# Chapter 11
# Anisakid Nematode (Ascaridoidea) Life Cycles and Distribution: Increasing Zoonotic Potential in the Time of Climate Change?

**Sven Klimpel and Harry W. Palm**

**Abstract** Parasitic nematodes are known as important pathogens that cause problems for human and animal health. Some of them naturally inhabit the marine environment, where they are widespread and can be found in a variety of different hosts. Food-borne zoonoses via aquatic animals are most often linked to anisakid nematodes of the genera *Anisakis* Dujardin, 1845, *Contracaecum* Railliet and Henry, 1912, and *Pseudoterranova* Mozgovoi, 1951. These are commonly found in the digestive tract of marine mammals, and infect aquatic invertebrates and vertebrates as intermediate hosts. The most widely distributed whale worms *Anisakis* spp. involve cetaceans as final and planktonic copepods, euphausiids, squids and teleosts as intermediate or paratenic hosts. Painful infections of the digestive tract in humans originate through consumption of raw or semi-raw fisheries products, for example fish and squid. Recent molecular studies revealed the existence of morphologically similar but genetically different cryptic species ('sibling species') within the anisakids. Among these, *A. simplex* (s.s.) is responsible for the highest number of recorded human infections. Molecular studies of *Anisakis* larvae from various parts of the world Oceans demonstrate an uneven species distribution, with *A. simplex* (s.s.) being limited to the northern hemisphere. Another species, *A. typica*, has not yet been connected to this disease, and seems to be restricted to the tropical regions. This chapter presents the present state of knowledge about this widespread group of fish parasites, including the importance as human pathogens, their life cycle biology, biogeography and phylogeny. The distribution of the currently recognized *Anisakis*

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species is summarized and combined with the number of known cases of human anisakiasis. We suggest that pathogenicity for humans is different among the *Anisakis* siblings, providing a possible explanation for uneven disease records worldwide. The possibility of a changing risk of anisakidosis in the time of climate change is discussed.

## 11.1 Introduction

Parasitism, a form of symbiosis, is one of the most successful modes of life (Palm and Klimpel 2007). More than half of all plant and animal species on earth are parasites, and probably no organism avoids parasitic infection during its lifetime (Palm and Klimpel 2007). Including approximately 256 families and more than 40,000 known species, the phylum Nematoda is one of the most species rich and abundant invertebrate Taxon (Anderson 2000; McClelland 2005). Beside free-living nematodes in freshwater, marine and terrestrial habitats (McClelland 2005), their parasitic forms use plants, animals and humans as host organisms at a global scale (e.g. Blaxter et al. 1998).

Gastrointestinal parasitic nematodes are known to cause a wide range of diseases and have consequences for human and animal health. They impose a significant economic burden as parasites of domestic animals, reduce productivity, and require elaborate and expensive control methods (e.g. Parkinson et al. 2004; Audicana and Kennedey 2008). Infections of humans cause substantial mortality and morbidity, resulting in about 2.9 billion infected people worldwide (Parkinson et al. 2004). Most important are the hookworms (e.g. *Ancylostoma* spp.), ascarids (*Ascaris* spp.), whipworms (e.g. *Trichuris trichiura*) and filarial nematodes that cause lymphatic filariasis (e.g. *Brugia malayi*) or elephantiasis (*Wuchereria bancrofti*) and African river blindness (e.g. *Onchocerca volvulus*) (e.g. Parkinson et al. 2004). Humans also become accidental hosts for nematodes that cannot complete their life cycles inside them, but can cause disease problems or initiate immune hypersensitivity states or allergies. The consumption of raw or undercooked fish regularly leads to food-borne zoonoses, most commonly caused by larvae of the anisakid nematode genera *Anisakis*, *Contracaecum* and *Pseudoterranova* (Sakanari and McKerrow 1989; Kaneko 1991; Audicana et al. 2002; Palm 2004).

Since the 1960s, the term anisakiasis had been used for a human disease caused by the third-stage larvae (L3) of members of the family Anisakidae. In 1988, a standardized nomenclature recommended three different terms: (1) anisakidosis caused by any members of the family Anisakidae, (2) anisakiasis caused by members of the genus *Anisakis*, and (3) pseudoterranovosis caused by members of the genus *Pseudoterranova* (e.g. Audicana et al. 2003; Audicana and Kennedey 2008). The first case of a human infection with *Anisakis* sp. was reported for the Netherlands (Van Thiel 1962) from an eosinophilic intestinal lesion in a patient. Ishikura and Kikuchi (1990) recorded 12,586 cases of anisakiasis between 1968 and 1989 in Japan. The number of cases is increasing worldwide, with ~50 cases annually in the USA and

~500 cases in Europe, over 95% of them from The Netherlands, Germany, France and Spain (e.g. Plath et al. 2001; Audicana et al. 2002; Fuentes et al. 2002). To date, over 14,000 anisakiasis cases have been reported, approximately 95% from Japan (Audicana et al. 2002). The parasite transmission is clearly related to the consumption of raw or semi-cooked fish. Especially Japanese sushi and sashimi, Dutch salted or smoked herring, Nordic gravlax (dry, cured salmon), Hawaiian lomi-lomi (raw salmon), German rollmop (rolled fillet of marinated/pickled herring), South American cebiche and Spanish boquerones en vinagre (pickled anchovies) are regular pathways of infection (e.g. Petersen et al. 1993; Audicana et al. 2002; Palm 2004).

The present communication summarizes the current state of knowledge on zoonotic anisakid nematodes, their pathogenicity, life cycle biology, biogeography and phylogeny. We suggest that in addition to different special dishes and food preferences, a characteristic distribution pattern of the currently recognized *Anisakis* species is responsible for an unequal regional distribution of known cases of anisakiasis so far. We assess the risk that might result from potentially changing infection levels in the time of increasing anthropogenic influence and climate change. This is especially important for an increasing number of people that use marine food products for their daily needs.

## 11.2 Genetic Identification

An accurate identification of nematodes at any particular life cycle stage is essential for the diagnosis of nematode infections, and consequently an important part of disease surveillance and control. Identification of nematodes from marine vertebrates has been based on morphological characters, such as the size and shape of the spiculae (sexual organs) in adult males, and head structures and papillae that regularly occur on the body surface. Larval identification used the orientation of the excretory pores, the arrangement and separation of the digestive tract into oesophagus, ventricle and attaching structures such as caeca and appendices, and the shape of the tail (Fig. 11.1). Even accompanied with morphometric information, generic and especially species identification has been difficult, leading to a high number of erroneous identifications. This promoted molecular methods for a better and more reliable species diagnosis.

Molecular techniques have the advantage that they allow analyses of the parasite DNA, securing species identification and providing data for phylogenetics. Genomic DNA sequences evolve at different rates, with non-coding, non-transcribed sequences of ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) evolving faster than those that encode essential proteins or nuclear DNA (nDNA), respectively. Molecular anisakid nematode identification started with allozyme analyses including restriction fragment length polymorphism techniques (PCR-RFLPs of ITS-DNA, e.g. D'Amelio et al. 2000; Kijewska et al. 2002; Pontes et al. 2005). The next approach was direct sequencing of rDNA, including the highly variable

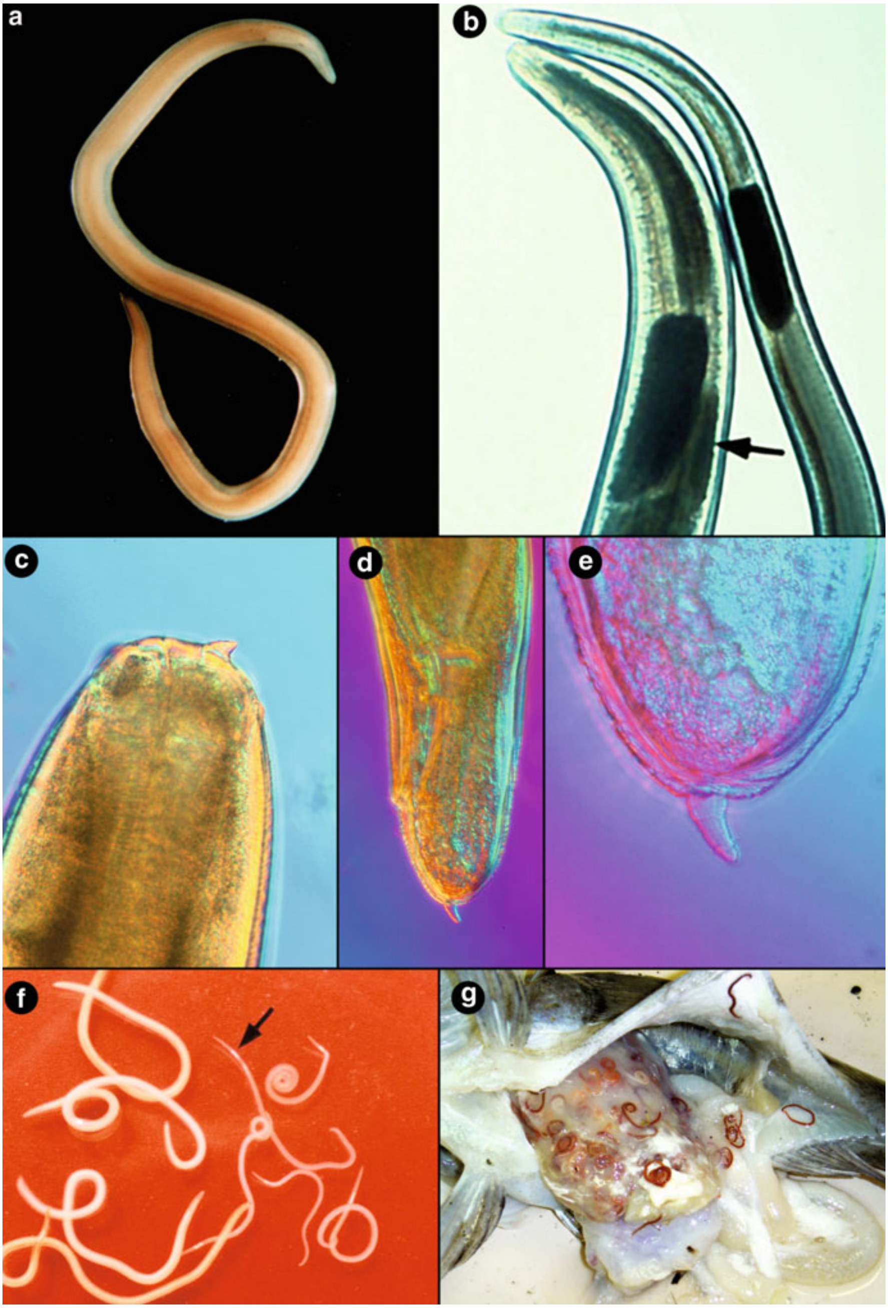

**Fig. 11.1** (**a**) Habitus of the anisakid nematode *Pseudoterranova decipiens* E isolated from the liver of the icefish *Chaenocephalus aceratus*. (**b**) Anterior end of the third-stage larvae (L3) of *P*. *decipiens* s.l. (*left*) from smelt (*Osmerus eperlanus*) and *Anisakis simplex* s.l. from herring

internal transcribed spacers ITS1-2 and the conserved 5.8S rDNA region; direct sequencing of the 28S (LSU rDNA) and complete internal transcribed spacer (ITS-1, 5.8S, ITS-2) rDNA (e.g. Li et al. 2005; Nadler et al. 2005; Zhu et al. 2000a, b, 2001, 2002) and mitochondrial cytochromoxidase 1 and 2 (mtDNA *cox1*, *cox2*) sequence analyses (e.g. Valentini et al. 2006; Cross et al. 2007; Mattiucci and Nascetti 2008; Mattiucci et al. 2008a, b) followed. Also micro-satellites can be used to distinguish the species among populations. These studies identified the existence of "sibling species" within the ascaridoids, being morphologically very similar but genetically different, having distinct host preferences, life cycles and geographical distribution (e.g. Mattiucci et al. 1997, 2005; Zhu et al. 2002; Nadler et al. 2005; Marques et al. 2006; D'Amelio et al. 2007; Klimpel et al. 2007, 2008, 2010; Mattiucci and Nascetti 2008).

Within the family Anisakidae the genus *Contracaecum* includes two sibling species complexes, the (1) *C. osculatum* complex with the five species *C. osculatum* A, B, C (*C. osculatum* s.s.), D, E, and the (2) *C. ogmorhini* complex with the two species *C. ogmorhini* (s.s.) and *C. margolisi*, and additionally *C. radiatum* (e.g. Mattiucci and Nascetti 2008; Shamshi et al. 2009a, b). The *Pseudoterranova decipiens* complex consists of six different species (*P. decipiens* s.s. = *P. decipiens* B, *P. krabbei*, *P. bulbosa*, *P. azarasi*, *P. decipiens* E, *P. cattani*) (Mattiucci et al. 2007; Mattiucci and Nascetti 2008). Most recently Klimpel et al. (2007, 2008, 2010) and Palm et al. (2008) studied the species identity within the genus *Anisakis* by using the following protocol.

Genomic DNA isolation and purification followed amplification of the rDNA region (ITS-1, 5.8S, ITS-2), and flanking sequences (=ITS+), using the primers NC5 (5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′) and NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′) (Zhu et al. 2000a). The PCR reaction (50 μL) includes 25 μL of Master-Mix (Peqlab Biotechnology GmbH, Erlangen, Germany) containing dNTPs, $MgCl_2$, buffer, and Taq-Polymerase, 3 μL of each primer, 14 μL aqua dest. and 5 μL genomic DNA. Each PCR reaction is performed in a thermocycler (Biometra or Peqlab, Germany) under the following conditions: after initial denaturation at 91°C for 1 min, 40 cycles of 94°C for 45 s (denaturation), 55°C for 45 s (annealing), and 72°C for 45 s (extension), followed by a final extension at 72°C for 10°min. PCR products were examined on 1% agarose gels. A 100-bp ladder marker (peqGOLD, Erlangen, Germany) is used to estimate the size of the PCR products. For anisakid nematode identification, the PCR products must be purified with an E.Z.N.A. Cycle-Pure Kit (Peqlab Biotechnology GmbH, Erlangen, Germany), followed by sequencing of a total volume of 7 μL, including 2 μL of primer (individually) and 5 μL of the PCR product (250 ng/μL). Both spacers and the 5.8S gene from each PCR product are sequenced in both directions,

**Fig. 11.1** (continued) (*Clupea harengus*). (**c**) Anterior end of the third-stage larva of *A. typica* with the boring tooth. (**d**, **e**) Posterior end of *A. typica* with the mucron. (**f**) Nematode larvae (L3) isolated from Wadden Sea fish; *left* – *P. decipiens* s.l., *right* – *A. simplex* s.l. (**g**) Numerous anisakid nematode larvae in the viscera of the icefish *C. aceratus*

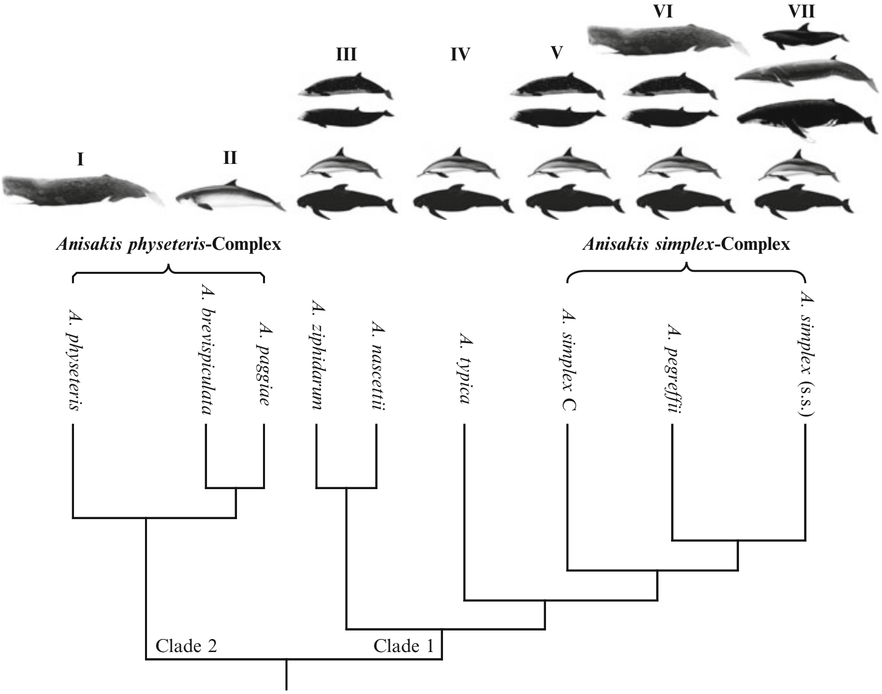

**Fig. 11.2** *Anisakis* spp. final cetacean host distribution in the *A. physeteris* and *A. simplex* complexes (Cetacea-families; I Physeteridae; II Kogiidae; III Ziphiidae, Delphinidae; IV Delphinidae; V Ziphiidae, Delphinidae; VI Physeteridae, Ziphiidae, Neobalaenidae, Delphinidae; VII Phocoenidae, Balaenopteridae, Monodontidae, Delphinidae)

using the primers NC5, NC13 (forward; 5′-ATC GAT GAA GAA CGC AGC-3′), NC13R (reverse; 5′-GCT GCG TTC TTC ATC GAT-3′), XZ1R (reverse; 5′-GGA ATG AAC CCG ATG GCG CAA T-3′), and NC2.

These studies revealed the existence of nine species, six belonging to two sibling species complexes, the (1) *A. simplex* complex with *A. simplex* (s.s.), *A. pegreffii*, and *A. simplex* C, the (2) *A. physeteris* complex with *A. physeteris*, *A. brevispiculata* and *A. paggiae*, and the three species *A. typica*, *A. ziphidarum* und *A. nascettii* (e.g. Klimpel et al. 2008, 2010; Mattiucci and Nascetti 2008; Fig. 11.2).

## 11.3 Life Cycle Ecology of Anisakid Nematodes

According to Anderson (1984, 1996), parasitic nematodes first evolved in terrestrial hosts and were only able to invade aquatic environments after the development of heteroxeny (the use of intermediate hosts) and paratenesis (the use of transport hosts). Intermediate hosts support larval growth and development to a stage where the nematode is capable of infecting its definitive host. Both intermediate and paratenic hosts participate in the temporal and spatial dispersal of the parasite, thereby increasing the likelihood of transmission into the final host (e.g. McClelland 2005). Heteroxeny is the common life cycle pattern of marine ascaridoid nematodes such as *Anisakis*, *Contracaecum*, and *Pseudoterranova*. Transmission pathways are habitat-dependent and usually involve a broad spectrum of invertebrates and intermediate or paratenic fish hosts (McClelland 2005; Klimpel and Rückert 2005; Palm and Klimpel 2007).

The life cycle of anisakid nematodes follows the general nematode life cycle pattern, including four larval stages (L1–L4) and the adults in the final host. The heteroxenous life cycle involves a variety of hosts that are transferred through the marine food chain. Most important are the three genera *Anisakis* (whales), *Contracaecum* (bird, seals), and *Pseudoterranova* (seals), that can be distinguished morphologically and according to their final host spectrum (e.g. Klimpel et al. 2008, 2010; Mattiucci and Nascetti 2008). Life cycle studies of these nematodes have been limited by difficulties in maintaining them alive in the laboratory, culturing sufficient numbers of parasite-free experimental hosts, and creating effective exposure (e.g. Køie and Fagerholm 1995; Køie et al. 1995; Køie 2001; Klimpel et al. 2004, 2008, 2010; Mattiucci and Nascetti 2008). However, empirical studies on the distribution and abundance of anisakid larvae in the intermediate and final hosts have revealed important insights into the life cycle biology of these parasites. Being responsible for anisakiasis and of high importance for human health, the whaleworms *Anisakis* spp. mainly infect toothed whales and a range of pelagic schooling fish worldwide. Thus, the life cycle can be considered to take place in the pelagic environment, with some seals and baleen whales getting accidentally infected (e.g., Køie et al. 1995; Hays et al. 1998a,b; Køie 2001; Klimpel et al. 2004, 2010). Life cycle stages include four larval stages (L1–L4), within the eggs (L1–L3) and subsequently in the intermediate or paratenic hosts (L3), and as preadults (L4) and adults in the cetacean final hosts (Fig. 11.3). The nematode eggs are excreted with the faeces and embryonate in seawater (Køie 2001). Køie et al. (1995) found larvae surrounded by two cuticles prior to hatching. They were surrounded by sheaths with lateral extensions and were able to float, enabling them to use mainly pelagic crustaceans as intermediate hosts (Køie et al. 1995). During ingestion by the crustacean first intermediate host, the larvae are most probably released from the second stage cuticle by the action of the mouthparts. This allows the third stage larvae to penetrate the gut prior to establishing themselves in the haemocoel (Køie et al. 1995). Larger invertebrates (mainly copepods, euphausiids) and smaller fish are thought to be important second intermediate hosts, and various predatory fish species and cephalopods serve as paratenic hosts. If small fishes are preyed upon by larger piscivorous fishes, the larvae are capable of re-infecting the latter without further moulting. Consequently, piscivorous hosts may accumulate enormous numbers of larvae (Lile 1998). Cetaceans acquire the nematodes by preying upon the intermediate hosts. To date, a total of 34 cetacean species have been found to harbour *Anisakis* spp. (e.g. Klimpel et al. 2008; Mattiucci and Nascetti 2008; Fig. 11.4).

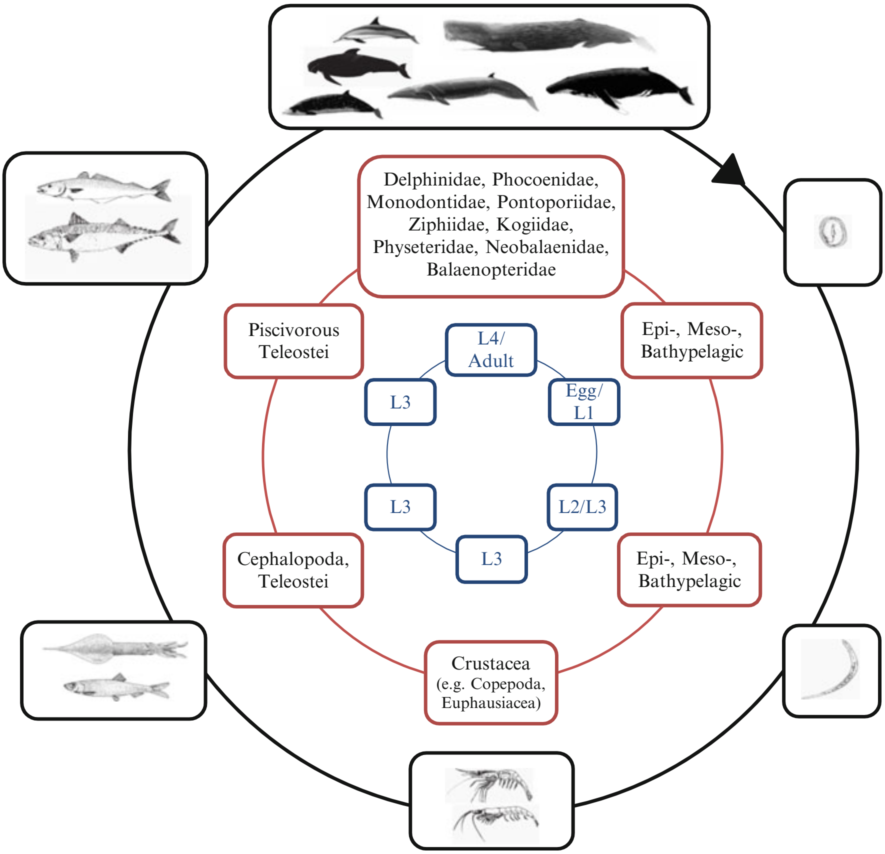

**Fig. 11.3** Schematic life cycle of *Anisakis* species. The pelagic life cycle of *Anisakis* spp. follows the general nematode life cycle pattern, including four larval stages (L1–L4) and the adults in the cetacean final host. The heteroxenous life cycle involves a variety of invertebrate and vertebrate hosts that are transferred through the marine food chain (Kuhn 2010)

In contrast to the whaleworms, the sealworms of the genus *Pseudoterranova* seem to be restricted to a benthic life cycle (e.g. Palm et al. 1994; Køie et al. 1995; Palm 1999; McClelland 2002). Partially embryonated eggs, passed in the faeces of a seal, settle on the sea bed where they complete development to the third stage larvae (L3) and hatch. Newly hatched larvae are still ensheathed in the cuticle of the previous second larval stage (L2) and attach to the substrate caudally (e.g. Køie et al. 1995; Anderson 2000; McClelland 2002, 2005). When ingested by benthic crustaceans (e.g. amphipods, gammarids, isopods, harpacticoid copepods), they exsheath inside the first intermediate host, penetrate into the haemocoel and begin to grow. These hosts serve to enhance transmission to a larger array of benthic macro-invertebrates as second intermediate hosts, where the larval sealworms grow in length (e.g. Anderson 2000, McClelland 2002, 2005). At this point they become infective to fish and also to seals. The invertebrate hosts are usually ingested by

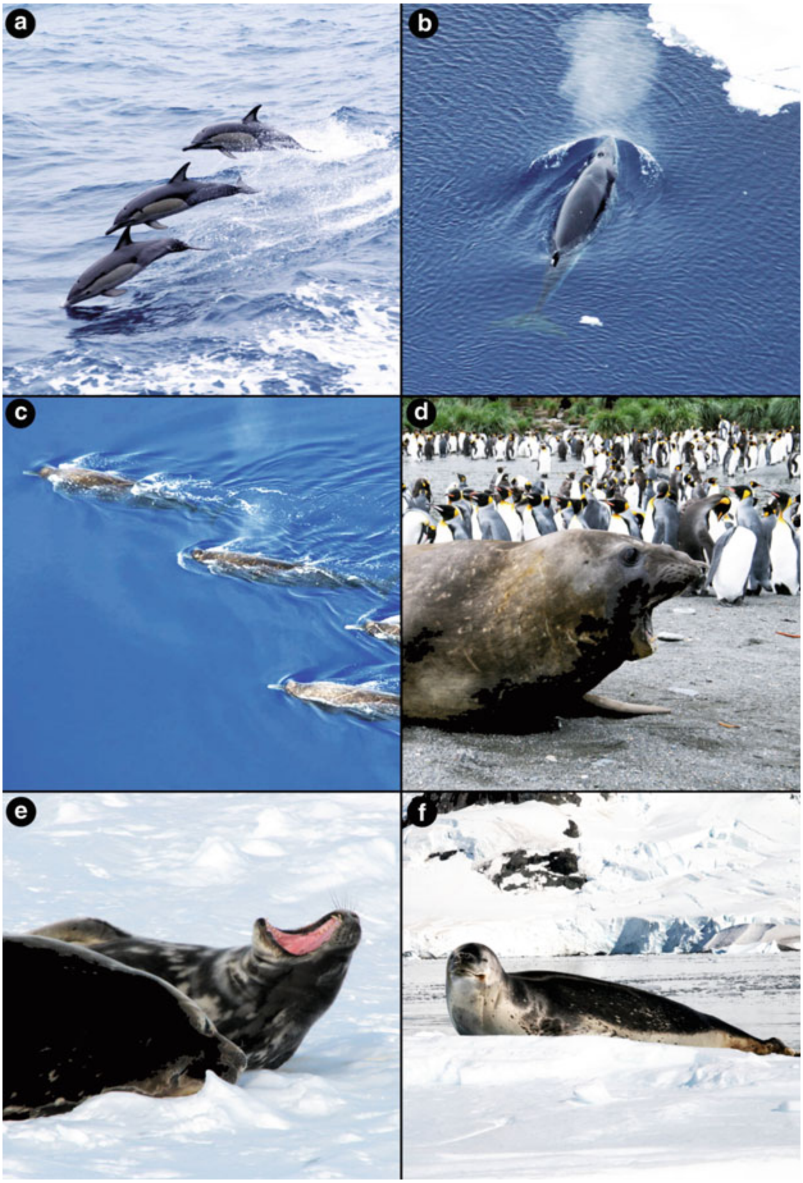

**Fig. 11.4** Cetacean and pinniped final hosts of anisakid nematode species. (**a**) Dolphins (Fam. Delphinidae) are the most common final hosts of *Anisakis typica* from subtropical and tropical marine waters. (**b**) Minke whale (*Balaenoptera bonaerensis*, Fam. Balaenopteridae) as potential final hosts for *Anisakis* spp. in the Southern Ocean (Antarctica). (**c**) Beaked whales (Fam. Ziphiidae) in the Southern Ocean (Antarctica) are final hosts of *Aniskais ziphidarum*. (**d**) Southern elephant seal (*Mirounga leonina*), (**e**) Weddell seal (*Leptonychotes weddellii*) and (**f**) Leopard seal (*Hydrurga leptonyx*) are final hosts of the nematode genera *Contracaecum* and *Pseudoterranova* in the Southern Ocean (Antarctica)

primary benthic teleosts, including juveniles of larger demersal fish species. The larvae penetrate the gut wall of the fish and establish themselves in the internal organs or the musculature, where they continue to grow in length. Large, piscivorous fish may serve as second/third fish or paratenic hosts that accumulate the larval worms (Palm 1999; Anderson 2000; McClelland 2002, 2005). Following ingestion by the seal definitive host, infective third stage larvae (L3) escape from the bodies of the fish or invertebrate, embed their anterior part into the gastric mucosa, mature and reproduce. Ten marine mammal species belonging to the Otariidae and Phocidae have been recorded as final hosts (e.g. Mattiucci and Nascetti 2008; Fig. 11.4).

Nematodes of the genus *Contracaecum* seem to have equally complex life cycles involving benthic and pelagic invertebrates (e.g. crustaceans, squid) and fish (e.g. Klöser et al. 1992; Køie and Fagerholm 1995; Køie et al. 1995). They are also transmitted to pelagic and demersal fish by for example euphausiids, shrimps and small fish that are found near bottom in daytime but feed pelagically at night. In total 12 marine mammal species including specimens from the families Ottariidae and Phocidae and different fish-eating sea birds (e.g. of the genera *Larus*, *Pelecanus*, *Phalacrocorax*) have been identified as final hosts in the *Contracaecum* life cycle (e.g. Torres et al. 1983; Farjallah et al. 2008; Mattiucci and Nascetti 2008; Shamshi et al. 2009a; Fig. 11.4).

## 11.4 Phylogeny and Host Range of Anisakid Nematodes (*Anisakis* spp.)

Traditional nematode taxonomy was based on a limited number of criteria, such as the shape of the oesophagus, male and female reproductive organs and life cycle patterns (e.g. Wijova et al. 2006). Recent morphology-based classifications split the nematodes into the classes Secernentea (= Phasmidea, = Rhabditea) and Adenophorea ( = Aphasmidea, = Enoplea), including most terrestrial and parasitic species and most marine species, respectively (Dorris et al. 1999). There were considerable obstacles for an accurate identification especially of the larval forms. For this reason, earlier classification systems were not compatible with each other, and there was no universally accepted nematode phylogeny during the last century (e.g. Blaxter et al. 2000; De Ley and Blaxter 2002; Meldal et al. 2007). A study of the small subunit (SSU) or 18S rRNA for a wide range of major nematode taxa concluded convergent evolution in many lineages, requiring revision of the morphology-based higher-level classification (Blaxter et al. 1989; Dorris et al. 1999). Although most recent datasets of the SSU rRNA genes comprise more than 300 taxa (Blaxter 2003), sampling remains strongly biased towards some groups and poor for other important taxa.

The nematodes divide into the three major clades Dorylaimia (clade I, free-living, invertebrate, vertebrate and plant parasites from the marine and terrestrial environment), Enoplia (clade II, marine and plant parasite species) and Chromadoria, which include the Rhabditida (marine free-living species, few terrestrial/freshwater representatives). The Rhabditida separate into the Spirurina (clade III, only animal parasites), Tylenchina (clade IV, animal-, plant- and fungus-parasitic and free-living groups) and Rhabditina (clade V, free-living and parasitic species) (e.g. De Ley and Blaxter 2002; Parkinson et al. 2004; De Ley 2006). Parasitism of both plants and animals seems to have arisen multiple times within nematode evolution, and all major clades include parasites (Blaxter et al. 1989; De Ley and Blaxter 2002; Parkinson et al. 2004). The Spirurina include five infraorders (Ascaridomorpha, Spiruromorpha, Rhigonematomorpha, Oxyuridomorpha, Gnathostomatomorpha) and one additional superfamily, the Dracunculoidea. With exception of the invertebrate parasites of the Rhigonematomorpha, all others include both invertebrate and vertebrate parasites (De Ley 2006; Wijova et al. 2006). The Ascaridomorpha are most closely related to the Spiruromorpha and Rhigonematomorpha, a clade that also comprises terrestrial and marine parasites (De Ley and Blaxter 2002; De Ley 2006), among these the family Anisakidae with *Anisakis, Contracaecum* and *Pseudoterranova*. While the former infects whales as final hosts, *Pseudoterranova* occurs in seals and *Contracaecum* in a range of aquatic hosts, including seals and birds.

Most *Anisakis* siblings have been identified from toothed whales, especially from the Delphinidae and Ziphiidae. The phylogenetic relationships within *Anisakis* together with their most common final hosts are illustrated in Fig. 11.2. The nine known species divide according to their host range into two major clades, the *A. physeteris* sibling species complex and the other six species. Three of them form the clade of the *A. simplex* sibling species complex. They are sister to the other three species, *A. typica*, *A. ziphidarum* and *A. nascettii*, the latter two combined on a single clade. *Anisakis typica* is restricted to dolphins (Delphinidae) from subtropical and tropical waters and to a single species of the family Pontoporidae (e.g. Mattiucci et al. 2002, 2005; Klimpel et al. 2008; Palm et al. 2008). *Anisakis ziphidarum* and *A. nascettii* have been reported so far only from the Ziphiidae. The *A. simplex* sibling species complex typically infects toothed but also baleen whales. *Anisakis simplex* (s.s.) parasitizes oceanic cetaceans of the families Delphinidae, Monodontidae, Phocoenidae, and Balaenopteridae mainly in the North Atlantic and Pacific Oceans. *Anisakis pegreffii* also utilizes the family Delphinidae as final hosts, however, additionally infecting the Ziphiidae, Physeteridae, and Neobalaenidae (Mattiucci et al. 1997) mainly in the entire Atlantic and Mediterranean but also in Australia. *Anisakis simplex* C infects toothed whales of the families Delphinidae and Ziphiidae in the southern hemisphere, extending its range of distribution into the North Pacific. Species within the *A. physeteris* sibling species complex are host specific for the Kogiidae and Physeteridae. *Anisakis brevispiculata* and *A. paggiae* have been recorded from kogiids mainly in the Mid- and Southern Atlantic Ocean (Mattiucci and Nascetti 2006; Valentini et al. 2006), and the cosmopolitan *A. physeteris* is known from physeterids (Fig. 11.5).

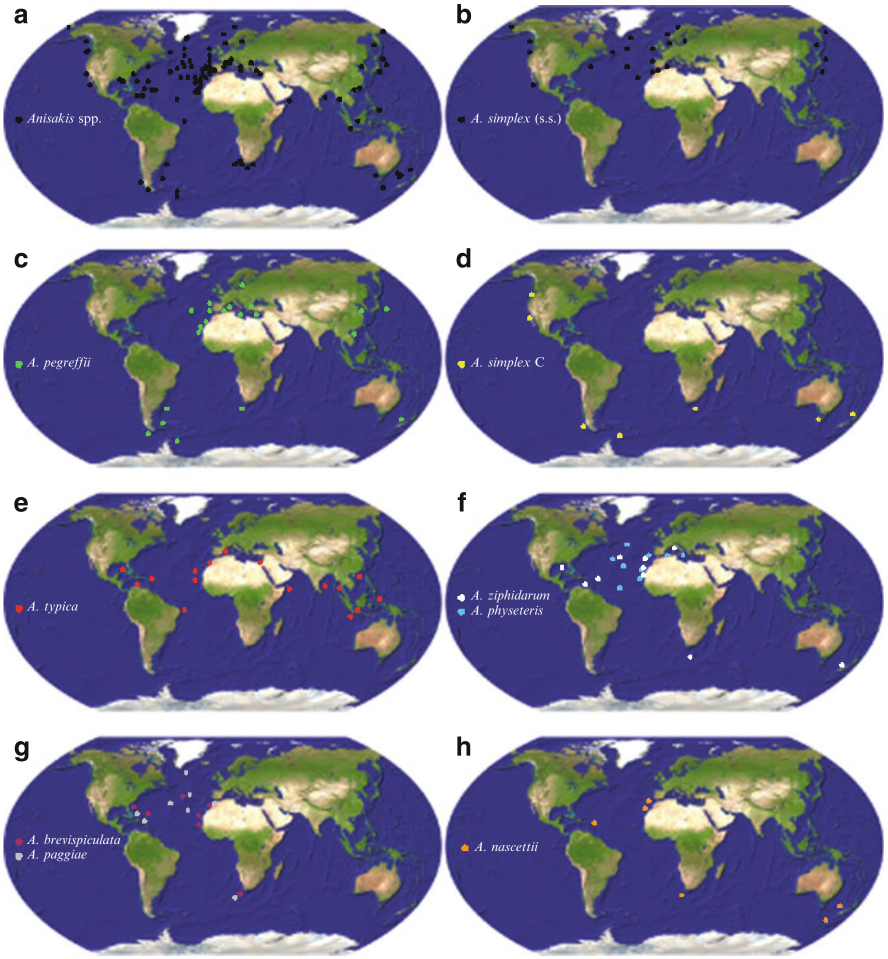

**Fig. 11.5** Geographical distribution of *Anisakis* species. (**a**) *Anisakis* spp.; (**b**) *A. simplex* (s.s.); (**c**) *A. pegreffii*; (**d**) *A. simplex* C; (**e**) *A. typica*; (**f**) *A. ziphidarum*, *A. physeteris*; (**g**) *A. brevispiculata*, *A. paggiae*; (**h**) *A. nascettii*

## 11.5 Biography of *Anisakis* spp.

Species of the genus *Anisakis* are distributed worldwide. The biogeography of *Anisakis* spp. follows a variety of factors that, in combination, lead to zoogeographical distribution patterns: (1) the final host distribution, (2) the host specificity in the final and intermediate hosts, (3) migration patterns of second and paratenic hosts, and (4) the characteristic life cycle (Kellermanns et al. 2007; Klimpel et al. 2008, 2010; Palm et al. 2008). These factors enable *Anisakis* siblings to explore all kinds of marine environments, from the shallow seas and open ocean into the deep-sea (e.g. Palm and Klimpel 2008). Most *Anisakis* siblings have been reported from the temperate, subtropical, and tropical waters between the equator and 35° North and South, while some species seem to be most common in the boreal regions of the Atlantic and Pacific (Fig. 11.5). Within Antarctic waters (Southern Ocean), these nematodes are at the most southern range of their distribution and to our knowledge extremely scarce (Klimpel et al. 2010).

Most detailed records on the zoogeography have been reported for *A. simplex* (s.s.), a common and highly abundant nematode in the North Atlantic and Pacific Oceans. According to the genetic identification, this species is limited to the northern hemisphere. The most closely related species, *A. pegreffii*, is known from the central Atlantic and the Mediterranean Sea, with some records from the most southern tips of the South American, African and Australian continents. Klimpel et al. (2010) identified specimens from migrating myctophids from the Southern Ocean off the South Shetlands that were genetically identical to specimens from China. The third species within the *A. simplex* complex, *A. simplex* C, has been also recorded from South America, Africa and Australia and around the South Shetland Islands in the Antarctic Ocean. Klimpel et al. (2010) analyzed the ITS-1, 5.8S and ITS-2 rDNA regions of *A. simplex* C specimens from the Antarctic Ocean, finding them identical to specimens from Pacific Canada and California, confirming the extensive range of distribution for this species. Because the parasites were found only in migrating myctophids coupled with rare findings from other teleosts in the Antarctic Ocean (also *A. pegreffii*), the authors concluded that these specimens originated from outside the Antarctic. Consequently, they can be considered at the most southern range of distribution in the Southern Ocean, and an earlier molecular record of *A. simplex* C from the elephant seal *Mirounga leonina* was interpreted as an accidental case of infection.

The two most closely related species within the *A. physeteris* complex, *A. paggiae* and *A. brevispiculata*, have been recorded so far only from the Atlantic Ocean, with most records in the northern hemisphere and a single record from the South African coast. *Anisakis physeteris* has been recorded only from the central and north Atlantic and the Mediterranean. However, because this species typically infects sperm whales that are known for an extensive zoogeographical distribution, the parasite might follow the distribution pattern of their final hosts. According to Klimpel et al. (2008) and Mattiucci and Nascetti (2008), *A. ziphidarum* and *A. nascettii* are typical parasites of ziphiid whales. The former has been recorded from the same localities as *A. paggiae* and *A. brevispiculata*, only from the Atlantic Ocean, and only a few records have reported *A. nascettii* from the waters of the central Atlantic Ocean, South Africa (SE Atlantic Ocean) and New Zealand (SW Pacific Ocean) (Mattiucci and Nascetti 2008; Mattiucci et al. 2009). A unique distribution pattern is known so far for *A. typica*, a species that has been described as circumtropical. According to Palm et al. (2008), several genotypes exist for this anisakid, however, there is no information on morphological differences between them. It can be concluded that following the extensive range of distribution of their mammalian final hosts and the low host specificity in migrating intermediate and paratenic hosts, anisakid nematodes have extensive ranges of distribution.

This may explain why they are among the most common fish parasites recorded during common fish parasitological examinations.

## 11.6 Pathogenicity and Zoonotic Potential

The research interest in anisakid nematodes is based on the ability of the parasite larvae to survive in humans when ingested alive. Besides having zoonotic potential, anisakid larvae in the teleosts as well as the adults in marine mammals are several centimetres in length, and can cause pathological effects in their hosts. According to Dailey (2001), gastritis or ulcers have been often found in association with aggregations of L3, L4 and adult stages of anisakids (*Anisakis*, *Contracaecum*, *Pseudoterranova*) in the stomach and upper intestine of pinnipeds, cetaceans and sea otters. The symptoms of heavy infections include diarrhoea, dehydration and anaemia (e.g. McClelland 2005). Intestinal perforations leading to peritonitis and death have been attributed to *Contracaecum* and *Pseudoterranova* infections in sea lions and sea otters, respectively.

Nematode parasites of marine vertebrates may also be pathogenic to their intermediate hosts. Larval *Pseudoterranova* spp. caused erratic behaviour and death in experimentally infected marine crustaceans (McClelland 1990). Various larval anisakids (*Anisakis, Contracaecum, Pseudoterranova*) have been connected to mechanical compression or necrosis of the liver, lesions in the gut wall, viscera and musculature, depletion of lipids and mortality in heavily infected marine fish (Rohde 1984; Williams and Jones 1994; McClelland 2005). However, even a high intensity of infestation with *Pseudoterranova decipiens* and *Contracaecum* spp. in Antarctic fish (Klöser et al. 1992; Palm 1999; Palm et al. 1994, 1998, 2007) or the frequent infestation of the Atlantic and Baltic Sea herring (e.g. Szostakowska et al. 2002; Levsen and Lunestad 2010) with *A. simplex* (s.s.) has no visible effect on the host's fitness. Some fish populations are commonly associated with very high anisakid burden, closely related to the abundance of their final hosts in the area of investigation (e.g. Des Clers 1991; Des Clers and Andersen 1995; Lile 1998).

Most recent research activities relate to the zoonotic potential of anisakids to infest humans. Epidemiological studies in Japan have indicated that anisakiasis was most frequent in coastal human populations (Audicana et al. 2002). Most common transmission routes are raw, undercooked and lightly marinated seafood (see Petersen et al. 1993; Palm 2004), for example of the spotted chub mackerel (*Scomber japonicus*) and Japanese flying squid (*Todarodes pacificus*) in Japan (Audicana et al. 2002; Audicana and Kennedey 2008). In western Europe, herring (*Clupea harengus*) is the main species involved, and in Spain, most cases can be related to the consumption of pickled anchovies (*Engraulis encrasicolus*) and raw sardines (*Sardina pilchardus*) (Audicana et al. 2002; Audicana and Kennedey 2008). Archetypal cases of anisakiasis or anisakiosis (Couture et al. 2003), involving the penetration of the alimentary tract and associated organs and causing clinical symptoms (e.g. nausea, severe epigastric pain, vomiting, allergy, diarrhoea), have been reported largely from Japan and other Asian countries (e.g. McClelland 2005). *Anisakis* larvae have been diagnosed as the disease-causing pathogen in most cases, the remainder being attributed to an infection with *Pseudoterranova* larvae (McClelland 2005). According to Smith (1999) and McClelland (2005) most cases of *Pseudoterranova* infection in Europe and the US have been largely asymptomatic, being diagnosed after the expulsion of the nematodes by coughing, vomiting or defaecation.

Helminth infections often induce chronic rather than acute disease, even in cases of very high levels of parasites. This results from parasite's adaptations to evade the host immune response to secure their own survival. Human anisakidosis is peculiar because these parasites are not adapted to humans, and more than 90% of cases are caused by a single larva (Kagei and Isogaki 1992; Daschner et al. 2000; Audicana and Kennedey 2008). Differences may therefore be expected between *A. simplex* pathogenesis and that caused by other helminths in humans. An example of this is filariasis, in which there is a high and persistent burden of parasites, possibly resulting from host–parasite coevolution in order to optimize their mutual survival (e.g. Mitchell 1991; Taylor et al. 2005; Audicana and Kennedey 2008). Overt hypersensitivity reactions are rare unless provoked by natural or drug-induced death of the parasites residing in tissues. This contrasts with *Anisakis* infections, where allergic reactions seem to be common in humans.

Over the last few years, studies have indicated that, as with other helminth infections, the pathological changes occurring within the gastrointestinal tract are the combined result of the direct action of the larva during tissue invasion and the complex interaction between the host immune system and the substances released by, or contained within, the parasite. Allergies caused by the anisakid larvae in fish consumers have been of major concern. In the reported allergic cases of people from northern Spain, cooked hake (*Merluccius merluccius*) closely followed anchovies as the main pathway of infection. In Germany especially rolled fillet of marinated herring (rollmop) and fried smelt (*Osmerus eperlanus*) are a common source of infection with allergic reactions to *A. simplex* (s.s) in the former and *P. decipiens* in the latter.

Human infection by anisakid nematodes, especially *Anisakis* species, induces stimulation of both T helper type 1 and 2 (Th1, Th2) responses, and provokes a strong specific immune response by antibody isotypes, the immunoglobulin (Ig) IgE, IgG, IgA and IgM (Kennedey 2000; Cho et al. 2005; Audicana and Kennedey 2008). According to Anadón et al. (2009) more than 10.0% of gastrointestinal anisakiasis may be accompanied by allergic symptoms. Some studies have detected the presence of anti-*Anisakis* IgE antibodies in more than 10.0% of healthy subjects, suggesting the existence of a high number of infected patients who do not develop clinical symptoms (Anadón et al. 2009). In contrast to marine mammals, *Anisakis* larvae do not usually reach the adult stage in humans and the larvae die over a specific period after infection. Therefore, it is likely that the immune response against *Anisakis* allergens from the third and/or fourth-stage larvae occurs in response to two consecutive antigenic stimuli, for example (1) the excretory/secretory (ES) and cuticle antigens while the larvae is alive and (2) the cuticle and protease-resistant somatic and ES antigens, after the larvae die (Anadón et al. 2009). Previous studies have shown that the *Anisakis* ES allergens are the most clinically important, as they are targeted by most of the anti-*Anisakis* IgE antibodies induced during infections by this parasite (Anadón et al. 2009). To date several ES and somatic *Anisakis* allergens have been characterized and cloned, including Anis s 1 and Anis s 7 as probably the most important ES allergens, and have been reported in 85–100.0% of infected humans (Anadón et al. 2009). The Anis s 2 (paramyosin) and Ani s 3 (tropomyosin) are somatic *Anisakis* allergens that cross-react with other common allergens. Other allergens such as Ani s 4 (cystain), Ani s 6 (serine protease inhibitor), Ani s 5, Anis s 8 and Ani s 9 (the latter three among to the SXR/RAL-2 family proteins) are minor ES allergens which were reported from fewer than 50.0% of infected humans (Anadón et al. 2009).

## 11.7 Ascaridoid Nematodes and Climate Change

We are living in a period of climate change. Temperatures have increased by at least 0.33°C since 1990 (ocean and land combined), and ice fields on Greenland and parts of the Antarctic continent, for example the Larsen and Wilkins shelf ice, are melting at alarming rates (e.g. Rahmstorf et al. 2007). Though proceeding at a moderate pace in terms of human life span, climate change is transforming the world's oceans by increasing the temperature and acidity of seawater and altering atmospheric and oceanic circulation. This has consequences for species distribution and composition in the marine ecosystems, changing the biogeography and biodiversity in aquatic habitats.

The natural variability of abiotic factors such as water temperatures (resulting e.g. in frontal zones, e.g. Klimpel and Rückert 2005) and ocean circulations is relatively high, often following non-linear or cyclic patterns. Similarly, aquatic habitats suffer significant anthropogenic habitat change, mainly caused by overexploitation and unsustainable use. Because these fluctuations overlay the so far subtle effects of temperature changes that are caused by anthropogenic-induced global warming, direct studies of the future consequences for the major ecosystems are difficult. However, the study of the effects of natural climate variability on selected organisms and environments can provide valuable insights into the possible impact of global warming. Compared to terrestrial systems, marine ecosystems are expected to react more sensitively and quickly to changes in climatic conditions, with unpredictable consequences for the species composition, spatial population shifts, or a restructuring of the food webs involved (e.g. Steele 1998, 2004; Hsieh et al. 2005; Jiao 2009). For example, many Atlantic and Pacific fish stocks exhibit a close correlation with climate patterns over many decades (Klyashtorin 2001). Even small natural climatic changes can have significant effects on the marine ecosystems and their organisms.

Fish parasites can be used as biological indicators for environmental impact and change (Palm 2010). However, their potential to indicate global change scenarios has not been tested. Palm (2010) suggested that oceanic and remote marine ecosystems such as the central Pacific, mid Atlantic, Antarctic or the Polar Sea are the best candidate localities to link an effect of a changing climate directly to aquatic parasite communities at a larger scale. These regions are less affected by factors such as anthropogenic species introduction, pollutants and for example seasonal migrations. Most informative are metazoan helminths as parasite bioindicators because they are embedded within the marine food web and live in oceanic and remote environments. Ascaridoid nematodes combined with larval cestodes and possibly acanthocephalans are useful as biological indicators for host abundance (Palm 2010). The anisakids especially, due to their omnipresence, wide distribution and dependence on the availability of the large predatory final hosts such as seals and whales, are potential candidates to indicate environmental change at a larger scale. According to Marcogliese (2008), warming of coastal waters will result in a higher number of pelagic fish species that follow warmer currents northwards, resulting in increasing *Anisakis* spp. infection of fish. Other effects are a general shift in host ranges and the introduction of pathogens into formerly uninfected regions.

Climate change might have a direct affect on the parasite species but also indirect effects through changes in the distribution and abundance of their intermediate and final hosts (Marcogliese 2008). Especially parasites with complex life cycles, or those in poikilothermic hosts, may be disproportionately affected by global warming (Marcogliese 2008). Induced ice melting in most northern and southern habitats must have consequences for the polar seal and whale populations, and also their parasites. Under global change scenarios, increased sea surface temperature in the northern Arctic might shrink seal populations, reducing the number of final hosts and consequently the number of worms in the fish. On the other hand, more ice-free waters allow the large migrating whale populations to extend their range of distribution into more northern and southern habitats, into formerly ice-covered regions. It has been demonstrated that *A. simplex* C and *A. pegreffii* are at the most southern range of distribution in the Southern Ocean (Klimpel et al. 2010). Higher water temperatures in the high Antarctic might lead to a higher abundance of the whaleworm *Anisakis* in formerly unrepresented regions. Consequently, while the numbers of the sealworms *Pseudoterranova* and possibly *Contracaecum* decrease under shrinking and changing final host populations, other anisakids (*Anisakis*) might be able to extend their numbers. This would also have consequences for the zoonotic potential of these worms. A higher worm abundance of *A. simplex* (s.s.) in fish of the North Atlantic and Pacific waters will result in higher transmission rates to humans, and probably cause an increasing conflict potential through the consumption of marine fish.

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