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ORIGINAL PAPER

The role of pelagic swarm fish (Myctophidae: Teleostei) in the oceanic life cycle of *Anisakis* sibling species at the Mid-Atlantic Ridge, Central Atlantic

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Abstract First information is provided on the parasitation and feeding ecology of the myctophid fish species *Myctophum punctatum* and *Notoscopelus kroyeri* from the Mid-Atlantic Ridge (MAR), Central Atlantic. Four different parasite species were found in both fish with a similar high prevalence and intensity of infestation. The digeneans *Gonocerca phycidis* and *Lethadena* sp. were isolated as adults from the stomach, larval tetraphyllidean cestodes (*Scolex pleuronectis*) from the intestine, and genetically identified larval anisakid nematodes of *Anisakis simplex* (*s.s.*) from the body cavity. No further *Anisakis* sibling species could be identified. Both myctophids had small pelagic crustaceans, mainly copepods and hyperiids, within their stomach contents. Ostracods, euphausiids, decapods, and amphipods were minor food components, demonstrating the pelagic environment for both fish. The recorded parasites including the anisakid *A. simplex* (*s.s.*) perform pelagic life cycles within the region, benefiting from extensive diurnal vertical migrations of their fish hosts. Comparison of the host range among the anisakis sibling species suggests that the *A. simplex* complex has low host specificity, infecting toothed and baleen whales on their extensive oceanic migrations. This contrasts the *Anisakis physeteris* complex that is restricted to toothed whales of the families Kogiidae and Physeteridae. Specificity in the teleost intermediate hosts for both complexes seems to be low, and sympatric occurrence of different siblings within the same intermediate hosts is likely. Myctophid swarm fish as important copepod feeders at the MAR significantly contribute to the oceanic anisakid nematode life cycle, especially considering the 100% prevalence and high intensity of infestation. Further genetic identification of *Anisakis* nematodes is needed in order to understand the sibling species distribution, along the MAR and within other oceanic environments.

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Introduction

Information on oceanic ecosystems is scarce in comparison to shallow coastal zones or the continental shelf regions. The open oceans have been compared with terrestrial deserts due to low primary production that is caused by the lack of nutrients especially in the surface layers. However, in regions such as the Mid-Atlantic Ridge system (MAR), local upwelling situations as well as complex currents and frontal systems can create favorable conditions for increased primary and secondary production in the top 200 m surface layer. Thus, the oceanic ecosystem is far more complex and needs detailed investigation.

Deep-sea lantern fishes of the family Myctophidae consist of more than 235 species belonging to 32 genera (e.g., Yamaguchi et al. 2000; Froese and Pauly 2008). Together with the Gonostomatidae and different crustaceans, they usually dominate the meso- and upper bathypelagic zones (approximately 200 to 2,500 m depth; Gjøsaeter and Kawaguchi 1980; Foxton 1970). The majority of these swarm fishes exhibit extensive vertical migration from water depths below 1500 m at daytime into the more productive surface layers at night (e.g., Pusch et al. 2004; Klimpel et al. 2006a). Diet analyses of meso- and upper bathypelagic fishes have been concentrated on the numerically abundant families Myctophidae, Gonostomatidae, Stomiidae, and Sternoptychidae (e.g., Gartner and

Zwerner 1989; Klimpel et al. 2006a). Though having considerable diet variations, their principal prey items are small crustaceans belonging to the Copepoda, Euphausiacea, Amphipoda, and Decapoda (Mauchline and Gordon 1984; Sutton and Hopkins 1996). Thus, myctophids and other pelagic swarm fishes are an important link between the low trophic levels and higher predators, such as piscivorous fishes and whales (Pusch et al. 2004).

Species of the nematode genus *Anisakis* are worldwide distributed fish parasites and typically infect cetaceans or pinnipeds as final hosts. This genus previously included three species (Davey 1971). Beside readily described morphospecies (e.g., *Anisakis schupakovi*), the analyses of allozymes, RFLPs of ITS-rDNA, SSCP of ITS-rDNA, and mtDNA*cox-2* data have revealed that this genus currently includes eight distinct sibling species, *Anisakis simplex* (*s.s.*), *A. pegreffii*, and *A. simplex* C (representing the *A. simplex*-complex) together with *A. typica*, *A. ziphidarum*, *A. paggiae*, *A. brevispiculata*, and *A. physeteris* (the latter three representing the *A. physeteris*-complex; Valentini et al. 2006; Mattiucci and Nascetti 2007). All these sibling species are morphologically very similar but genetically different and have distinct host preferences, life cycles, and zoogeographical distribution (Valentini et al. 2006; Klimpel et al. 2007; Kellermanns et al. 2007; Mattiucci et al. 2007). Life cycle studies of these nematodes have been limited by difficulties in maintaining *Anisakis* spp. alive, culturing sufficient numbers of parasite-free experimental hosts, and creating effective exposure in the laboratory. In general, the life cycle of *Anisakis* spp. is heteroxenous, involving several different invertebrate and/or vertebrate intermediate hosts and cetaceans and sometimes pinnipeds as final host (e.g., Køie et al. 1995; Hays et al. 1998a, b; Køie 2001; Klimpel et al. 2004). Life cycle stages include four larval stages (L1–L4), within the eggs (L1–L3), and subsequent in the intermediate or paratenic hosts (L3), and as preadults (L4) and adults in the final hosts.

The zoogeography of *Anisakis* spp. depends on a variety of different factors that, in combination with each other, lead to different zoogeographical distribution: (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. These factors enable *Anisakis* siblings to explore different marine environments, such as shallow seas, the open ocean, or the deeper waters. Most *Anisakis* siblings have been recorded from temperate, subtropical, and tropical waters between the equator and 35° North and South, while some species typically occur in the boreal regions of the Atlantic and Pacific. Within Antarctic waters (Southern Ocean), these nematodes are extremely rare.

The most abundant fish species in the central Atlantic oceanic waters along the MAR are the myctophids *Myctophum punctatum* and *Notoscopelus kroyeri* (Sutton et al. 2008). Both species undergo extensive vertical (diurnal) migrations. While average peak abundance during the day ranges between 225–750 m for *M. punctatum* and 350–1000 m for *N. kroyeri*, at night, peaks are more usually between 10–125 m, respectively (Froese and Pauly 2008). Both species feed mainly on copepods, amphipods, and euphausiids (Froese and Pauly 2008). Earlier parasitological studies recorded few metazoan fish parasites in *M. punctatum* and *N. kroyeri* (e.g., Mordvinova 2000; Klimpel et al. 2001).

The aim of the present study was (1) to investigate the typical parasite fauna and prey items of both fish species along the MAR, (2) to genetically identify the collected *Anisakis* larvae, (3) to identify the transfer mechanism of the recorded anisakids along the MAR, and finally (4) to discuss patterns of parasite–host association within this oceanic environment.

Materials and methods

Sample collection

Myctophid fish species were sampled during the MAR-ECO (Census of Marine Life) research cruise of the RV G. O. Sars at June to August 2004 at the MAR. Fish sampling was conducted with different pelagic trawls (e.g., Krill-Trawl, Åkra-Trawl, Egersund-Trawl) at the sampling period. At station 339 (position 52°55′ N 34°39′ W; sampling depth 300–800 m) in the northern region of the MAR, a total of 89 *M. punctatum* and 74 *N. kroyeri* were captured to study metazoan parasites and stomach contents. All specimens were directly sorted from the catch during sampling processing in the ship laboratory and identified to the lowest possible taxon. All fish specimens were deep frozen at −40°C for later studies in the wet laboratory on board. Prior examination each fish specimen was defrosted at 0–1°C. Morphometric data including the total length and total weight were recorded to the nearest 0.1 cm and 0.001 g.

Parasitological examination

The presence of metazoan parasites within all organs was studied by using a stereomicroscope. Ectoparasite infestation was examined while the fish was still in a partly frozen state. Inspection included the skin, fins, eyes, nasal cavities, gills, and the buccal and branchial cavity. The body cavity was opened to examine the liver, stomach, pyloric caeca, intestine, and gonads microscopically for endoparasites, and the stomach contents were removed. Isolated parasites were fixed in 4% borax-buffered formalin and preserved

in 70% ethanol–5% glycerine for detailed identification. For identification purposes, nematodes were dehydrated in a gradated ethanol series and transferred to 100% glycerine. Digenean and cestode species were stained with Acetic Carmine, dehydrated, cleared with Eugenol or Creosote, and mounted in Canada Balsam. Parasite identification literature included original description. The ecological terminology (prevalence, intensity, mean intensity) follows Bush et al. (1997). Prevalence is the number of infected fish with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species (commonly expressed as a percentage); intensity (I) is the number of individuals of a particular parasite species in a single infected host (expressed as a numerical range); and mean intensity (mI) is the average intensity; in other words, it is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite. Furthermore, we use the following parasitological terms and definitions: (1) final host—where a parasite reaches sexual maturity, (2) intermediate host—required by a parasite to complete its life cycle; usually, it undergoes considerable morphological or physiological change, (3) paratenic host/transport host—not required by a parasite to complete its life cycle without detectable morphological change.

Stomach content analyses

The stomach contents were sorted and food items were identified to the lowest possible taxon and grouped into taxonomic categories. In order to determine the relative importance of food items, the frequency of occurrence of each prey item *i* (F %) and its percentage by number (N %) was calculated (Hyslop 1980; Amundsen et al. 1996). The frequency of occurrence was determined as the number of stomachs with prey item *i* compared to all nonempty stomachs. Numerical percentage was calculated as the number of prey item *i* compared to the total number of all prey items (Hyslop 1980).

Polymerase chain reaction amplification and sequencing of ITS-1, 5.8S, and ITS-2

Nematodes isolated from the examined fish were identified morphologically by existing keys and descriptions as belonging to the genus *Anisakis*. After isolation and identification, the nematodes were freed from host tissue and stored in 100% ethanol. Four anisakid nematode larvae from both myctophid fish species were used for molecular identification. Genomic DNA was isolated and purified from individual larvae using a genomic DNA extraction kit (Peqlab Biotechnology GmbH, Erlangen, Germany) according to the instructions of the manufacturer. The rDNA region comprising the ITS-1, 5.8S, ITS-2, and flanking sequences (=ITS+) was amplified using the previously described 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. 2000). Polymerase chain reaction (PCR) (26 μl) included 13 μl Master-Mix (Peqlab Biotechnology GmbH, Erlangen, Germany) containing dNTP, $MgCl_2$, buffer, and Taq-Polymerase, 3 μl of each primer, 2 μl dest. water, and 5 μl genomic DNA. Each PCR reaction were performed in a thermocycler (Biometra, Germany) under the following conditions: after an initial denaturation at 95°C for 15 min, 30 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), 72°C for 1 min (extension), followed by a final extension at 72°C for 5 min. Samples without DNA were included in each PCR run. PCR products were examined on 1% agarose gels. A 100-bp ladder marker (peqGOLD, Erlangen, Germany) was used to estimate the size of the PCR products. To identify the anisakid nematodes, the PCR products were purified with E.Z.N.A. Cycle-Pure Kit (Peqlab Biotechnology GmbH, Erlangen, Germany). Afterwards a total volume of 7 μl, including 2 μl primer (individually) and 5 μl of the PCR product (250 ng/μl) were sequenced by Seqlab (Goettingen GmbH, Germany). Both spacers and the 5.8S gene from each PCR product were sequenced in both directions using 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. The obtained sequences were identified via GenBank and aligned with previously characterized sequences of anisakid nematodes (AJ937671 *A. simplex* (*s.s.*) from Europe and China; AB277823 *A. pegreffii* from Japan; AY821739 *A. simplex* C from northern Pacific), using CLUSTAL W (1.83) Multiple Sequence Alignments (Thompson et al. 1994).

Results

Parasite composition

The parasite fauna of both myctophid fish species was similar in species composition and infestation rates. Four different parasite species were found in *M. punctatum* and *N. kroyeri*, including two digenean, one cestode, and one nematode species (Tables 1 and 2). The digeneans *Gonocerca phycidis* and *Lethadena* sp. were adult and located in the stomach. Larval tetraphyllidean cestodes (*Scolex pleuronectis*) were detected in the intestine with relatively high infestation rates. These tetraphyllideans are cosmopolitan distributed and have been found in various

Table 1 Prevalence (P), intensity (I), mean intensity (mI) and site of infestation as well as frequency of occurrence (F%) and numerical percentage of prey *i* (N%) of the food items identified from the stomach contents of the myctophid fish species *Myctophum punctatum* from the Mid-Atlantic Ridge

Parasite	Uninfested/infested	a/l	P [%]	I (mI)	Site of infestation	*n* Stomachs with food	*n* Food item i	F [%]	N [%]
DIGENEA									
Gonocerca phycidis [a]	89/3	a	3.4	1 (1.0)	Stomach				
Lethadena sp. [a,b]	89/7	a	7.9	1–2 (1.2)	Stomach				
CESTODA									
Tetraphyllidea indet. *Scolex pleuronectis)*	89/21	l	23.6	1 (1.0)	Intestine				
NEMATODA									
Anisakis simplex (*s.s.*)[a]	89/89	l	100.0	1–8 (3.2)	Organs of body cavity				
Stomach contents									
Prey group									
Copepoda				1–30 (6.9)		89	617	100.0	73.02
Ostracoda				1–2 (1.2)		6	7	6.7	0.83
Hyperiidae				1–10 (4.7)		40	187	44.9	22.13
Amphipoda				1–3 (1.3)		17	22	19.1	2.60
Decapoda				1–2 (1.3)		9	12	10.1	1.42

n Number, *a* adult, *l* larva
[a] New host record
[b] New locality record.

fish species. However, further identification is not possible without strobila characters, knowledge of the life cycle, and molecular data. The anisakid nematode *Anisakis simplex* (*s.s.*) was found in high numbers and represented the predominant parasite species in both examined myctophids at a prevalence of 100.0%, respectively. The third-stage larvae (L3) of this anisakid nematode (molecular identification; see below) was found in or on the organs of the body cavity. Three

Table 2 Prevalence (P), intensity (I), mean intensity (mI) and site of infestation as well as frequency of occurrence (F%) and numerical percentage of prey *i* (N%) of the food items identified from the stomach contents of the myctophid fish species *Notoscopelus kroyeri* from the Mid-Atlantic-Ridge

Parasite	Uninfested/infested	a/l	P [%]	I (mI)	Site of infestation	*n* Stomachs with food	*n* Food item i	F [%]	N [%]
DIGENEA									
Gonocerca phycidis[a]	74/2	a	2.7	1 (1.0)	Stomach				
Lethadena sp.[a,b]	74/6	a	8.1	1 (1.0)	Stomach				
CESTODA									
Tetraphyllidea indet. *Scolex pleuronectis)*	74/15	l	20.3	1 (1.0)	Intestine				
NEMATODA									
Anisakis simplex (*s.s.*)[a]	74/74	l	100.0	1–6 (3.0)	Organs of body cavity				
Stomach contents									
Prey group									
Copepoda				2–39 (14.2)		74	1048	100.0	82.85
Ostracoda				2–4 (3.0)		2	6	2.7	0.74
Hyperiidae				1–15 (2.7)		63	167	85.1	13.20
Amphipoda				1–3 (1.4)		13	18	17.6	1.42
Euphausiacea				1–2 (1.1)		17	19	23.0	1.50
Decapoda				1 (1.0)		7	7	9.5	0.55

n Number, *a* adult, *l* larva
[a] New host record
[b] New locality record

new host and one new locality record for the MAR are established. There was no significant correlation between the fish length and number of ingested copepods and accumulated *A. simplex* (*s.s.*) (Fig. 1a–d).

Stomach contents

Most prey items of both fish species were small pelagic crustaceans, mainly copepods and hyperiids. Ostracods, euphausiids, decapods and amphipods were minor components. Copepods were numerically predominant (N%), with 73.02% for *M. punctatum* and 82.85% for *N. kroyeri*, followed by hyperiids with values of 22.13% and 13.20%, respectively. The frequencies of occurrence (F%) of copepods in both fish species were the same with 100.0%, respectively. Values of F (%) for hyperiids and amphipods for *M. punctatum* were 44.9% and 19.1%, with values of 85.1% for hyperiids and 17.6% for amphipods for *N. kroyeri* (Tables 1 and 2).

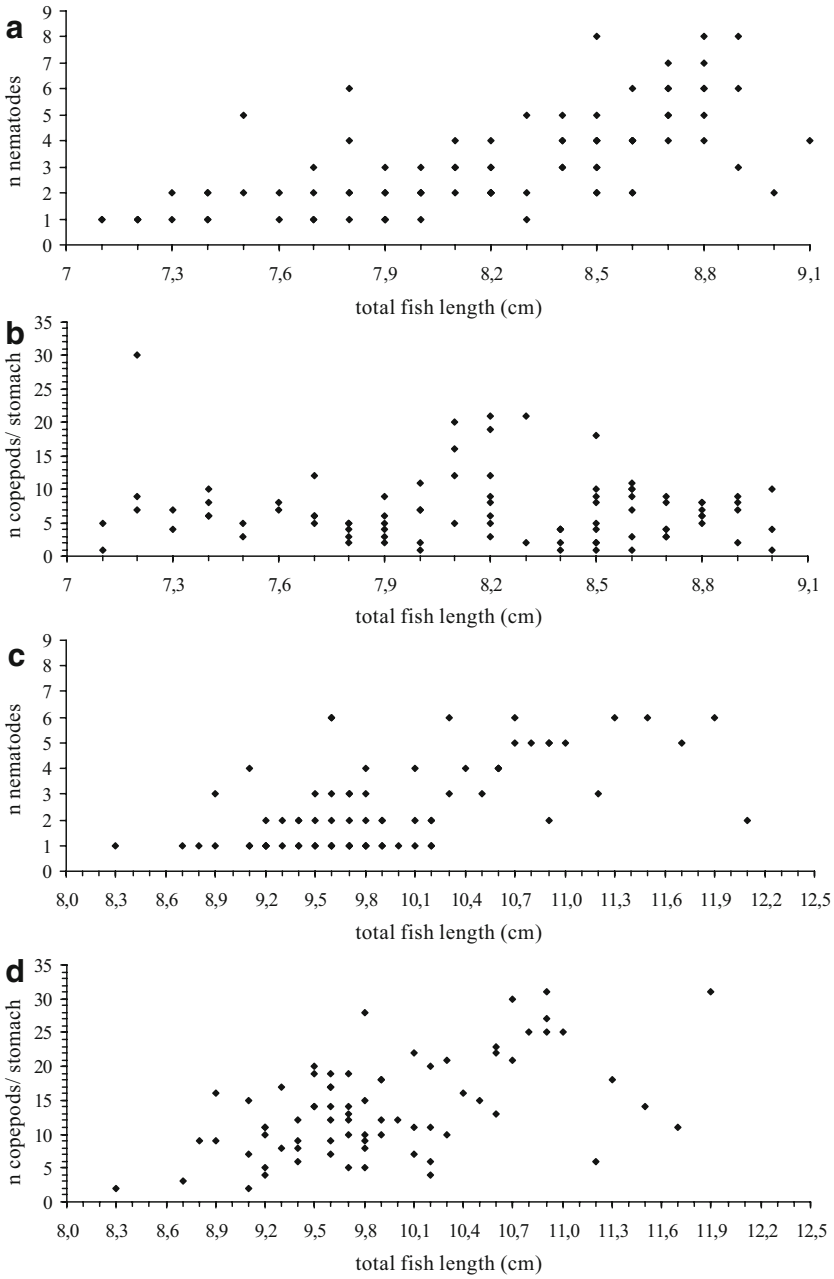

Fig. 1 **a**–**d** Random distribution of *A. simplex* (*s.s.*) in *Myctophum punctatum* (**a**) and *Notoscopelus kroyeri* (**c**) over the size range and number of copepods within the stomach contents of both fish species (**b**, **d**)

Genetic identification

The ITS-1, 5.8S, and ITS-2 sequences were determined for four anisakids from *M. punctatum* (AsMP1–AsMP4) and four anisakids from *N. kroyeri* (AsNK1–AsNK4). No size variation was detected for any of the three rDNA regions on the agarose gel. Identification via GenBank demonstrated that all six specimens belonged to *A. simplex* (*s.s.*). The G + C contents for the three regions of rDNA of all individuals ranged from 44.7% to 45.4%. The length of the ITS-1 sequence was 365 bp for all samples whereas the ITS-2 sequence was 308 bp long. The length of the 5.8S sequence of the samples was 157 bp, respectively. The alignment of the ITS-1, 5.8S, and ITS-2 consensus sequence of a single specimen of *A. simplex* (*s.s.*) from *M. punctatum* and from *N. kroyeri* is given in Fig. 2. No sequence differences between ITS-1, 5.8S, and ITS-2 (*M. punctatum* 0.0%, *N. kroyeri* 0.0%) could be detected.

Discussion

The present study provides the first information on the parasitation and feeding ecology of *M. punctatum* and *N. kroyeri* from the Central Atlantic along the MAR. In accordance with earlier investigations of myctophid fish, the detected parasites belonged to the digeneans, tetraphyllidean cestodes, and anisakid nematodes (Klimpel et al. 2001). Together with low species richness and the presence of different nematode and cestode larvae combined with adult digeneans, this seems to be the typical parasite fauna of myctophid fish from the meso- or bathypelagic zone (e.g., Mordvinova 2000; Klimpel et al. 2001, 2006a).

All isolated helminth parasites are known to utilize planktonic invertebrates as intermediate hosts within their life cycle. The predominant digenean trematode species was *Lethadena* sp. Members of the family Hemiuridae are distributed in both shallow waters and in the upper deep-sea (e.g., Bray 2004). *Lethadena profunda* has previously been

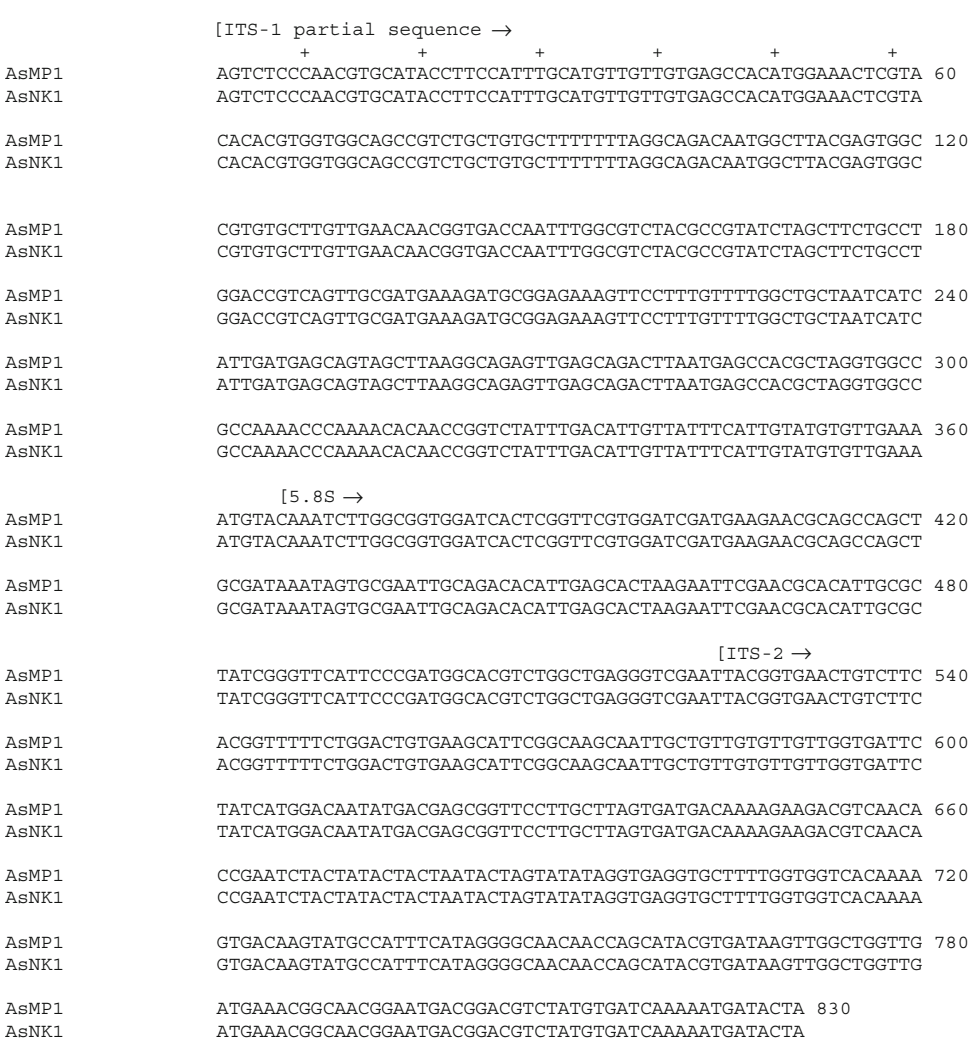

Fig. 2 Alignment of the ITS-1 (partial sequence), 5.8S, and ITS-2 (complete sequence) consensus sequence for third stage larvae of *A. simplex* (*s.s.*) from *M. punctatum* (AsMP1) and *N. kroyeri* (AsNK1) collected from the MAR. The *numbers* refer to the alignment position

recorded from myctophid fish species such as *Ceratoscopelus warmingii* and *Notoscopelus resplendens* (Klimpel et al. 2001). The life cycle of *Lethadena* species is entirely unknown. However, a heteroxenous life cycle with gastropods (molluscs) as first intermediate and especially pelagic invertebrates (e.g., copepods, euphausiids, chaetognaths) as second intermediate hosts is most common in the family Hemiuridae (e.g., Gibson et al. 2002; Klimpel et al. 2006b). With the observed minor infection rates, both myctophid species seem to have no significant importance for the life cycle of this digenean species. The other digenean, *G. phycidis*, is a widely distributed pelagic fish parasite especially in mid-oceanic ecosystems. Its depth range has been recorded from the epipelagic zone down to several hundred meters into the abyssopelagial (Gibson et al. 2002; Bray 2004). Its life cycle is unknown, however, most likely also includes pelagic invertebrates. In Faeroese waters, *G. phycidis* gradually replaces another wide ranging trematode, *Derogenes* sp., at depths from 400 to 500 m (Køie 2000).

The presence of Cestoda of the order Tetraphyllidea (*S. pleuronectis*) is also not surprising since these parasites are common in large numbers and in different fish species (Klimpel et al. 2001). These larvae are offshore and inshore distributed and represent a composite of different taxa that also might have different life cycles. Along the MAR, most probably, pelagic Crustacea (copepods) are involved as first intermediate hosts, myctophids as second/paratenic intermediate, and different elasmobranchs as final hosts. Further comments on these cosmopolitan cestodes are not possible without strobila characters, specific knowledge of the life cycle and/or genetic analyses.

Both sampled myctophid species are typical diurnal vertical migrants and distributed between depths of 225–750 m for *M. punctatum* and 350–1000 m for *N. kroyeri* during daytime and between 10–125 m at night, respectively (Froese and Pauly 2008). Migratory myctophids are known to actively feed in the epipelagic layer at night and typically exhibit obvious diurnal feeding periodicity (e.g., Moku et al. 2000). In highly productive regions such as the MAR, seamounts, and upwelling or slope regions, migratory myctophids and other fish species have been reported to feed both at night and during the day (e.g., Klimpel et al. 2006b). Our specimens were sampled during daytime at 300–800 m water depth. Because of the stomach content and the nondigested condition of several prey items, our results support active daytime feeding of *M. punctatum* and *N. kroyeri* at the MAR. It is, thus, likely that the infected *M. punctatum* and *N. kroyeri* have acquired some of their recorded parasites, such as the pelagic *G. phycidis* or anisakid nematodes (see below) during their feeding activity at daytime and within upper water layers of the mesopelagic zone.

The analyzed anisakid nematodes from both myctophid species could be identified as *Anisakis simplex* (*s.s.*). This species has been earlier recorded from MAR by Kellermanns et al. (2007) in *Macrourus berglax* (Fam. Macrouridae) and by Klimpel et al. (2007) in *Maurolicus*

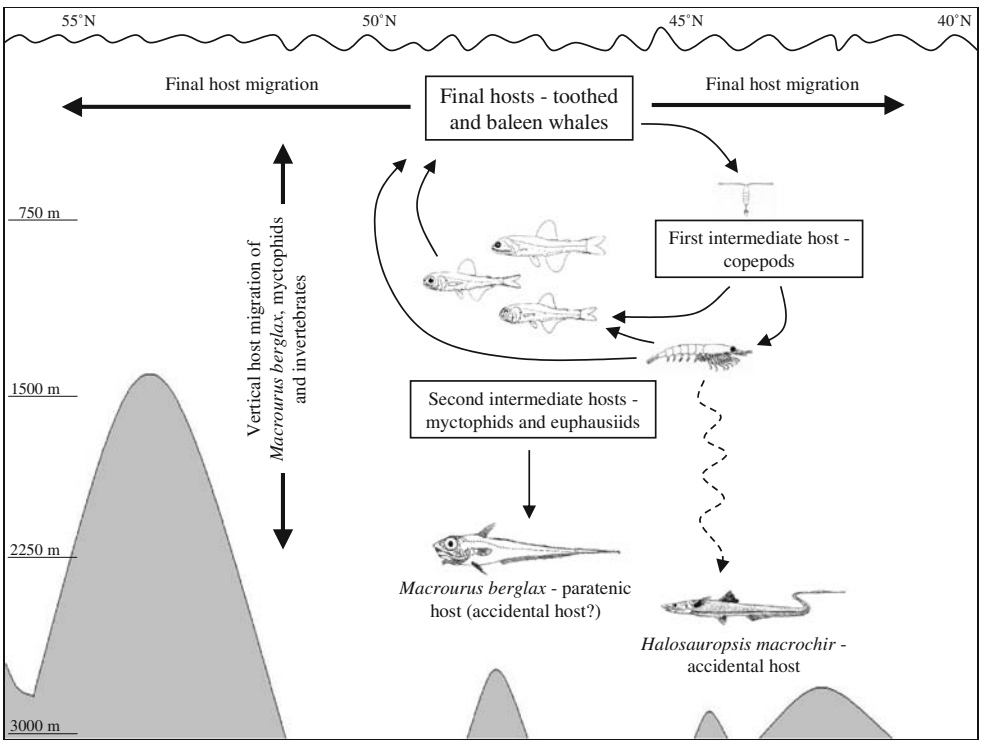

Fig. 3 Schematic illustration of the pelagic life cycle of *A. simplex* (*s.s.*) at the MAR. Migrating final hosts (also see Fig. 4) transport adult nematodes into the region. Copepods serve as first and myctophids (e.g., *M. punctatum*, *N. kroyeri*) as well as euphausiids as second intermediate hosts. Deep-sea fish (*M. berglax*, *H. macrochir*) has access to infective third stage larvae through vertical migration into upper surface layers or diurnal migrations of first and second intermediate hosts

Table 3 Host records of *Anisakis* species within their cetacean final hosts. Data are taken from the literature

Family	Whale species	parasite species							
		A. simplex (*s.s.*)	*A. pegreffii*	*A. simplex* C	*A. typica*	*A. ziphidarum*	*A. brevispiculata*	*A. paggiae*	*A. physeteris*
Delphinidae	*Delphinus delphis*	*	*		*				
	Feresa attenuata				*				
	Globicephala macrorhynchus				*				
	Globicephala melas	*		*	*				
	Lagenorhynchus albirostris	*							
	Lagenorhynchus obscurus				*				
	Lissodelphis borealis			*					
	Orcinus orca	*							
	Peponocephala electra				*				
	Pseudorca crassidens	*		*					
	Stenella attenuata				*				
	Stenella coeruleoalba	*			*				
	Stenella frontalis				*				
	Stenella longirostris				*				
	Sotalia fluviatilis				*				
	Steno bredanensis				*				
	Tursiops truncatus		*		*				
Pontoporiidae	*Pontoporia blainvillei*				*				
Monodontidae	*Delphinapterus leucas*	*							
Phocoenidae	*Phocoena phocoena*	*							
Ziphiidae	*Mesoplodon densirostris*					*			
	Mesoplodon europaeus					*			
	Mesoplodon layardii			*		*			
	Ziphius cavirostris		*			*			
Physeteridae	*Physeter macrocephalus*		*						*
Kogiidae	*Kogia breviceps*						*	*	
	Kogia sima							*	
Neobalaenidae	*Caperea marginata*		*						
Balaenopteridae	*Balaenoptera acutorostrata*	*							

Major references: D'Amelio et al. 2000; Mattiucci and Nascetti 2007; Mattiucci et al. 1997, 2002, 2005, 2007; Nadler et al. 2005; Paggi et al. 1998; Valentini et al. 2006

muelleri (Fam. Sternoptychidae), with *Anisakis* sp. occurring in *Halosauropsis macrochir* (Fam. Halosauridae) (Klimpel et al. 2008). Specimens of *Anisakis* spp. are considered to follow a pelagic life cycle, involving pelagic invertebrate and vertebrate intermediate hosts. It is, thus, likely that the mesopelagic myctophids are commonly infected with these nematodes, supporting the parasite's life cycle in oceanic environments. The random distribution of the number of *A. simplex* (*s.s.*) larvae within the studied myctophids demonstrates that these fish can be considered as true second intermediate hosts, in the sense of Palm (1999; Fig. 3). Recent data demonstrate that the genus *Anisakis* includes eight distinct species, *A. simplex* (*s.s.*), *A. pegreffii*, *A. simplex* C (representing the *A. simplex*-complex), *A. paggiae*, *A. brevispiculata*, *A. physeteris* (representing the *A. physeteris*-complex), *A. typica*, and *A. ziphidarum*. However, only a single sibling (*A. simplex* (*s.s.*)) could be recorded within the present and earlier investigations (e.g., Kellermanns et al. 2007; Klimpel et al. 2007). According to Mattiucci et al. (2007) and Mattiucci and Nascetti (2007), *A. simplex* (*s.s.*) and *A. pegreffii* have been found within the Atlantic Ocean, also suggesting a potential occurrence of the latter sibling around the MAR region. It has been suggested that the different *Anisakis* siblings ecologically separated by invading different final and teleost intermediate hosts (e.g., Kellermanns et al. 2007). This seems to also be the case at the studied locality, where the teleost intermediate hosts for the other *Anisakis* sibling species are yet unknown.

Molecular techniques for delimiting and identifying nematodes of the genus *Anisakis* have markedly influenced our understanding of their systematics, zoogeography, and biodiversity (e.g., Nadler et al. 2005). In a detailed morphologically based revision of *Anisakis* spp. conducted by Davey (1971), the author recognized three valid species (*A. simplex*, *A. typica*, and *A. physeteris*) and retained four others as *species inquirendae* (*A. dussumierii*, *A. insignis*, *A. schupakovi*, and *A. alexandri*). *A. dussumierii* was described from the final hosts *Physeter macrocephalus* (Physeteridae) and *Delphinus delphis* (Delphinidae; e.g., Nadler et al. 2005), while *A. insignis* were isolated only from the Amazon dolphin (*Inia geoffrensis*, Iniidae) and was redescribed by Petter (1972). Sprent (1982) erraneously transferred *A. insignis* into *Peritrachelius* Diesing, 1851, giving account to the lip and spicule structure. Different authors previously reported *A. alexandri* from *Stenella frontalis* (Delphinidae), but this species was originally described from *Sousa chinensis* (Delphinidae; Mignucci-Giannoni et al. 1998; Perrin 2002). Previous studies confirm the validity of *A. schupakovi*, a parasite of the pinniped *Phoca caspica* (Phocidae) limited to the Caspian Sea (e.g., D'Amelio et al. 2000). The morphological approach, however, still needs verification using molecular data.

According to the available literature and the present results, most *Anisakis* siblings have been identified from toothed whales, especially from the Delphinidae and Ziphiidae (Table 3, Fig. 4). The host distribution of *A.*

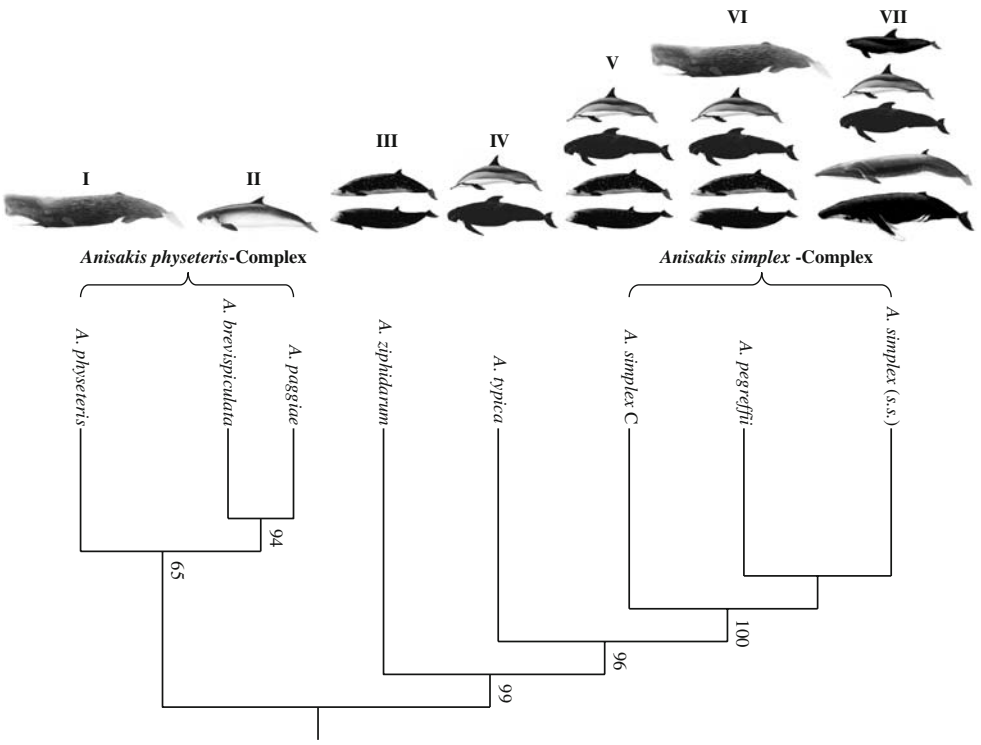

Fig. 4 Host distribution of *Anisakis* species demonstrating low host specificity within the *A. simplex* species complex. Genetic relationships of *Anisakis* spp. (adapted from Mattiucci and Nascetti 2006) mapped with the cetacean (families) final hosts (*I* Physeteridae; *II* Kogiidae; *III* Ziphiidae; *IV* Delphinidae, Pontoporiidae; *V* Delphinidae, Ziphiidae; *VI* Delphinidae, Ziphiidae, Physeteridae, Neobalaenidae; *VII* Delphinidae, Monodontidae, Phocoenidae, Balaenopteridae). For further information see text and host records see Table 3

typica is restricted to dolphins (Delphinidae) from subtropical and tropical waters and to a single species of the toothed whale family Pontoporidae (Mattiucci et al. 2002, 2005; Palm et al. 2008). *A. ziphidarum* has been reported only from the Ziphiidae on the southern hemisphere, while *A. brevispiculata* and *A. paggiae* are specific for the Kogiidae, mainly in the Mid- and Southern Atlantic Ocean (Mattiucci and Nascetti 2006; Valentini et al. 2006). The latter two siblings together with *A. physeteris*, being specific for the Physeteridae (cosmopolitan), form the *A. physeteris* sibling species complex. These *Anisakis* siblings have been solely reported from toothed whales. This contrasts the *Anisakis simplex* sibling species complex, where typically toothed but also baleen whales appear to belong to the final host spectrum. *A. simplex* (*s.s.*) parasitizes oceanic cetaceans of the families Delphinidae, Monodontidae, Phocoenidae, and Balaenopteridae and has been genetically identified mainly in the North Atlantic and Pacific Oceans. *A. pegreffii* also utilizes the family Delphinidae as final hosts, however, also infecting the Ziphiidae, Physeteridae, and Neobalaenidae (Mattiucci et al. 1997) mainly in the entire Atlantic and Mediterranean but also from Australia. *A. simplex C* uses toothed whales of the families Delphinidae and Ziphiidae on the southern hemisphere and also from the North Pacific to complete its life cycle (Table 3, Fig. 4). The host range and zoogeographical distribution pattern of the known *Anisakis* siblings demonstrate that far few records exist to conclude about host specificity and distribution patterns. However, toothed whales are the common hosts for the known *Anisakis* spp., and besides the low latitude restricted *A. typica*, all other *Anisakis* species have the potential to occur from (sub) tropical into boreal and also high latitude regions. This, most likely, is connected to the extensive final host migration. Final host specificity for the *A. simplex* complex seems to be low while that for the *A. physeteris*, complex appears to be high. Specificity in the teleost intermediate hosts for both complexes seems to be low (see e.g., Palm et al. 2008), and sympatric occurrence of different siblings within the same intermediate hosts seems to be likely. Further genetic identification is needed for a better understanding of the host–parasite associations and coevolution within this interesting group of marine parasitic nematodes.

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References

Amundsen PA, Gabler HM, Staldvik FJ (1996) A new approach to graphical analysis of feeding strategy from stomach contents data—modification of the Costello (1990) method. J Fish Biol 48:607–614

Bray RA (2004) The bathymetric distribution of the digenean parasites of deep-sea fishes. Folia Parasitol 51:268–274

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

D'Amelio S, Mathiopoulos KD, Santos CP, Pugachev ON, Webb SC, Picanço M, Paggi L (2000) Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidae) defined by polymerase chain reaction-based restriction fragment length polymorphism. Int J Parasitol 30:223–226

Davey JT (1971) A revision of the genus *Anisakis* Dujardin, 1875 (Nematoda: Ascaridata). J Helminthol 45:51–72

Foxton P (1970) The vertical distribution of pelagic decapods (Crustacea: Natantia) collected on the SOND cruise 1965. J Mar Biol Assoc UK 50:961–1000

Froese R, Pauly D (2008) FishBase. World Wide Web electronic publication. http://www.fishbase.org, version (04/2008)

Gartner JV, Zwerner DE (1989) The parasite faunas of meso- and bathypelagic fishes of Norfolk Submarine Canyon, western North Atlantic. J Fish Biol 34:79–95

Gibson DI, Jones A, Bray RA (2002) Keys to the trematodes, vol. 1. CAB International, Wallingford

Gjøsaeter J, Kawaguchi K (1980) A review of the world resources of mesopelagic fish. FAO Fish Tech Pap 193:1–151

Hays R, Measures LN, Huot J (1998a) Euphausiids as intermediate hosts of *Anisakis simplex* in the St. Lawrence estuary. Can J Zool 76:1226–1235

Hays R, Measures LN, Huot J (1998b) Capelin (*Mallotus villosus*) herring (*Clupea harengus*) as paratenic hosts of *Anisakis simplex*, a parasite of beluga (*Delphinapterus leucas*) in the St. Lawrence estuary. Can J Zool 76:1411–1417

Hyslop EJ (1980) Stomach content analysis - a review of methods and their application. J Fish Biol 17:411–429

Kellermanns E, Klimpel S, Palm HW (2007) Molecular identification of ascaridoid nematodes from the deep-sea onion-eye grenadier (*Macrourus berglax*) from the East Greenland Sea. Deep-Sea Res Pt I 54:2194–2202

Klimpel S, Seehagen A, Palm HW, Rosenthal H (2001) Deep-water metazoan fish parasites of the world. Logos, Berlin

Klimpel S, Palm HW, Rückert S, Piatkowski U (2004) The life cycle of *Anisakis simplex* in the Norwegian Deep (northern North Sea). Parasitol Res 94:1–9

Klimpel S, Palm HW, Busch MW, Kellermanns E, Rückert S (2006a) Fish parasites in the Arctic deep-sea: Poor diversity in pelagic fish species vs. heavy parasite load in a demersal fish. Deep-Sea Res Pt I 53:1167–1181

Klimpel S, Rückert S, Piatkowski U, Palm HW, Hanel R (2006b) Diet and metazoan parasites of silver scabbard fish *Lepidopus caudatus* from the Great Meteor Seamount (North Atlantic). Mar Ecol Prog Ser 315:249–257

Klimpel S, Kellermanns E, Palm HW, Moravec F (2007) Zoogeography of fish parasites of the pearlside (*Maurolicus muelleri*), with genetic evidence of *Anisakis simplex* (*s.s.*) form the Mid-Atlantic Ridge. Mar Biol 152:725–732

Klimpel S, Palm HW, Busch MW, Kellermanns E (2008) Fish parasites in the bathyal zone: The halosaur *Halosauropsis macrochir* (Günther, 1878) from the Mid-Atlantic Ridge. Deep-Sea Res Pt II 55:229–235

Køie M (2000) Metazoan parasites of teleost fishes from Atlantic waters off the Faroe Islands. Ophelia 52:25–44

Køie M (2001) Experimental infections of copepods and sticklebacks *Gasterosteus aculeatus* with small ensheated and large third-stage larvae of *Anisakis simplex* (Nematoda, Ascaridoidea, Anisakidae). Parasitol Res 87:32–36

Køie M, Berland B, Burt MDB (1995) Development to third-stage larvae occurs in the eggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). Can J Fish Aquat Sci 52:134–139

Mattiucci S, Nascetti G, Cianchi R, Paggi L, Arduino P, Margolis L, Brattey J, Webb S, D'Amelio S, Orecchia P, Bullini L (1997) Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). J Parasitol 83:401–416

Mattiucci S, Paggi L, Nascetti G, Portes Santos C, Costa G, Di Beneditto AP, Ramos R, Argyrou M, Cianchi R, Bullini L (2002) Genetic markers in the study of *Anisakis typica* (Diesing, 1860): larval identification and genetic relationships with other species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae). Syst Parasitol 51:159–170

Mattiucci S, Nascetti G, Dailey M, Webb SC, Barros NB, Cianchi R, Bullini L (2005) Evidence for a new species of *Anisakis* Dujardin, 1845: morphological description and genetic relationships between congeners (Nemtoda: Anisakidae). Syst Parasitol 61:151–171

Mattiucci S, Nascetti G (2006) Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: an update. Parasite 13:99–113

Mattiucci S, Nascetti G (2007) Genetic diversity and infection levels of anisakid nematodes parasitic in fish and marine mammals from Boreal and Austral hemispheres. Vet Parasitol 148:43–57

Mattiucci S, Paoletti M, Damiano S, Nascetti G (2007) Molecular detection of sibling species in anisakid nematodes. Parassitologia 49:147–153

Mauchline J, Gordon JDM (1984) Incidence of parasitic worms in stomachs of pelagic and demersal fish of the Rockall Trough, northeastern Atlantic Ocean. J Fish Biol 24:281–285

Mignucci-Giannoni AA, Hoberg EP, Siegel-Causey D, Williams EH (1998) Metazoan parasites and other symbionts of cetaceans in the Caribbean. J Parasitol 84:939–946

Moku M, Kawaguchi K, Watanabe H, Ohno A (2000) Feeding habits of three dominant myctophid fishes, *Diaphus theta*, *Stenobrachius leucopsarus* and *S. nannochir*, in the subarctic and transitional waters of the western North Pacific. Mar Ecol Prog Ser 207:129–140

Mordvinova TN (2000) Helminthofauna of myctophid fishes from the world ocean. Ecol Morya 53:59–63 (In Russian)

Nadler SA, D'Amelio S, Dailey MD, Paggi L, Siu S, Sakanari JA (2005) Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from northern Pacific marine mammals. J Parasitol 91:1413–1429

Paggi L, Nascetti G, Webb SC, Mattiucci S, Cianchi R, Bullini L (1998) A new species of *Anisakis* Dujardin, 1845 (Nematoda, Anisakidae) from beaked whales (Ziphiidae): allozyme and morphological evidence. Syst Parasitol 40:161–174

Palm HW (1999) Ecology of *Pseudoterranova decipiens* Krabbe, 1878 (Nematoda: Anisakidae) from Antarctic waters. Parasitol Res 85:638–656

Palm HW, Damriyasa IM, Linda Oka IBM (2008) Molecular genotyping of *Anisakis* Dujardin, 1845 (Nematoda: Ascaridoidea: Anisakidae) larvae from marine fish of Balinese and Javanese waters, Indonesia. Helminthologia 45:3–12

Perrin WF (2002) *Stenella frontalis*. Mamm Spec 702:1–6

Petter AG (1972) Redescription of *Anisakis insignis* (Diesing) (Ascaridoidea), parasite of the Amazone dolphin *Inia geoffrensis*. Invest Cetacea 4:93–99

Pusch C, Beckmann A, Porteiro FM, Westernhagen von H (2004) The influence of seamounts on mesopelagic fish communities. Arch Fish Mar Res 51:165–186

Sprent JF (1982) Ascaridoid nematodes of South American mammals, with a definition of a new genus. J Helminthol 56:275–295

Sutton TT, Hopkins TL (1996) Trophic ecology of the stomiid (Pisces: Stomiidae) fish assemblage of the eastern Gulf of Mexico: strategies, selectivity and impact of a top mesopelagic predator group. Mar Biol 127:179–192

Sutton TT, Porteiro FM, Heino M, Byrkjedal I, Langhelle G, Anderson CIH, Horne J, Søiland H, Falkenhaug T, Godø OR, Bergstad OA (2008) Vertical structure, biomass and topographic association of deep-pelagic fishes in relation to a mid-ocean ridge system. Deep-Sea Res Pt II 55:161–184

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Valentini A, Mattiucci S, Bondanelli P, Webb SC, Mignucci-Giannone AA, Colom-Llavina MM, Nascetti G (2006) Genetic relationship among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *cox2* sequences, and comparison with allozym data. J Parasitol 92:156–166

Yamaguchi M, Miya M, Okiyama M, Nishida M (2000) Molecular phylogeny and larval morphological diversity of the lanternfish genus *Hygophum* (Teleostei: Myctophidae). Mol Phyl Evol 15:103–114

Zhu X, Gasser RB, Jacobs DE, Hung GC, Chilton NB (2000) Relationships among some ascaridoid nematodes based on ribosomal DNA sequence data. Parasitol Res 86:738–744