easily be resolved when having adult specimens available, particularly considering that immunological or molecular methods for the diagnosis of human fasciolopsiasis have not been developed (Toledo et al. 2014).

Heterophyids A large number of species included in this family have been reported to parasitize humans, among which *Heterophyes heterophyes*, *Metagonimus yokogawai*, and *Haplorchis taichui* are generally considered the most important species implicated in human infections, commonly found throughout Asia (Yu and Mott 1994; Chai et al. 2005, 2010; Toledo et al. 2014).

Diagnosis is basically made by the recovery of the eggs in fecal examinations (Fig. 14.3c, d), which are small, ovoid, operculated, yellow-brown, and embryonated when passed in feces, measuring 20–30 µm in length and 15–17 µm in

width (*M. yokogawai* 26–28 × 15–17 μm, *H. heterophyes* 28–30 × 15–17 μm, and *H. taichui* 20–30 × 14–17 μm) (Ditrich et al. 1992; Sukontason et al. 1999; Ash and Orihel 2007). However, establishing a specific differentiation is very complicated, even at genus level, within this helminth group due to their similar morphology, and their reduced egg size (Lee et al. 1984, 2012a; Tesana et al. 1991; Ditrich et al. 1992; Sukontason et al. 1999; Chai et al. 2015). Therefore, the term "*heterophyid fluke infection*" is recommended in their diagnosis (Chai and Lee 2002). As a consequence of the marked morphological similarity of these eggs to those of *Opisthorchis* and *Clonorchis*, or even those of lecithodendriid species, other terms such as "opisthorchiid-like egg" or "minor intestinal flukes (MIFs)" are used in the definitive diagnosis especially in areas where these species coexist (Chai 2007; Chai et al. 2007, 2015).

However, several publications have helped to establish criteria for the differential diagnosis through parasitological techniques (Lee et al. 1984, 2012a; Tesana et al. 1991; Ditrich et al. 1992; Sukontason et al. 1999). Surface morphology, the shape and the size of eggs, in the latter case using different parameters, mainly the values of the length-width ratio and the Faust-Meleney index (= length of the egg by the square of the width of the egg), have been used by different authors to facilitate egg differentiation in light microscopy and scanning electron microscopy among various heterophyid species and even in relation to *Opisthorchis* and *Clonorchis* (Lee et al. 1984, 2012a; Ditrich et al. 1992). Thus, *Haplorchis* spp. eggs can be recognized by the presence of filamentous mesh-like structures or thread-like ridges on the shell surface distinctly different from the wrinkles or muskmelon-like structure on the shell surface of opisthorchiid eggs (Tesana et al. 1991; Ditrich et al. 1992). When potassium permanganate temporary staining in fecal examinations is used, *H. taichui* eggs show a light striae pattern on the surface markedly different from the muskmelon-like prominent ridges of the surface of *O. viverrini* eggs as well as of the smooth egg shell of lecithodendriid *Phaneropsolus bonnie* eggs (Sukontason et al. 1999).

In spite of the different characteristics reported to be useful for the morphological differentiation of heterophyid eggs in fecal samples, adequate specific and generic discrimination is very difficult and may require the recovery of adult worms after anthelmintic treatment and purgation with magnesium salt, or even surgery or autopsy (Chai 2007; Chai et al. 2009a, 2015; Sohn et al. 2014).

From an immunological and molecular diagnostic point of view, several techniques have been developed. Serological tests such as ELISA are helpful in the human diagnosis of *M. yokogawai* and *H. taichui* (Chai et al. 1989; Ditrich et al. 1991; Lee et al. 1993) although the 100-kDa somatic antigen from *M. yokogawai* commonly reacts with other trematodes (*Clonorchis, Fasciola*, and *Paragonimus*). Moreover, several PCR tests have also been applied in different hosts to discriminate *Haplorchis* spp. from *O. viverrini*, with different sensitivity and specificity (Thaenkham et al. 2007; Lovis et al. 2009; Wongsawad and Wongsawad 2009, 2012; Wongsawad et al. 2009b, 2012; Sato et al. 2009, 2010; Buathong et al. 2017), and specific primers have been successfully developed to identify *H. taichui* and to discriminate it from *O. viverrini*, using high annealing temperature random amplified polymorphic DNA (HAT-RAPD) (Wongsawad et al. 2009a). Recently, a combined conventional PCR and real-time PCR-based assay for detecting and discriminating between *H. taichui* and *O. viverrini* infections in human stools has been developed, which has a good potential for diagnostic application in areas where multiple parasites coexist (Lamaningao et al. 2017).

Gastrodiscids The only common amphistome of humans is *Gastrodiscoides hominis*, which causes a serious zoonosis throughout the Old World: India, Southeast Asia, Kazakhstan, the Volga Delta in Russia and even in Africa (Mas-Coma et al. 2005; Toledo et al. 2014). Diagnosis of human infection is based on the finding of its characteristic eggs in feces (Fig. 14.3e), which are unembryonated and large, measuring 127–160 × 62–75 μm (mean 146 × 66 μm) (Ash and Orihel 2007). The eggs of fasciolids (*Fasciola*

and *Fasciolopsis*) and echinostomatids (i.e., *Echinostoma*) are similar to these eggs although gastrodiscid eggs are broad and tapered toward the opercular end, with a pale greenish-brown color, and do not present a thickening at the abopecular end. Diagnosis based on expelled adult worms is more accurate (Gupte et al. 2014) as no immunological and molecular methods have been developed for the diagnosis of this uncommon human trematodiasis.

Lecithodendriids *Prosthodendrium molenkampi* and *Phaneropsolus bonnie* are the two species of this family mainly reported in humans from Thailand and Laos (Chai 2007; Toledo et al. 2014). The eggs of both species are included in the SIF group, measuring 23–32 μm in length and 11–16 μm in width (*P. bonnie* eggs being thinner and bigger than *P. molenkampi* eggs) (Fig. 14.3f), being morphologically similar to embryonated *O. viverrini* eggs (Kaewkes et al. 1991; Tesana et al. 1991).

The differentiation between them under light microscope may be possible by the presence of an iodophilic body, a large mass at the posterior end of the miracidium that turns brown in 0.2% iodine solution, in lecithodendriid eggs (Kaewkes et al. 1991). Furthermore, under light microscopy, even when using potassium permanganate staining for the surface morphology, as well as scanning electron microscopy, lecithodendriid eggs have smooth eggshells and indistinct or small shoulders and knobs contrasting with the rough eggshells (the so-called muskmelon pattern), the prominent aboperculum knobs, and the distinct operculum and shoulders presented by *O. viverrini* eggs (Tesana et al. 1991; Sukontason et al. 1999). Nonetheless, morphological studies of adult worms obtained from expulsion chemotherapy, autopsy, or surgery are recommendable for the definitive diagnosis. Immunological and molecular techniques have not been developed for the diagnosis of these uncommon human intestinal flukes.

Blood flukes The species of the genus *Schistosoma*, causative of human schistosomiasis, or bilharziasis, concretely *S. mansoni, S. japonicum, S. mekongi*, *S. intercalatum, and S. guineensis*, responsible for intestinal schistosomiasis, and S. *haematobium*, responsible for vesical schistosomiasis, are included in this group.

Genus *Schistosoma* Common and conclusive means of diagnosing schistosome infection is egg detection. The most distinctive features of eggs used in the recognition are: being non-operculate, containing a miracidium when passed in feces and/or urine, with a transparent shell and the presence of a spine. Within the genus, eggs can be distinguished from another on the basis of morphology, size, and location of the spine:

- *S. mansoni*: Eggs are large and elongate (114–175 × 45–68 μm), present in feces, and occasionally in urine, with a prominent subterminal spine (Fig. 14.4a). However, in some coverslip preparations, it will occasionally be hidden from view (Fig. 14.4b);
- *S. japonicum* and *S. mekongi*: Eggs are round, passed in feces, having a small and inconspicuous spine (Fig. 14.4c). Eggs of both species are similar, except that the former are bigger in size (70–100 × 50–65 μm vs 51–78 × 39–66 μm). Fecal debris tends to adhere to the shells of these eggs that may be obscured and overlooked in fecal preparations, and the small spine may be inapparent and difficult to see, especially when the spine lies on the underside. Nevertheless, some strains of *S. japonicum* appear to have a more prominent spine than others.
- *S. intercalatum/S. guineensis*: Eggs are large and elongate (140–240 × 50–85 μm), and are found exclusively in fecal specimens, not in urine. The equatorial bulge varies in prominence, and the terminal spine is usually longer, slightly curved, and more sharply pointed (Fig. 14.4d) than the terminal spine in *S. haematobium*. Eggs of *S. guineensis* a recently described species are anatomically indistinguishable from those of *S. intercalatum* (Pagès et al. 2003). The geographic origin of the infection should be considered in the differentiation of both species: Democratic Republic

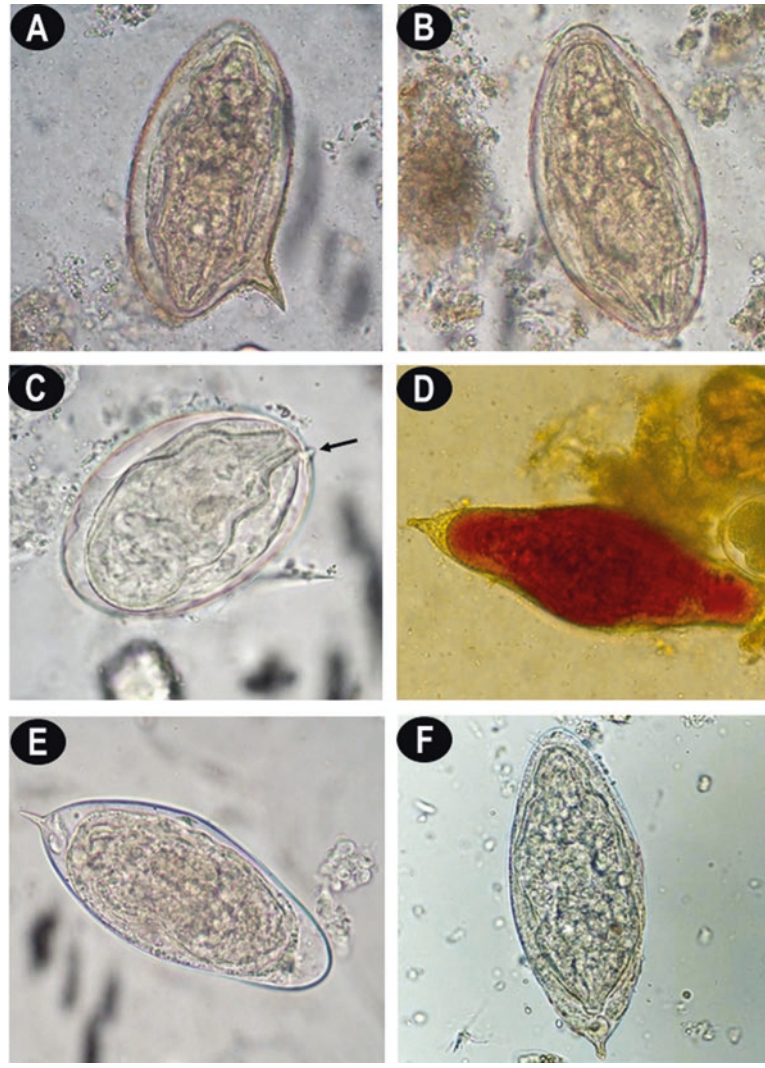

Fig. 14.4 Photomicrographs of various eggs of blood flukes of the genus *Schistosoma* detected in formalin- and MIF-preserved human fecal (**a**–**c**, **f** and **d**, respectively) and urine (**e**) samples: (**a**) *S. mansoni* showing the typical prominent lateral spine, which occasionally may not be visible (**b**) (size: 137.5 × 52.5 μm); (**c**) *S. japonicum* showing the small and inconspicuous spine (arrow) (87.5 × 62.5 μm); (**d**) *S. intercalatum* (140.0 × 67.5 μm); and (**e**, **f**) *S. haematobium* (135.0 × 62.5 μm and 125.0 × 52.5 μm, respectively)

of Congo in *S. intercalatum* vs Cameroon, Equatorial Guinea, Gabon, Nigeria, and Sao Tomé in *S. guineensis* (Webster et al. 2006). Moreover, sporadic cases of human parasitation by *S. bovis* are noteworthy, which have been correlated in some instances with spurious infections acquired by the ingestion of infected livers from cattle or other animals. However, these eggs are very large, 230–380 × 70–90 μm, with a terminal spine and an equatorial bulge invariable in prominence (Ash and Orihel 2007).

– *S. haematobium*: Eggs are large and elongate (112–170 × 40–70 μm), found in urine (Fig. 14.4e) and occasionally in feces (Fig. 14.4f), with a prominent terminal spine, variable in size and prominence. In some instances, cells in urine adhere to the eggs and obscure them although the large size of these eggs usually makes them readily visible.

Schistosomiasis can also be proven through the detection of eggs in tissues obtained by rectal, intestinal, hepatic, prostatic, or vesical biopsies. In ectopic infections (in the lungs, skin, central nervous system) or in light infections, a biopsy may provide the first clue of a schistosome infection. In all these cases, the tissue, preferably

unfixed, is examined microscopically pressed between two glass slides. This is more sensitive than histological examination and allows a more accurate assessment of the species. This technique is also effective in the detection of eggs in fixed or unfixed surgical or autopsy specimens. Eggs may also be recovered by hydrolysis of tissues in 4% KOH for 18 h at 37 °C for fresh tissues and 56 °C for fixed tissues (Cheever and Neafie 2000).

Specific identification of eggs within the characteristic-appearing pseudotubercle (egg granulomas) in histological examination depends on morphological features, but also on epidemiological considerations. In geographic areas where *S. mansoni* and *S. haematobium* overlap in distribution, finding schistosome egg granulomas without the characteristic spines can make diagnosis difficult. When a modified Ziehl-Neelsen staining technique is used, the eggs of *S. haematobium* are not acid fast, whereas those of both *S. mansoni* and *S. japonicum* are (Orihel and Ash 1995). Nevertheless, this differentiation must be made with caution as the shells and spines sometimes fail to stain as expected (Cheever and Neafie 2000). *S. haematobium* eggs can also easily be confused with those of *S. intercalatum* in tissue sections, as both have a terminal spine and are similar in size. However, *S. intercalatum* eggs are usually longer (140–240 μm) and have a larger and more conspicuous spine. Terminal spines of *S. intercalatum* are 3–4 μm wide at the base, pointed at the tip, and 10–20 μm long (Cheever and Neafie 2000).

Computer-directed visual identification using minimicroscopes constructed from inexpensive imaging devices, such as mobile phones or web cams, with samples placed directly on the image sensor has been used for the direct detection of schistosome eggs (Linder et al. 2013).

Imaging techniques are other tools for the definition of the clinical manifestations and pathological outcomes of schistosomiasis although they are restricted to hospitals. Indeed, various imaging techniques, such as myelography, US, CT, and MRI can be useful (Lambertucci et al. 2008; Olveda et al. 2014). Cystoscopy, radiological and endoscopy examinations are also applied (Gryseels et al. 2006), and more recently, confocal laser scanning microscopy has been used, in combination with standard cystoscopy, to detect *S. haematobium* eggs directly in the urothelium of a patient with urinary schistosomiasis (Fritzsche et al. 2012). In addition, a portable ultrasonographic equipment has markedly facilitated schisotosomiasis diagnosis (Hatz 2001; Ohmae et al. 2004).

Additional supportive laboratory findings of schistosome infection might include evidence of peripheral-blood eosinophilia, anemia (iron-deficiency anemia, anemia of chronic disease, or macrocytic anemia), hypoalbuminemia, elevated urea and creatinine levels, and hypergammaglobulinemia (Ross et al. 2002; Osakunor et al. 2018). Reagent strips for microhematuria and simple questionnaires for red urine are cheap, easy, and effective tools for the screening and rapid epidemiological assessment of urinary schistosomiasis (Lengeler et al. 2002; Bassiouny et al. 2014; Atalabi et al. 2017; Osakunor et al. 2018). Measurement of soluble tumor necrosis factor receptors II and intracellular adhesive molecule 1 levels could serve as additional markers for assessment of schistosome-induced liver fibrosis (Ellis et al. 2008).

During the last two decades, a great amount of literature on the immunological and molecular diagnosis of schistosomiasis has been published (McManus et al. 2010; Muth et al. 2010; Zhu et al. 2010; Gray et al. 2011; Weerakoon et al. 2015, 2018). Serological methods have been extensively developed, being based on the detection of Ig G, Ig M, or Ig E against soluble worm or egg antigens mainly by circumoval precipitin test, cercarien Hüllen reaction, indirect hemagglutination test, indirect immunofluorescence assay, and above all ELISA (Zhu et al. 2010; Wang et al. 2012; Zhao et al. 2012; Weerakoon et al. 2015), and are important for the diagnosis in travellers, migrants, and other occasionally exposed subjects returning home from disease-endemic areas (van Gool et al. 2002; Bierman et al. 2005; Bottieau et al. 2006; Cavalcanti et al. 2013; Marchese et al. 2018). A magnetic affinity ELISA based on soluble egg antigens, with a higher sensitivity, has been developed for the diagnosis of

schistosomiasis japonicum in individuals with low-intensity infections (Yu et al. 2012).

Serological methods have also been implemented for antigen detection. Adult worm antigens, soluble egg antigen as well as circulating cathodic and circulating anodic antigens (CCA and CAA, respectively) have been evaluated (Ashton et al. 2011; Shane et al. 2011; Navaratnam et al. 2012; Tchuem Tchuenté et al. 2012; Colley et al. 2013; Coulibaly et al. 2013a; Grenfell et al. 2013; Weerakoon et al. 2015).

The CCA-based assay is used as a point-of-care test to diagnose *S. mansoni* (Ochodo et al. 2015; Ortu et al. 2017: Bezerra et al. 2018; Chernet et al. 2018), but does not work in the case of *S. haematobium* (Sanneh et al. 2017) although it can lead to false-positive results in case of hematuria, urinary tract infections, or the presence of certain glycoproteins in the urine (Polman et al. 2000). The optimization of the CCA point-of-care cassette test could be assessed by using image analysis, being able to quantify the color of the lines strip of the cassette (Casacuberta et al. 2016; Sanneh et al. 2017).

An up-converting phosphor technology-based lateral flow assay for the detection of CAA in serum for both *S. mansoni* and *S. haematobium* has been developed (Corstjens et al. 2008; Knopp et al. 2015; Ochodo et al. 2015; van Grootveld et al. 2018), but has not as yet proven as effective with other schistosome species.

A rapid diagnostic test incorporating *S. mansoni* cercarial transformation fluid has been employed for the detection of antibodies in blood samples (Coulibaly et al. 2013b), and mass spectrometry has been applied to identify and capture various oligosaccharides originating from *S. mansoni* eggs, using specific monoclonal antibodies (Robijn et al. 2008; Zhao et al. 2012). However, further studies are required to validate its actual clinical applications.

The need to have a test available for diagnosing schistosomiasis in all phases of the clinical disease, including the capacity to diagnose Katayama syndrome and active disease, prompted several groups to develop molecular testing using DNA-based methods (Gray et al. 2011). The current status of the knowledge of the main DNA detection methods that have been employed in the diagnosis of human schistosomiasis has been compiled by Weerakoon et al. (2015, 2018). In this sense, the application of real-time quantitative PCR and droplet digital PCR for the detection of circulating cell-free parasite DNA using different clinical samples (feces, urine, saliva, cerebrospinal fluid, and serum/plasma), and the development of isothermal amplification assays such as LAMP, multiple LAMP, and recombinase polymerase amplification techniques should be mentioned (ten Hove et al. 2008; Obeng et al. 2008; Lier et al. 2009b; Wichmann et al. 2009; Gomes et al. 2010; Xu et al. 2010; Cnops et al. 2012, 2013; Zhao et al. 2012; Aryeetey et al. 2013; Cavalcanti et al. 2013; Mwangi et al. 2018).

A prospective European-wide multicenter study has demonstrated the applicability of a blood-based real-time PCR test for the detection of *S. mansoni* DNA in patients with acute schistosomiasis who acquired their infection in various endemic regions (Wichmann et al. 2013). Recently, egg-based systematic evolution of ligands by exponential enrichment (egg-SELEX) was employed and identified two ssDNA aptamers (LC6 and LC15), which exhibited strong binding to and specific recognition of *S. japonicum* eggs (Long et al. 2016).

14.4 Key to Digenetic Trematode Eggs

A quick key for the identification of digenetic trematode eggs found in feces and body fluids is included to assist the microscopist in the differential diagnosis of parasite species when eggs of unusual characteristics are found.

- ***Egg in urine sediment***
  - » Egg large, non-operculate with prominent terminal spine and with smooth shell; contains a miracidium; measures 112–170 µm by 40–70 µm
    Schistosoma haematobium

- ***Egg in sputum***
  - » Egg moderately large, with operculum, and broadly ovoid with thick shell and golden-

brown color; unembryonated; measures 80–120 µm by 45–70 µm
Paragonimus spp.

- ***Egg in feces***
  - » egg with operculum
    - ✓ egg unembryonated
      - egg large and indistinct operculum
        - egg broadly elipsoidal with a light yellowish-brown color; with or without roughened or irregular area at abopercular end of egg; measures 100–182 µm by 64–106 µm
          Fasciola spp.
        - difficult visualization of operculum; shell not blemished at abopercular end; measures 130–140 µm by 80–85 µm
          Fasciolopsis buski
        - egg thickened or wrinkled at abopercular end of shell; measures 77–130 µm by 52–80 µm depending on the species
          Echinostoma spp.
        - egg broad and tapered toward the opercular end; pale greenish-brown color; measures 127–160 µm by 62–75 µm
          Gastrodiscoides hominis
      - egg moderately large and operculum distinct; egg broad ovoid with thick shell; shell thickened at abopercular end
        Paragonimus spp.
    - ✓ egg small, ovoid and embryonated, contains a miracidium
      - yellow-brown color; moderately thick shell and a seated operculum; usually a small knob at abopercular end; measures 21–36 µm by 10–22 µm
        Clonorchis sinensis
        Opisthorchis spp.
      - yellow brown color with indistinct operculum; measures 20–30 µm by 14–17 µm
        Heterophyes heterophyes
        Metagonimus yokogawai
        Haplorchis spp.
      - yellow brown color; operculum distinct or not; overlap in size with other Heterophyid species and *Clonorchis*/*Opisthorchis* species
        Prosthodendrium spp.
      - thick and golden-brown shell; measures 35–45 µm by 22–30 µm
        Dicrocoelium spp.
  - » egg with spine, non-operculate and contains a miracidium
    - terminal spine and transparent shell
      - egg measures 112–170 µm by 40–70 µm
        Schistosoma haematobium
      - egg with equatorial budge; spine long, slightly curved and sharply pointed; measures 140–240 µm by 50–85 µm
        Schistosoma intercalatum
        Schistosoma guineensis
    - lateral spine
      - prominent lateral spine; measures 114–175 µm by 45–70 µm
        Schistosoma mansoni
      - small and inconspicuous lateral spine; round egg; fecal debris often adheres to shell surface
        - measures 70–100 µm by 50–65 µm
          Schistosoma japonicum
        - measures 51–78 µm by 39–66 µm
          Schistosoma mekongi

14.5 Concluding Remarks

The public health relevance of human trematodiases is unquestionable, and therefore the correct diagnosis of these trematode infections is crucial, mainly for assessing drug efficacy and monitoring the impact of control interventions.

The gold standard for the diagnosis of trematodiases is the detection of eggs through parasitological techniques, although some may have a certain lack of sensitivity, require an intensive effort and can be time-consuming, requiring,

moreover, experienced technicians. Yet, sometimes it is difficult to establish a diagnosis due to morphological similarities among various eggs. For all these reasons, specialists are concentrating their efforts on the development of more sensitive and specific diagnostic methods, focusing mainly on the immunology and molecular biology. The increased use of genomic, transcriptomic, and proteomic approaches offers a promising future for the diagnosis of these human fluke infections.

International travel, migratory flows, and other demographic changes in a globalized world imply a risk linked to the diagnosis of these human trematode infections mainly in non-endemic countries, and it should be kept in mind that the direct microscopic examination of clinical samples together with the identification of trematode eggs remains the gold standard in diagnosis. Consequently, it is indispensable to invest in the training of personnel to acquire the relevant skills and experience in parasitological diagnosis, which should go hand in hand with the quest for the development of more sensitive and specific techniques to be applied in the field and, above all, in clinical practice.

Acknowledgements This work was supported by the projects PROMETEO/2009/081 and PROMETEO2014-083 Fase II of Conselleria d'Educació, Generalitat Valenciana (Valencia, Spain); by project SN07.A126 of Cooperación al Desarrollo de la Universitat de València (Valencia, Spain); by projects BFU2013-41040 and BFU2016-75639-P of Ministerio de Economía y Competitividad (Madrid, Spain); and by projects No. RD12/0018/0013 (IV National Program of I+D+I 2008-2011) and RD16/0027/0023 (IV National Program of I+D+I 2017-2021) of the Red de Investigación Cooperativa en Enfermedades Tropicales-RICET, ISCIII-Subdirección General de Redes y Centros de Investigación Cooperativa of Ministerio de Sanidad y Consumo (Madrid, Spain).

References

Aguayo V, Valdes B, Espino AM (2018) Assessment of *Fasciola hepatica* glutathione S-transferase as an antigen for serodiagnosis of human chronic fascioliasis. Acta Trop 186:41–49

Ahn CS, Na BK, Chung DL, Kim JG, Kim JT, Kong Y (2015) Expression characteristics and specific antibody reactivity of diverse cathepsin F members of *Paragonimus westermani*. Parasitol Int 64:37–42

Ai L, Chen MX, Alasaad S, Elsheikha HM, Li J, Li HL, Lin RQ, Zou FC, Zhu XQ, Chen JX (2011) Genetic characterization, species differentiation and detection of *Fasciola* spp. by molecular approaches. Parasit Vectors 4:101

Alasaad S, Soriguer RC, Abu-Madi M, El Behairy A, Baños PD, Píriz A, Fickel J, Zhu XQ (2011) A fluorescence-based polymerase chain reaction-linked single-strand conformation polymorphism (F-PCR-SSCP) assay for the identification of *Fasciola* spp. Parasitol Res 108:1513–1517

Ali NM (2012) Development and evaluation of a dipstick assay in diagnosis of human fasciolosis. Parasitol Res 110:1649–1654

Allam G, Bauomy IR, Hemyeda ZM, Sakran TF (2012) Evaluation of a 14.5 kDa-*Fasciola gigantica* fatty acid binding protein as a diagnostic antigen for human fascioliasis. Parasitol Res 110:1863–1871

Allen AVH, Ridley DS (1970) Further observations on the formol-ether concentration technique for faecal parasites. J Clin Pathol 23:545–546

Alva A, Cangalaya C, Quiliano M, Krebs C, Gilman RH, Sheen P, Zimic M (2017) Mathematical algorithm for the automatic recognition of intestinal parasites. PLoS One 12(4):e0175646

Arimatsu Y, Kaewkes S, Laha T, Hong SJ, Sripa B (2012) Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification. Parasitol Int 61:178–182

Arimatsu Y, Kaewkes S, Laha T, Sripa B (2015) Specific diagnosis of *Opisthorchis viverrini* using loop-mediated isothermal amplification (LAMP) targeting parasite microsatellites. Acta Trop 141(Pt B):368–371

Armignacco O, Caterini L, Marucci G, Ferri F, Bernardini G, Natalini Raponi G, Ludovisi A, Bossù T, Gomez Morales MA, Pozio E (2008) Human illnesses caused by *Opisthorchis felineus* flukes, Italy. Emerg Infect Dis 14:1902–1905

Armignacco O, Ferri F, Gomez-Morales MA, Caterini L, Pozio E (2013) Cryptic and asymptomatic *Opisthorchis felineus* infections. Am J Trop Med Hyg 88:364–366

Aryeetey YA, Essien-Baidoo S, Larbi IA, Ahmed K, Amoah AS, Obeng BB, van Lieshout L, Yazdanbakhsh M, Boakye DA, Verweij JJ (2013) Molecular diagnosis of *Schistosoma* infections in urine samples of school children in Ghana. Am J Trop Med Hyg 88:1028–1031

Ash LR, Orihel TC (1991) Parasites: a guide to laboratory procedures and identification. ASCP Press, American Society of Clinical Pathologists, Chicago

Ash LR, Orihel TC (2007) Atlas of human parasitology, 5th edn. American Society for Clinical Pathology Press, Chicago

Ash LR, Orihel TC, Savioli L (1994) Bench Aids for the diagnosis of intestinal parasites. World Health Organization, Geneva

Ashrafi K, Bargues MD, O'Neill S, Mas-Coma S (2014) Fascioliasis: a worldwide parasitic disease of importance in travel medicine. Travel Med Infect Dis 12(6 Pt A):636–649

Ashton RA, Stewart BT, Petty N, Lado M, Finn T, Brooker S, Kolaczinski JH (2011) Accuracy of circulating cathodic antigen tests for rapid mapping of *Schistosoma mansoni* and *S. haematobium* infections in Southern Sudan. Tropical Med Int Health 16:1099–1103

Atalabi TE, Adubi TO, Lawal U (2017) Rapid mapping of urinary schistosomiasis: an appraisal of the diagnostic efficacy of some questionnaire-based indices among high school students in Katsina State, northwestern Nigeria. PLoS Negl Trop Dis 11(4):e0005518

Barda BD, Rinaldi L, Ianniello D, Zepherine H, Salvo F, Sadutshang T, Cringoli G, Clementi M, Albonico M (2013) Mini-FLOTAC, an innovative dirtect diagnostic technique for intestinal parasitic infections: experience from the field. PLoS Negl Trop Dis 7:e2344

Bassiouny HK, Hasab AA, El-Nimr NA, Al-Shibani LA, Al-Waleedi AA (2014) Rapid diagnosis of schistosomiasis in Yemen using a simple questionnaire and urine reagent strips. East Mediterr Health J 20:242–249

Bergquist R, Johansen MV, Utzinger J (2009) Diagnostic dilemmas in helminthology: what tools to use and when? Trends Parasitol 4:151–156

Bezerra FSM, Leal JKF, Sousa MS, Pinheiro MCC, Ramos AN Jr, Silva-Moraes V, Katz N (2018) Evaluating a point-of-care circulating cathodic antigen test (POC-CCA) to detect Schistosoma mansoni infections in a low endemic area in north-eastern Brazil. Acta Trop 182:264–270

Bierman WFW, Wetsteyn JCFM, van Gool TV (2005) Presentation and diagnosis of imported schistosomiasis: relevance of eosinophilia, microscopy for ova, and serology. J Travel Med 12:9–13

Blagg W, Schloegel EL, Mansour NS, Khalaf GI (1955) A new concentration technic for the demonstration of protozoa and helminth eggs in feces. Am J Trop Med Hyg 4:23–28

Blair D, Xu ZB, Agatsuma T (1999) *Paragonimus* and paragonimiasis. Adv Parasitol 3:99–158

Blair D, Agatsuma T, Wang W (2007) Paragonimiasis. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites, vol 11. Springer, New York, pp 117–150

Bottieau E, Clerinx J, De Vega MR, Van den Enden E, Colebunders R, Van Esbroeck M, Vervoort T, Van Gompel A, Van den Ende J (2006) Imported Katayama fever: clinical and biological features at presentation and during treatment. J Infect 52:339–345

Buathong S, Leelayoova S, Mungthin M, Ruang-Areerate T, Naaglor T, Suwannahitatorn P, Piyaraj P, Taamasri P, Tan-Ariya P (2017) Molecular discrimination of *Opisthorchis*-like eggs from residents in a rural community of central Thailand. PLoS Negl Trop Dis 11(11):e0006030

Cabada MM, Malaga JL, Castellanos-Gonzalez A, Bagwell KA, Naeger PA, Rogers HK, Maharsi S, Mbaka M, White AC Jr (2017) Recombinase polymerase amplification compared to real-time polymerase chain reaction test for the detection of *Fasciola hepatica* in human stool. Am J Trop Med Hyg 96:341–346

Cabaret J, Bayssade-Dufour C, Tami G, Albaret JL (1999) Identification of African Paragonimidae by multivariate analysis of the eggs. Acta Trop 72:79–89

Cabeza-Barrera I, Cabezas-Fernández T, Salas Coronas J, Vázquez Villegas J, Cobo F (2011) *Dicrocoelium dendriticum*: an emerging spurious infection in a geographic area with a high level of immigration. Ann Trop Med Parasitol 105:403–406

Cabrera BD (1984) Paragonimiasis in the Philippines: current status. Arzneimittelforschung 34:1188–1192

Cai XQ, Xu MJ, Wang YH, Qiu DY, Liu GX, Lin A, Tang JD, Zhang RL, Zhu XQ (2010) Sensitive and rapid detection of *Clonorchis sinensis* infection in fish by loop-mediated isothermal amplification (LAMP). Parasitol Res 106:1379–1383

Cai XQ, Yu HQ, Bai JS, Tang JD, Hu XC, Chen DH, Zhang RL, Chen MX, Ai L, Zhu XQ (2012) Development of a TaqMan based real-time PCR assay for detection of *Clonorchis sinensis* DNA in human stool samples and fishes. Parasitol Int 61:183–186

Calvani NED, Windsor PA, Bush RD, Slapeta J (2017) Scrambled eggs: a highly sensitive molecular diagnostic workflow for *Fasciola* species specific detection from faecal samples. PLoS Negl Trop Dis 11(9):e0005931

Casacuberta M, Kinunghi S, Vennervald BJ, Olsen A (2016) Evaluation and optimization of the Circulating Cathodic Antigen (POC-CCA) cassette test for detecting *Schistosoma mansoni* infection by using image analysis in school children in Mwanza Region, Tanzania. Parasit Epidemiol Control 1:105–115

Castilla EA, Jessen R, Sheck DN, Procop GW (2003) Cavitary mass lesion and recurrent pneumothoraces due to *Paragonimus kellicotti* infection—North American paragonimiasis. Am J Surg Pathol 27:1157–1160

Cavalcanti MG, Silva LF, Peralta RHS, Barreto MG, Peralta JM (2013) Schistosomiasis in áreas of low endemicity: a new era in diagnosis. Trends Parasitol 29:75–82

Chai JY (2007) Intestinal flukes. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites, vol 11. Springer, New York, pp 53–115

Chai JY (2009) Echinostomes in humans. In: Fried B, Toledo R (eds) The biology of Echinostomes. From the molecule to the community. Springer, New York, pp 147–183

Chai JY (2013) Paragonimiasis. Handb Clin Neurol 114:283–296

Chai JY, Lee SH (2002) Food-borne intestinal trematode infections in the Republic of Korea. Parasitol Int 51:129–154

Chai JY, Yu JR, Lee SH, Jung HC, Song IS, Cho SY (1989) An egg-negative patient of acute metagonimiasis diagnosed serologically by ELISA. Seoul J Med 30:138–142

Chai JY, Darwin Murrell K, Lymbery AJ (2005) Fish-borne parasitic zoonoses: status and issues. Int J Parasitol 35:1233–1254

Chai JY, Han ET, Guk SM, Shin EH, Sohn WM, Yong TS, Eom KS, Lee KH, Jeong HG, Ryang YS, Hoang

EH, Phommasack B, Insisiengmay B, Lee SH, Rim HJ (2007) High prevalence of liver and intestinal fluke infections among residents of Savannakhet Province in Laos. Korean J Parasitol 45:213–218

Chai JY, Han ET, Shin EH, Sohn WM, Yong TS, Eom KS, Min DY, Um JY, Park MS, Hoang EH, Phommasack B, Insisiengmay B, Lee SH, Rim HJ (2009a) High prevalence of *Haplorchis taichui, Phaneropsolus molenkampi*, and other helminth infections among people in Khammouane province, Lao PDR. Korean J Parasitol 47:243–247

Chai JY, Shin EH, Lee SH, Rim HJ (2009b) Foodborne intestinal flukes in Southeast Asia. Korean J Parasitol 47:S69–S102

Chai JY, Yong TS, Eom KS, Min DY, Shin EH, Banouvong V, Insisiengmay B, Insisiengmay S, Phommasack B, Rim HJ (2010) Prevalence of the intestinal flukes *Haplorchis taichui* and *H. yokogawai* in a mountainous area of Phongsaly Province, Lao PDR. Korean J Parasitol 48:339–342

Chai JY, Jung BK, Kim DG, Kim JL, Lim H, Shin EH, Lee KH, Kim MR, Han SJ, Yeom JH, Park SM, Hwang JS (2015) Heterophyid trematodes recovered from people residing along the Boseong River, South Korea. Acta Trop 148:142–146

Chang ZS, Wu B, Blair D, Zhang YN, Hu L, Chen SH, Chen MG, Feng Z, Davis GM (2000) Gene sequencing for identification of *Paragonimus* eggs from a human case. Chin J Parasitol Parasit Dis 18:213–215

Cheever AW, Neafie C (2000) Schistosomiasis. In: Meyers WM, Neafie RC, Marty AM, Wear DJ (eds) Pathology of infectious diseases, vol I. Helmintiases. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, pp 23–48

Chen M, Lu Y, Hua X, Mott KE (1994) Progress in assessment of morbidity due to *Clonorchis sinensis* infection: a review of recent literature. Trop Dis Bull 91:R7–R65

Chen MX, Ai L, Zhang RL, Xia JJ, Wang K, Chen SH, Zhang YN, Xu MJ, Li X, Zhu XQ, Chen JX (2011) Sensitive and rapid detection of *Paragonimus westermani* infection in humans and animals by loop-mediated isothermal amplification (LAMP). Parasitol Res 108:1193–1198

Cheng MG (1989) Schistosomiasis control program in the People's Republic of China: a review. Southeast Asian J Trop Med Public Health 20:511–517

Chernet A, Neumayr A, Hatz C, Kling K, Sydow V, Rentsch K, Utzinger J, Probst-Hensch N, Marti H, Nickel B, Labhardt ND (2018) Spectrum of infectious diseases among newly arrived Eritrean refugees in Switzerland: a cross-sectional study. Int J Public Health 63:233–239

Choi D, Hong ST (2007) Imaging diagnosis of clonorchiasis. Korean J Parasitol 45:77–85

Choi D, Hong ST, Lim JH, Cho SY, Rim HJ, Ji Z, Yuan R, Wang S (2004a) Sonographic findings of active *Clonorchis sinensis* infection. J Clin Ultrasound 32:17–23

Choi BI, Han JK, Hong ST, Lee KH (2004b) Clonorchiasis and cholangiocarcinoma: etiologic relationship and imaging diagnosis. Clin Microbiol Rev 17:540–552

Cnops L, Tannich E, Polman K, Clerinx J, Van Esbroeck M (2012) *Schistosoma* real-time PCR as diagnostic tool for international travellers and migrants. Trop Med Int Health 17:1208–1216

Cnops L, Soentjens P, Clerinx J, Van Esbroeck M (2013) A *Schistosoma haematobium*-specific real-time PCR for diagnosis of urogenital schistosomiasis in serum samples of international travelers and migrants. PLoS Negl Trop Dis 7(8):e2413

Coelho PMZ, Jurberg AD, Oliveira AA, Katz N (2009) Use of a saline gradient for the diagnosis of schistosomiasis. Mem Inst Oswaldo Cruz 104:720–723

Colley DG, Binder S, Campbell C, King CH, Tchuem Tchuenté LA, N'Goran EK, Erko B, Karanja DM, Kabatereine NB, van Lieshout L, Rathbun S (2013) A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of *Schistosoma mansoni*. Am J Trop Med Hyg 88:426–432

Corstjens PL, van Lieshout L, Zuiderwijk M, Kornelis D, Tanke HJ, Deelder AM, van Dam GJ (2008) Up-converting phosphor technology-based lateral flow assay for detection of *Schistosoma* circulating anodic antigen in serum. J Clin Microbiol 46:171–176

Coulibaly JT, N'Gbesso YK, Knopp S, N'Guessan NA, Silué KD, van Dam GJ, N'Goran EK, Utzinger J (2013a) Accuracy of urine circulating cathodic antigen test for the diagnosis of *Schistosoma mansoni* in preschool-aged children before and after treatment. PLoS Negl Trop Dis 7:e2109

Coulibaly JT, N'Goran EK, Utzinger J, Doenhoff MJ, Dawson EM (2013b) A new rapid diagnostic test for detection of anti-*Schistosoma mansoni* and anti-*Schistosoma haematobium* antibodies. Parasit Vectors 6:29

Cringoli G (2006) FLOTAC, a novel apparatus for a multivalent faecal egg count technique. Parassitologia 48:381–384

Cringoli G, Rinaldi L, Maurelli MP, Utzinger J (2010) FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of intestinal parasites in animals and humans. Nat Protoc 5:503–515

Devi KR, Narain K, Bhattacharya S, Negmu K, Agatsuma T, Blair D, Wickramashinghe S, Mahanta J (2007) Pleuropulmonary paragonimiasis due to *Paragonimus heterotremus*: molecular diagnosis, prevalence of infection and clinicoradiological features in an endemic area of northeastern India. Trans R Soc Trop Med Hyg 101:786–792

Ditrich O, Kopacek P, Giboda M, Gutvirth J, Scholz T (1991) Serological differentiation of human small fluke infections using *Opisthorchis viverrini* and *Haplorchis taichui* antigens. Southeast Asian J Trop Med Public Health 22:174–178

Ditrich O, Giboda M, Scholz T, Beer SA (1992) Comparative morphology of eggs of the Haplorchiinae (Trematoda: Heterophyidae) and some other medically important heterophyid and opisthorchiid flukes. Folia Parasitol 39:123–132

Doanh PN, Nawa Y (2016) *Clonorchis sinensis* and *Opisthorchis* spp. in Vietnam: current status and prospects. Trans R Soc Trop Med Hyg 110:13–20

Doanh PN, Shinohara A, Horii Y, Habe S, Nawa Y, Le NT (2007) Description of a new lung fluke species, *Paragonimus vietnamensis* sp. nov. (Trematoda, Paragonimidae), found in northern Vietnam. Parasitol Res 101:1495–1501

Doanh PN, Shinohara A, Horii Y, Habe S, Nawa Y (2008) Discovery of *Paragonimus westermani* in Vietnam and its molecular phylogenetic status in *P. westermani* complex. Parasitol Res 104:1149–1155

Doanh PN, Dung do T, Thach DT, Horii Y, Shinohara A, Nawa Y (2011) Human paragonimiasis in Viet Nam: epidemiological survey and identification of the responsible species by DNA sequencing of eggs in patients' sputum. Parasitol Int 60:534–537

Duenngai K, Sithithaworn P, Rudrappa UK, Iddya K, Laha T, Stensvold CR, Strandgaard H, Johansen MV (2008) Improvement of PCR for detection of *Opisthorchis viverrini* DNA in human stool samples. J Clin Microbiol 46:366–368

Duenngai K, Boonmars T, Sithithaworn J, Sithithaworn P (2013) Diagnosis of early infection and post chemotherapeutic treatment by copro-DNA detection in experimental opisthorchiasis. Parasitol Res 112:271–278

Dusak A, Onur MR, Cicek M, Firat U, Ren T, Dogra VS (2012) Radiological imaging features of *Fasciola hepatica* infection—a pictorial review. J Clin Imaging Sci 2:2

Duthaler U, Rinaldi L, Maurelli MP, Vargas M, Utzinger J, Cringoli G, Keiser J (2010) *Fasciola hepatica*: comparison of the sedimentation and FLOTAC techniques for the detection and quantification of faecal egg counts in rats. Exp Parasitol 126:161–166

Eberl M, Al-Sherbiny M, Hagan P, Ljubojevic S, Thomas AW, Wilson RA (2002) A novel and sensitive method to monitor helminth infections by faecal sampling. Acta Trop 83:183–187

Ellis MK, Li Y, Hou X, Chen H, McManus DP (2008) sTNFR-II and sICAM-1 are associated with acute disease and hepatic imflammation in schistosomiasis japonica. Int J Parasitol 38:717–723

Ephraim RK, Duah E, Cybulski JS, Prakash M, D'Ambrosio MV, Fletcher DA, Keiser J, Andrews JR, Bogoch II (2015) Diagnosis of *Schistosoma haematobium* infection with a mobile phone-mounted Foldscope and a reversed-lens CellScope in Ghana. Am J Trop Med Hyg 92:1253–1256

Esteban JG, Muñoz-Antolí C (2009) Echinostomes: systematics and life cycles. In: Fried B, Toledo R (eds) The biology of Echinostomes. From the molecule to the community. Springer, New York, pp 1–34

Esteban JG, Bargues MD, Mas-Coma S (2008) Geographical distribution, diagnosis and treatment of human fascioliasis: a review. Res Rev Parasitol 58:13–42

Figueroa-Santiago O, Delgado B, Espino AM (2011) *Fasciola hepatica* saposin-like protein-2-based ELISA for the serodiagnosis of chronic human fascioliasis. Diagn Microbiol Infect Dis 70:355–361

Fischer PU, Curtis KC, Marcos LA, Weil GJ (2011) Molecular characterization of the North American lung fluke *Paragonimus kellicotti* in Missouri and its development in Mongolian gerbils. Am J Trop Med Hyg 84:1005–1011

Fischer PU, Curtis KC, Folk SM, Wilkins PP, Marcos LA, Weil GJ (2013) Serological diagnosis of North American paragonimiasis by Western blot using *Paragonimus kellicotti* adult worm antigen. Am J Trop Med Hyg 88:1035–1040

Fried B, Haseeb MA (1991) Plathyhelminthes: Aspidogastrea, Monogenea, and Digenea. In: Harrison FW, Bogitish BJ (eds) Microscopic anatomy of invertebrates, vol III. Plathyhelminthes and Nemertinea. Wiley-Liss Inc., New York, pp 141–209

Fried B, Graczyk TK, Tamang L (2004) Food-borne intestinal trematodiases in humans. Parasitol Res 93:159–170

Fritzsche C, Stachs O, Holtfreter MC, Nohr-Łuczak C, Guthoff RF, Reisinger EC (2012) Confocal laser scanning microscopy, a new in vivo diagnostic tool for schistosomiasis. PLoS One 7(4):e34869

Fürst T, Keiser J, Utzinger J (2012a) Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. Lancet Infect Dis 12:210–221

Fürst T, Sayasone S, Odermatt P, Keiser J, Utzinger J (2012b) Manifestation, diagnosis, and management of foodborne trematodiasis. BMJ 344:e4093

Gan X, Shi X, Wang Y, Wang P, Shen H, Zhu M, Tang Y, Zhang S (2005) Development of rapid diagnostic kit (Dot Immunogold Filtration Assay) for detection of antibodies against *Paragonimus westermani*. Chin J Zoonoses 21:988–990

Glinz D, Silué KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, Rinaldi L, Cringoli G, N'Goran EK, Utzinger J (2010) Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for *Schistosoma mansoni* and soil-transmitted helminths. PLoS Negl Trop Dis 4:e754

Gomes LI, Dos Santos Marques LH, Enk MJ, de Oliveira MC, Coelho PM, Rabello A (2010) Development and evaluation of a sensitive PCR-ELISA system for detection of *Schistosoma* infection in feces. PLoS Negl Trop Dis 4(4):e664

Gomez-Morales MA, Ludovisi A, Amati M, Pozio E (2013) Validation of an excretory/secretory antigen based-Elisa for the diagnosis of *Opisthorchis felineus* infection in humans from low trematode endemic areas. PLoS One 8(5):e62267

Gong Z, Miao R, Shu M, Zhu Y, Wen Y, Guo Q, Liao Q, Wan C (2017) Paragonimiasis in Children in Southwest

China: a retrospective case reports review from 2005 to 2016. Medicine (Baltimore) 96(25):e7265
Gonzales Santana B, Dalton JP, Vasquez Camargo F, Parkinson M, Ndao M (2013) The diagnosis of human fascioliasis by enzyme-linked immunosorbent assay (ELISA) using recombinant cathepsin L protease. PLoS Negl Trop Dis 7(9):e2414
Gottstein B, Schneeberger M, Boubaker G, Merkle B, Huber C, Spiliotis M, Müller N, Garate T, Doherr MG (2014) Comparative assessment of ELISAs using recombinant saposin-like protein 2 and recombinant cathepsin L-1 from Fasciola hepatica for the serodiagnosis of human fasciolosis. PLoS Negl Trop Dis 8(6):e2860
Graczyk TK, Fried B (2007) Human waterborne trematode and protozoa infections. Adv Parasitol 64:111–160
Graczyk TK, Gilman RH, Fried B (2001) Fasciolopsiasis: is it a controllable food-borne disease? Parasitol Res 87:80–83
Gray DJ, Ross AG, Li YS, McManus DP (2011) Diagnosis and management of schistosomiasis. BMJ 342:d2651
Grenfell R, Harn DA, Tundu S, Da'dara A, Siqueira L, Coelho PM (2013) New approaches with different types of circulating cathodic antigen for the diagnosis of patients with low *Schistosoma mansoni* load. PLoS Negl Trop Dis 7(2):e2054
Gryseels B, Polman K, Clerinx J (2006) Human schistosomiasis. Lancet 368:1106–1118
Gualdieri L, Rinaldi L, Petrullo L, Morgoglione ME, Maurelli MP, Musella V, Piemonte M, Caravano L, Coppola MG, Cringoli G (2011) Intestinal parasites in immigrants in the city of Naples (southern Italy). Acta Trop 117:196–201
Gupte A, Shah C, Koticha A, Shukla A, Kuyare S, Bhatia S (2014) *Gastrodiscoides hominis* infestation of colon: endoscopic appearance. Gastrointest Endosc 79:549–550
Habtamu K, Degarege A, Ye-Ebiyo Y, Erko B (2011) Comparison of the Kato-Katz and FLOTAC techniques for the diagnosis of soil-transmitted helminth infections. Parasitol Int 60:398–402
Harinasuta T, Pungpak S, Keystone J (1993) Trematode infections. Opisthorchiasis, clonorchiasis, fascioliasis and paragonimiasis. Infect Dis Clin N Am 7:699–716
Hatz CF (2001) The use of ultrasound in schistosomiasis. Adv Parasitol 48:225–284
Henry TS, Lane MA, Weil GJ, Bailey TC, Bhalla S (2012) Chest CT features of North American paragonimiasis. Am J Roentgenol 198:1076–1083
Hong ST, Fang Y (2012) *Clonorchis sinensis* and clonorchiasis, an update. Parasitol Int 61:17–24
Huang SY, Tang JD, Song HQ, Fu BQ, Xu MJ, Hu XC, Zhang H, Weng YB, Lin RQ, Zhu XQ (2012a) A specific PCR assay for the diagnosis of *Clonorchis sinensis* infection in humans, cats and fishes. Parasitol Int 61:187–190
Huang SY, Zhao GH, Fu BQ, Xu MJ, Wang CR, Wu SM, Zou FC, Zhu XQ (2012b) Genomics and molecular genetics of *Clonorchis sinensis*: current status and perspectives. Parasitol Int 61:71–76
Hung NM, Madsen H, Fried B (2013) Global status of fish-borne zoonotic trematodiasis in humans. Acta Parasitol 58:231–238
Intapan PM, Sanpool O, Thanchomnang T, Imtawil K, Pongchaiyakul C, Nawa Y, Maleewong W (2012) Molecular identification of a case of *Paragonimus pseudoheterotremus* infection in Thailand. Am J Trop Med Hyg 87:706–709
Iwagami M, Rajapakse RP, Paranagama W, Okada T, Kano S, Agatsuma T (2008) Ancient divergence of *Paragonimus westermani* in Sri Lanka. Parasitol Res 102:845–852
Jang JS, Kim KH, Yu JR, Lee SU (2007) Identification of parasite DNA in common bile duct stones by PCR and DNA sequencing. Korean J Parasitol 45:301–306
Jeandron A, Abdyldaieva G, Usubalieva J, Ensink JH, Cox J, Matthys B, Rinaldi L, Cringoli G, Utzinger J (2010) Accuracy of the Kato-Katz, adhesive tape and FLOTAC techniques for helminth diagnosis among children in Kyrgyzstan. Acta Trop 116:185–192
Jeandron A, Rinaldi L, Abdyldaieva G, Usubalieva J, Steinmann P, Cringoli G, Utzinger J (2011) Human infections with *Dicrocoelium dendriticum* in Kyrgyzstan: the tip of the iceberg? J Parasitol 97:1170–1172
Johansen MV, Sithithaworn P, Bergquist R, Utzinger J (2010) Towards improved diagnosis of zoonotic trematode infections in Southeast Asia. Adv Parasitol 73:171–195
Johansen MV, Lier T, Sithithaworn P (2015) Towards improved diagnosis of neglected zoonotic trematodes using a One Health approach. Acta Trop 141(Pt B):161–169
Jurberg AD, de Oliveira AA, Lenzi HL, Coelho PM (2008) A new miracidia hatching device for diagnosing schistosomiasis. Mem Inst Oswaldo Cruz 103:112–114
Kaewkes S, Elkins DB, Sithithaworn P, Haswell-Elkins MR (1991) Comparative studies on the morphology of the eggs of *Opisthorchis viverrini* and Lecithodendriid trematodes. Southeast Asian J Trop Med Public Health 22:623–630
Kaewkong W, Intapan PM, Sanpool O, Janwan P, Thanchomnang T, Laummaunwai P, Lulitanond V, Doanh PN, Maleewong W (2013) Molecular differentiation of *Opisthorchis viverrini* and *Clonorchis sinensis* eggs by multiplex real-time PCR with high resolution melting analysis. Korean J Parasitol 51:689–694
Kang S, Sultana T, Loktev VB, Wongratanacheewin S, Sohn WM, Eom KS, Park JK (2008) Molecular identification and phylogenetic analysis of nuclear rDNA sequences among three opisthorchiid liver fluke species (Opisthorchiidae: Trematoda). Parasitol Int 57:191–197
Katz N, Chaves A, Pellegrino J (1972) A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop São Paulo 14:397–400
Kazantseva L, García Lázaro MDP, Herrera-Velit P, Espinoza JR (2017) Anti-Fas2 IgM antibodies in

Fasciola hepatica infected patients with positive IgG serology. Trans R Soc Trop Med Hyg 111:102–106

Keiser J, Utzinger J (2005) Emerging foodborne trematodiasis. Emerg Infect Dis 11:1507–1514

Keiser J, Utzinger J (2009) Food-borne trematodiases. Clin Microbiol Rev 22:466–483

Keiser J, Duthaler U, Utzinger J (2010) Update on the diagnosis and treatment of food-borne trematode infections. Curr Opin Infect Dis 23:513–520

Khan MAH, Ullah R, Rehman A, Rehman L, Shareef PA, Abidi SMA (2017) Immunolocalization and immunodetection of the excretory/secretory (ES) antigens of *Fasciola gigantica*. PLoS One 12(10):e0185870

Kim TI, Na BK, Hong SJ (2009) Functional genes and proteins of *Clonorchis sinensis*. Korean J Parasitol 47:S59–S68

Kim YJ, Lee SM, Choi GE, Hwang SH, Kim HH, Lee EY, Chang CL (2010) Performance of an enzyme-linked immunosorbent assay for detection of *Clonorchis sinensis* infestation in high- and low-risk groups. J Clin Microbiol 48:2365–2367

Kim KU, Lee K, Park HK, Jeong YJ, Yu HS, Lee MK (2011) A pulmonary paragonimiasis case mimicking metastatic pulmonary tumor. Korean J Parasitol 49:69–72

Knight WB, Hiatt RA, Cline BL, Ritchie LS (1976) A modification of the formol-ether concentration technique for increased sensitivity in detecting Schistosoma mansoni. Am J Trop Med Hyg 55:818–823

Knopp S, Rinaldi L, Khamis IS, Stothard JR, Rollinson D, Maurelli MP, Steinmann P, Marti H, Cringoli G, Utzinger J (2009a) A single FLOTAC is more sensitive than triplicate Kato-Katz for the diagnosis of low-intensity soil-transmitted helminth infections. Trans R Soc Trop Med Hyg 103:347–354

Knopp S, Glinz D, Rinaldi L, Mohammed KA, N'Goran EK, Stothard JR, Marti H, Cringoli G, Rollinson D, Utzinger J (2009b) FLOTAC: a promising technique for detecting helminth eggs in human faeces. Trans R Soc Trop Med Hyg 103:1190–1194

Knopp S, Corstjens PL, Koukounari A, Cercamondi CI, Ame SM, Ali SM, de Dood CJ, Mohammed KA, Utzinger J, Rollinson D, van Dam GJ (2015) Sensitivity and specificity of a urine circulating anodic antigen test for the diagnosis of *Schistosoma haematobium* in low endemic settings. PLoS Negl Trop Dis 9(5):e0003752

Koh EJ, Kim SK, Wang KC, Chai JY, Chong S, Park SH, Cheon JE, Phi JH (2012) The return of an old worm: cerebral paragonimiasis presenting with intracerebral hemorrhage. J Korean Med Sci 27:1428–1432

Lall M, Sahni AK, Rajput AK (2013) Pleuropulmonary paragonimiasis: mimicker of tuberculosis. Pathog Glob Health 107:40–42

Lamaningao P, Kanda S, Laimanivong S, Shimono T, Darcy AW, Phyaluanglath A, Mishima N, Nishiyama T (2017) Development of a PCR assay for diagnosing trematode (*Opisthorchis* and *Haplorchis*) infections in human stools. Am J Trop Med Hyg 96:221–228

Lambertucci JR, dos Santos Silva LC, Andrade LM, de Queiroz LC, Carvalho VT, Voieta I, Antunes CM (2008) Imaging techniques in the evaluation of morbidity in schistosomiasis mansoni. Acta Trop 108:209–217

Lane MA, Marcos LA, Onen NF, Demertzis LM, Hayes EV, Davila SZ, Nurutdinova DR, Bailey TC, Weil GJ (2012) *Paragonimus kellicotti* flukes in Missouri, USA. Emerg Infect Dis 18:1263–1267

Le TH, Van De N, Blair D, Sithithaworn P, McManus DP (2006a) *Clonorchis sinensis* and *Opisthorchis viverrini*: development of a mitochondrial-based multiplex PCR for their identification and discrimination. Exp Parasitol 112:109–114

Le TH, Van De N, Blair D, McManus DP, Kino H, Agatsuma T (2006b) *Paragonimus heterotremus* Chen and Hsia (1964), in Viet Nam: a molecular identification and relationships of isolates from different hosts and geographical origins. Acta Trop 98:25–33

Le TH, Hguyen NTB, Truong NH, De NV (2012a) Development of mitochondrial loop-mediated isothermal amplification for detection of the small liver fluke *Opisthorchis viverrini* (Opisthorchiidae; Trematoda; Platyhelminthes). J Clin Microbiol 50:1178–1184

Le TH, Nguyen KT, Nguyen NT, Doan HT, Le XT, Hoang CT, De NV (2012b) Development and evaluation of a single-step duplex PCR for simultaneous detection of *Fasciola hepatica* and *Fasciola gigantica* (family Fasciolidae, class Trematoda). J Clin Microbiol 50:2720–2726

Lee SH, Hwang SW, Chai JY, Seo BS (1984) Comparative morphology of eggs of heterophyids and *Clonorchis sinensis* causing human infections in Korea. Korean J Parasitol 22:171–180

Lee SC, Chung YB, Kong Y, Kang SY, Cho SY (1993) Antigenic protein fractions of *Metagonimus yokogawai* reacting with patient sera. Korean J Parasitol 31:43–48

Lee EG, Na BK, Bae YA, Kim SH, Je EY, Ju JW, Cho SH, Kim TS, Kang SY, Cho SY, Kong Y (2006) Identification of immunodominant excretory-secretory cysteine proteases of adult *Paragonimus westermani* by proteome analysis. Proteomics 6:1290–1300

Lee JS, Lee J, Kim SH, Yong TS (2007) Molecular cloning and characterization of a major egg antigen in *Paragonimus westermani* and its use in ELISA for the immunodiagnosis of paragonimiasis. Parasitol Res 100:677–681

Lee JJ, Jung BK, Lim H, Lee MY, Choi SY, Shin EH, Chai JY (2012a) Comparative morphology of minute intestinal fluke eggs that occur in human stools in the Republic of Korea. Korean J Parasitol 50:207–213

Lee MR, Kim YJ, Kim DW, Yoo WG, Cho SH, Hwang KY, Ju JW, Lee WJ (2012b) The identification of antigenic proteins: 14-3-3 protein and propionyl-CoAcarboxylase in *Clonorchis sinensis*. Mol Biochem Parasitol 182:1–6

Lengeler C, Utzinger J, Tanner M (2002) Screening for schistosomiasis with questionnaires. Trends Parasitol 18:375–377

Levy C, Lymp J, Angulo P, Gores GJ, Larusso N, Lindor KD (2005) The value of serum CA 19-9 in predicting cholangiocarcinomas in patients with primary sclerosing colangitis. Dig Dis Sci 50:1734–1740

Li Y, Hu X, Liu X, Huang Y, Xu J, Zhao J, Wu Z, Yu X (2012) Serological diagnosis of clonorchiasis: using a recombinant propeptide of cathepsin L proteinase from *Clonorchis sinensis* as a candidate antigen. Parasitol Res 110:2197–2203

Liang T, Liang G, Du Y, Wang X, Wang Y, Chen A, Chen Z, Du J, Li H, Yu L (2018) Scrotal Paragonimiasis in adults: two case reports and review of literature. Medicine (Baltimore) 97(14):e0328

Lier T, Simonsen GS, Wang T, Lu D, Haukland HH, Vennervald BJ, Johansen MV (2009a) Low sensitivity of the formol-ethyl acetate sedimentation concentratiom technique in low-intensity *Schistosoma japonicum* infections. PLoS Negl Trop Dis 3(2):e386

Lier T, Simonsen GS, Wang T, Lu D, Haukland HH, Vennervald BJ, Hegstad J, Johansen MV (2009b) Real-time polymerase chain reaction for detection of low-intensity *Schistosoma japonicum* infections in China. Am J Trop Med Hyg 81:428–432

Lim JH, Mairiang E, Ahn GH (2008) Biliary parasitic diseases including clonorchiasis, opisthorchiasis and fascioliasis. Abdom Imaging 33:157–165

Linder E, Lundin M, Thors C, Lebbad M, Winiecka-Krusnell J, Helin H, Leiva B, Isola J, Lundin J (2008) Web-based virtual microscopy for parasitology: a novel tool for education and quality assurance. PLoS Negl Trop Dis 2(10):e315

Linder E, Grote A, Varjo S, Linder N, Lebbad M, Lundin M, Diwan V, Hannuksela J, Lundin J (2013) On-chip imaging of *Schistosoma haematobium* eggs in urine for diagnosis by computer vision. PLoS Negl Trop Dis 7(12):e2547

Liu GH, Yan HB, Otranto D, Wang XY, Zhao GH, Jia WZ, Zhu XQ (2014) *Dicrocoelium chinensis* and *Dicrocoelium dendriticum* (Trematoda: Digenea) are distinct lancet fluke species based on mitochondrial and nuclear ribosomal DNA sequences. Mol Phylogenet Evol 79:325–331

Long Y, Qin Z, Duan M, Li S, Wu X, Lin W, Li J, Zhao Z, Liu J, Xiong D, Huang Y, Hu X, Yang C, Ye M, Tan W (2016) Screening and identification of DNA aptamers toward *Schistosoma japonicum* eggs via SELEX. Sci Rep 6:24986

López-Caballero J, Oceguera-Figueroa A, León-Règagnon V (2013) Detection of multiple species of human *Paragonimus* from Mexico using morphological data and molecular barcodes. Mol Ecol Resour 13(6):1125–1136

Lovis L, Mak TK, Phongluxa K, Soukhathammavong P, Sayasone S, Akkhavong K, Odermatt P, Keiser J, Felger I (2009) PCR diagnosis of *Opisthorchis viverrini* and *Haplorchis taichui* infections in a Lao community in an area of endemicity and comparison of diagnostic methods for parasitological field surveys. J Clin Microbiol 47:1517–1523

Lumbreras H, Cantella R, Burga R (1962) Acerca de un procedimiento de sedimentación rápida para investigar huevos de *Fasciola hepatica* en las heces, su evaluación y su uso en el campo. Rev Méd Perú 31:167–174. (in Spanish).

Mairiang E, Laha T, Bethony JM, Thinkhamrop B, Kaewkes S, Sithithaworn P, Tesana S, Loukas A, Brindley PJ, Sripa B (2012) Ultrasonography assessment of hepatobiliary abnormalities in 3359 subjects with *Opisthorchis viverrini* infection in endemic areas of Thailand. Parasitol Int 61:208–211

Maleewong W (1997) Recent advances in diagnosis of paragonimiasis. Southeast Asian J Trop Med Public Health 28(Suppl 1):134–138

Marchese V, Beltrame A, Angheben A, Monteiro GB, Giorli G, Perandin F, Buonfrate D, Bisoffi Z (2018) Schistosomiasis in immigrants, refugees and travellers in an Italian referral centre for tropical diseases. Infect Dis Poverty 7:55

Marco-Flores V, Marcos-Raymundo L, Terashima Iwashita A, Samalvides-Cuba F, Miranda-Sanchez E, Espinoza-Babilon J, Gotuzzo-Herencia E (2002) Fas-2 ELISA and the rapid sedimentation technique modified by Lumbreras for *Fasciola hepatica* infection diagnosis. Rev Méd Herediana 13:49–57

Martínez-Sernández V, Muiño L, Perteguer MJ, Gárate T, Mezo M, González-Warleta M, Muro A, Correia da Costa JM, Romarís F, Ubeira FM (2011) Development and evaluation of a new lateral flow immunoassay for serodiagnosis of human fasciolosis. PLoS Negl Trop Dis 5:e1376

Marty AM, Neafie RC (2000) Paragonimiasis. In: Meyers WM, Neafie RC, Marty AM, Wear DJ (eds) Pathology of infectious diseases, vol I. Helmintiases. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, pp 49–67

Mas-Coma S, Bargues MD (1997) Human liver flukes: a review. Res Rev Parasitol 57:145–218

Mas-Coma S, Bargues MD, Esteban JG (1999) Human fasciolosis. In: Dalton JP (ed) Fasciolosis. CAB International, Wallingford, Oxon, pp 411–434

Mas-Coma S, Bargues MD, Marty AM, Neafie RC (2000) Hepatic trematodiases. In: Meyers WM, Neafie RC, Marty AM, Wear DJ (eds) Pathology of infectious diseases, vol 1. Helminthiases. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, pp 69–92

Mas-Coma S, Bargues MD, Valero MA (2005) Fascioliasis and other plant-borne trematode zoonoses. Int J Parasitol 35:1255–1278

Mas-Coma S, Bargues MD, Valero MA (2007) Plant-borne trematode zoonoses: fascioliasis and fasciolopsiasis. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites, vol 11. Springer, New York, pp 293–334

Mas-Coma S, Valero MA, Bargues MD (2009) *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolu-

tionary genetics, molecular epidemiology and control. Adv Parasitol 69:41–146

Mas-Coma S, Bargues MD, Valero MA (2014a) Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. Parasitology 141:1918–1946

Mas-Coma S, Valero MA, Bargues MD (2014b) Fascioliasis. In: Toledo R, Fried B (eds) Digenetic trematodes, 1st edn. Springer Science + Business Media, New York, pp 77–114

Mas-Coma S, Bargues MD, Valero MA (2018) Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. Parasitology 145:1665–1699

Mazidur Rahman SM, Choi MH, Bae YM, Hong ST (2012) Coproantigen capture ELISA for detection of *Clonorchis sinensis* infection in experimentally infected rats. Parasitol Int 61:203–207

McManus DP, Gray DJ, Li Y, Feng Z, Williams GM, Stewart D, Rey-Ladino J, Ross AG (2010) Schistosomiasis in the People's Republic of China: the era of the Three Gorges Dam. Clin Microbiol Rev 23:442–466

Meyers WM, Neafie RC, Marty AM, Wear DJ (2000) Pathology of infectious diseases, vol I. Helmintiases. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC

Mirzadeh A, Valadkhani Z, Yoosefy A, Babaie J, Golkar M, Esmaeili Rastaghi AR, Kazemi-Rad E, Ashrafi K (2017) Expression, purification and in vitro refolding of the recombinant truncated Saposin-like protein 2 antigen for development of diagnosis of human fascioliasis. Acta Trop 171:163–171

Mokhtarian K, Akhlaghi L, Mohammadi M, Meamar AR, Razmjou E, Khoshmirsafa M, Falak R (2016a) Evaluation of anti-Cathepsin L1: a more reliable method for serodiagnosis of human fasciolosis. Trans R Soc Trop Med Hyg 110:542–550

Mokhtarian K, Akhlaghi L, Meamar AR, Razmjou E, Manouchehri Naeini K, Gholami S, Najafi Samei M, Falak R (2016b) Serodiagnosis of fasciolosis by fast protein liquid chromatography-fractionated excretory/secretory antigens. Parasitol Res 115:2957–2965

Mordvinov VA, Yurlova NI, Ogorodova LM, Katokhin AV (2012) *Opisthorchis felineus* and *Metorchis bilis* are the main agents of liver fluke infection of humans in Russia. Parasitol Int 61:25–31

Moure Z, Zarzuela F, Espasa M, Pou D, Serre-Delcor N, Treviño B, Bocanegra C, Molina I, Pumarola T, Sulleiro E (2017) *Dicrocoelium dendriticum*: an unusual parasitological diagnosis in a reference international health unit. Am J Trop Med Hyg 96:355–357

Mulvenna J, Yonglitthipagon SB, Sripa B, Brindley PJ, Loukas A, Bethony JM (2012) Banking on the future: biobanking for "omics" approaches to biomarker discovery for *Opisthorchis*-induced cholangiocarcinoma in Thailand. Parasitol Int 61:173–177

Muth S, Sayasone S, Odermatt-Biays S, Phompida S, Duong S, Odermatt P (2010) *Schistosoma mekongi* in Cambodia and Lao People's Democratic Republic. Adv Parasitol 72:179–203

Mwangi IN, Agola EL, Mugambi RM, Shiraho EA, Mkoji GM (2018) Development and evaluation of a loop-mediated isothermal amplification assay for diagnosis of *Schistosoma mansoni* infection in faecal samples. J Parasitol Res 2018:1267826

Navaratnam AM, Mutumba-Nakalembe MJ, Stothard JR, Kabatereine NB, Fenwick A, Sousa-Figueiredo JC (2012) Notes on the use of urine-CCA dipsticks for detection of intestinal schistosomiasis in preschool children. Trans R Soc Trop Med Hyg 106:619–622

Nkouawa A, Okamoto M, Mabou AK, Edinga E, Yamasaki H, Sako Y, Nakao M, Nakaya K, Blair D, Agatsuma T, Enyong P, Shibahara T, Moyou-Somo R, Ito A (2009) Paragonimiasis in Cameroon: molecular identification, serodiagnosis and clinical manifestations. Trans R Soc Trop Med Hyg 103:255–261

Obeng BB, Aryeetey YA, de Dood CJ, Amoah AS, Larbi IA, Deelder AM, Yazdanbakhsh M, Hartgers FC, Boakye DA, Verweij JJ, van Dam GJ, van Lieshout L (2008) Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium* in urine samples from Ghana. Ann Trop Med Parasitol 102:625–633

Ochodo EA, Gopalakrishna G, Spek B, Reitsma JB, van Lieshout L, Polman K, Lamberton P, Bossuyt PM, Leeflang MM (2015) Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. Cochrane Database Syst Rev (3):CD009579

Ohmae H, Sinuon M, Kirinoki M, Matsumoto J, Chigusa Y, Socheat D, Matsuda H (2004) *Schistosomiasis mekongi*: from discovery to control. Parasitol Int 53:135–142

Olveda DU, Olveda RM, Lam AK, Chau TN, Li Y, Gisparil AD II, Ross AG (2014) Utility of diagnostic imaging in the diagnosis and management of Schistosomiasis. Clin Microbiol 3(2):142–155

Orihel TC, Ash LR (1995) Parasites in human tissues. ASCP Press, American Society of Clinical Pathologists, Chicago

Ortu G, Ndayishimiye O, Clements M, Kayugi D, Campbell CH, Lamine MS, Zivieri A, Magalhaes RS, Binder S, King CH, Fenwick A, Colley DG, Jourdan PM (2017) Countrywide reassessment of *Schistosoma mansoni* infection in Burundi using a urine-circulating cathodic antigen rapid test: informing the National Control Program. Am J Trop Med Hyg 96:664–673

Osakunor DNM, Woolhouse MEJ, Mutapi F (2018) Paediatric schistosomiasis: what we know and what we need to know. PLoS Negl Trop Dis 12(2):e0006144

Pagès JR, Jourdane J, Southgate VR, Tchuem Tchuenté LA (2003) Reconnaissance de deux espèces jumelles au sein du taxon *Schistosoma intercalatum* Fisher, 1934, agent de la schistosomose humaine rectale en Afrique. Description de *Schistosoma guineensis* n. sp. In: Combes C, Jourdane J (eds) Taxonomy, ecology

and evolution of metazoan parasites, vol II. Presses Universitaires de Perpignan, Perpignan, pp 139–146

Patel NU, Bang TJ, Dodd GD 3rd (2016) CT findings of human *Fasciola hepatica* infection: case reports and review of the literature. Clin Imaging 40:251–255

Pauly A, Schuster R, Steuber S (2003) Molecular characterization and differentiation of opisthorchiid trematodes of the species *Opisthorchis felineus* (Rivolta, 1884) and *Metorchis bilis* (Braun, 1790) using polymerase chain reaction. Parasitol Res 90:409–414

Petney TN, Andrews RH, Saijuntha W, Wenz-Mücke A, Sithithaworn P (2013) The zoonotic, fish-borne liver flukes *Clonorchis sinensis*, *Opisthorchis felineus* and *Opisthorchis viverrini*. Int J Parasitol 43:1031–1046

Polman K, Diakhate MM, Engels D, Nahimana S, Van Dam GJ, Falcão Ferreira ST, Deelder AM, Gryseels B (2000) Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. Trop Med Int Health 5:534–537

Pozio E, Armignacco O, Ferri F, Gomez Morales MA (2013) *Opisthorchis felineus* an emerging infection in Italy and its implication for the European Union. Acta Trop 126:54–62

Prasad PK, Goswami LM, Tandon V, Chatterjee A (2011) PCR-based molecular characterization and insilico analysis of food-borne trematode *parasites Paragonimus westermani*, *Fasciolopsis buski* and *Fasciola gigantica* from Northeast India using ITS2 rDNA. Bioinformation 6:64–68

Procop GW (2009) North American paragonimiasis (caused by *Paragonimus kellicotti*) in the context of global paragonimiasis. Clin Microbiol Rev 22:415–446

Qiu XG, Nakamura-Uchiyama F, Nawa Y, Itoh M (2016) A tool for mass-screening of paragonimiasis: an enzyme-linked immunosorbent assay with urine samples. Trop Med Health 44:19. https://doi.org/10.1186/s41182-016-0019-4

Quiao TQ, Zheng PM, Ma RH, Luo XB, Luo ZL (2012) Development of a real-time PCR assay for the detection of *Clonorchis sinensis* DNA in gallbladder bile and stone samples from patients with cholecystolithiasis. Parasitol Res 111:1497–1503

Rahimi MT, Ashrafi K, Koosha S, Abdi J, Rokni MB (2011) Evaluation of Fast-ELISA versus standard-ELISA to diagnose human fasciolosis. Arch Iran Med 14:18–21

Rim HJ (1982) Opisthorchiasis. In: Hillyer GV, Hopla CE (eds) Handbook series in zoonoses. Section C: Parasite zoonoses, vol III. CRC Press, Boca Raton, pp 109–121

Rim HJ (2005) Clonorchiasis: an update. J Helminthol 79:269–281

Ritchie LS (1948) An ether sedimentation technique for routine stool examinations. Bull U S Army Med Dep 8:326

Robijn ML, Planken J, Kornelis D, Hokke CH, Deelder AM (2008) Mass spectrometric detection of urinary oligosaccharides as markers of *Schistosoma mansoni* infection. Trans R Soc Trop Med Hyg 102:79–83

Rokni MB, Mirhendi H, Mizani A, Mohebali M, Sharbatkhori M, Kia EB, Abdoli H, Izadi S (2010) Identification and differentiation of *Fasciola hepatica* and *Fasciola gigantica* using a simple PCR-restriction enzyme method. Exp Parasitol 124:209–213

Ross AGP, Sleigh AC, Li Y, Davis GM, Williams GM, Jiang Z, Feng Z, McManus DP (2001) Schistosomiasis in the People's Republic of China: prospects and challenges for the 21st century. Clin Microbiol Rev 14:270–295

Ross AGP, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, McManus DP (2002) Schistosomiasis. N Engl J Med 346:1212–1220

Sandoval H, Manga-González MY, Castro JM (2013) A tool for diagnosis of *Dicrocoelium dendriticu*m infection: hatching eggs and molecular identification of the miracidium. Parasitol Res 112:1589–1595

Sanneh B, Joof E, Sanyang AM, Renneker K, Camara Y, Sey AP, Jagne S, Baldeh I, Ceesay SJ, Sambou SM, Ogoussan K (2017) Field evaluation of a schistosome circulating cathodic antigen rapid test kit at point-of-care for mapping of schistosomiasis endemic districts in The Gambia. PLoS One 12(8):e0182003

Sanpool O, Intapan PM, Thanchomnang T, Janwan P, Lulitanond V, Doanh PN, Van Hien H, Dung do T, Maleewong W, Nawa Y (2012) Rapid detection and differentiation of *Clonorchis sinensis* and *Opisthorchis viverrini* eggs in human fecal samples using a duplex real-time fluorescence resonance energy transfer PCR and melting curve analysis. Parasitol Res 111:89–96

Sarkari B, Khabisi SA (2017) Immunodiagnosis of human fascioliasis: an update of concepts and performances of the serological assays. J Clin Diagn Res 11:OE05–OE10

Sato M, Thaenkham U, Dekumyoy P, Waikagul J (2009) Discrimination of *O. viverrini*, *C. sinensis*, *H. pumilio* and *H. taichui* using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. Acta Trop 109:81–83

Sato M, Pongvongsa T, Sanguankiat S, Yoonuan T, Dekumyoy P, Kalambaheti T, Keomoungkhoun M, Phimmayoi I, Boupha B, Moji K, Waikagul J (2010) Copro-DNA diagnosis of *Opisthorchis viverrini* and *Haplorchis taichui* infection in an endemic area of Lao PDR. Southeast Asian J Trop Med Public Health 41:28–35

Sawangsoda P, Sithithaworn J, Tesana S, Pinlaor S, Boonmars T, Mairiang E, Yongvanit P, Duenngai K, Sithithaworn P (2012) Diagnostic values of parasite-specific antibody detections in saliva and urine in comparison with serum in opisthorchiasis. Parasitol Int 61:196–202

Shafiei R, Sarkari B, Sadjjadi SM (2015) Performance of a 27 kDa *Fasciola hepatica* antigen in the diagnosis of human fascioliasis. J Lab Phys 7:17–20

Shalaby S, El-Bahy M, Hassan A, Shalaby H, Gupta N (2015) Diagnotic value of some *Fasciola gigantica* antigens. J Parasit Dis 39:479–486

Shane HL, Verani JR, Abudho B, Montgomery SP, Blackstock AJ, Mwinzi PN, Butler SE, Karanja DM, Secor WE (2011) Evaluation of urine CCA assays for

detection of *Schistosoma mansoni* infection in Western Kenya. PLoS Negl Trop Dis 5:e951

Shim SS, Kim Y, Lee JK, Lee JH, Song DE (2012) Pleuropulmonary and abdominal paragonimiasis: CT and ultrasound findings. Br J Radiol 85:403–410

Shin SH, Hsu A, Chastain HM, Cruz LA, Elder ES, Sapp SG, McAuliffe I, Espino AM, Handali S (2016) Development of two FhSAP2 recombinant-based assays for immunodiagnosis of human chronic fascioliasis. Am J Trop Med Hyg 95:852–855

Sithithaworn P, Yongvanit P, Tesana S, Pairojkul C (2007) Liver flukes. In: Murrell KD, Fried B (eds) Food-borne parasitic zoonoses: fish and plant-borne parasites, world class parasites, vol 11. Springer, New York, pp 3–52

Slesak G, Inthalad S, Basy P, Keomanivong D, Phoutsavath O, Khampoui S, Grosrenaud A, Amstutz V, Barennes H, Buisson Y, Odermatt P (2011) Ziehl-Neelsen staining technique can diagnose paragonimiasis. PLoS Negl Trop Dis 5(5):e1048. https://doi.org/10.1371/journal.pntd.0001048

Slusarewicz P, Pagano S, Mills C, Popa G, Chow KM, Mendenhall M, Rodgers DW, Nielsen MK (2016) Automated parasite faecal egg counting using fluorescence labelling, smartphone image capture and computational image analysis. Int J Parasitol 46:485–493

Sohn WM, Yong TS, Eom KS, Min DY, Lee D, Jung BK, Banouvong V, Insisiengmay B, Phommasack B, Rim HJ, Chai JY (2014) Prevalence of *Haplorchis taichui* among humans and fish in Luang Prabang Province, Lao PDR. Acta Trop 136:74–80

Sowerby SJ, Crump JA, Johnstone MC, Krause KL, Hill PC (2016) Smartphone microscopy of parasite eggs accumulated into a single field of view. Am J Trop Med Hyg 94:227–230

Sripa B, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, Pairojkul C, Bhudhisawasdi V, Tesana S, Thinkamrop B, Bethony JM, Loukas A, Brindley PJ (2007) Liver fluke induces cholangiocarcinoma. PLoS Med 4:1148–1155

Sripa B, Kaewkes S, Intapan PM, Maleewong W, Brindley PJ (2010) Food-borne trematodiases in Southeast Asia epidemiology, pathology, clinical manifestation and control. Adv Parasitol 72:305–350

Sripa B, Bethony JM, Sithithaworn P, Kaewkes S, Mairiang E, Loukas A, Mulvenna J, Laha T, Hotez PJ, Brindley PJ (2011) Opisthorchiasis and *Opisthorchis*-associated cholangiocarcinoma in Thailand and Laos. Acta Trop 120S:S158–S168

Sripa J, Brindley PJ, Sripa B, Loukas A, Kaewkes S, Laha T (2012) Evaluation of liver fluke recombinant cathepsin B-1 protease as a serodiagnostic antigen for human opisthorchiasis. Parasitol Int 61:191–195

Stensvold CR, Saijuntha W, Sithithaworn P, Wongratanacheewin S, Strandgaard H, Ornbjerg N, Johansen MV (2006) Evaluation of PCR based coprodiagnosis of human opisthorchiasis. Acta Trop 97:26–30

Stoll NR (1923) Investigations on the control of hookworm disease. XV. An effective method of counting hookworm eggs in feces. Am J Hyg 3:59–70

Sukontason K, Piangjai S, Sukontason K, Chaithong U (1999) Potassium permanganate staining for differentiation the surface morphology of *Opisthorchis viverrini*, *Haplorchis taichui* and *Phaneropsolus bonnie* eggs. Southeast Asian J Trop Med Public Health 30:371–374

Sun J, Xu J, Liang P, Mao Q, Huang Y, Lv X, Deng C, Liang C, de Hoog GS, Yu X (2011) Molecular identification of *Clonorchis sinensis* and discrimination with other opisthorchid liver fluke species using multiple ligation-depended probe amplification (MLPA). Parasit Vectors 4:98

Tang ZL, Huang Y, Yu XB (2016) Current status and perspectives of *Clonorchis sinensis* and clonorchiasis: epidemiology, pathogenesis, omics, prevention and control. Infect Dis Poverty 5:71

Tantrawatpan C, Intapan PM, Janwan P, Sanpool O, Lulitanond V, Srichantaratsamee C, Anamnart W, Maleewong W (2013) Molecular identification of *Paragonimus* species by DNA pyrosequencing technology. Parasitol Int 62:341–345

Tantrawatpan C, Intapan PM, Thanchomnang T, Sanpool O, Janwan P, Lulitanond V, Sadaow L, Maleewong W (2014) Development of a PCR assay and pyrosequencing for identification of important human fish-borne trematodes and its potential use for detection in fecal specimens. Parasit Vectors 7:88

Tantrawatpan C, Saijuntha W, Manochantr S, Kheolamai P, Thanchomnang T, Sadaow L, Intapan PM, Maleewong W (2016) A singleplex real-time fluorescence resonance energy transfer PCR with melting curve analysis for the differential detection of *Paragonimus heterotremus*, *Echinostoma malayanum* and *Fasciola gigantica* eggs in faeces. Trans R Soc Trop Med Hyg 110:74–83

Tchuem Tchuenté LA, Kueté Fouodo CJ, Kamwa Ngassam RI, Sumo L, Dongmo Noumedem C, Kenfack CM, Gipwe NF, Nana ED, Stothard JR, Rollinson D (2012) Evaluation of circulating cathodic antigen (CCA) urine-tests for diagnosis of *Schistosoma mansoni* infection in Cameroon. PLoS Negl Trop Dis 6:e1758

Teimoori S, Arimatsu Y, Laha T, Kaewkes S, Sereerak P, Tangkawattana S, Brindley PJ, Sripa B (2015) Immunodiagnosis of opisthorchiasis using parasite cathepsin F. Parasitol Res 114:4571–4578

Teixera CF, Neuhauss E, Ben R, Romanzini J, Graeff-Teixeira C (2007) Detection of *Schistosoma mansoni* eggs in feces through their interaction with paramagnetic beads in a magnetic field. PLoS Negl Trop Dis 1:e73

ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Dieye L, van Lieshout L (2008) Multiplex real-time PCR for the detection and quantification of *Schistosoma mansoni* and *S. haematobium* infection in stool samples collected in northern Senegal. Trans R Soc Trop Med Hyg 102:179–185

Terashima A, Marcos L, Maco V, Canales M, Samalvides F, Tello R (2009) Spontaneous sedimentation in tuve technique (SSTT) for diagnosis of intestinal parasites. Rev Gastroenterol Peru 29:305–310

Tesana S, Srisawanwonk T, Kaewkes S, Sithithaworn P, Kanla P, Arunyanart C (1991) Egg shell morphology of the small eggs of human trematodes in Thailand. Southeast Asian J Trop Med Public Health 22:631–636

Thaenkham U, Visetsuk K, Dung DT, Waikagul J (2007) Discrimination of *Opisthorchis viverrini* from *Haplorchis taichui* using COI sequence marker. Acta Trop 103:26–32

Toledo R, Esteban JG (2016) An update on human echinostomiasis. Trans R Soc Trop Med Hyg 110:37–45

Toledo R, Esteban JG, Fried B (2006) Immunology and pathology of intestinal trematodes in their definitive hosts. Adv Parasitol 63:285–365

Toledo R, Esteban JG, Fried B (2012) Current status of food-borne trematode infections. Eur J Clin Microbiol Infect Dis 31:1705–1718

Toledo R, Muñoz-Antolí C, Esteban JG (2014) Intestinal trematode infections. In: Toledo R, Fried B (eds) Digenetic trematodes, 1st edn. Springer Science + Business Media, New York, pp 201–240

Traub RJ, Macaranas J, Mungthin M, Leelayoova S, Cribb T, Murrell KD, Thompson RC (2009) A new PCR-based approach indicates the range of *Clonorchis sinensis* now extends to Central Thailand. PLoS Negl Trop Dis 3:e367

Ubeira FM, Muiño L, Valero MA, Periago MV, Pérez-Crespo I, Mezo M, González-Warleta M, Romarís F, Paniagua E, Cortizo S, Llovo J, Más-Coma S (2009) MM3-ELISA detection of *Fasciola hepatica* coproantigens in preserved human stool samples. Am J Trop Med Hyg 81:156–162

Upatham ES, Viyanant V (2003) *Opisthorchis viverrini* and opisthorchiasis: a historical review and future perspective. Acta Trop 88:171–176

Utzinger J, Rinaldi L, Lohourignon LK, Rohner F, Zimmermann MB, Tschannen AB, N'Goran EK, Cringoli G (2008) FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. Trans R Soc Trop Med Hyg 102:84–90

Valero MA, Pérez-Crespo I, Periago MV, Khoubbane M, Mas-Coma S (2009a) Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. Acta Trop 111:150–159

Valero MA, Ubeira FM, Khoubbane M, Artigas P, Muiño L, Mezo M, Pérez-Crespo I, Periago MV, Mas-Coma S (2009b) MM3-ELISA evaluation of coproantigen release and serum antibody production in sheep experimentally infected with *Fasciola hepatica* and *F. gigantica*. Vet Parasitol 159:77–81

Valero MA, Periago MV, Pérez-Crespo I, Rodríguez E, Perteguer MJ, Gárate T, González-Barberá EM, Mas-Coma S (2012a) Assessing the validity of an ELISA test for the serological diagnosis of human fascioliasis in different epidemiological situations. Tropical Med Int Health 17:630–636

Valero MA, Periago MV, Pérez-Crespo I, Angles R, Villegas F, Aguirre C, Strauss W, Espinoza JR, Herrera P, Terashima A, Tamayo H, Engels D, Gabrielli AF, Mas-Coma S (2012b) Field evaluation of a coproantigen detection test for fascioliasis diagnosis and surveillance in human hyperendemic areas of Andean countries. PLoS Negl Trop Dis 6(9):e1812

van Gool T, Vetter JCM, Vervoort T, Doenhoff MJ, Wetsteyn J, Overbosch D (2002) Serodiagnosis of imported schistosomiasis by a combination of a commercial indirect hemagglutination test with *Schistosoma mansoni* adult worm antigens and enzyme-linked immunosorbent assay with *S. mansoni* egg antigens. J Clin Microbiol 40:3232–3237

van Grootveld R, van Dam GJ, de Dood C, de Vries JJC, Visser LG, Corstjens PLAM, van Lieshout L (2018) Improved diagnosis of active *Schistosoma* infection in travellers and migrants using the ultra-sensitive in-house lateral flow test for detection of circulating anodic antigen (CAA) in serum. Eur J Clin Microbiol Infect Dis 37:1709–1716

Vanijanonta S, Radomyos P, Bunnag D, Harinasuta T (1981) Pulmonary paragonimiasis with expectoration of worms: a case report. Southeast Asian J Trop Med Public Health 12:104–106

Wang W, Li Y, Li H, Xing Y, Qu G, Dai J, Liang Y (2012) Immunodiagnostic efficacy of detection of *Schistosoma japonicum* human infections in China: a meta-analysis. Asian Pac J Trop Med 5:15–23

Watwiengkam N, Sithithaworn J, Duenngai K, Sripa B, Laha T, Johansen MV, Sithithaworn P (2013) Improved performance and quantitative detection of copro-antigens by a monoclonal antibody based ELISA to diagnose human opisthorchiasis. Acta Trop 128:659–665

Webb CM, Cabada MM (2018) Recent developments in the epidemiology, diagnosis, and treatment of Fasciola infection. Curr Opin Infect Dis 31:409–414

Webster BL, Southgate VR, Littlewood DTJ (2006) A revision of the interrelationships of *Schistosoma* including the recently described *Schistosoma guineensis*. Int J Parasitol 36:947–955

Weerakoon KG, Gobert GN, Cai P, McManus DP (2015) Advances in the diagnosis of human schistosomiasis. Clin Microbiol Rev 28:939–967

Weerakoon KG, Gordon CA, McManus DP (2018) DNA diagnostics for schistosomiasis control. Trop Med Infect Dis 3(3):E81

WHO (1991) Basic laboratory methods in medical parasitology. World Health Organization, Geneva

Wichmann D, Panning M, Quack T, Kramme S, Burchard GD, Grevelding C, Drosten C (2009) Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. PLoS Negl Trop Dis 3:e422

Wichmann D, Poppert S, Von Thien H, Clerinx J, Dieckmann S, Jensenius M, Parola P, Richter J, Schunk M, Stich A, Zanger P, Burchard GD, Tannich E (2013) Prospective European-wide multicentre study on a blood based real-time PCR for the diagnosis of acute schistosomiasis. BMC Infect Dis 13:55

Wolfe MS (2007) *Dicrocoelium dendriticum* or *Dicrocoelium hospes*. Clin Infect Dis 44:1522

Wongkham C, Intapan PM, Maleewong W, Miwa M (2005) Evaluation of human IgG subclass antibodies

in the serodiagnosis of *Paragonimiasis heterotremus*. Asian Pac J Allergy Immunol 23:205–211

Wongratanacheewin S, Pumidonming W, Sermswan RW, Pipitgool V, Maleewong W (2002) Detection of *Opisthorchis viverrini* in human stool specimens by PCR. J Clin Microbiol 40:3879–3880

Wongratanacheewin S, Sermswan RW, Sirisinha S (2003) Immunology and molecular biology of *Opisthorchis viverrini* infection. Acta Trop 88:195–207

Wongsawad P, Wongsawad C (2009) Development of PCR-based diagnosis of minute intestinal fluke, *Haplorchis taichui*. Southeast Asian J Trop Med Public Health 40:919–923

Wongsawad C, Wongsawad P (2012) *Opisthorchis viverrini* and *Haplorchis taichui*: development of a multiple PCR assay for their detection and differentiation using specific primers derived from HAT-RAPD. Exp Parasitol 132:237–242

Wongsawad C, Wongsawad P, Chai JY, Anuntalabhochai S (2009a) *Haplorchis taichui*, Witenberg, 1930: development of a HAT-RAPD marker for the detection of minute intestinal fluke infection. Exp Parasitol 123:158–161

Wongsawad C, Wongsawad P, Chuboon S, Anuntalabhochai S (2009b) Copro-diagnosis of *Haplorchis taichui* infection using sedimentation and PCR-based methods. Southeast Asian J Trop Med Public Health 40:924–928

Wongsawad C, Phalee A, Noikong W, Chuboon S, Nithikathkul C (2012) Co-infection with *Opisthorchis viverrini* and *Haplorchis taichui* detected by human fecal examination in Chomtong district, Chiang mai province, Thailand. Parasitol Int 61:56–59

Xia Y, Chen J, Ju Y, You C (2016) Characteristic CT and MR imaging findings of cerebral paragonimiasis. J Neuroradiol 43:200–206

Xu J, Rong R, Zhang HQ, Shi CJ, Zhu XQ, Xia CM (2010) Sensitive and rapid detection of *Schistosoma japonicum* DNA by loop-mediated isothermal amplification (LAMP). Int J Parasitol 40:327–331

Yahiro S, Habe S, Duong V, Odermatt P, Barennes H, Strobel M, Nakamura S (2008) Identification of the human paragonimiasis causative agent in Lao People's Democratic Republic. J Parasitol 94:1176–1177

Yang SH, Park JO, Lee JH, Jeon BH, Kim WS, Kim SI, Yun KJ, Jeong ET, Lee KW, Kim YM, Lee MH, Park H (2004) Cloning and characterization of a new cysteine proteinase secreted by *Paragonimus westermani* adult worms. Am J Trop Med Hyg 71:87–92

Yang Y, Li M, Pan C, Yang Y, Chen X, Yao C, Du A (2018) A duplex PCR for the simultaneous detection of *Fasciola hepatica* and *Clonorchis sinensis*. Vet Parasitol 259:1–5

Yoonuan T, Nuamtanong S, Dekumyoy P, Phuphisut O, Adisakwattana P (2016) Molecular and immunological characterization of cathepsin L-like cysteine protease of *Paragonimus pseudoheterotremus*. Parasitol Res 115:4457–4470

Yossepowitch O, Gotesman T, Assous M, Marva E, Zimlichman R, Dan M (2004) Opisthorchiasis from imported raw fish. Emerg Infect Dis 10:2122–2126

Yu S, Mott K (1994) Epidemiology and morbidity of food-borne intestinal trematode infections. Trop Dis Bull 91:R125–R152

Yu Q, Yang H, Feng Y, Zhu Y, Yang X (2012) Magnetic affinity enzyme-linked immunoassay for diagnosis of schistosomiasis japonicum in persons with low-intensity infection. Am J Trop Med Hyg 87:689–693

Yu S, Zhang X, Chen W, Zheng H, Ai G, Ye N, Wang Y (2017) Development of an immunodiagnosis method using recombinant PsCP for detection of *Paragonimus skrjabini* infection in human. Parasitol Res 116:377–385

Zarrin-Khameh N, Citron DR, Stager CE, Laucirica R (2008) Pulmonary paragonimiasis diagnosed by fine-needle aspiration biopsy. J Clin Microbiol 46:2137–2140

Zhao GH, Li J, Blair D, Li XY, Elsheikha HM, Lin RQ, Zou FC, Zhu XQ (2012) Biotechnological advances in the diagnosis, species differentiation and phylogenetic analysis of *Schistosoma* spp. Biotechnol Adv 30:1381–1389

Zhou BJ, Yang BB, Doanh PN, Yang ZQ, Xiang Z, Li CY, Shinohara A, Horii Y, Nawa Y (2008) Sequence analyses of ITS2 and CO1 genes of *Paragonimus proliferus* obtained in Yunnan province, China and their similarities with those of *P. hokuoensis*. Parasitol Res 102:1379–1383

Zhou YB, Zheng HM, Jiang QW (2011) A diagnostic challenge for schistosomiasis japonica in China: consequences on praziquantel-based morbidity control. Parasit Vectors 4:194

Zhu HP, Yu CH, Xia X, Dong G, Tang J, Fang L, Du Y (2010) Assessing the diagnostic accuracy of immunodiagnostic techniques in the diagnosis of schistosomiasis japonica: a meta-analysis. Parasitol Res 107:1067–1073

Index

R. Toledo, B. Fried (eds.), *Digenetic Trematodes*, Advances in Experimental Medicine and Biology 1154, https://doi.org/10.1007/978-3-030-18616-6