Apicomplexa

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Jan Votýpka, David Modrý, Miroslav Oborník, Jan Šlapeta, and Julius Lukeš

Abstract

The phylum Apicomplexa is a large group of parasitic protists with more than 6,000 described and possibly thousands of undescribed species. All species are obligatory parasites, and potentially every vertebrate and majority of invertebrates host at least one apicomplexan species. More frequently apicomplexans are specialists with rather high host specificity; nevertheless, generalists with low host specificity exist. Many species are highly pathogenic to their host including human and domestic animals and from medical perspective represent the most important eukaryotic parasites. Coccidians are omnipresent in vertebrates, e.g., virtually all poultry and rabbits are infected by several host-specific *Eimeria* spp.;

J. Votýpka
Department of Parasitology, Faculty of Sciences, Charles University, Prague, Czech Republic

Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic
e-mail: jan.votypka@natur.cuni.cz

D. Modrý
Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic

Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
e-mail: modryd@vfu.cz

M. Oborník
Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic

Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
e-mail: obornik@paru.cas.cz

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theileriosis is responsible for enormous losses in cattle farming; about 20% of global human population is infected by *Toxoplasma gondii*; and, finally, *Plasmodium falciparum* and other *Plasmodium* species cause globally distributed malaria, which kills millions of people in tropical countries.

The phylum Apicomplexa includes morphologically and ecologically diverse protists, such as the gregarines, cryptosporidia, coccidia, haemosporidia, and piroplasms. The life cycle of majority of Apicomplexa involves sexual and asexual multiplication in the parasitized host and an environmentally resilient cyst forms. Transmission strategies are diverse, from direct transmission to intricate cycles in trophic webs between predators and their prey or involving arthropod vectors.

The phylum is highly successful, thanks to morphological and molecular adaptations. The name is derived from two Latin words, *apex* (top) and *complexus* (infolds), and refers to a set of organelles composed from spirally arranged microtubules, polar ring(s), and secretory bodies, such as rhoptries and micronemes. Apical complex structures mediate entry of the parasite into the host cells, where they usually survive inside a parasitophorous vacuole. Most apicomplexans possess a unique organelle called the apicoplast, which is a highly reduced non-photosynthetic plastid, which retains few functions essential for a parasite survival. The phylum evolved from a photosynthetic flagellate, and core apicomplexans form a sister group to a free-living marine and freshwater protists (*Chromera*, *Vitrella*, and *Colpodella*).

Keywords

Alveolata • Apicoplast • Endosymbiosis • Intracellular • Micronemes • Pathogens • Parasites • Protozoa • Rhoptries

J. Šlapeta
Sydney School of Veterinary Science and School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney, NSW, Australia
e-mail: jan.slapeta@sydney.edu.au

J. Lukeš (✉)
Biology Centre, Institute of Parasitology, Czech Academy of Sciences, České Budějovice, Czech Republic

Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Canadian Institute for Advanced Research, Toronto, ON, Canada
e-mail: jula@paru.cas.cz

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Introduction

General Characteristics

The Apicomplexa (Telosporea, Sporozoa) are parasitic.[1] heterotrophic protists that form uniformly banana-shaped uninucleate stages. Apicomplexans move by gliding motion, and at least one stage is characterized by apical secretory organelles releasing their content through a microtubule-anchored ring. The rod-shaped micronemes and bulk-shaped rhoptries are both essential components typifying the phylum Apicomplexa. A majority of species have complex parasitic life cycles, with alternating asexual and sexual multiplication. They either possess a respiring mitochondrion or a non-respiring mitosome. Apicomplexa contain a multimembranous compartment, now known to be a modified chloroplast, termed apicoplast, acquired via endosymbiosis of a photosynthetic alga. The apicoplast is neither photosynthetically active nor present in all extant members of the phylum; however, if present, it is an indispensable organelle.

The core of Apicomplexa is traditionally divided into three major obligatory parasitic classes (hematozoa, coccidia, and *gregarines*). The sister group of the core apicomplexans has been widely debated over the past 30 years. Colpodellids, free-living predatory protists of previously uncertain status, are now considered a sister group to the monophyletic hematozoans, coccidians, and gregarines.

[1]Parasitism is a type of symbiotic relationship between two different organisms – parasite and host. Three distinct types of parasitism are considered: biotroph, hemibiotroph, and necrotroph. Apicomplexans should be classified as biotrophs and partially as hemibiotrophs. Necrotrophs utilize dead animal tissues as a source of nutrients, while apicomplexans benefit from a prolonged, close association with the living host cells only.

Parasitic apicomplexans have a bad reputation for causing malaria, toxoplasmosis, coccidiosis, and other serious diseases of humans and animals. Recently discovered photosynthetic alveolates *Chromera velia* and *Vitrella brassicaformis* are together with heterotrophic colpodellids closely related to the core apicomplexans.

History of Knowledge

The symptoms of malaria were first described more than 5,000 years ago in Egyptian papyri, and this plague appears in historical records of Greeks and Romans (Cox 2010). Yet the first apicomplexan was spotted by Antony van Leeuwenhoek, who in 1674 observed under his famous microscope coccidian oocysts in the bile of a rabbit. However, thanks to their size, gregarines were the first apicomplexans to attract interest of early protozoologists. The genus *Gregarina* from an insect was described by Leon Dufour in 1828, and they already appeared as a protistan group in the classification of Ernst Haeckel in 1866.

Meanwhile, research on malaria, one of the major scourges of humankind, gathered momentum. In 1880, Charles Laveran became convinced that the pigment in erythrocytes of his patients is a parasite. Within the same decade, Alexander Danilewski discovered several other intracellular parasites in the blood of vertebrates and called them haemosporidians, while Ilya Metchnikow recognized their relationship with coccidians. It was, however, not firmly established until 1897, when Ronald Ross proved that the parasites causing malaria are transmitted by mosquitoes, for which he was awarded the Nobel Prize in 1902. A major contribution to the understanding of the malarial life cycle came also from Giovanni Battista Grassi ("there is no malaria without *Anopheles*"). One more Nobel Prize for research on malaria went to Julius von Wagner-Jauregg, who in 1917 discovered that syphilitic patients can be treated by controlled malaria infection. William Trager is credited for seminal discoveries, such as continuous cultivation of the erythrocytic stages. The last Nobel Prize for research on malaria went in 2015 to Youyou Tu for her discoveries concerning a novel therapy.

The first piroplasmid was described from the blood of cattle during the 1880s epidemic of the Texas cattle fever in the USA. Intracellular blood stages, later included in the genus *Babesia*, were described in 1888 by Victor Babeş. Only a few years later, Theobald Smith and Frank Kilbourne successfully transmitted a related organism to a noninfected cattle via a tick, being first to show that invertebrates can serve as vectors of a parasitic disease. Another Nobel Prize was awarded in 1951 to Max Theiler for his breakthrough studies of the life cycle of *Theileria*. The most widespread apicomplexan, *Toxoplasma gondii*, was first observed in 1908 by Charles Nicolle in a semidesert rodent, the common gundi (*Ctenodactylus gundi*), which was being used for leishmaniasis research in the laboratory of the Pasteur Institute in Tunis (Dubey 2014). Yet it took most of the twentieth century to decipher its intriguing life cycle, because the cat was successfully identified as the host shedding the oocysts only in 1970.

Since their description, the systematics of the apicomplexans had undergone periodical changes. Early influential reviews were published more than a hundred years ago by Labbé (in 1899) and Minchin (in 1903), later followed by the system proposed by Wenyon (in 1926). The pre-electron microscopy era was summarized by Pierre-Paul Grassé (in 1953). The intense studies by electron microscopy in the 1960s and 1970s that resulted in the identification of common ultrastructural features at the apical end prompted Norman Levine to propose the name Apicomplexa for these protists.

Mutual relationships of major groups within the phylum were differently assessed by influential authors, such as Emile Vivier and Isabelle Desportes (in 1980). An exhaustive list of all named species was compiled by Norman Levine (1988), and a recent account of the classification of the parasitic Apicomplexa is reviewed by Frank Perkins et al. (2000). Avian blood parasites have been reviewed exhaustively by Gediminas Valkiūnas (2004).

Practical Importance

Apicomplexans represent an obligatory parasitic lineage with an enormous diversity and more than 6,000 named species infecting invertebrates and mostly vertebrates. Even though under natural conditions most parasitoses are asymptomatic, some Apicomplexa are causative agents of serious human and animal diseases. With no doubt, the main importance rests in the pathogenic character of the species described below (see also Seeber and Steinfelder 2016).

Although the majority of haemosporidians are parasites of wild animals (reptiles, birds, and mammals) exerting only a negligible effect on their hosts, some are responsible for very serious, even fatal diseases. Most notorious are several species of *Plasmodium*, the causative agents of malaria, responsible for enormous human suffering and economic loss in most tropical countries. Human malaria used to be widespread also in the temperate zone, from where it was successfully eradicated after the Second World War. *Plasmodium* is considered one of the most frequent agents of deaths in the history of humankind, even now killing about half a million people annually, particularly children in sub-Saharan Africa (Gething et al. 2011). For good reasons, it is one of the most well-studied protists, yet only a few effective drugs and no fully protective and effective vaccine against human malaria are available.

The east coast fever (theileriosis) and bovine tropical theileriosis in cattle and water buffaloes are caused by *Theileria parva* and *T. annulata*, respectively (Bishop et al. 2004). Several *Babesia* species are responsible for babesiosis of cattle, horses, dogs, and rarely also humans. Poor growth, low milk production, and mortality of infected animals resulted in several efforts to control piroplasmoses. Before implementation of successful eradication programs focused on vectors, the costs of the piroplasmosis were estimated at more than 100 million dollars in direct and indirect annual losses in the USA only. While under control in developed countries, these

diseases still cause serious economic loss in tropical and subtropical countries. Since the eradication of tick vectors is not realistic in most tropical countries, there is a demand for effective control of piroplasmoses by alternative approaches. Vaccines using live attenuated *Babesia bovis* and *B. bigemina* are commercially available and millions of doses of the combined vaccine have been used in the New World and Australia (Jackson et al. 2001). The development of live vaccines against bovine babesiosis was prompted by early observations indicating that cows that recovered from natural *Babesia* spp. infections developed long-lasting immunity. However, vaccines using live *Theileria* parasites, soluble antigen from *Babesia* species (e.g., the vaccine for canine babesiosis was marketed in parts of Europe), or vaccines composed of subunits are being developed or have even reached the stage of clinical trials but have yet to be tested on a large scale.

Toxoplasma gondii, causing toxoplasmosis, is the most widespread protozoan parasite capable of infecting virtually every mammalian (and bird) host species including man, with 15–70% of the human population seropositive (Tenter et al. 2000). Most infections in humans are asymptomatic or mild, even in the acute phase. Yet on the other hand, congenital toxoplasmosis in fetuses can result in serious eye (chorioretinitis) and brain damage (encephalitis and hydrocephalus). Equally important may be the impact of chronic toxoplasmosis on human behavior (Flegr 2007). Neosporosis, caused by *Neospora caninum*, a parasite closely related to *T. gondii*, is found worldwide in dogs, cattle, and other mammals. Relatively recently, *N. caninum* has been implicated as an important cause of abortion in cattle due to congenital infection (Reichel et al. 2013). Numerous *Sarcocystis* species form cystic stages in muscular tissues of various wild animals and under certain circumstances make these hosts more vulnerable to their predators, which represent definitive hosts.

Several *Eimeria* species causing coccidiosis are widespread in poultry farms and represent a major cause of morbidity and decreased weight gain implying economic losses to the industry by direct mortality, decreasing food conversion rate and expenses connected to anticoccidial medication or vaccination. With about 40 billion chickens raised annually worldwide, the disease is estimated to cost upward of 800 million US dollars per annum. Management of coccidiosis through anticoccidial drugs and vaccines using live attenuated *Eimeria* species has critical implications for the poultry industry, while other species negatively affect rabbits and farm ruminants (Allen and Fetterer 2002). *Cystoisospora suis* is the causative agent of an acute diarrhea in piglets. The waterborne *Cyclospora* and *Cryptosporidium* species are important for public health as the causes of diarrhea. Recent Global Enteric Multicenter Study identified *Cryptosporidium* as the second most common pathogen in infants in developing countries (Kotloff et al. 2013). Cryptosporidiosis may cause, under favorable conditions, diarrhea of epidemic proportions even in developed countries. Several *Cryptosporidium* species cause watery diarrhea in humans and are held responsible for gastrointestinal disease and morbidity of HIV-infected patients.

Similar to other infectious diseases and pathogens, several apicomplexan parasites have been introduced to nonnative continents, with their subsequent spreading

through the new areas, as in the case of avian sarcosporidiosis (*Sarcocystis rileyi*) introduced to Europe from North America. Moreover, some etiological agents could be considered as emerging diseases, as in the case of small intraerythrocytic piroplasm *Cytauxzoon felis* in domestic cats or cyst-forming sarcosporidia, *Besnoitia besnoiti*, an emerging pathogen of cattle coursing besnoitiosis mainly in Europe.

Several poorly studied species are also known to infect invertebrates. The most common among them are gregarines, which could inflict serious damage to insect farms or in laboratory colonies. At the same time, various apicomplexan parasites have a potential as agents for biological warfare against the crop, animal, and human pests and vectors, yet for such applications, they have never been put into effect on a large scale.

Habitat and Ecology

Apicomplexans are obligatory parasites, fully dependent on their hosts throughout most of their life cycle. As highly sophisticated parasites, apicomplexans benefit from their prolonged and close association with the host, which they exploit for food, habitat, and dispersal in order to increase their fitness. The act of parasitism reduces host fitness in causing pathology or altering the behavior or social status of the host. In the wild the pathology of most species is low, and the infected hosts usually show no signs of the disease. However, under intensive farming conditions or after the introduction into new susceptible hosts in non-endemic areas, these parasites may cause high morbidity and mortality.

Obligatory Dependence on the Host

The Apicomplexa obtain food (nutrient sources) from the host. Being dependent on a host requires tools and mechanisms to access its metabolites. The apical complex with its repertoire of secretory organelles is the key to the global success of this group of protists. While the apical complex is the unifying morphological feature of the phylum, the means of host exploitation are enormously diverse. By attaching to the host cell via their apical end, gregarines remain extra- or epicellular, with the host cell remaining virtually unaltered. In the case of cryptosporidia, the host cell envelopes the parasite with its flat membrane folds, while the only contact zone between both cells, termed the feeder organelle, is a highly modified interface. Coccidians and hematozoans are intracellular parasites, usually with a complex life cycle, undergoing remarkable morphological transformations allowing them to persist in diverse locations within their hosts. For example, *Plasmodium* is capable of flourishing in both mosquitoes and humans, where it can modify the surface of the infected red blood cells by exporting its proteins through membranes and the lumen of the host cell.

Localization in the Host

In the initial stage of invasion, the motile zoite (Fig. 1) will find the target tissue and establish the infection (Fig. 2). During the development, the intra- and extracellular phases may alternate, although a vast majority of species develop inside of the host cell (Bartošová-Sojková et al. 2015). The life cycle is terminated by a stage resistant to unfavorable conditions that is usually excreted during the host's life, or is released into the environment after its death. Some heteroxenous species do not form any exogenous stages as they are transmitted via ingestion/inoculation by blood-feeding arthropods.

Extracellular parasites. All extracellular species belong to the gregarines. They develop mostly in the digestive tract but can also be found in the respiratory and excretory organs. Even the extracellular gregarines are almost permanently attached to the host cell, this association is being terminated only prior to the extrusion of the parasite into the environment. The epicellular localization represents a transitional form between the extra- and intracellular parasitism. It is characteristic

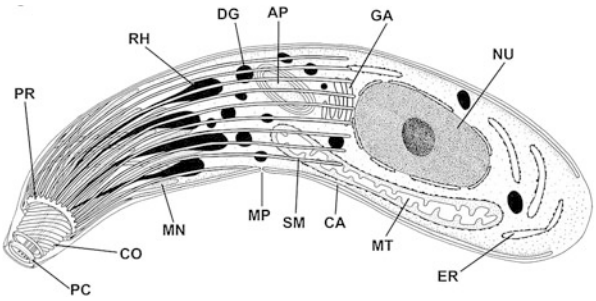

Fig. 1 3D structure of a typical sporozoite or merozoite. *AP* apicoplast, *CA* cortical alveoli, *CO* conoid, *DG* dense granules, *ER* endoplasmic reticulum, *GA* Golgi apparatus, *MN* micronemes, *MP* microporus, *MT* mitochondrion, *NU* nucleus, *PC* pre-conoidal rings, *PR* polar ring, *RH* rhoptries, *SM* subpellicular microtubules

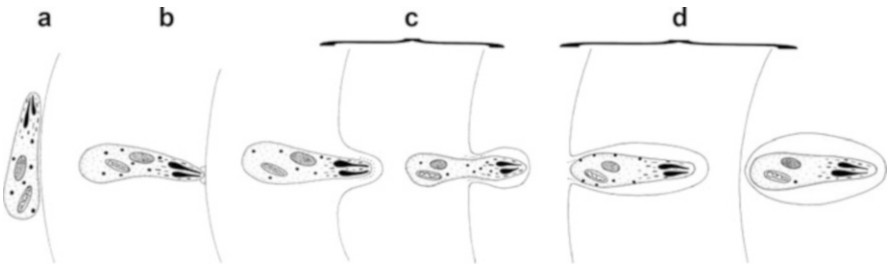

Fig. 2 Invasion of the apicomplexan zoite into a host cell. Primary contact of the zoite without orientation (**a**); attachment followed by the apical reorientation (**b**); induction of the parasitophorous vacuole (**c**); translocation of the zoite into the vacuole (**d**)

for cryptosporidians, which communicate with the host cell via a specialized feeder organelle, which closely resembles the attachment site of some gregarines. Many intracellular species have an extracellular phase in their life cycle, during which a cystic stage is released into the environment, where it awaits ingestion by a new host.

Intracellular parasites. The Apicomplexa are able to invade almost any cell type, *T. gondii* being a prime example of such an indiscriminate strategy. The parasites are either in direct contact with the host cell cytoplasm or are encircled by a "parasitophorous vacuole" formed by components of both the host and parasite cells. Several types of location within the host cell can be distinguished: (i) intracytoplasmic location is typical for most coccidians and hematozoans; (ii) extracytoplasmic location on the periphery of the epithelial cell facing the lumen, during which direct communication with the host cell cytoplasm is maintained, is characteristic for some coccidians of cold-blooded vertebrates; (iii) some coccidians are localized inside the host cell nucleus.

Furthermore, the intracellular stages can be subdivided based on their activity. Usually, upon host cell invasion, the zoite starts to divide and the life cycle proceeds fairly rapidly. However, when the zoite transforms into a dormant stage (dormozoite), the growth becomes arrested, and the stage can persist unchanged for years. It will, however, be awakened by stimuli, such as a change in the health state of the host or by the ingestion of the host by a predator. Another life form, the oocyst, is usually released into the external environment, where it may exist for a long period of time without growth, supporting itself from storage organelles until the next host is encountered.

Multiple Species in One Host

It has been predicted that each multicellular organism hosts at least one apicomplexan species, yet this simplified view is incorrect. Detailed studies of the medically and veterinary important hosts revealed that a single host species may be exploited by more than a dozen of distinct apicomplexan species affecting different host tissues. However, for most host organisms, only an incomplete record exists, keeping the diversity of apicomplexans largely unknown.

Fowl coccidia, the major problem in the poultry industry and the cause of chicken coccidiosis, are an example of such multispecies phenomenon. At least seven distinct *Eimeria* species are found in chicken, each occupying a specific habitat within the gastrointestinal tract (Clark et al. 2016). The most devastating species is *Eimeria tenella*, a parasite of caeca (Sharman et al. 2010). The enormous capacity to propagate is illustrated by the fact that each oocyst of *E. tenella* is theoretically capable of producing 2.5×10^6 oocysts within just 5 days. Besides their specific location within the intestinal tract, individual species invade mucosal cells either at the tips of the villi or in the crypts, while others found the interior of the villi as the most suitable environment. A complete set of economically significant coccidia infecting rabbits along with their specific location with the host is shown in Fig. 3

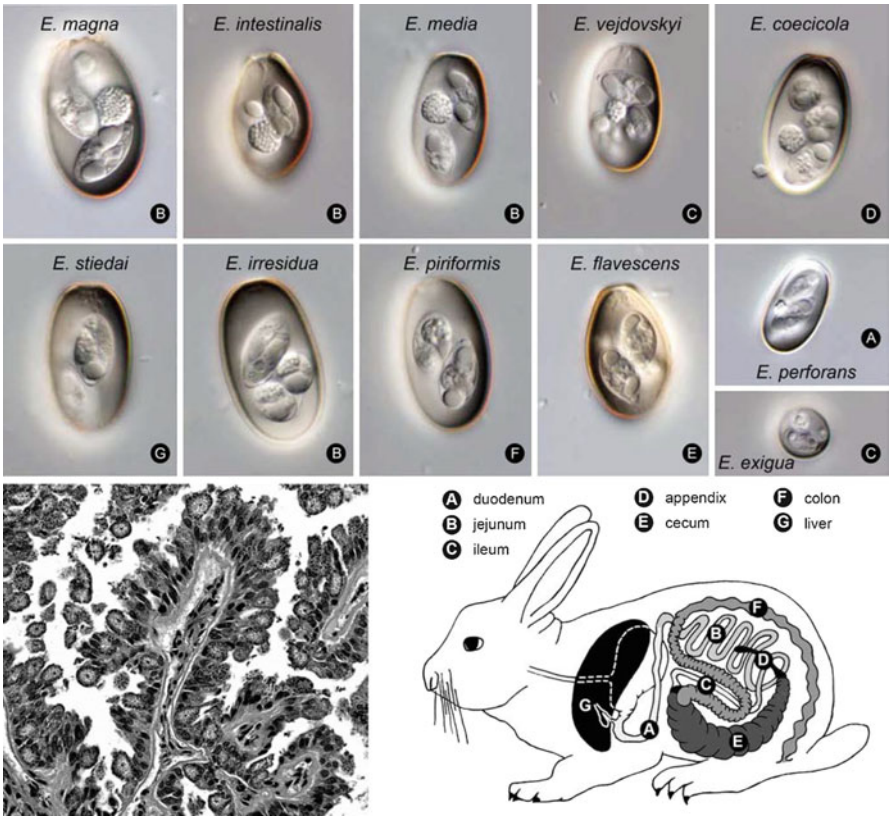

Fig. 3 Multiple *Eimeria* species infecting rabbit. Sporulated oocysts of 11 named *Eimeria* species parasitizing rabbits. Morphologically similar oocysts are distinguished by their size, shape, the presence of the micropyle, and the presence/absence and characteristic structure of the oocyst residuum. Individual species differ in the location in and pathogenicity for the host. The picture in the lower left corner shows proliferative changes in bile ducts with multiple gamogonial stages of *E. stiedae*

(Duszynski and Couch 2013). Thus, each parasite secures its distinct niche within the host organism. *Cryptosporidium* species exploit both the gastric mucosa and the intestinal mucosa. Similarly, human malaria is caused by four distinct worldwide distributed species – *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* – circulating among humans via the *Anopheles* mosquito vectors; however, at least one more species, *P. knowlesi*, sometimes called the fifth human malarial parasite, which is principally a pathogen commonly found in nonhuman primates in Southeast Asia, may also infect humans (Tenter et al. 2000; Singh and Daneshvar 2013). Each human *Plasmodium* is characterized by a distinct life cycle pattern in the host. By far the most devastating is *P. falciparum*, causing malignant tertian malaria with indefinite multiplication of asexual stages in the red blood cells.

Parasitism and/or Mutualism

The apicomplexans often affect their hosts in highly sophisticated ways. In the two-host life cycles and particularly in those exploiting the predator-prey relationship, the success of the parasite is directly linked to the consumption of the infected prey by the predator. Thus, any mechanism that increases susceptibility to predation enhances the parasite's fitness (Vorisek et al. 1998). On the other hand, (very) low virulence of the parasite for the predator, as often seen in species such as *Toxoplasma* and *Sarcocystis*, can be considered as commensal or even mutualistic rather than parasitic. Since mutualism is a form of coexistence (symbiosis) enhancing the fitness of both partner organisms, it is not surprising that in evolutionary terms the long relationship between coccidians and their hosts frequently developed from parasitism into commensalism or mutualism. For example, *Sarcocystis* (syn. *Frenkelia*) *microti* and *S. glareoli* circulate between buzzards and small rodents, its definitive and intermediate hosts, respectively. While in buzzards it causes no symptoms, large cysts in the brains of rodents make them more vulnerable to the predator. The mechanisms behind such an increased susceptibility do not seem to be associated with cellular pathology, but the parasite most likely changes the social status or behavior of the infected individual. In another example, rats and mice infected with *T. gondii* lose fear of the odor of feline urine. Importantly, humans may also be manipulated by the ubiquitous *Toxoplasma* that may alter our behavior, psyche, and response to certain stimuli (Flegr 2013). However, the extent of alterations during human toxoplasmosis remains controversial.

The development of some monoxenous coccidians seems to follow circadian rhythms (Martinaud et al. 2009). It has been shown that oocysts of *Isospora* are significantly more prevalent in the feces of passerine birds excreted in the afternoon as compared to other parts of the day. Preferential shedding of the environmentally resistant oocysts in the afternoon can be explained by the avoidance of initial and/or prolonged harmful desiccation and UV.

Distribution

The Apicomplexa are virtually omnipresent. As parasites of the majority of vertebrates and invertebrates, they are distributed on all continents, on the ocean floor as well as in the air. Many species produce environmentally resistant envelopes that protect the parasite for months or years, either in the soil or water. *Cryptosporidium* is a typical example of a waterborne pathogen, the oocysts of which contaminate water sources and are passively transmitted to large areas, spreading the disease to new locations. Because of their minute size, these resistant stages can even be passively carried by insects. Blood-feeding arthropods also play a key role in the life cycles of medically and veterinary important hematozoans and piroplasms, the distribution of which follows the geographical range of their vectors. In fact some

estimates consider the Apicomplexa as the most specious group of eukaryotes, predicting the existence of million(s) of species (Pawlowski et al. 2012).

In the past, human colonization of remote places dramatically affected the distribution of many apicomplexan parasites. Transmission of the bird malaria caused by *Plasmodium relictum* is impossible without the compulsory vector mosquito *Culex quinquefasciatus*. Its introduction to Hawaii in 1826 triggered outbreaks of the avian malaria imported to the islands either by exotic birds released in the late nineteenth century or by migratory birds breeding in the Arctic. The total lack of immunity of the endemic Hawaiian birds to malaria caused by thousands of years of the absence of malaria resulted in epidemic mortality bringing some endemic species to the margin of extinction. Similarly, the appearance of avian malaria on the Bermuda Islands is directly linked to human encroachment. A Spanish sailor shipwrecked in 1603 on uninhabited Bermuda reported a total absence of mosquitoes. It is thus likely that the extinction of the endemic passerine birds in Bermuda was caused by the introduction of mosquitoes and alien passerines with malaria that had a similar devastating effect on the resident birds. Bovine babesiosis which has probably haunted farmers since the beginning of livestock production in warm regions of the Old World was later introduced into the New World by early settlers with imported cattle and the first documented reports date from around 1810 in North America.

One of the most successful parasites is undoubtedly *T. gondii* propagated in the form of an asexual clonal population. It is highly prevalent in all warm-blooded vertebrates, its success being supported by several key adaptations of its life cycle. The disease caused by asexually multiplying *Toxoplasma* is in most cases mild and self-limiting, leading to formation of dormant cystic stages in tissues. However, the host will remain an infectious passive carrier for life. Importantly, unlike other cyst-forming coccidians, *Toxoplasma* is able to bypass sexual development. The dormant cysts are capable of inducing an infection in any predator or scavenger munching on the animal tissues containing parasite cysts. The astonishing success of *T. gondii* has been recently explained by unique and ancient North and South American dichotomy of its former population that occurred prior to the reconnection of the Panamanian land and was coupled with a recent global sweep of few clonal populations. More than 95% of isolated strains in North America and Europe belong to just three clonal lineages (Howe and Sibley 1995) that have arisen ~10,000 years ago.

Characterization and Recognition

The Apicomplexa are distinguished by the complex and characteristic organization of the apical part of the invasive stages (the zoites, usually present both in sporozoites and merozoites, which alternate in the life cycle) and by the presence of a small inconspicuous organelle in the cytoplasm of all developmental stages – the apicoplast.

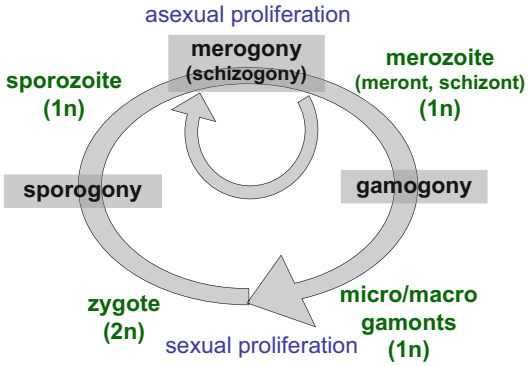

Fig. 4 Schematic drawing of a typical apicomplexan life cycle. See text for explanation

The Life Cycle

The life cycle of apicomplexans is rather complex and comes in several significantly different forms characteristic for main subgroups of the phylum. Its most simple form is known in gregarines, where it is composed of gamogony (the sexual phase) and sporogony (the asexual phase) (Fig. 4) (Ferguson et al. 2008). The life cycle of coccidians and haemosporidians contains asexual multiplication – merogony.

The life cycle usually commences with the release of a sporozoite from the oocyst/sporocyst (see below), an event that often takes place in the intestinal content of the host. The gliding sporozoite has a relatively short time to find a host cell, which it will penetrate by means of its apical complex and thus initiates the infection (Fig. 2). Shortly thereafter, organelles of the apical complex undergo resorption, and the elongate sporozoite transforms into an oval meront that starts growing. Upon reaching a critical size, the meront divides into a dozen to hundreds of merozoites (Fig. 5). These are similar in ultrastructure to the sporozoite and are destined to spread the infection to other host cells, where the cycle proceeds by a new generation(s) of meronts and merozoites. The next phase is characterized as gamogony, since some merozoites are predetermined to become female macrogametocytes, while the rest evolve into male microgametocytes (Fig. 6). The life cycle proceeds by fusion of a small flagellated microgamete with a large and nonmotile macrogamete. This conversion from a haploid into diploid phase is termed sporogony (Figs. 6 and 7) and is characterized by a species-specific number of cell divisions, leading to the formation of sporozoites, usually enclosed in a resistant sporocyst and/or oocyst wall (Figs. 6, 7, and 8). Upon the release of the sporozoites under favorable conditions (Fig. 9), the life cycle is completed, as their function is to transmit the infection into a new host.

Host Cell Invasion and Parasite Multiplication

The Apicomplexa are experts in host cell manipulation and immune evasion. *Toxoplasma gondii*, *Theileria* spp., *Plasmodium* spp., and others secrete different

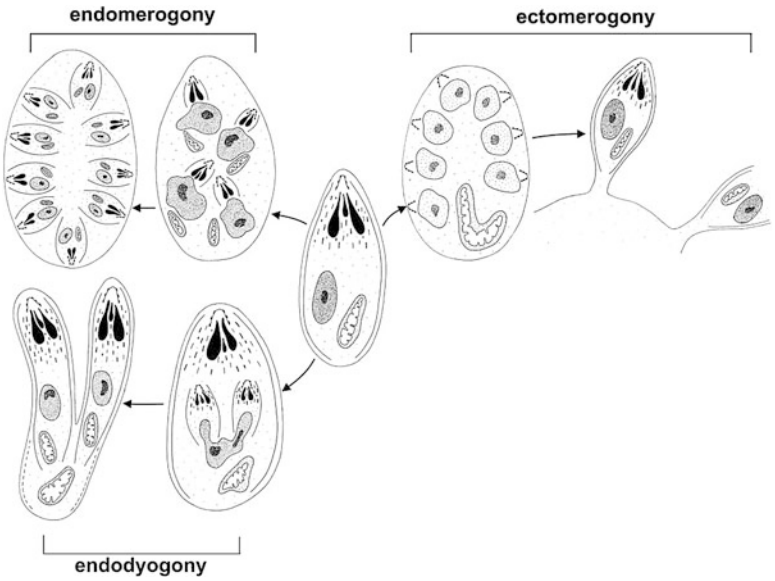

Fig. 5 Schematic drawing of main types of merogonial division. Merogonial division can proceed either via endomerogony (*upper left*), ectomerogony (*upper right*), or endodyogony (*lower left*)

effector molecules into the host cell to reach this aim. Invasion of an apicomplexan into the host cell is a complex action, some parts of which have yet to be elucidated (Baum et al. 2008). Generally, it consists of four phases: (i) primary contact without orientation, (ii) attachment followed by apical reorientation (with the exception of the genus *Theileria*), (iii) induction of the parasitophorous vacuole, and (iv) translocation of the parasite into the vacuole (Fig. 2). Attachment to the host cell via the apical end is followed by establishment of a connection through sequential secretion from the secretory organelles of the parasite. These unique extrusive organelles, represented by few claviform rhoptries, numerous filamentous micronemes, and round dense bodies contain molecules required for the interaction with the host cell (Besteiro et al. 2009). They are deployed in the course of the invasion and play various roles during intracellular development. Apically secreted adhesins from the micronemes are translocated along the parasite length and are shed at the site of the moving junction. This circumferential zone of moving junction is associated with a constriction of the parasite that moves from its apex to the posterior end. The parasite enters the nascent parasitophorous vacuole by capping the moving junction down its body, and components from the rhoptries are secreted into this newly formed compartment (Shen and Sibley 2012). Ultimately, the apicomplexan cell becomes enclosed within a cavity delimited by the invaginated host cell membrane. This protects the parasite against host immune mechanisms. On the other hand, brisk trade of nutrients is in motion among the parasite's surface, inner

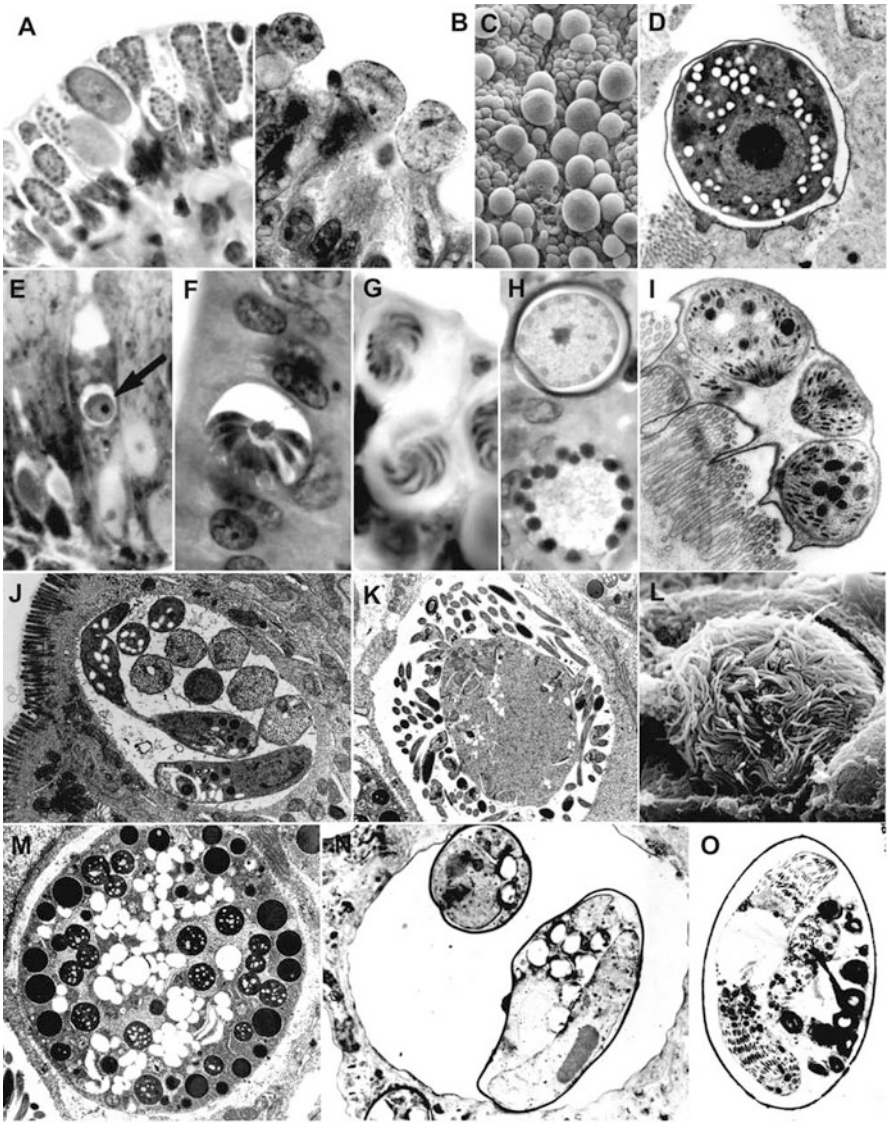

Fig. 6 Representative morphology of coccidian life cycle stages in the intestine, gall bladder, and spleen of various vertebrate hosts. Intestinal epithelium heavily infected by intracellular gamogonial stages of *Eimeria neodebliecki* from a pig (**A**); early gamogonial stages of *Choleoeimeria hirbayah* in the gall bladder of a chameleon; note that the infected cells are displaced toward the lumen (**B**); similar situation showing displaced cells with stages of *Choleoeimeria baltrocki* in the gall bladder of a skink (**C**); early extracytoplasmic meront of *Epieimeria anguillae* from an eel (**D**); young meront of *Goussia bohemica* initiating infection in the goblet cell of a gudgeon (**E**); ectomerogonial division of *Eimeria zuhairamri* from the intestine of a field mouse (**F**); mature microgametocytes of *Eimeria neodebliecki* from a pig, containing prominent flagellated microgametes (**G**); early (*upper* cell) and mature macrogametocytes containing well-visible wall-forming bodies (*lower* cell) and

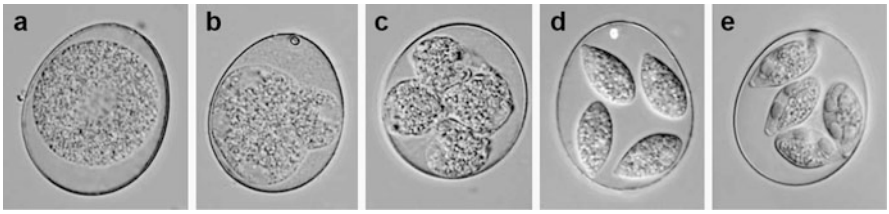

Fig. 7 Sporulation of *Eimeria maxima* from the intestine of a chicken. Unsporulated oocysts are shed with feces into the environment (**a**). When exposed to oxygen in an environment of appropriate humidity and suitable temperature, they undergo sporulation (it takes about 48 h at 25 °C before they become infectious). Upon asexual division through four sporoblasts (**b**, **c**), four sporocysts are formed initially full of granular material (**d**) that during sporulation wanes, until mature infectious sporozoites with remaining sporocyst residuum appear (**e**); note also process of formation of prominent Stieda bodies on poles of sporocysts (**d**, **e**)

membrane of the parasitophorous vacuole, and outer membrane of the infected cell (and thus with the surrounding environment).

Apicomplexan parasites replicate by internal budding termed merogonial division or merogony (schizogony in older literature) to create either two daughter cells (endodyogony) or multiple progeny (endopolygony, multiple synchronized endopolygony, and ectomerogony) that differ mainly in the preservation or loss of the maternal cell (see below) (Striepen et al. 2007). The apicomplexan nucleus divides by cryptomitosis (the nuclear membrane remains intact throughout the process), and karyokinesis occurs without chromosomal condensation.

The Sporozoite

This is the most characteristic stage of the phylum. The sporozoite is invariably elongated with a polar organization of its intracellular structures (Morrissette and Sibley 2002). Its size varies from less than 1 μm to about 25 μm in length. The tapered end is equipped with a conserved and specialized set of structural and secretary organelles labeled the apical complex (Fig. 1). Their extraordinary

Fig. 6 (continued) young oocyst (*upper* cell) of *Eimeria cahirinensis* from a spiny mouse (**H**); extracytoplasmic "spiderlike" meront of *Goussia pannonica* containing three merozoites from a white bream (**I**); numerous mature merozoites of *Eimeria arvalis* from a vole (**J**); mature microgametocyte of *E. arvalis* containing microgametes on its periphery (**K**); mature microgametocyte of *Eimeria vermiformis* from a mouse, containing microgametes on its periphery (**L**); mature macrogametocyte of *E. arvalis*, containing various wall-forming bodies (**M**); sporulating oocyst of *Goussia metchnikovi* from the spleen of a gudgeon, with cross-sectioned sporocysts containing immature sporozoites and large residual body (**N**); sporulated sporocyst of *G. metchnikovi* with a mature sporozoite filled with micronemes and dense bodies (**O**). Histological sections (**A**, **B**, **C**–**H**) and scanning (**C**, **I**) and transmission electron microscopy (**D**, **I**–**K**, **M**–**O**)

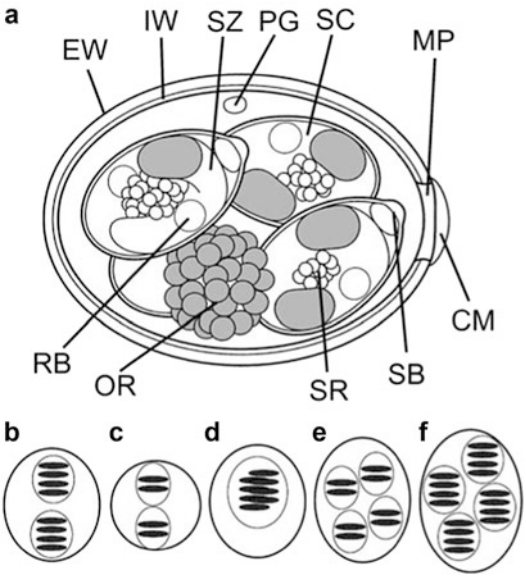

Fig. 8 **Schematic drawing of a typical coccidian oocyst**. *Eimeria* (**a**) *CM* cap of the micropyle, *IW* internal oocyst wall, *EW* external oocyst wall, *MP* micropyle, *OR* oocyst residuum, *PG* polar granule, *SB* Stieda body, *SC* sporocyst, *SR* sporocyst residuum, *SZ* sporozoite, *RB* sporozoite refractile body. Several types of the inner organization of the oocysts are shown: *Isospora*-like (two sporocysts, each with four sporozoites) (**b**); *Cyclospora* (two sporocysts, each with two sporozoites) (**c**); *Caryospora* (single sporocyst, containing eight sporozoites) (**d**); *Eimeria*-like (four sporocysts, each with two sporozoites) (**e**); *Wenyonella* (four sporocysts, each with four sporozoites) (**f**)

combination constitutes the very effective invasion apparatus responsible for an enormous evolutionary success of these parasites. The apical tip contains a polar ring, to which subpellicular microtubules are attached that stretch into the cell. Adjacent to the conoid are one or two apical rings composed of spirally arranged microtubules. The most prominent components of the apical complex are rhoptries and micronemes, secretory organelles full of molecules important for the invasion (see below). This cargo is of protein and lipid nature, specialized for intracellular parasitism. Rhoptries are often very prominent club-shaped organelles, whereas micronemes are usually rather thin, prolonged, and abundant ducts. Dense granules, usually located more distal from the conoid, have also recently been implicated with invasion. The specialized parasitophorous vacuole is formed with their help and its main purpose is to protect the apicomplexan from host attack, while the parasite can still obtain nutrients from the host. The most intensely studied organelle of the sporozoite is the apicoplast (see "Evolutionary History").

Sporozoites, same as all the other apicomplexan stages, also contain standard equipment of the eukaryotic cell, such as the nucleus, Golgi apparatus, endoplasmic reticulum, plasma membrane, and mitochondrion (Fig. 1). At first, the omnipresent

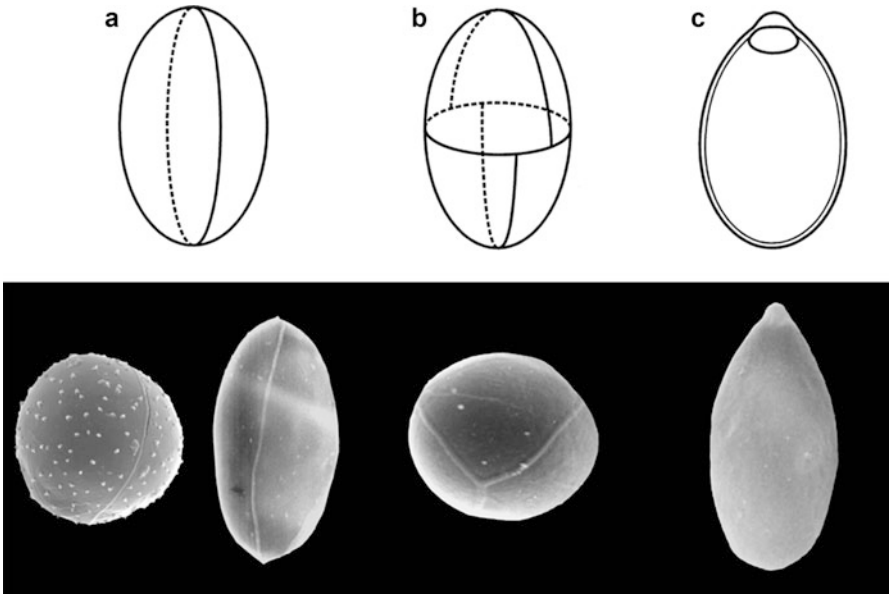

Fig. 9 Excystation structures of coccidian; schematic drawings and scanning electron microscopy of sporocysts. Probably ancestral opening of the sporocyst along a single longitudinal slit (*Goussia janae*, *Aggregata octopiana*) (**a**). Alternative opening by sutures in the sporocyst wall composed of four valves (*Cystoisospora suis*) (**b**). In the eimeriid coccidian (*Eimeria tenella*), sporozoites are released after the plug formed by the Stieda and substieda bodies is dissolved (**c**)

mitochondrion appeared to be missing from the *Cryptosporidium* species. Only recently, it was shown that this organelle is present in an extremely reduced form, termed mitosome, sandwiched between the nucleus and the crystalloid body (Keithly et al. 2005). The position of genus *Cryptosporidium* on the evolutionary tree of Apicomplexa is unresolved largely due to its highly divergent genome and unusual cellular biology (see below).

With the exception of some gregarines, sporozoites enter the host cell, and an intracellular development proceeds. The sporozoites of gregarines also penetrate the cell wall, and the apical portion of the cell develops into a family-specific attachment apparatus. While gregarine sporozoites of the family Ganymenidae and Lecudinidae attach via the so-called mucron, the remaining gregarines develop into a morphologically prominent epimerite, via which they penetrate into the host cell (Figs. 10 and 11). With most of its body extracellular, the sporozoite feeds on the epithelial cells, substantially increases its size, and develops characteristic longitudinal folds that likely propel its movement thru the intestine to other epithelial cells (Fig. 11).

Sporozoites are motile and for host cell invasion utilize gliding motility, which is propelled by the actin cytoskeleton and myosin motors. During the motility phase, micronemes secrete adhesions onto the apical part of the parasite's surface; hence, they are gradually translocated by an actomyosin-based complex. Visualization of the adhesions deposited during a sporozoite's movement can reveal its gliding

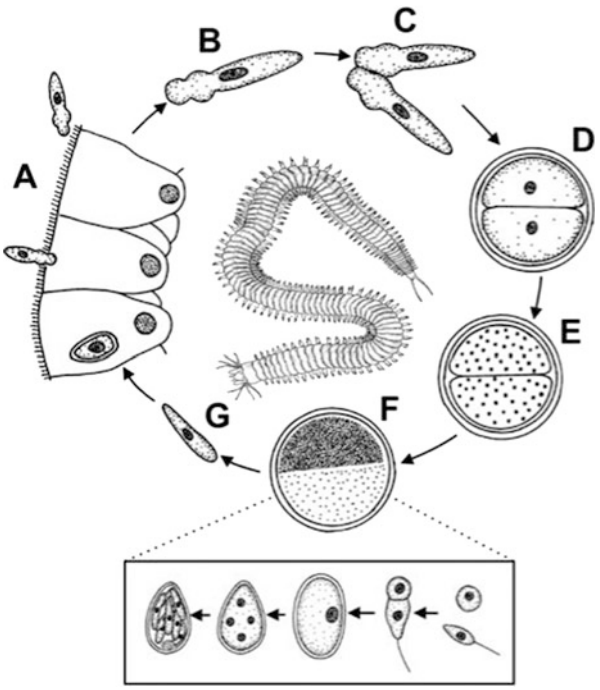

Fig. 10 Schematic drawing of the life cycle of the gregarine *Lecudina* in a Polychaete. Extracellular trophozoite attached to the host epithelium via an epimerit (*A*); free trophozoite (*B*); syzygy (*C*); early gametocyst (*D*); formation of gametes (*E*); cyst filled with separated mature gametes of both sexes (*F*); the inset shows development in the cyst from morphologically distinguishable female and male gametes that upon fusion grow into an oocyst, to a mature oocyst containing infectious sporozoites; free sporozoite initiating new infection (*G*)

movement. In apicomplexans, host cell invasion always initiates by the attachment of the apical end to the host cell, and with just a few exceptions, in the next step, the parasitic cell induces invagination of the plasma membrane. This host membrane transforms into the parasitophorous vacuole enclosing the parasite and subsequently becomes massively altered by the insertion of various proteins and lipids primarily secreted by the rhoptries.

Meront and Merogonial Development

The intracellularly established meront starts intense feeding on the host cell via numerous micropores. In intracellular gregarines and all coccidians, the increase in size is characterized by the accumulation of amylopectin and lipid granules in the cytoplasm and nuclear division(s). The merogonial division (also called schizogony in older literature) may lead to the formation of only two cells within an intact, fully polarized mother cell, a process termed endodyogony (Fig. 5). Nuclear division in the polarized mother cell is followed by the formation of two buds, each composed of newly formed membrane complex and subpellicular microtubules (Striepen et al. 2007). The mature daughter cells finally appear from the mother cell.

However, in the course of more frequent merogonial division through multiple divisions, dozens to thousands of merozoites are formed, their number being usually

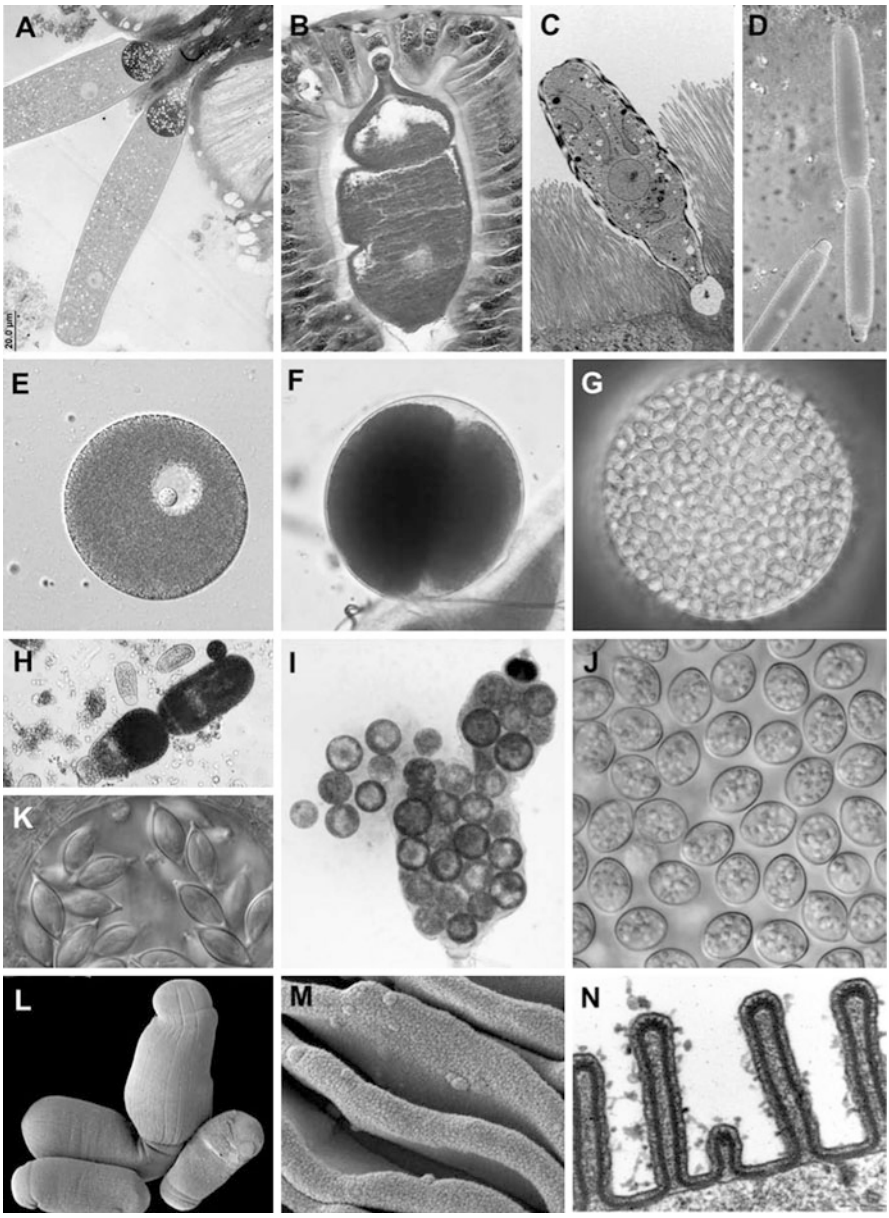

Fig. 11 Representative morphology of life cycle stages of gregarines. Mature trophozoites of *Gregarina polymorpha* (**a**) and *Gregarina garnhami* (**b**) attached to the host cell; mature trophozoite of *Gregarina steini* with a well-developed epimerite, invading a host cell (**c**); caudo-frontal syzygy of *G. polymorpha* (**d**); aseptate gamont of *Ascogregarina chagasi* containing a large nucleus and a prominent nucleolus (**e**); maturing gametocyst of *A. chagasi* with two gamonts separated by partition (**f**) and a gametocyst filled with numerous oocysts (**g**); gamonts of *Gregarina katherina* associated by caudo-frontal syzygy (**h**); sporulated biconical oocysts of *Monocystis* sp. (**i**); flea's

characteristic for a given species. The merogonial division exists either as endomerogony, during which the merozoites are formed within the mother cell, while exomerogony is characterized by merozoites that bud outside of the dividing mother cell (Figs. 5 and 6). The formation of progeny in the merogonial stage usually occurs in the host cell cytoplasm, although this phase of the life cycle may also be located in nucleoplasm of the host cell.

Upon their release from the ruptured host cells, polarized merozoites rely on energy sources that support only a short-time search (usually a few minutes) for a new host cell that they will invade. Following the invasion, the apical complex disassembles, the cell becomes oval and increases in size, the nucleus repeatedly divides, and another generation of merozoites is formed. The number of merogonial generations is usually species specific, ranging from one to a dozen or up to hundreds. Subtle morphological differences often allow assignment of given meront/merozoite to specific generations. The merogonial stages are responsible for rapid proliferation and most of the pathogenicity.

Sexual (Gamogonial) Development

The last generation of meronts enters the sexual phase of the life cycle by evolving into either micro- or macrogametes (Fig. 6); however, in hematozoans and piroplasms, gamonts emerge continuously as a product of specific part of meront populations. Sexual (gamogonial) development results in the production of large numbers of microgametes, or male sexual cells, and a much lower quantity of female macrogametes. Isogamous apicomplexans, haemogregarines and gregarines, where gamonts of each sex are equally numerous, represent exceptions. Young microgamonts contain numerous peripherally arranged nuclei and a homogeneous cytoplasm full of ribosomes, cisternae of endoplasmic reticulum, while lipid inclusions and other granules are small and relatively scarce. During maturation, the nuclei are juxtaposed to the cell surface, and the cell membrane forms dense thickening at the contact site, termed perforatorium anlagen. Mature microgamonts are usually large oval cells with invaginations on their surface. From their periphery, microgamonts emerge, equipped with an apical dense protrusion (perforatorium), one to three prominent flagella, a mitochondrion, and a dense elongate nucleus (Fig. 6). Each flagellum is supported by microtubules in the classical eukaryotic 9 + 2 arrangement (peripheral doublets with two centrally located microtubules).

Fig. 11 (continued) midgut filled with unsporulated gametocyst (**j**) and developing elliptical oocysts of a undescribed gregarine (**k**); two single gamonts of *G. garnhami* and two gamonts associated in caudo-frontal syzygy (**l**); epicytic folds of the apical region of protomerite of *G. garnhami* (**m**); cross-sectioned epicytic folds of *G. garnhami* with a newly formed fold rising between them (**n**). Histological sections (**a**, **b**), light microscopy (**d**, **e**–**k**), and scanning (**l**, **m**) and transmission electron microscopy (**c**, **n**)

The number of microgametes emerging from a single microgamont varies from dozens to thousands.

Early macrogamonts are large oval cells with a central nucleus and prominent nucleolus. The cytoplasm usually contains abundant, concentrically arranged cisternae of endoplasmic reticulum, electron-lucent amylopectin granules, lipid inclusions, electron-dense membrane-bound vesicles of various sizes, and mitochondria (Fig. 6). The amount of amylopectin inclusions as well as various vesicles increases in more advanced stages, and the cytoplasm becomes denser due to high number of ribosomes. Each macrogamont matures into a single macrogamete, which is fertilized by a microgamete, propelled by its flagella. All stages described so far were haploid, but fusion of the nuclei of micro- and macrogametes leads to a zygote, the status of which is diploid.

Sporogonial Development

In most cases fertilization occurs in the same tissue where the merogonial development is located, and this is also the site of the sporogonial phase, or sporogony (Fig. 7), with hematozoans and piroplasms representing exceptions. The zygote forms a protective wall around itself and sporogony, another process of asexual multiplication, yields a sporozoite-filled oocyst. Within the oocyst there are sporocysts, and within these are the sporozoites (Figs. 7 and 8). The oocyst and/or sporocyst wall is composed of several layers which possess a key role in the protection of the infectious and motile sporozoites that in many aspects resemble the merozoites (Figs. 1 and 8) (Belli et al. 2006). The sporozoites are usually released when the sporulated oocyst is eaten by another host. The morphology of oocysts, sporocysts, and sporozoites is one of the key characters of the taxonomy of most apicomplexan groups (Figs. 8 and 9).

Methods of Recognition

Absolute majority of apicomplexans are tiny protists not exceeding dozens of μm in size. They may be recognized with classical light microscopy, which usually suffices for taxonomic diagnosis. Exact species assignment requires the presence of morphologically informative stages. In gregarines and coccidian, these are in most cases trophozoites and oocysts, respectively. The determination of hematozoans is based on the morphology of stages in the blood cells. Thanks to their size reaching hundreds of μm, gregarines such as those infecting seminal vesicles of earthworms can be observed even by the dissecting stereomicroscope. Similarly, the cystic stages of some cyst-forming coccidia can sometimes be observed even with the naked eye, for example, as white nodules in the esophagus of infected sheep (e.g., *Sarcocystis gigantea*) or white fine threads in skeletal muscles (e.g., *Sarcocystis rileyi*).

Diseases caused by apicomplexans have been well recognized prior to the identification of the causative agent. Due to their impact on human health, certain apicomplexans are routinely associated with specific clinical symptoms in given endemic areas. Malaria is a prime suspect in all cases of cyclic fevers in tropical areas. Cryptosporidiosis is globally suspected in travelers' diarrhea. Coccidiosis manifested as diarrhea is always a threat in crowded conditions of intense farming, particularly of young animals.

For observing ultrastructural features, which are sometimes necessary for exact determination, the transmission and scanning electron microscopy are preferred approaches. Morphology is often insufficient, so host specificity is thus considered a leading criterion for species assignment. Completion of the life cycle, observation of the entire development, and elucidation of the host range are in many instances necessary. Public health authorities are particularly interested in the reservoir hosts for the zoonotic species and identification of the complete spectrum of vectors. Increasingly, molecular biology methods based on polymerase chain reaction (PCR), barcoding, and recently also next-generation sequencing (NGS) are being widely applied for detection and identification of apicomplexan species important in human and veterinary medicine. Genetic signatures linked to zoonotic transmission and clinical syndrome play an important role in current epidemiological investigations. In human malarias, fostered by multiple genome sequences and single nucleotide polymorphism (SNP) maps, gene modifications are being linked to antimalarial drugs.

Classification of Apicomplexa

The Apicomplexa comprise five principal working groups: gregarines, haemogregarines, coccidia, hematozoans (malarial parasites), and piroplasms, traditionally grouped into four classes. Besides these dominant groups, there is a myriad of small transitional groups or species, for example, the medically important cryptosporidia forming an independent group. The phylum classification is presented down to the suborder level. We annotate only major families, and for those with single or few representatives, we refer the reader to the work of Perkins et al. (2000), Tenter et al. (2002), and Adl et al. (2012).

The vividly discussed ancestry of the parasitic apicomplexans from predatory non-photosynthetic colpodellids can be found in Adl et al. (2012). Yet it is the photosynthetic chromerid species isolated from stony corals by Moore et al. (2008) that are currently the most favored as the closest living relative to the common ancestor of the phylum. Within this chapter, colpodellids and chromerids are considered "relict apicomplexa", while all the parasitic species are considered "core apicomplexa" (Table 1). The core apicomplexans represent a solid and well-defined group of eukaryotes, in contrary to their sister groups, here represented by, but not exclusive to, the two aforementioned groups. The quest for the basal relict apicomplexan is far from over (Table 1).

Table 1 Classification of Apicomplexa

Phylum: **Apicomplexa** Levine, 1970 emend. Adl et al., 2005	
Core apicomplexans (obligatory parasites)	
Subphylum: **Conoidasida** Levine, 1988	
Class: **Gregarinida** Dufour, 1828	Gregarines
Class: **Coccidia** Leuckart, 1879	Coccidia, haemogregarines
Cryptosporidida *incertae sedis*	Cryptosporidia
Subphylum: **Aconoidasida** Mehlhorn, Peters and Haberkorn, 1980	Hematozoa
Class: **Haemosporidia** Danielewsky, 1885	Haemosporidia (malarial parasites)
Class: **Piroplasmida** Wenyon, 1926	Piroplasms
Relict apicomplexans (free-living)	
Colpodellida Cavalier-Smith, 1993 *incertae sedis*	Colpodellids
Chromerida Moore et al., 2008 *incertae sedis*	Chromerids

Note: Commonly used vernacular names are indicated on the right. Colpodellida are ranked at the same level as Conoidasida and Aconoidasida in Adl et al. (2012)

Phylum Apicomplexa Levine, 1970 Emend. Adl et al., 2005

Apical complex generally consisting of polar ring(s), rhoptries, micronemes, conoid and subpellicular microtubules present at some stage; micropores present at some stage; cilia absent; all species parasitic; about 6,000 named species. The Apicomplexa has become more frequent in recent literature but not exclusive.

Subphylum Conoidasida Levine, 1988

Complete apical complex, including a conoid in all or most asexual motile stages; flagella, where present, found exclusively in microgametes (male gametes); with the exception of microgametes, motility generally via gliding with the possibility of body flexion and undulation of longitudinal pellicular ridges; heteroxenous or homoxenous. This group is not monophyletic. Subdivisions are artificial and unclear at this time.

Class Gregarinida Dufour, 1828 (Syn. Gregarinia Dufour, 1828, Gregarinasina Dufour, 1828)

Mature gamonts extracellular, large; mucron or epimerite, derived from conoid, ordinarily present in mature organism; sexuality usually by syngamy of gamonts; gametes generally very similar; similar number of male and female gamonts produced, zygotes form oocysts within gametocysts; life cycle consists of gametogony and sporogony; parasites of digestive tract or body cavity of invertebrates or lower chordates; generally homoxenous; about 1,800 named species. (*Gregarina*, *Lecudina*, *Mattesia*, *Monocystis*, *Selenidium*).

Class Coccidia Leuckart, 1879 (Syn. Coccidiasina Leuckart, 1879)

Mature gamonts intracellular, small; conoid not modified into mucron or epimerite; syzygy generally absent (if present involves gametes); sexual stages generally very different; different number of male and female gametes; microgametes without flagella; zygote forms oocyst from fertilized macrogametocyte; homoxenous or heteroxenous life cycles consist of merogony, gamogony, and sporogony; parasites of vertebrates and invertebrates about 3,500 names species (Adeleorina Léger, 1911: *Adelina*, *Haemogregarina*, *Hepatozoon*, *Klossiella*; Eimeriorina Léger, 1911: *Caryospora*, *Cyclospora*, *Eimeria*, *Goussia*, *Isospora*, *Lankesterella*, *Neospora*, *Sarcocystis* (syn. *Frenkelia*), *Toxoplasma*; *Aggregata*, *Lankesterella*).

Subphylum Aconoidasida Mehlhorn, Peters and Haberkorn, 1980 (Syn. Hematozoa Vivier, 1982)

Secondarily incomplete apical complex; conoid absent in asexual motile stages (some motile zygotes [ookinetes] contain conoid); formation of macrogametes and microgametes independent; heteroxenous.

Class Haemosporida Danilewsky, 1885

Motile zygote [ookinete] with conoid; flagellated microgametes produced by merogony; oocyst with sporozoites; heteroxenous; parasites of vertebrates; invertebrates serve as vectors, in which sporogony occurs; about 500 named species (*Haemoproteus*, *Leucocytozoon*, *Plasmodium*).

Class Piroplasmida Wenyon, 1926

Conoid and flagella absent in all stages; piriform, round, rod shaped, or amoeboid; no oocyst; sexual stages still uncertain but probably associated with the formation of the large axopodium-like stages; heteroxenous; parasites of vertebrates (in blood cells); ticks serve as vectors; about 200 named species (*Babesia*, *Theileria*).

Gregarines

Gregarines represent an extremely large and highly abundant group of early-branching apicomplexans that exploit exclusively invertebrate hosts, such as annelids, mollusks, nemerteans, phoronids, echinoderms, sipunculids, crustaceans, hemichordates, appendicularians, and insects (Fig. 10). Gregarines have monoxenous life cycles consisting almost exclusively of gamogony and sporogony, since only very few species display merogony. The life cycle of most gregarines commences by the release of young trophozoite from a sporocyst engulfed by the host. The trophozoite generally attaches to the epithelial tissue in the gut lumen of the host and occupies it (Fig. 11). However, some species can be found in coelomic cavities and tissues associated with the reproductive system. After an enormous increase in size, the trophozoite is released from the host tissue into the gut lumen. It transforms into a

gamont that will attach to a partner in a species-specific orientation (head-to-head, tail-to-tail, or head-to-tail) in a process called syzygy (Figs. 10 and 11). In the next step, the gametocyst enclosing both gamonts is formed. Several mitotic divisions inside the gametocyst give rise to hundreds of gametes. Next, the gametes fuse with their partners from the other gamont and produce numerous zygotes. Newly formed sporocyst (oocyst) wall confines each zygote, and subsequent meiosis produces four or more sporozoites per sporocyst (oocyst). Gametocysts filled with mature sporocysts (oocyst) are then released into the environment and ingurgitated by a new host, repeating the cycle. This general scheme has numerous genus- or species-specific modifications, a feature not surprising for organisms displaying such an extreme diversity. The gregarine trophozoites can move and change direction through a mechanism unique among eukaryotes, called gliding motility. This may be accomplished via a cytoskeleton composed of actin and myosin. Gregarines seem to lack the apicoplast, as do the closely related parasitic cryptosporidia.

Gregarines are taxonomically subdivided into three orders: basal archigregarines, advanced eugregarines, and neogregarines. Such a branching order has been inferred from life cycles of these parasites and is, at least to some extent, supported by molecular phylogeny (Leander et al. 2003a, b; Leander 2008; Desportes and Schrével 2013).

Order Archigregarinida Grassé, 1953

This order contains extracellular intestinal parasites of annelids, sipunculids, hemichordates, and ascidians. Their trophozoites are anchored in the host epithelium via the epimerite (or mucron). They are characterized by the absence of septa (aseptate), the persistence of zoite organelles, the pairing of trophozoites (syzygy), and the encystment of gamonts. Sporocysts contain four to eight or even more sporozoites. Archigregarines are parasites of marine invertebrates, with the life cycle completed within the intestinal lumen of a single host. Trophozoites of some species may use myzocytosis-based feeding. This ability, together with the number of infective sporozoites, links archigregarines to colpodellids, free-living biflagellated predators that form a sister group to the parasitic apicomplexans.

Order Eugregarinida Léger, 1900 (Syn. Eugregarinorida Grassé, 1953)

Extracellularly parasitic eugregarines represent the most abundant and best studied group within the class Gregarinida. The trophozoites use epimerite (septate gregarines) or mucron (aseptate gregarines) for their attachment to the host epithelium. Pairing of trophozoites is, same as in Archigregarinida, followed by the encystment of gamonts, producing sporocysts each with eight sporozoites. Eugregarinida

comprises over 1600 species belonging to about 240 genera containing aseptate or septate species.

Order Neogregarinida Grassé, 1953 (Syn. Neogregarinorida Grassé, 1953)

Neogregarines develop intracellularly in the host tissue. After invading the host, they undergo multiple rounds of merogony. The resulting stages known as merozoites spread the infection to other tissues of the host, such as gonads. Usually small gamonts produce a low number of gametes and neogregarinid sporocysts contain eight sporozoites. The order is subdivided into six families of insect parasites.

Cryptosporidia

The genus *Cryptosporidium* was established to accommodate tiny epicellular parasites found in the mouse gastric glands (*C. muris*) and intestine (*C. parvum*). Following their discovery almost 100 years ago, Tyzzer experimentally verified the life cycle and correctly speculated about an autoinfection within the host (Šlapeta 2009). Yet it was only in the 1980s that cryptosporidia were identified as causative agents of cryptosporidiosis, an important waterborne human disease. In 1993 a large waterborne outbreak affected an estimated 400,000 persons in Milwaukee, mostly infected by contaminated water (MacKenzie et al. 1995). The cryptosporidiosis manifests as potentially devastating diarrhea, for which no effective therapy is currently available.

After being released from oocysts in the gastrointestinal tract, the infective sporozoites attach themselves to the host cell membrane and become enveloped by its extended folds (Fig. 12) (Valigurová et al. 2008). A specialized structure called the feeder organelle is formed at the attachment site to facilitate the uptake of nutrients from the host cell by the parasite (Fig. 13). *Cryptosporidium* then undergoes asexual and sexual reproductions, which both have the potential for autoinfection, leading to persistent infection with massive shedding of oocysts in the feces (Fig. 13).

Environmentally resistant oocysts measure 4–8 μm in diameter and are characterized by a single suture at one pole (Fig. 12). *Cryptosporidium* completes the development within a single host, and the oocysts are fully infectious when excreted. The oocysts are spread via host-to-host transmission and indirectly as the waterborne or food-borne pathogens. There are 30 named species affecting virtually all vertebrates. Genotyping of diverse isolates revealed a diverse spectrum of host-specific and zoonotic genotypes. Cattle are considered to be the reservoir for the zoonotic (animal-to-human) transmission.

The traditional classification of *Cryptosporidium* within the coccidians has now been securely rejected, based on comparative ultrastructural and genomic data. The current view holds that the phylogenetic position of cryptosporidia is at the base of the core apicomplexan and gregarine divergence (Morrison 2009).

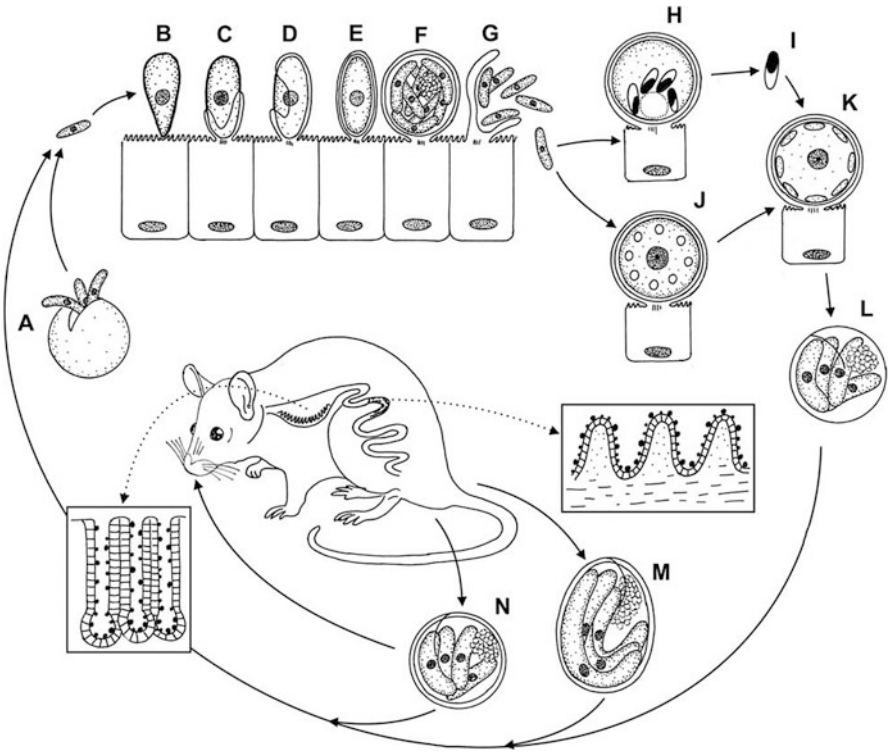

Fig. 12 Schematic drawing of the life cycle of *Cryptosporidium parvum* and *Cryptosporidium muris* in a mouse. Sporozoites are released from a mature oocyst through an open suture (*A*); upon contact with the host epithelium (*B*), sporozoites are enveloped by extended folds of the host membrane (*C–E*); upon epicellularly located merogony (*F*), merozoites are released (*G*) and transform into either microgametocytes (*H*), which produces microgametes (*I*) or macrogametocyte (*J*); upon their fusion (*K*) four sporozoites are formed during sporogony (*L*). Mouse can be infected with either *C. parvum* (*M*) and/or *C. muris* (*N*), confined to the intestine and gastric glands (insets), respectively

Coccidia

Order Eucoccidiorida Léger and Duboscq, 1910

Merogony is present, mostly parasites of vertebrates and less frequently of invertebrates. Besides the order Eucoccidiorida, there are some 20 named species from marine invertebrates classified into separate classes Agamococcidiorida and Protococcidiorida, distinguished by the absence of merogony and/or gamogony, respectively.

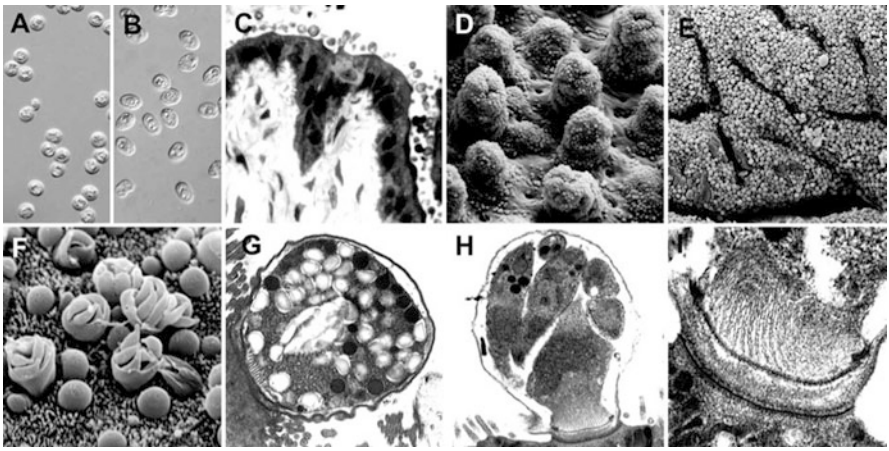

Fig. 13 Representative morphology of cryptosporidian life cycle stages. Spherical oocysts of intestinal *Cryptosporidium saurophilum* (**a**) and ellipsoidal oocysts of gastric *Cryptosporidium muris* (**b**) from a lizard and a mouse, respectively; surface of the intestinal mucosa showing numerous developmental stages of *C. saurophilum* (**c**); surface of the swine intestinal mucosa with prominent villi virtually covered with stages of *C. parvum* (**d**); detail of the gecko intestinal mucosa, heavily infected with *C. saurophilum* (**e**); various developmental stages of *C. saurophilum*, with merozoites undergoing liberation (**f**); macrogamont of *C. parvum* on the surface of an infected enterocyte, revealing the feeder organelle (**g**); developing meront of *C. parvum* (**h**) and detail of the feeder organelle of the same species (**i**). Light microscopy (**a**–**c**) and scanning (**d**–**f**) and transmission electron microscopy (**g**–**i**)

Suborder Adeleorina Léger, 1911

Two groups belong to this suborder: (i) monoxenous coccidians of invertebrates (herein referred to as adelines) and (ii) heteroxenous coccidians cycling between blood-feeding invertebrates (definitive hosts) and various vertebrates (intermediate hosts), usually referred to as haemogregarines. The genus *Klossiella* (Klossiellidae), involving monoxenous coccidia of mammals and reptiles, represents an exception. Phylogenetic studies indicate that entire group is monophyletic, characterized also by several morphological and developmental features. Microgamonts produce usually only one to four microgametes, which associate with the macrogamete in syzygy. Other characteristic features of Adeleorina are the absence of endodyogony and the enclosure of sporozoites in sporocysts and/or oocysts.

So far, there are ~500 named species, almost certainly a great underestimate of the real diversity. Members of the genera *Adelina* and *Adelea* infect mostly insects, whereas *Klossia* is a well-studied coccidium from mollusks. The haemogregarines (Hepatozoidae, Haemogregarinidae, and Dactylosomatidae) comprise several genera, including pathogens of vertebrates, such as *Hepatozoon* from carnivores and reptiles and *Haemogregarina* from fish and turtles (Karadjian et al. 2015). In any case, invertebrates play a role of the definitive host with gamogony in their digestive

system. Then, basically two modes of transmission occur: (i) the inoculative way (*Haemogregarina*, *Dactylosoma*), when the infectious sporozoites enter the vertebrate host during blood feeding, and (ii) alternatively, the parasite is transmitted via the ingestion of an infected definitive (invertebrate) host by the appropriate vertebrate host (*Hepatozoon*, *Haemolivia*, *Karyolyssus*). The latter mode of transmission may even involve a paratenic host (Fig. 14). Regardless of the mode of transmission, the merogonial division of haemogregarines usually takes place in the parenchymatous organs of vertebrates, followed by the formations of infective gametocytes in the circulating red (in the case of *Hepatozoon* also white) blood cells. The next definitive host is infected exclusively by blood feeding (Fig. 14).

Suborder Eimeriorina Léger, 1911

Macrogametes and microgametes develop independently and syzygy is absent. Anisogamous microgamonts produce a large number of flagellated motile microgametes, while the zygote is invariably nonmotile. Sporozoites are always enclosed in a sporocyst. For ~2,500 named species, homoxenous or heteroxenous life cycles have been recorded. Two families comprise species of economic and medical importance.

Family Eimeriidae Minchin, 1903

This family traditionally contains the monoxenous coccidians and arguably is one of the most diversified protist taxa. The formation of environmentally resistant oocysts, usually expelled in host feces, is one of the principal features of Eimeriidae. The general morphology of this easily detectable stage, and especially the numbers of sporocysts and sporozoites within the oocyst, has been widely used to define individual coccidian genera (Figs. 8 and 15). Results of recent phylogenetic studies, however, correlate only poorly with current taxonomy. They also showed that several diagnostic features considered hitherto unique are in fact synapomorphies, shared by several non-related genera.

Life cycle of a typical eimeriid coccidium starts by the ingestion of a sporulated, environmentally resistant oocyst. Following an immediate excystation in the proximal part of the digestive tract, upon invasion of the host epithelia, the sporozoites transform into meronts. These produce numerous merozoites that are consequently released from the ruptured host cell and initiate the next round of merogonial division. Usually there are two to seven asexual generations that differ in the number and morphology of merozoites. The last generation of merozoites eventually becomes intracellular macro- and microgamonts. Macrogamonts can be distinguished by the presence of numerous electron-dense wall-forming bodies, thought to contribute to the formation of the oocyst wall during a later stage of development (Fig. 6). Coccidia of aquatic hosts usually lack this feature, which is attributed to the

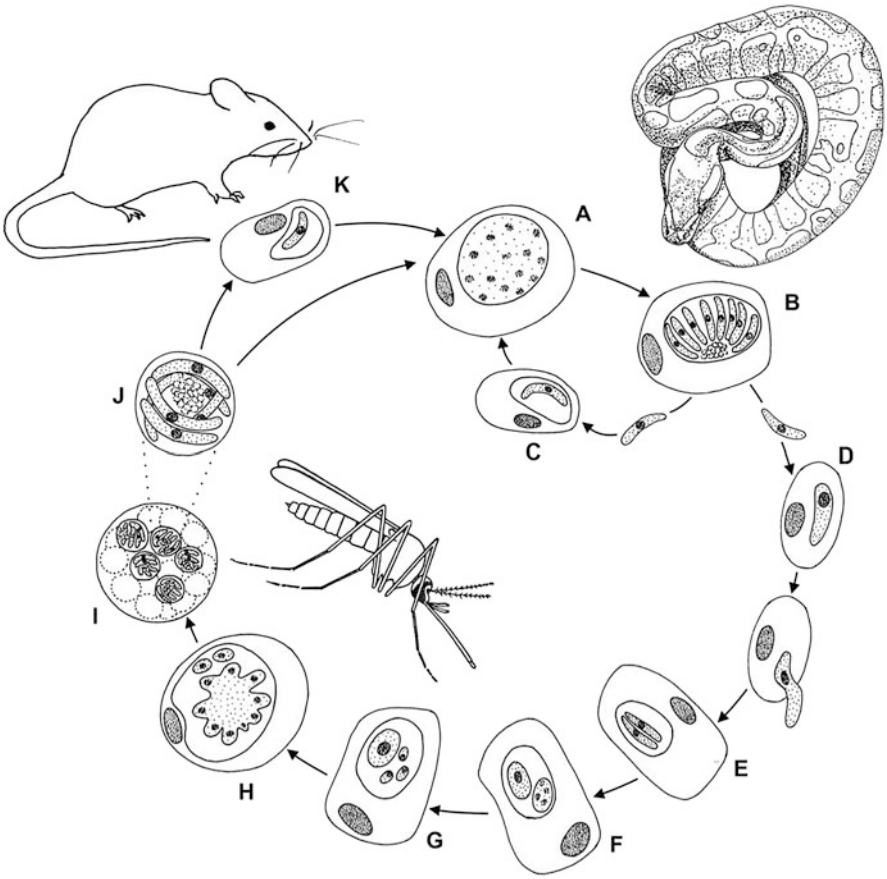

Fig. 14 Schematic drawing of the life cycle of mosquito transmitted *Hepatozoon ayorgbor* in a snake and a mouse. After ingestion of the first intermediate host, merogony takes place in the liver and kidneys of a royal python (*A*, *B*); released merozoites form dormant stages (hypnozoites, *C*) that can probably initiate further merogonial division; merozoites enter red blood cells and transform into gametocytes (*D*); after ingestion by mosquito definitive host, gametocytes enter the fat body cells in host hemocoel, where they associate in pairs in so-called syzygy (*E*); microgamont divides into low number of microgametes (*F*), one of which fuses with the macrogametocyte (*G*) and together form a zygote or young oocyst (*H*); sporoblasts, formed during the asexual division inside the oocyst (*I*), finally develop into sporocysts (*J*); each sporocyst contains several elongated sporozoites and a residual body; an infected mosquito is ingested either by the intermediate host (a phyton) or by the paratenic host (a mouse), in which dormant stages develop (*K*) and wait for the ingestion by a phyton

absence of a prominent oocyst wall in these species. Young oocysts are usually expelled in feces unsporulated and noninfective, as their development is only terminated in the external environment, where further divisions of their contents lead to the formation of sporozoites enveloped by sporocysts (Fig. 7). The entire life cycle is usually completed within 1–3 weeks.

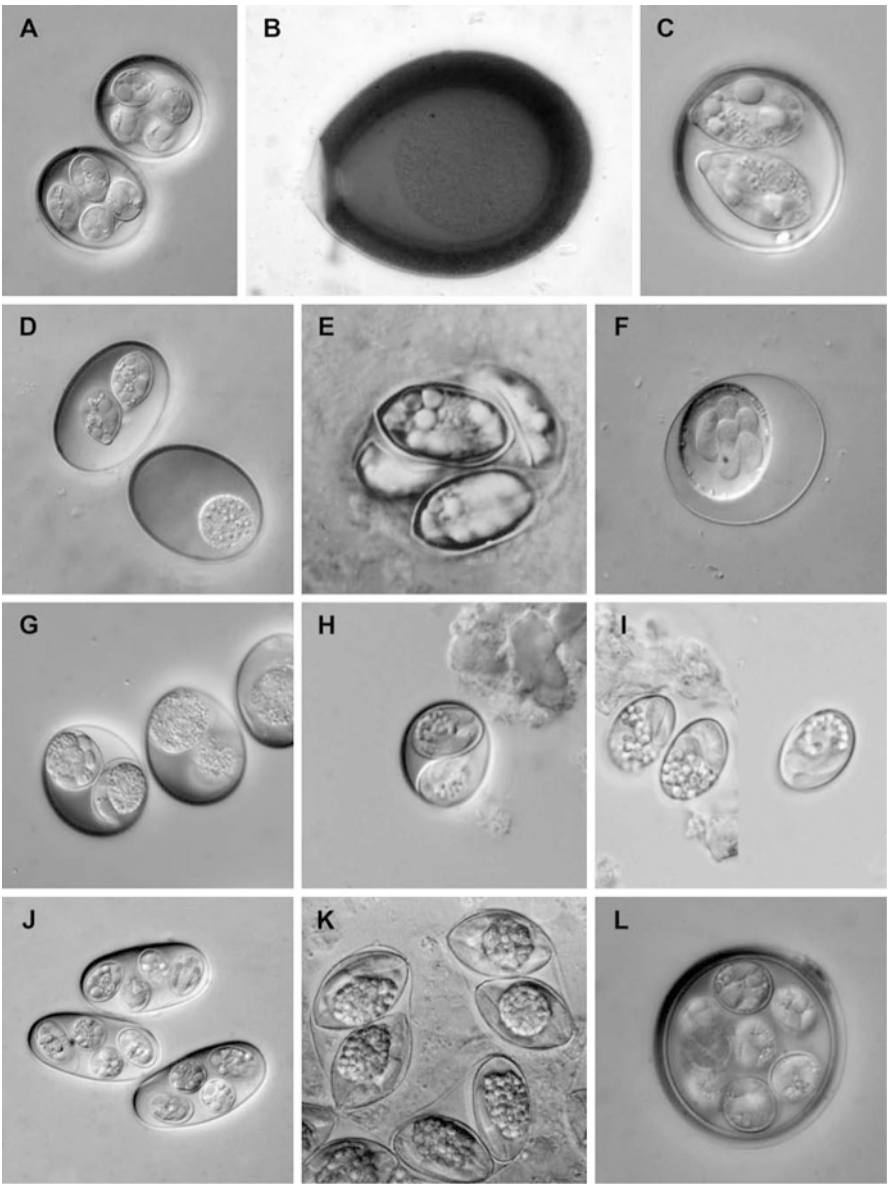

Fig. 15 Representative morphology of sporulated coccidian oocysts from the intestinal content (a–d, f–i, l) or other organs (e, k, j) of various hosts. *Eimeria elephantuli* from a rufous elephant shrew (**a**); giant oocyst of *Eimeria cameli* from a Bactrian camel (**b**); *Isospora* sp. from a passeriform bird (**c**); *Isospora jaracimrmani* from a chameleon (**d**); *Goussia alburni* from the fat body of a perch (**e**); *Caryospora kutzeri* from a kestrel (**f**); *Cystoisospora vulpina* from a fox (**g**); *Toxoplasma gondii* from a domestic cat (**h**); oocyst (*left*) and free sporocyst (*right*) of *Sarcocystis* sp. from a domestic dog (**i**); *Choleoeimeria hirbayah* from the gall bladder of a Yemen chameleon (**j**); *Hyaloklossia lieberkuehni* from the kidney of a green frog (**k**); *Adelina dimidiata* from a centipede of the genus *Scolopendra* (**l**)

Genus *Eimeria*

With ~1,700 described species, this genus is one of the most diversified eukaryotic genera. Traditional definition of the genus is simple and straightforward – it comprises monoxenous coccidians with tetrasporocystic oocysts and dizoic sporocysts (Figs. 7, 8, and 15). However, recent studies based on morphology as well as molecular phylogeny do not support this sensu lato delimitation of monoxenous coccidians. To solve the paraphyly of *Eimeria*, several genera (*Acroeimeria*, *Choleoeimeria*, *Epieimeria*, *Goussia*) have been established. The current view holds that the presence or absence of the Stieda body distinguishes *Eimeria* sensu stricto from other coccidians with *Eimeria*-like oocysts.

The striking diversity of the genus *Eimeria* may be the result of its high host specificity. Poly-infections with several *Eimeria* species are typical for many hosts (ruminants, rodents, lagomorphs, gallinaceous birds), and organ specificity and ecological within-host niche partitioning (Fig. 3) further contribute to the diversity of this genus. Although most *Eimeria* develop in the host intestinal epithelium, bile ducts, kidneys, and even placenta serve as sites of development for some species.

Thanks to features such as direct life cycle, short-generation interval, very high production of oocysts, and intracellular multiplication inside the host, *Eimeria* qualifies as one of the most detrimental parasites of domestic animals. As a result, intensive animal husbandry, especially in the case of domestic fowl, is virtually impossible without efficient control of coccidioses, either by vaccination or anticoccidial medication. However, *Eimeria* has developed drug resistance against most anticoccidials used today which has led to the requirement for an effective vaccine strategy. Attenuated strains of several coccidia were developed and are widely used for vaccination of domestic fowl. Pathogenicity for domestic mammals and birds is mainly caused by high density of animals of the same age in an artificial environment, where the transmission is substantially facilitated, as coccidians of their wild ancestors are usually only mild pathogens.

Other Genera of the Eimeriidae

Monoxenous coccidians of the genera *Caryospora*, *Cyclospora*, *Isospora*, *Tyzzeria*, and *Wenyonella* also exploit vertebrates having life cycle similar to *Eimeria* (Fig. 15). Among them, genus *Isospora,* possessing bisporocystic oocysts with tetrazoic sporocysts and Stieda bodies, is by far the most numerous, comprising ~200 species found mainly avian and reptilian hosts. Exceptionally, some avian *Isospora* (formerly assigned to *Atoxoplasma*) form extraintestinal stages, detectable in the blood cells and parenchymatous organs. In contrast to the simplicity of a typical monoxenous life cycle, some *Caryospora* cycle between snakes and rodents in a rather complicated manner, involving the intestinal merogony and gamogony in the predator and further merogony and gamogony in the connective tissue of the prey.

Despite the fact that many species were described already in the nineteenth century, monoxenous eimeriids of invertebrates (genera *Barrouxia*, *Caryotropha*,

Diaspora, *Dorisa*, *Mantonella*, *Ovivora*) belong to the least known coccidians. So far, no sequences are available from these obscure and neglected organisms.

Family Sarcocystidae Poche, 1913

Traditionally, the family Sarcocystidae comprised heteroxenous coccidians of vertebrates, with the merogony and gamogony located in the intermediate vertebrate prey and final carnivorous predator, respectively. The family accommodates ~350 named species, some of great medical and veterinary significance, with *Sarcocystis* and *Toxoplasma* being the most prominent members. The family is well defined by the unique morphology of its bisporocystous tetrazoic oocysts. The sporocyst wall is composed of four plates joined by sutures that enable the excystation in host's intestine (Fig. 16).

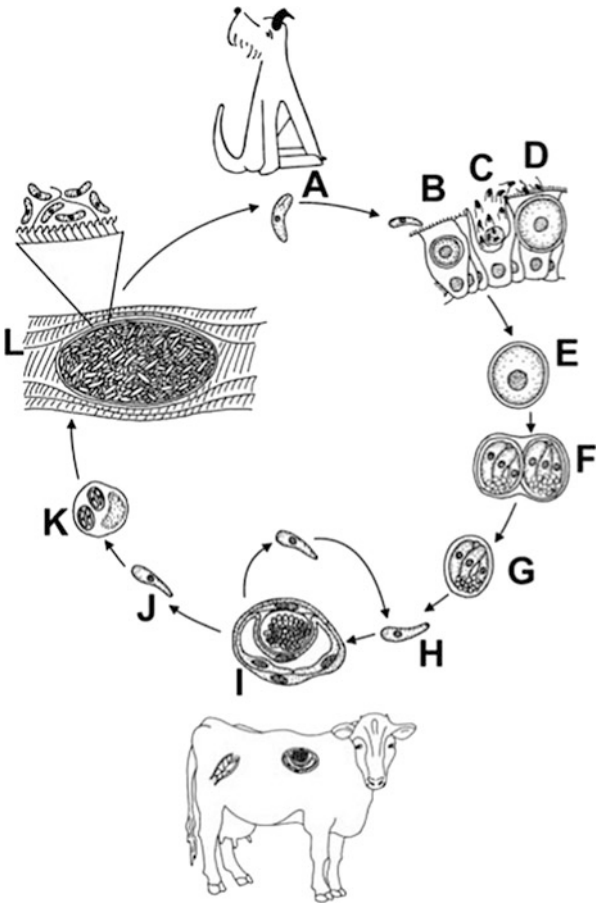

Fig. 16 Schematic drawing of the life cycle of *Sarcocystis cruzi* cycling between cattle and dogs. After ingestion of infected meat by definitive host (dog), cystozoites are released from a tissue cyst during digestion (*A*); cystozoite invades intestinal mucosa, where macrogametocytes (*B*), microgametocytes (*C*), and zygotes (*D*) are formed; unsporulated oocysts (*E*) sporulate in situ (*F*); upon rupture of the oocyst wall, sporocysts are released into the environment and subsequently ingested by the intermediate host (*G*); released sporozoites (*H*) undergo cyclic merogony in endothelial cells (*I*); liberated merozoites (*J*) then spread via macrophages (*K*) to striated muscles, where the tissue cysts develop (*L*); inset shows detail of the cyst wall with invaginations dividing the content into chambers

Phylogenetically, the family is split into two major branches: (i) a well-defined monophyletic assemblage of *Sarcocystis* (including the former genus *Frenkelia*) referred to as Sarcocystidae and (ii) a group consisting of closely related *Toxoplasma*, *Hammondia*, and *Neospora* and somewhat less related *Besnoitia*, *Cystoisospora*, and *Hyaloklossia*. Moreover, recent phylogenetic studies revealed surprisingly close relatedness of some monoxenous coccidians, such as *Cystoisospora* from the intestine of carnivores and *Hyaloklossia* from the kidneys of frogs (Fig. 15), with the above-mentioned heteroxenous genera.

Genus *Sarcocystis*

The most species-rich genus within the Sarcocystidae contains invariably heteroxenous members, cycling between predator (definitive host) and prey (intermediate host). Although the causative agent of macroscopically visible cysts in muscles of various animals was named *Sarcocystis* by Lankester in 1882, the life cycle of these widespread parasites was not deciphered until the 1970s. The definitive host becomes infected by ingestion of meat containing tissue cysts with cystozoites. Directly after that, gamogony takes place in deeper layers of the intestinal mucosa, often close to the lamina propria, and sporulation typically occurs in situ. In most cases, the oocyst wall ruptures in the intestine, and liberated sporocysts are shed in the feces of the definitive host (Fig. 16). Upon ingestion of sporocysts with contaminated food or water by the intermediate host, merogony occurs in its parenchymatous organs, followed by the formation in muscles of tissue cysts, often of macroscopic dimensions and species-specific morphology. Within the cysts, the parasite multiplies by endodyogony or endopolygony, resulting into the formation of metrocytes and later thousands of infectious cystozoites. On the ultrastructural level, the primary wall of the sarcocysts usually bears distinct micro-ornamentation, often with bizarre protrusions (Fig. 17). Numerous invaginations stretching inside the sarcocysts divide its content into chambers, in which cystozoites develop. Cystozoites are characterized by numerous closely packed micronemes (Fig. 17).

The life cycle is based exclusively on predator-prey trophic relationships. The spectrum of definitive hosts comprises carnivorous mammals (especially canids, felids, and marsupials), raptorial birds and owls, and a variety of snakes. Preys of these predators represent intermediate hosts (Fig. 16). For example, dogs (and other canines) are definitive hosts of several *Sarcocystis* species, cycling through goats, sheep, cattle, camels, etc. The so-called dihomoxenous development has been described for *Sarcocystis* affecting lizards in isolated island ecosystems, where the same host species alternatively serves as intermediate and definitive host. In many cases, host specificity of individual *Sarcocystis* species is poorly understood or even unknown. Moreover, more than 50% of species are known only from the intermediate host. At least one species – *Sarcocystis hominis* – is cycling between humans (definitive host) and cattle (intermediate host). Some species possess a remarkable affinity for the central nervous system, where either tissue cysts (species formerly referred to as *Frenkelia*) or meronts (*Sarcocystis neurona* in the brains of its aberrant

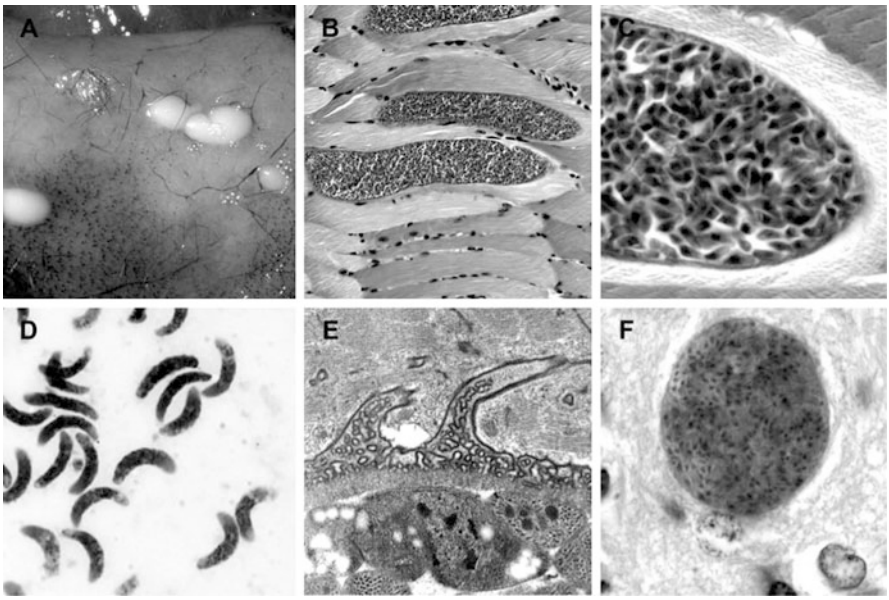

Fig. 17 Representative morphology of sarcosporidian stages in tissues of intermediate hosts. Macroscopically visible cysts of *Sarcocystis dirumpens* from the connective tissue of a rodent (**a**); elongated microscopic cysts of *Sarcocystis dispersa* from the muscles of a mouse (**b**); periphery of mature cyst of *S. dispersa* packed with cystozoites (**c**); cystozoites released from smashed cyst of *Sarcocystis muris* (**d**); species-specific structure of protrusions of primary cyst wall of *Sarcocystis lacertae* from a wall lizard (**e**); characteristic rounded cyst of *Toxoplasma gondii* from brain (**f**). Light microscopy (**a**, **d**), histological sections (**b**, **c**, **f**), and transmission electron microscopy (**e**)

hosts, horses) develop. However, in most cases, clinical significance of *Sarcocystis* infections is generally low both for the definitive and intermediate hosts (Fig. 17).

Genus *Toxoplasma* (and Related Genera)

Since humans are intermediate hosts for *Toxoplasma gondii*, it is understandably the most studied coccidian (Weiss and Kim 2014). The parasite was described already in the beginning of the twentieth century from the brain of a North African rodent (common gundi), but its life cycle remained unknown until 1970, when the domestic cat was identified as its definitive host.

After oral ingestion of sporulated oocysts, asexual multiplication occurs in the intermediate host (Fig. 18). In the so-called "acute" phase, the merogonial development (by endodyogony) occurs in various tissues, leading to the formation of short tachyzoites organized in pseudocysts. This process is repeated many times, ending by the penetration of tachyzoites into the neural (and other) tissues, where cysts are formed. In contrast to the *Sarcocystis* cysts, the tissue cysts of *Toxoplasma* are noticeably smaller and nonseptate (Fig. 17). Inside the cysts, continuous

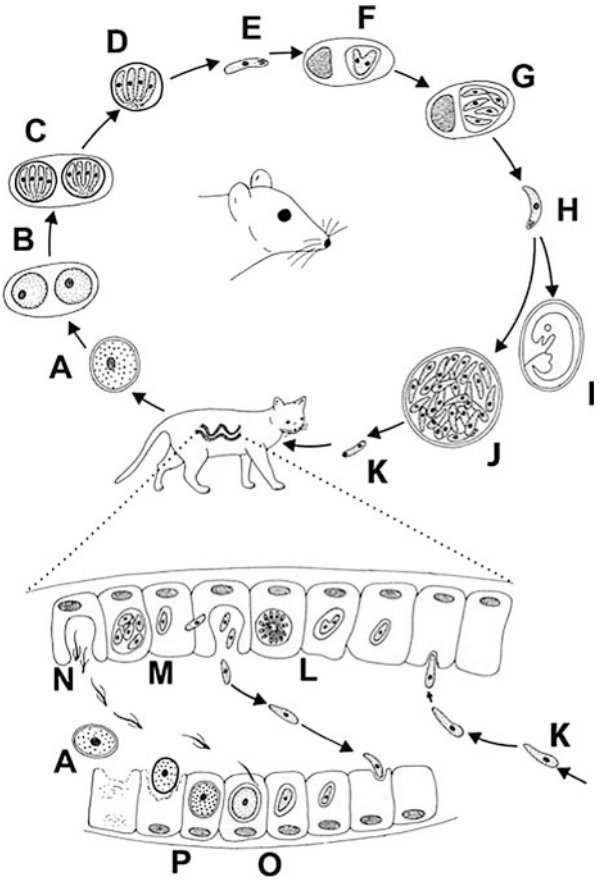

Fig. 18 Schematic drawing of the life cycle of *Toxoplasma gondii* in a mouse and a cat. Unsporulated oocyst in fecal content (*A*); upon sporulation (*B*), two sporocysts each with four sporozoites are formed (*C*); oocyst wall usually ruptures at this stage (*D*); after ingestion by an intermediate host, sporozoites (*E*) invade host cells, where they multiply by endodyogony (*F*), gradually fill the cell (*G*) and form extracellular tachyzoites (*H*); these can be transmitted transplacentally into embryos (*I*); tachyzoites invade new cells and form another generation of tissue cysts (*J*), where bradyzoites are formed (*K*). The inset shows intestinal development infected with bradyzoites (*K*) released from cysts from ingested meal; after several rounds of merogony (*L*, *M*), gamogony takes place (*N*–*P*) and unsporulated oocysts are released from the final host (*A*)

endodyogony occurs, producing prolonged bradyzoites. In this dormant stage, the parasite may survive for years, perhaps even decades. Cat as the definitive host typically acquires the infection by ingesting cysts with bradyzoites in the tissue of the intermediate host. After several merogonial generations in the cat's intestine, gamogony takes place in its intestinal epithelia, and unsporulated oocysts are expelled in feces. Importantly, *T. gondii* can be transmitted among intermediate hosts without involving the definitive one, representing a classical example of opportunism in the transmission mode. For example, humans (as well as any other intermediate host) become infected by several alternative routes: (i) by the ingestion of oocysts from the environment, (ii) by the ingestion of bradyzoites in tissue cysts from meat, (iii) by the transfer of tachyzoites transplacentally, and (iv) rarely by the transmission of tachyzoites in milk (Fig. 18). Along with the domestic cat, wild felids also serve as definitive hosts. When intermediate hosts are concerned, *T. gondii* infects hundreds of mammalian species, less often also birds and rarely some reptiles. Without any doubt, *T. gondii* is the most prevalent parasite of

humankind and one of the most widely distributed parasites of homeotherms in general.

There are two coccidian genera closely related to *Toxoplasma*: *Neospora caninum* exploits dogs as the definitive hosts, while the species of *Hammondia* cycle either through cats or dogs. Only after molecular techniques allowed distinguishing between *T. gondii* and *N. caninum*, the latter turned out to be a potentially serious pathogen of ruminants and dogs. Interestingly, despite its wide distribution and intense research, its life cycle was elucidated only in 1998.

Family Aggregatidae Labbé, 1899

This is a relatively small family (~20 named species) of heteroxenous coccidia from marine invertebrates. The type species *Aggregata eberthi* circulates between crabs and cuttlefish or octopus. Gamogony takes place within the intestine of definitive host (cephalopod), where macroscopically visible oocysts containing thousands of sporocysts are formed. Water contaminated with sporocysts is ingested by crabs, in which extraintestinal merogony occurs. Life cycle is finished by the ingestion of the infected crab by the cephalopod.

Family Lankesterellidae Nöller, 1920

A unique feature of this family is the absence of environmentally resistant oocysts. About 30 named species belonging to the genera *Lankesterella* and *Schellackia* invariably have a heteroxenous life cycle. Frogs and lizards serve as definitive hosts, in the intestine of which gamogony occurs. Oocysts lack sporocysts and harbor variable numbers of sporozoites, which upon exit in situ from the thin-walled oocysts enter the blood cells. The merogony, gamogony, and sporogony of *Lankesterella* occur in the frog's intestine, while sporozoites mature in leeches, which are thought to be principal vectors. *Schellackia* from lizards possesses the same morphological and developmental traits; however, it has only eight sporozoites per oocyst and is transmitted by mosquitoes (Fig. 20).

Haemosporidia

Haemosporidians and piroplasms constitute a phylogenetically well-defined group (e.g., Outlawa and Ricklefs 2011) with obligatory heteroxenous life cycles (Fig. 19). Haemosporidian genera can be distinguished on the basis of the erythrocytic stage morphology, localization of endogenous development in vertebrate host, and the type of invertebrate vector. Merogony of individual species occurs in vertebrate hosts (amphibians, reptiles, birds, and mammals) which serve as intermediate hosts, while sporogony takes place in a broad spectrum of blood-feeding Diptera. Parasites are taken up with the blood meal by the vector, where fertilization occurs and a

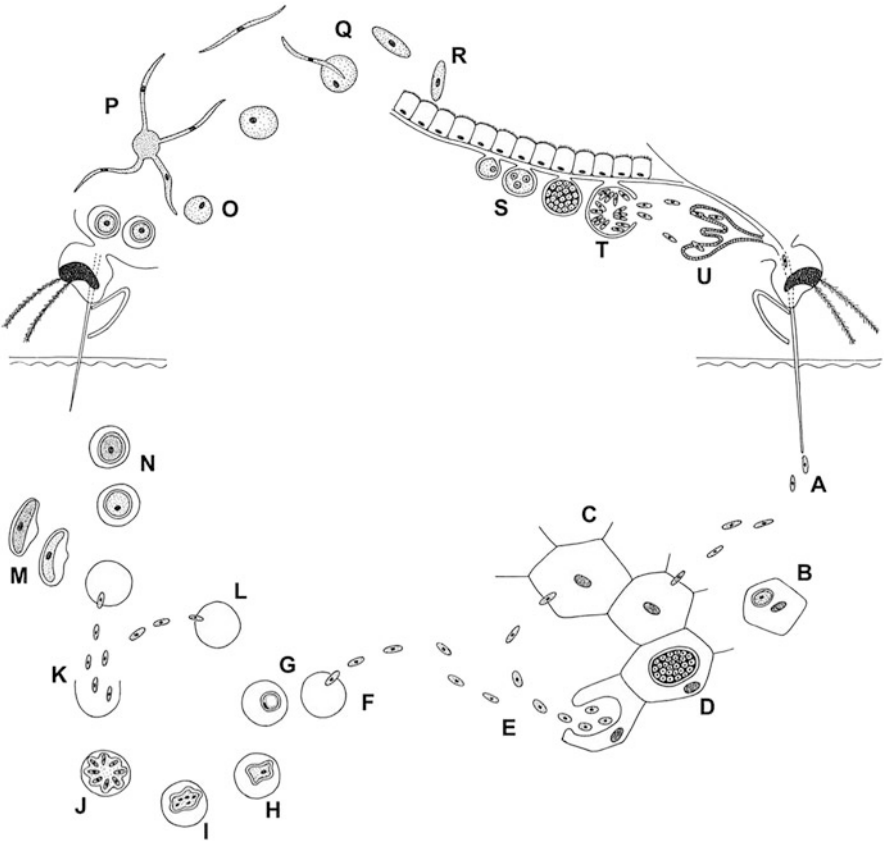

Fig. 19 Schematic drawing of the life cycle of *Plasmodium falciparum* in primate and mosquito hosts. During blood feeding by a mosquito, sporozoites (*A*) are injected into the blood; they enter hepatic cells and either turn into dormozoites (*B*) or active meronts (*C*), which undergo exoerythrocytic merogony (*D*); after release from the liver, merozoites (*E*) invade the red blood cells (*F*); from a characteristic ring stage (*G*), they produce through merogonial division (*H*–*J*), a species-specific number of merozoites (*K*) that either repeat the cycle (*L*) or transfer into gametes; the immature gametes, like other blood stages, have species-specific morphology. Here stages of *Plasmodium falciparum* (*M*) and *Plasmodium malariae* (*N*) are shown; during blood feeding, gametocytes are taken up by another mosquito where they turn into mature macrogametes (*O*) and microgametes (*P*) that copulate (*Q*); the ookinete (*R*) penetrates the intestinal wall and undergoes sporogony (*S*), in the course of which it substantially grows and produces numerous sporozoites (*T*); these invade the salivary glands (*U*) and during the next blood feeding enter another intermediate host

motile zygote (ookinete) is formed. The ookinete actively enters hemocoel by penetrating the midgut wall, rounds up, and transforms into the oocyst. Large oocysts of *Plasmodium* and *Haemoproteus* transmitted by mosquitoes and hippoboscid flies, respectively, subdivide their contents into several sporoblasts, from which hundreds of sporozoites bud off. Haemosporidians with small oocysts

– *Leucocytozoon* (transmitted by black flies) and species of *Parahaemoproteus* (transmitted by biting midges) – produce just one sporoblast with less than a hundred sporozoites. The oocyst ruptures, and the freed naked sporozoites with rudimentary apical complex migrate into salivary glands, where they develop organelles such as rhoptries and micronemes.

Sporozoites injected into the blood of vertebrate hosts by the vectors transform into the exoerythrocytic meronts (Figs. 19 and 20), known to develop most frequently in the liver but found also in the spleen, lungs, kidneys, heart, skeletal musculature, and endothelium of other organs. The megalomeront stage is characteristic of second-generation merogony of *Leucocytozoon*, *Hepatocystis*, and (*Para*) *Haemoproteus*. The prepatent period varies from 2 to 3 weeks. The process of transformation of sporozoites and exoerythrocytic merozoites into trophozoites inside host cells includes a rapid degeneration of the inner double-membrane layer, subpellicular microtubules, polar rings, rhoptries, and micronemes. Within erythrocytes, trophozoites of *Plasmodium* and (*Para*)*Haemoproteus* are localized in the parasitophorous vacuole and absorb host cell cytoplasmic content via a micropyle. Haemosporidians with the intraerythrocytic development (*Plasmodium* and (*Para*) *Haemoproteus*) turn host hemoglobin into a characteristic pigment hemozoin, easily discernible under the microscope. Rapid growth of trophozoites is finalized by the formation of meronts. Members of the genus *Leucocytozoon* depart from the general scheme, as they infect a significantly wider range of host cells, and when infecting erythrocytes digest hemoglobin without the formation of hemozoin granules. Gametocytes of haemosporidians develop only in the blood cells, and individual human malaria species can be distinguished based on their morphology. The life cycle is closed when the gametocytes enter the appropriate vector during blood feeding, where they undergo fertilization and formation of the ookinete (Figs. 19, 20, and 21).

Genus *Plasmodium*

After injection into host blood, sporozoites rapidly attack cells of various inner organs (e.g., hepatocytes in mammals), where the asexual exoerythrocytic division followed by transformation into merozoites occurs. After penetration into erythrocytes, merozoites initiate erythrocytic merogony and develop into meronts. Even though amplification via asexual reproduction in blood cells is not genetically limited in terms of the number of divisions, in each generation a certain number of merozoites develop into macrogamonts (macrogametocytes) and microgamonts (microgametocytes) after entering new erythrocytes. These stages then await ingestion by a mosquito, where each macrogamont matures into a macrogamete, while each microgamont produces six to eight flagellate microgametes (exflagellation) (Fig. 19).

It is often an overlooked fact that only less than 2% of known *Plasmodium* species infect humans, namely, the relatively rare *P. malariae*, *P. ovale*, and simian *P. knowlesi*, followed by *P. vivax* which is responsible for approximately 20% of human malaria worldwide and by far the most pathogenic species *P. falciparum*, which represents the majority of human cases. All four human *Plasmodium* species

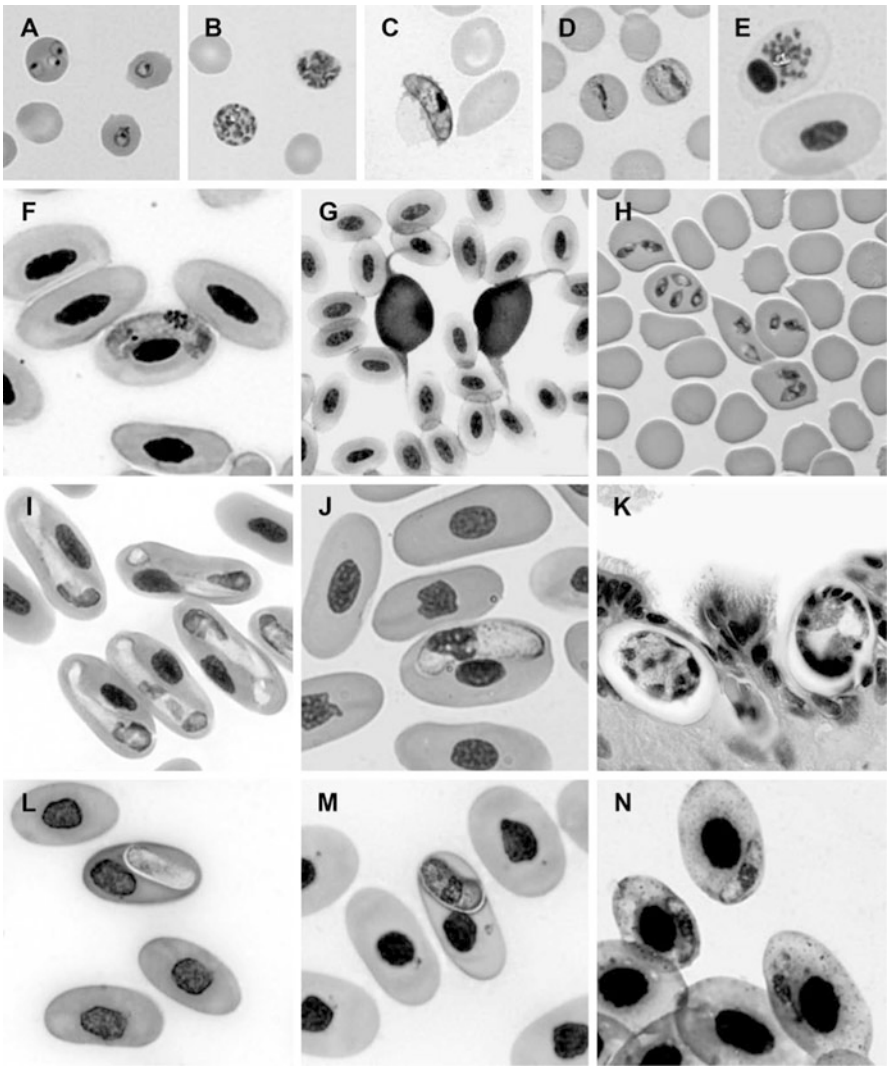

Fig. 20 Representative morphology of apicomplexan stages in the blood cells of vertebrates. Ring stages (**a**), merogonial rosette stages (**b**), and a gametocyte (**c**) of *Plasmodium falciparum* from human blood; characteristic striped meronts of *Plasmodium malariae* from human blood (**d**); *Plasmodium gallinaceum* in an erythrocyte of a fowl (**e**); gametocyte of *Haemoproteus* sp. from an avian host (**f**); gametocyte of *Leucocytozoon* sp. from an avian host (**g**); merozoites of *Babesia canis* from a domestic dog (**h**); gametocyte of *Hepatozoon* sp. from a blue-lipped sea krait (**i**); gametocyte of *Hepatozoon ayorgbor* from a ball python (**j**); merogonial stages of *Hepatozoon* sp. from the lungs of a blue-lipped sea krait (**k**); gametocyte of *Haemolivia mauritanica* from a Greek tortoise (**l**); gametocyte of *Haemogregarina stepanowi* from a swamp turtle (**m**); sporozoite of *Lankesterella minima* from a green frog (**n**). Light microscopy (**a**–**j**, **l**–**n**) and histological section (**k**)

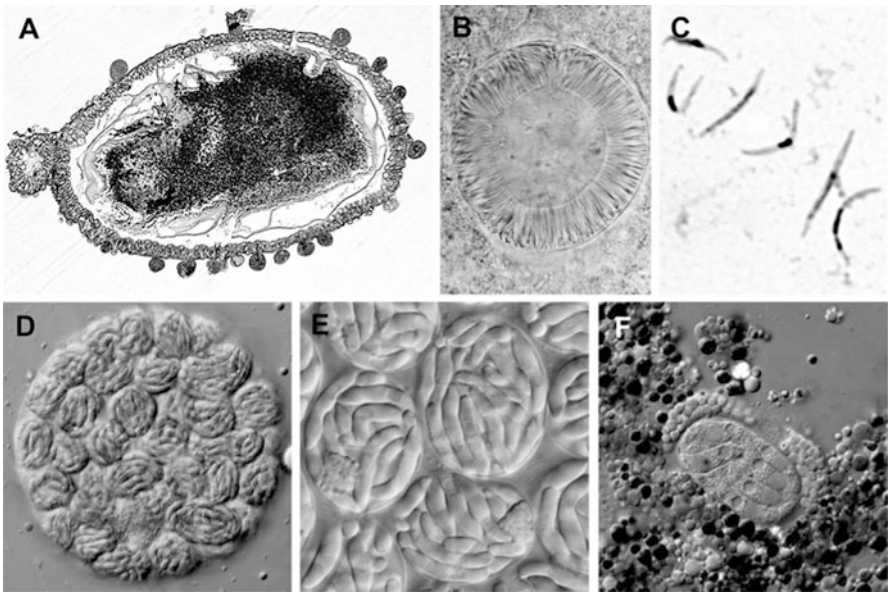

Fig. 21 Representative morphology of apicomplexan stages in the insect vector. The oocysts of *Plasmodium vivax* on the outer intestinal wall of anopheline mosquito female (**a**); the oocyst of *Plasmodium yoelii* with typical wheel-like formation of sporozoites and a central core (**b**, foto J. Vávra) and sporozoites (**c**) from vector salivary glands. Oocyst (**d**) and a detail of sporocysts (**e**) of *Hepatozoon ayorgbor* from the hemocoel of its mosquito vector; sporocyst of *Haemolivia mauritanica* from a hard-bodied tick (**f**). Histological section (**a**), fresh squash preparation (**b**, **d**–**f**), and light microscopy (**c**)

are closely related to various simian species, and *P. falciparum* seems to have been acquired by humans from gorillas only relatively recently (Prugnolle et al. 2011). Lately developed amplification of *Plasmodium* DNA from host feces allowed an insight into the diversity of *Plasmodium* species in African and Asian great apes, sharpening significantly the view on evolution of *Plasmodium* in humans and suggesting some level of cross-species transmission between humans and nonhuman primates. Almost 50 other *Plasmodium* species belonging to three subgenera are transmitted exclusively by anopheline mosquitoes (*Anopheles*) to various mammals, mostly rodents and primates. The remaining five subgenera comprise of more than 40 species attacking birds are transmitted mainly by the *Culex* mosquitoes. In general, avian species do not cause serious diseases in their hosts, with highly pathogenic species, such as *P. gallinaceum* in chicken and *P. relictum* in wild birds, being exceptions. With almost hundred species described to date, reptiles (mainly lizards) host about half of all named *Plasmodium* species. Vectors of *Plasmodium* parasitizing cold-blooded vertebrates are mosquitoes (*Culex*, *Aedes*), phlebotomine sand flies (*Phlebotomus*, *Lutzomyia*), and biting midges (*Culicoides*). Interestingly, so far only two species have been described from amphibian hosts.

Members of the genus *Hepatocystis* (25 species) are considered nonpathogenic and are found in reptiles, birds, and mammals, particularly in bats and monkeys. Phylogenetic studies indicate that *Hepatocystis* represents just an internal group among the mammalian plasmodia. Since oocysts of these less studied haemosporidians develop in the head and thorax of biting midges, the transmission is likely mediated by the ingestion of vectors.

Genera *Haemoproteus* and *Leucocytozoon*

Species of both genera undergo development similar to *Plasmodium* with the following exceptions. Asexual reproduction is limited to the exoerythrocytic merogony that occurs in the endothelial cells (*Haemoproteus*) or hepatocytes (*Leucocytozoon*). A unique feature of the life cycle of *Leucocytozoon* is that huge megaloschizonts develop in host macrophages, producing millions of merozoites. The erythrocytic merogony is absent, and merozoites enter erythrocytes (*Haemoproteus*) or leukocytes and immature erythrocytes (*Leucocytozoon*) only to develop into gametocytes (Fig. 20).

The genus *Haemoproteus* includes over 130 morphologically defined species of avian blood parasites (Valkiūnas 2004; Peirce 2005); however, some reptilian blood parasites are also accommodated within this genus. An absolute majority of species infecting birds belongs to the subgenus *Parahaemoproteus* and are transmitted by biting midges (Ceratopogonidae), whereas sporogony of six named species from the subgenus *Haemoproteus* takes place in hippoboscid flies (Hippoboscidae). The genus *Leucocytozoon* is also confined to the avian hosts and is subdivided into the subgenus *Leucocytozoon* with approximately 40 species transmitted by black flies (Simuliidae) and the monospecific subgenus *Aikiba*, the sporogony of which takes place in biting midges (Figs. 19 and 20).

Piroplasmida

This order is a diverse group of haemosporidians (sometimes called piroplasms or piroplasmids), owing their name to pear-shaped (piriform) intracellular stages formed in the host erythrocytes. Unique morphological features of piroplasmids are the absence of conoid and the reduction of the apical complex to the polar ring. Extreme reduction is characteristic for the family Theileriidae, which lacks subpellicular microtubules, the inner membrane complex, as well as the micronemes. After entering the host cell, piroplasms escape from the parasitophorous vacuole and, with few exceptions (*Theileria buffeli*, *T. separata*), digest host hemoglobin without producing any pigment or other visible residues. Heteroxenous life cycle is composed of merogony taking place in a wide range of mammals (to a lesser extent in birds and reptiles), with gamogony and sporogony occurring in the gut and salivary glands of invertebrates, respectively. So far, only hard ticks (Ixodidae) were identified as vectors, although for the majority of species vectors are yet to be found (Fig. 22).

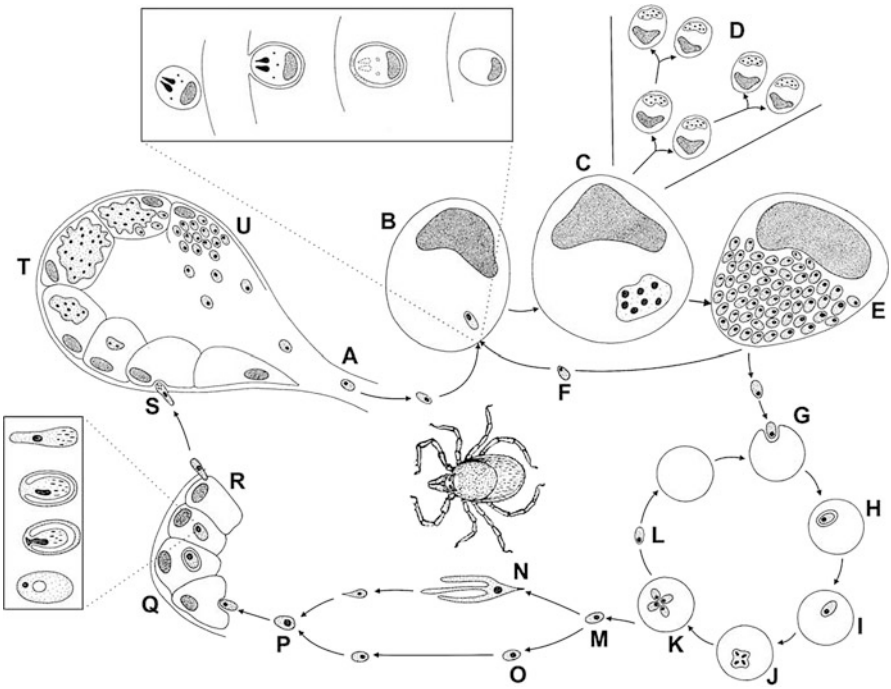

Fig. 22 Schematic drawing of the life cycle of *Theileria annulata* in a cow and a tick. Sporozoite injected with saliva of feeding tick (*A*) in the vertebrate host enters its macrophage (*B*); *inset* shows the invasion process characteristic for *Theileria* without reorientation and escape from the parasitophorous vacuole; merogonial division (*C*) induces a unique clonal expansion of the infected leukocytes (*D*); leukocytes full of merozoites (*E*), although known as Koch's bodies, rupture and released merozoites either repeat the cycle (*F*) or enter erythrocytes (*G*); merozoite also escapes there from the parasitophorous vacuole (*H*, *I*), and multiplication leads to the formation of the Maltese cross composed of four merozoites (*J*, *K*); there can be several merogonial divisions (*L*); upon engorgement during blood feeding by the tick (*M*), gamogony occurs in its intestine, where flagellated microgametocyte (*N*) fuses with macrogametocyte (*O*); motile zygote (*P*) invades epithelial cells, where it transforms into a motile kinete (*Q*); the peculiar transformation is shown in the inset; after traversing, the gut wall (*R*) enters salivary glands (*S*), where sporogony takes place (*T*), producing an enormous number of infectious sporozoites (*U*) terminating the cycle during following blood feeding

Family Babesiidae

In mammalian erythrocytes, trophozoites of the genus *Babesia* usually produce by binary fission two (rarely four) daughter merozoites, which enter new red blood cells (Schnittger et al. 2012). However, development within the invertebrate host is quite complicated and is known for only a few species. After ingestion by a tick, the parasites leave the blood cells and develop into pseudopodia-like gametes (spiky-rayed bodies), which fuse into motile zygote and form a primary kinete. Due to the penetration of the elongated kinetes (vermicules) into numerous internal organs of

the tick, including the ovaries, and in some species, the infection can be passed through transovarial transmission into the next generation, while the ticks can maintain the infection for two or more generations. Further development occurs in the hemocoel and various organs, where *Babesia* produces new secondary kinetes, some of which migrate to the salivary glands. During blood feeding that takes several hours or days, piroplasms rapidly multiply and eventually transform into sporonts and infectious sporozoites. Finally, sporozoites from the tick saliva are injected into the vertebrate host where they directly infect red blood cells and develop into the well-known piriform stages.

The genus *Babesia* contains more than 110 species; several globally distributed species (depending on the range of their tick vectors) are important pathogens of livestock, such as bovines (*B. bovis*, *B. bigemina*, and *B. divergens*), sheep, goats, horses, pigs, dogs (*B. canis*, *B. gibsoni*), cats, and rodents (Uilenberg 2006). Humans can also be accidentally infected with several species (mostly *B. divergens* or *B. microti* of rodents) (Fig. 20). Human babesiosis occurs mainly in the New World where it is a serious disease, especially in immunocompromised and splenectomized persons (Telford et al. 1993; Lobo et al. 2013).

Family Theileriidae

After injection into the vertebrate host, sporozoites enter the T and B lymphocytes or macrophages by a process significantly different from the invasion process known for the other apicomplexans (Fig. 22). Sporozoites as well as merozoites enter into host cells (lymphocytes and erythrocytes) by zippering in from any orientation. Importantly, the invasion does not require reorientation of the parasite's apical end toward the host cell membrane, with the internalization being much slower than in other apicomplexans. The completely surrounded and internalized sporozoites and merozoites release the contents of their secretory organelles (rhoptries and granular bodies), which apparently allows them to escape from the enclosing parasitophorous vacuole into the host cytoplasm. Once established in the host cytoplasm, the parasite grows and differentiates into a multinucleate schizont and, by a remarkable, yet largely unknown mechanism, transforms infected host lymphocytes into immortal cells, which leads to their clonal expansion (Fig. 22). Leukocytes filled with schizonts are called Koch's bodies. The released merozoites invade erythrocytes, where usually another round of division occurs, producing a generation of merozoites, which in turn infect new erythrocytes, particularly in species with limited or missing intralymphocytic multiplication. Multiplication in erythrocytes results in four merozoites forming characteristic tetrads (the Maltese cross), yet some species (*T. parva*) do not multiply in the red blood cells (erythrocytes), their multiple rounds of asexual division being confined only to lymphocytes. Gamogony occurs in the vector's intestine, where gametes fuse to produce a motile zygote. This stage invades epithelial cells, where it transforms into a single motile kinete similar to the haemosporidian ookinetes, and remains there during the development of the tick (trans-stadial transmission). However, unlike in *Babesia*, the kinete does not

multiply and ceases to further develop in the gut but transverses the gut wall and via the celom and hemolymph reaches and consequently penetrates the cells in salivary glands, where sporogony takes place. Feeding of the tick initiates rapid sporozoite development, and in the glandular epithelium, parasites rapidly multiply and produce an enormous number of sporozoites (up to 100,000 per each kinete) that escape into the salivary ducts (Fig. 22).

The two most important species are *T. parva*, which causes the east coast fever in Africa, and *T. annulata*, the causative agents of the tropical or Mediterranean theileriosis (Mans et al. 2015). About 40 *Theileria* species infect mainly ungulates in Africa and Asia but also Australia's marsupials, foxes, and other hosts. Species such as *T. ovis* are pathogenic to sheep and to other small ruminants; *T. equi* is an important pathogen of horses.

The classical difference between the genera *Theileria* and *Babesia* is the absence of extraerythrocytic asexual multiplication (schizogony) in the latter, while schizogony in *Theileria* occurs in lymph nodes and erythrocytes rather than in erythrocytes alone. Despite such a clear distinction, systematic affiliations of several species of piroplasms, even those with economic impact, remain unresolved. Small piroplasms of equines were recently transferred from the genus *Babesia* to the genus *Theileria*. Even more complicated is the case of *Babesia microti*, whose schizogony in lymphocytes and development and transmission in ticks are more similar to *Theileria*. Phylogenetic studies also indicate that the only two named species of the genus *Cytauxzoon* infecting felids including domestic cats represent just an internal group within the *Theileria-Babesia* clades. However, molecular evidence indicates that all these "problematic" parasites differ both from typical *Theileria* and *Babesia*. As in other apicomplexan groups, molecular tools are becoming increasingly important for phylogenetic delineation of the order Piroplasmida (Sivakumar et al. 2014).

Predatory and Photosynthetic Reminiscence of Apicomplexa

Colpodella was first described by Cienkowski in 1865 yet has not found its evolutionary home until the twenty-first century, when insight into its detailed ultrastructure and molecular phylogeny revealed its close relationship with the core Apicomplexa. *Colpodella* is a small (less than 20 μm long; Fig. 23), usually biflagellated free-living predator of other protists and algae, to which it attaches by its anterior tip containing the apical complex, through which it sucks the cellular content of its prey. After feeding, the organism withdraws flagella and forms a cyst. The major component of the apical complex of colpodellids is a pseudo-conoid, composed of an incomplete ring of subpellicular microtubules, micronemes, and elongated organelles reminiscent of rhoptries. All these organelles are considered plesiomorphies common to all apicomplexans. Furthermore, it has been suggested that the gliding motility used by *Colpodella* to penetrate prey cells is very similar to the mode of motility used by apicomplexans to invade host cells (Gubbels and Duraisingh 2012).

Chromerids are photosynthetic algae closely related to apicomplexans, branching in the frame of colpodellids. The group contains just two named species, *Chromera*

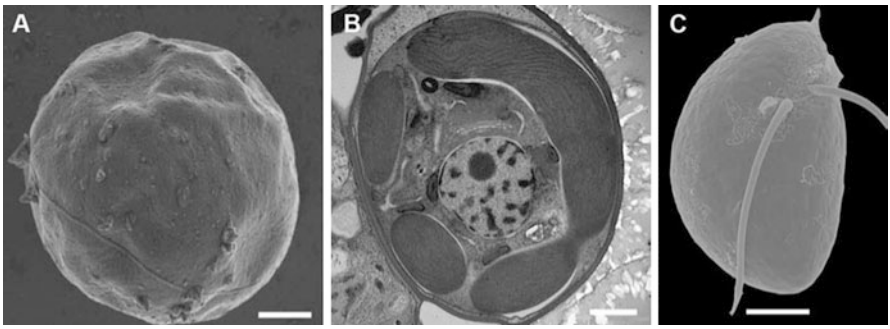

Fig. 23 Morphology of *Chromera velia* and *Colpodella edax*. SEM electromicrograph of *Chromera velia* cell with apparent suture in its cell wall (**a**); TEM electromicrograph of cross-sectioned cell of *C. velia* revealing a giant plastid (**b**); SEM electromicrograph of *Colpodella edax* with two flagella (**c**)

velia and *Vitrella brassicaformis*, isolated from stony corals in Australia (Fig. 23) (Moore et al. 2008; Oborník et al. 2011; Weatherby et al. 2011). The ecology of this organism and the nature of its association with the corals are not fully resolved; however, it has been shown that *C. velia* can infect coral larvae of the genus *Acropora* (Cumbo et al. 2013). The alga hosts a single secondary plastid per cell pigmented by chlorophyll *a*, a novel isoform of isofucoxanthin, and, surprisingly, lacks chlorophyll *c*. Primitive apical complex (pre-conoid) was found in *C. velia*; its presence or absence in *V. brassicaformis* remains to be established (Oborník et al. 2011; Oborník and Lukeš 2013; Portman and Šlapeta 2014; Oborník et al. 2016). Ultrastructural features, noncanonical genetic code in the plastid, and four membranes surrounding the plastid of *Chromera* resembling the non-photosynthetic apicoplast together indicate that *Chromera* possesses characteristics of the relict phototrophic organism leading to the extremely successful phylum Apicomplexa (Fig. 23) (Moore et al. 2008; Janouškovec et al. 2010; Oborník et al. 2016; Woo et al. 2015).

Maintenance and Cultivation

In vitro culture systems represent powerful tools for screening of potential drug candidates. Cultures of apicomplexan parasites such as *Cryptosporidium*, *Eimeria*, *Sarcocystis*, *Neospora*, *Toxoplasma*, *Besnoitia*, *Plasmodium*, *Babesia*, and *Theileria* have been documented, but they are often not productive and capable of sustaining the parasite for only a finite number of replication cycles. The mainstream apicomplexan cell cultures are *Toxoplasma* asexual stages (tachyzoites) in mammalian host tissue cells and *Plasmodium* asexual stages in mammalian red blood cells. The generation of sexual stages is still lacking for *Toxoplasma*, and in vitro animal experimentation is required to fulfill the life cycle (Müller and Hemphill 2013). Cell cultures for *Cryptosporidium* and *Eimeria* remain nonproductive.

Cultivation of any apicomplexan parasite in a good defined cellular system for studies on the proliferative stages is quite complicated because the complex nutritional and environmental characteristics of the host cells are difficult to mimic in vitro. The ultimate goal is to cultivate the parasites in a fully defined medium. In vitro cultivation of apicomplexans is further complicated by the tendencies of most life cycle stages to produce different stages (trophozoites and merozoites transform into merozoites and gamonts, respectively), transfer to a different host, and/or remain as encysted dormant tissue cyst or environmentally resistant oocysts. Only a few examples of life cycle stages have the ability to cycle indefinitely such as those in the mainstream culture systems for *Toxoplasma* and *Plasmodium*.

The availability of cultivation brought many benefits and remains a key research technique for the studies of hematozoans. The asexual stages of *Plasmodium* in the red blood cells are successfully exploited, and a wide array of genetic tools is now available to study malaria in vitro, including stable transfection to study roles of individual genes (de Koning-Ward et al. 2015). Continuous cultivation of *P. falciparum* in a medium containing red blood cells (not fully defined) is fundamental for drug screening and advanced studies of its molecular and cellular biology. Moreover, in 2002, a complete life cycle from sporozoite to sporozoite under in vitro conditions has been achieved for *Plasmodium berghei*, a model malaria species infecting rodents (Al-Olayan et al. 2002; Schuster 2002). Although each parasitic stage requires different cultivation conditions, the tissue culture RPMI-1640 remains the medium of choice, not only for *P. falciparum* and other human malarial parasites but also for piroplasms. However, recent findings have shown that a combination of three commercially available growth media (RPMI-1640, NCTC-135, and IMDM) supplemented with 10% bovine calf serum supports optimally long-term cultivation.

In spite of the fact that certain stages of avian coccidians as well as tachyzoites of *Toxoplasma* or *Neospora* can be readily cultivated in cell cultures, pharmaceutical compounds are still usually tested on parasites collected from experimentally infected hosts that are infected either orally (*Eimeria*), intraperitoneally (*Toxoplasma*), or via arthropod vectors (*Plasmodium*).

Evolutionary History

Origin of Apicomplexa

In the absence of a fossil record, apicomplexan evolution has been inferred from ultrastructural and morphological characters, coevolution with hosts, and molecular phylogenetic analyses. It has been generally supposed that the Apicomplexa first invaded marine invertebrates, as molecular dating places their origin between 600 and 800 million years ago, long before the emergence of vertebrates.

The Apicomplexa belongs to a group named Alveolata, which traditionally consists of three phyla: (i) the almost exclusively parasitic apicomplexans, (ii) the fully heterotrophic ciliates (Ciliophora), and (iii) the facultative photoautotrophic dinoflagellates (Dinophyta), which possess a complex (secondary or tertiary) plastid

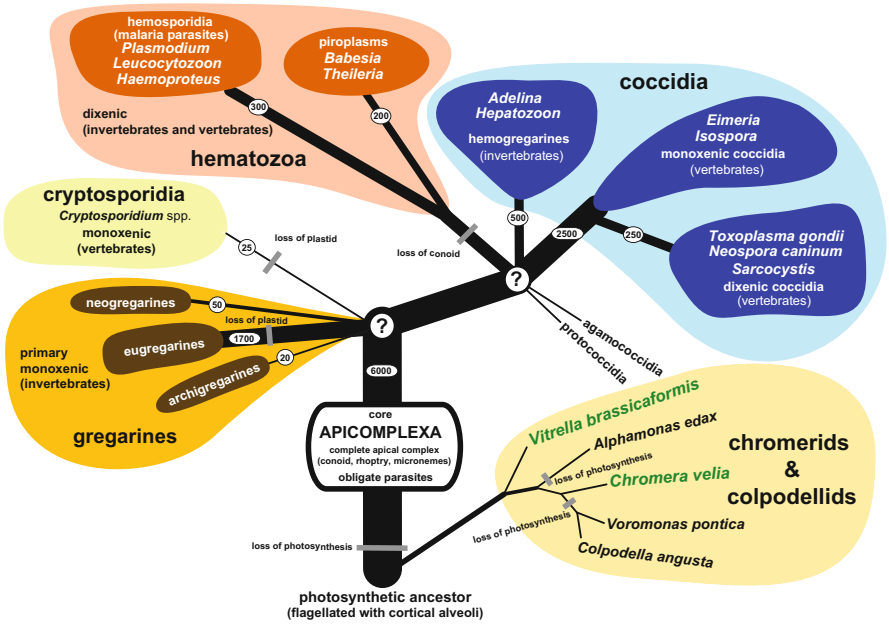

Fig. 24 Hypothetical tree of life of the Apicomplexa. The relationships are derived from morphology, biology, and molecular evolutionary studies based on current state of knowledge. The two major question marks denote the uncertainty of these key radiation events. The key to the common names is provided in Table 1. The branch thickness indicates the relative number of extant species also encircled at the branch

(Fig. 24). From the evolutionary standpoint, the ciliates are early-branching alveolates with the apicomplexans and dinoflagellates constituting an advanced sister groups. Finally, recently discovered chromerids contain coral-associated algae *Chromera velia* (Moore et al. 2008) and *Vitrella brassicaformis* (Oborník et al. 2012) that both appear more closely related to the apicomplexans than to the dinoflagellates (Moore et al. 2008; Janouškovec et al. 2010; Oborník and Lukeš, 2013; Janouškovec et al. 2015; Woo et al. 2015). It has been proposed that the entire group nowadays classified as the supergroup or kingdom SAR (Stramenopila + Alveolata + Rhizaria) (Adl et al. 2012) evolved through secondary and/or tertiary endosymbiotic event(s) between the red alga and a phagotrophic eukaryotic ancestor. However, the exact number of such events remains unknown, with proposals varying from a single endosymbiosis (Cavalier-Smith 1999) to multiple independent endosymbioses for each group of phototrophs (e.g., Falkowski et al. 2004) in the frame of the SAR supergroup. The proposed single secondary endosymbiosis has been dated to ~1.3 billion years ago, about 300–400 million years after the occurrence of the primary endosymbiosis between the heterotrophic eukaryote and cyanobacterium leading to the evolution of the primary plastids known from plants and rhodophytes (Fig. 24).

Although chromerids are closely related to the Apicomplexa, *C. velia* and *V. brassicaformis* do not form sister groups as anticipated but seem to be placed in

unrelated phylogenetic positions in the frame of colpodellids, with *Chromera* being affiliated with the *Colpodella* + *Voromonas* clade, while *Vitrella* is a sister to the genus *Alphamonas* (Gile and Slamovits 2013; Janouškovec et al. 2015; Oborník and Lukeš 2015). This suggests several possible independent losses of photosynthesis in this group. Although chromerids are not as closely related to each other as expected, they are known to possess noncanonical pathway for tetrapyrrole (heme and chlorophyll) synthesis, which is using the heterotrophic C4 route to form aminolevulinate, homologously to apicomplexan parasites and colpodellids (Kořený et al. 2011; Woo et al. 2015; Janouškovec et al. 2015). Since chromerids form relatively long branches, possibly resulting in artifacts in phylogenetic analyses, metabolic synapomorphies in the tetrapyrrole biosynthetic pathway between apicomplexans, colpodellids and chromerids represent one of the most convincing evidence for their common ancestry.

Regardless of the fact that both chromerid algae were isolated from similar environment, they substantially differ in morphology and life cycle. The isolated stage of *C. velia* is a coccoid vegetative cell containing a single large plastid surrounded by four membranes and numerous small mitochondria. Moreover, upon light exposure, large zoosporangia and consequently flagellated zoospores that highly resemble colpodellids are formed in the culture. Autosporangia of *C. velia* contain up to four autospores and zoosporangia up to ten zoospores, whereas the autosporangia and zoosporangia of *V. brassicaformis* are filled with dozens of spores (Oborník et al. 2011, 2012; Oborník and Lukeš 2013). Formation of zoospores in the zoosporangium of *C. velia* ultrastructurally resembles schizogony in Apicomplexa. It represents so far the only known developmental synapomorphy between photosynthetic chromerids and parasitic apicomplexans (Oborník et al. 2016).

Evolutionary Significance of the Apicoplast

The discovery of the apicoplast showed that the evolutionary history of the Apicomplexa is closely associated with the phenomenon of secondary endosymbiosis (Delwiche 1999; Foth and McFadden 2003; Keeling 2013). During this process, a eukaryotic alga was engulfed (or invaded) by a phagotrophic eukaryotic heterotroph and evolved into a multimembraneous complex plastid. Subsequently, this plastid lost its most important function – photosynthesis – and in hematozoans and coccidians, its genome has been reduced to a mere 35 kb circle. At the same time, the ancestral apicomplexan had to switch from autotrophy to heterotrophy, which may have coincided with the evolution of parasitism (Woo et al. 2015). Monophyletic origin of the apicoplast is generally accepted (Denny et al. 1998), yet two distinct lineages that slightly differ in plastid gene order, nucleotide composition, codon usage, and metabolic pathways have been distinguished (Oborník et al. 2002). Secondary endosymbiosis has deeply influenced the apicomplexan evolution by numerous replacements of the secondary host (exosymbiont) nuclear genes by their homologues from all three engulfed algal (endosymbiont) genomes (nuclear,

plastidial, and mitochondrial), likely through endosymbiotic gene transfer. This suggests that a substantial fraction of the apicomplexan genome can be composed of genes obtained from distantly related eukaryotes and their organelles.

However, not all members of the phylum Apicomplexa contain a plastid. This organelle is absent from the genus *Cryptosporidium* (Zhu et al. 2000; Keeling 2004), while its presence in gregarines is yet to be resolved. So far, a multimembraned apicoplast-like structure has been observed in the archigregarine *Selenidium hollandei* (Schrével 1971), whereas the eugregarine *Gregarina niphandrodes* seems to lack both the organelle and its genome (Tosso and Omoto 2007). It is likely that in this group the apicoplast has been lost multiple times, supporting the recent opinion that gregarines form a paraphyletic assembly at the base of the apicomplexan tree. It is plausible that some gregarines and the related genus *Cryptosporidium* lost their apicoplast early in the evolution, well before it became essential, as it is in *Plasmodium* and *Toxoplasma*, where cytosolic pathways were substituted by their plastidial counterparts (Oborník et al. 2009).

Besides predominating parasites, apicomplexans also include free-living marine predators called colpodellids, which use their apical complex for predation instead of parasitism (Leander et al. 2003b). The presence of a plastid in colpodellids has been recently confirmed (Gile and Slamovits 2013). While both photosynthetic alveolates branch within colpodellids, they contain photosynthetic plastids lacking chlorophyll *c*, the hallmark of the chromist and alveolate plastids. Interestingly, the plastid of *C. velia* (but not the one of *V. brassicaformis*) uses the noncanonical UGA triplet to encode tryptophan in the plastid-encoded proteins, which is a synapomorphy with the coccidian apicoplast (Moore et al. 2008). Plastid genomes of both chromerids contain roughly the same number of genes, but they display substantially different sizes and therefore also different levels of genome compaction. While the plastid genome of *C. velia* is linear and ~120 kb long, encoding highly divergent genes, the *V. brassicaformis* plastid genome is circular and compacted into ~80 kb (Janouškovec et al. 2010).

The apicomplexan cell carries unusual mitochondria, which either contain the smallest mitochondrial genome known or lost DNA altogether. While the linear mitochondrial genome of *P. falciparum* is only 5.9 kb long (Suplick et al. 1988; Feagin 1992), the DNA-lacking mitochondrion of *Cryptosporidium* has been reduced to a relic form resembling the mitosomes of microsporidia and diplomonads. Mitochondrial genome of *C. velia* is even smaller than those found in *Plasmodium* and *Toxoplasma*. It contains only two protein-coding genes (conserved *cox1* and highly divergent *cox3*) and fragmented rRNA genes. Consequently, the entire respiratory complex III (ubiquinol: cytochrome *c* oxidoreductase) was lost specifically from *C. velia*; homologously to apicomplexan parasites, *V. brassicaformis* and dinoflagellates, the complex I (NADH: ubiquinone oxidoreductase) is also absent in this chromerid alga. The electron transport function of the complex III is proposed to be substituted by L- and D-lactate cytochrome c oxidoreductases and L-galactono-1,4-lactone dehydrogenase. In contrast to *Chromera*, *Vitrella* still contains complex III; however, the mentioned newly proposed components of the respiratory chain are also present. Phylogenetic analyses showed that

these proteins are mostly of eukaryotic origins and have likely been lost from most of eukaryotic lineages (Flegontov et al. 2015; Oborník and Lukeš 2015).

Nuclear genomes of *C. velia* (194 Mb) and *V. brassicaformis* (73 Mb) were sequenced, and phylogenomic analyses confirmed phylogenetic position of chromerids on the root of Apicomplexa (Woo et al. 2015). It was also shown that massive gene loss (about 3,900 orthogroups) occurred during transition from a phototrophic ancestor to the apicomplexan parasites, while only dozens (80 orthogroups) were acquired. This suggests that the phototrophic ancestor of Apicomplexa already contained most of genes (or their ancestors) which are used for parasitism in apicomplexan parasites (Woo et al. 2015).

Evolutionary Diversity of Apicomplexa

The evolution of the apical complex and gliding motility opened an extremely successful obligatory parasitic niche for the apicomplexans (Portman and Šlapeta 2014; Heintzelman 2015; Keeling and Rayner 2015). The core parasitic Apicomplexa are monophyletic (Fig. 24). There are two alternative schools of thought in respect to relationships among the principal groups. The first school postulates that coccidians represent the ancestral polyphyletic group from which all the other major groups arose independently. This scenario assumes secondary hypertrophy of the gregarine trophozoites, as well as acquisition of extracellularity from primarily intracellular ancestral coccidians. An alternative scenario is that the gregarines are the most ancient paraphyletic group, from which monophyletic hematozoa and coccidia arose. Such a view finds support in the evolutionary relationships with hosts (invertebrates vs. vertebrates) and the complexity of life cycles (single host vs. multiple hosts). Unlike coccidia and hematozoa, gregarines are exclusively parasites of invertebrates and have simple life cycles.

The current knowledge of the gregarine evolution has recently been dramatically challenged by molecular ecology surveys of diverse oceanic and sediment samples (Leander 2008). A large proportion of phylotypes formerly unrelated to any known eukaryotic group have been shown to constitute an assembly of gregarine sequences monophyletic within the Apicomplexa. Their morphological identity remains unknown, but the link between phylotypes represents a challenging issue to be elucidated in the coming decades. Besides these relatively well-defined apicomplexan groups, there is a myriad of organisms of uncertain taxonomic placement, often found in diverse and obscure marine hosts. Organisms classified under agamococcidia (*Rhytidocystis*) from polychetes, protococcidea (*Gemmocystis*) from corals, or even parasites of squids and crabs (*Aggregata*) are just a few examples that challenge even the simplest traditional scenario of apicomplexan evolution.

Due to their effects on human and animal health, members of the monoxenic and cyst-forming coccidia have attracted substantially more attention than the other groups. The evolution of coccidia is traditionally based on the life cycle and number of sporocysts and sporozoites in the environmentally resistant oocysts. Stabilization of the number of sporocysts per oocyst and sporozoites per sporocyst seem to be key

events. However, the modes of excystation are arguably even more informative characters (Jirků et al. 2002). Feline and canine species infecting ruminants as intermediate hosts represent a classical examples of coevolution of the cyst-forming coccidia with their final hosts, where sexual development occurs. The two-host life cycle appears multiple times in coccidian evolution, and it has been hypothesized that homoxeny (single-host cycle) predated heteroxeny (two-host cycle).

The fact that gametogony of hematozoa takes place in vertebrate hosts implies that these parasites have evolved from coccidians of invertebrates rather than vertebrates. The common ancestral host of both avian malarial parasites (*Plasmodium* and *Haemoproteus*) appears to be reptile, and host switches between reptiles and birds are documented quite frequently. By contrast, the host shift from reptiles to mammals was a singular event. On a species level, fascinating recent evolutionary consequences are revealed about malarial parasites affecting humans. It has been postulated that 10,000 years ago a major geographic expansion of malaria took place in Africa. Mechanisms behind this expansion are wide adoption of more efficient agriculture resulting in increased population size and coinciding spread of the *P. falciparum* mosquito vector with climate change in sub-Saharan Africa after the last glacial period. Over the past 40 years, incidence of malaria is rapidly increasing, amplified by the rapid spread of antimalarial resistance, pesticide-resistant mosquitoes, increased population size, poverty, and global warming, all resembling the situation 10,000 years ago. In contrary, recent advances in malaria control led to eradication of significant reduction of impact of malaria in several tropical areas.

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