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RESEARCH ARTICLE

Zoogeography of fish parasites of the pearlside (*Maurolicus muelleri*), with genetic evidence of *Anisakis simplex* (*s.s.*) from the Mid-Atlantic Ridge

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Abstract A total of 200 *Maurolicus muelleri* from the Mid-Atlantic Ridge (MAR) and the Norwegian Deep (ND) were studied for parasitic helminths. Two different metazoans were isolated from the MAR and five species from the ND. The predominant parasite species in both areas were tetraphyllidean cestode larvae (*Scolex pleuronectis*) and the anisakid nematode *Anisakis simplex sensu stricto* (*s.s.*), the latter being identified using genetic analysis of the internal transcribed spacer (ITS-1, ITS-2) and 5.8S regions of the rDNA. The parasite fauna of *M. muelleri* from the MAR was less species rich in comparison to ND, due to the deep-sea and oceanic environment. The digeneans *Brachyphallus crenatus* and *Lecithaster confusus* as well as the raphidascarid fish nematode *Hysterothylacium aduncum* were only collected from the ND. This can be explained either by the deep origin of the sampled fish specimens or the lack of suitable intermediate or final hosts in the region. Based on the frequent occurrence of *A. simplex* (*s.s.*) around the MAR and the ND, a pelagic life cycle is suggested at both localities, involving baleen and toothed whales as final and pelagic and mesopelagic fish and invertebrates as intermediate or paratenic hosts.

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Introduction

Fish parasitological research in recent years mainly focused on the analysis and description of species that occur on or in the vicinity of the continental shelf regions. The deep-sea, the outer continental shelf regions or the Central Oceans have been less studied due to difficulties to obtain sufficient material (Klimpel et al. 2001). The Mid-Atlantic Ridge (MAR) system in the Atlantic Ocean is widely unknown in terms of parasites that inhabit oceanic or deep-sea fish in that region. A first fish parasitological examination from the MAR was carried out by Justine et al. (2002), who studied the zoarcid deep-sea hydrothermal vent fish *Pachycara thermophilum* from the Central Atlantic Ocean at 3,008–3,510 m water depth. The authors described a new nematode species, adding that only a single acanthocephalan has been described from another hydrothermal vent system, however, from the Central Pacific. No further records of the helminth fish parasite fauna from the MAR exist.

Helminth fish parasites can be widely distributed with an oceanic or even with a cosmopolitan distribution pattern. For example, the anisakid nematodes of the *Anisakis simplex* complex have been recorded from the North Atlantic and Pacific Ocean (Mattiucci et al. 1997). Genetic markers from multilocus allozyme electrophoresis have demonstrated that the morphospecies *A. simplex* is a complex of sibling species: (1) *A. simplex* (*s.s.*) from the North Atlantic and Pacific Ocean, (2) *A. pegreffii* from the Mediterranean Sea, North-East Atlantic and the Southern hemisphere and (3) *A. simplex* C from the Pacific coast of Canada and the Southern hemisphere (e.g. Mattiucci et al. 1997). These sibling species are morphologically very similar but genetically different and have distinct host preferences and geographical distributions (Marques et al. 2006). Mattiucci et al. (1997) suggested a mainly benthic or demersal life-cycle

for *A. simplex* (*s.s.*) and a mainly pelagic life-cycle for *A. pegreffii*. In contrast, Klimpel et al. (2004) proposed a pelagic life-cycle for *A. simplex* in the Norwegian Deep (ND), however, did not further identify the sibling species. From the Central Atlantic, e.g. around the MAR, no data on the occurrence of the *A. simplex* complex exist.

Mesopelagic fish species represent an important component of the oceanic community. The majority of these fishes exhibit extensive diurnal vertical migrations, from daytime water depths below 500 m into the productive surface layers at night (Pusch et al. 2004). They represent an important link between predators of higher trophic levels (e.g. whales, seabirds, piscivorous fishes) and zooplankton (Pusch et al. 2004), thus being important for the transmission of fish helminths into the final host. One of the most abundant fish species around the MAR is the mesopelagic pearlside *M. muelleri* (Sternoptychidae) (e.g. Badcock 1984; Bergstad 1990), feeding mostly on copepods and euphausiids (Rasmussen and Giske 1994; Klimpel et al. 2003). Previous parasitological investigations show that *M. muelleri* is heavily infested with different parasite species in the Herdlefjorden (western Norway) and the ND (Hamre and Karslbakk 2002; Klimpel et al. 2004).

The purpose of the present study was: (1) to identify the metazoan parasite fauna of *M. muelleri* from the MAR; (2) a genetic analysis of *A. simplex* (*s.s.*) from the MAR and from a new sample from the ND; (3) an analysis of the stomach content of both samples from the MAR and ND in order to (4) identify the transfer mechanism of the parasitic helminths at both localities. Finally, the parasite fauna from the MAR and the ND were compared in order to better understand the life-cycles and zoogeographical distribution of the collected parasites.

Materials and methods

Collection of samples

A total of 100 *M. muelleri* was sampled in June 2004 on board of the Norwegian research vessel G.O. Sars during the field phase of the international project MAR-ECO along the Mid-Atlantic Ridge (MAR). In May 2001, 100 *M. muelleri* were collected on board of the German research vessel RV Heinke during an annual cruise in the Norwegian Deep (ND) (see Klimpel et al. 2004). The Station of the MAR was located at 52°58N and 34°52W whereas the station in the ND was located south-east of Norway at 57°42N and 06°53E (Fig. 1). *M. muelleri* was collected at a water depth between 1,630 and1,650 m at the MAR and between 175 and 215 m at the ND, respectively. In both areas the fish was caught by using pelagic nets (Åkra trawl at MAR; Kombitrawl 10 at ND) and at a trawling time of approx. 60 min in MAR and approx. 30 min in ND. All fishes were deep frozen at −20°C immediately after the catch for subsequent examination in the laboratory.

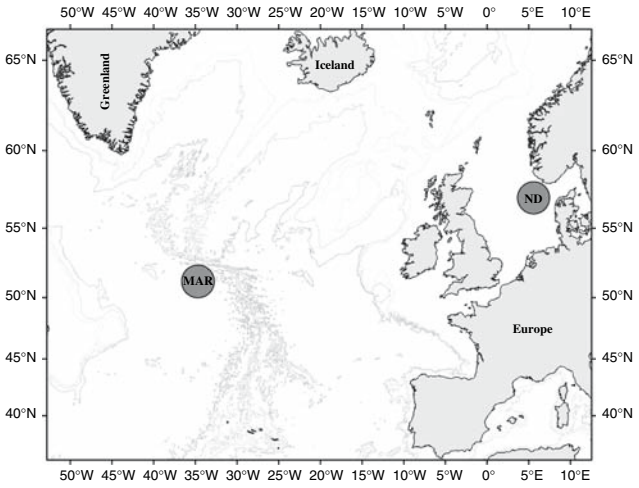

Fig. 1 Area of investigation with both sampling stations included the Mid-Atlantic Ridge (*MAR*) and the Norwegian Deep (*ND*)

Parasitological examination

Prior to fish examination the morphometrical fish data, total length (TL) and total weight (TW), were recorded to the nearest 0.1 cm and 0.001 g, respectively. The eyes, skin, fins, gills, nostrils and mouth cavity of each fish specimen were studied for ectoparasites. The body cavity was opened to examine microscopically the liver, stomach, pyloric caeca, intestine and gonads for endoparasites. The stomach content was removed and all food items were sorted and identified to the lowest possible taxonomic level, and grouped into taxonomic categories.

The isolated parasites were fixed in 4% borax-buffered formalin and preserved in 70% ethanol/5% glycerine or in the case of anisakid nematodes some specimens were stored in 96% ethanol. For identification purposes, some nematodes were dehydrated in a graded ethanol series and transferred to 100% glycerine (Riemann 1988). Digenea and Cestoda were stained with Acetic carmine, dehydrated, cleared with Eugenol or Creosote and mounted on Canada balsam. Parasite identification literature included Gibson et al. (2002) and Køie (1992) for Digenea, Khalil et al. (1994) for Cestoda and Køie (1993), Anderson (2000), Klimpel et al. (2004) and Abe et al. (2005) for Nematoda. The parasitological terminology used follows Bush et al. (1997).

PCR amplification and sequencing of ITS-1, 5.8S and ITS-2

Nematodes isolated from the examined *M. muelleri* were identified morphologically by existing keys and descriptions.

After isolation and identification the anisakid nematodes were fixed and stored in 96% ethanol. A total of 30 specimens of the nematode *A. simplex* (*s.s.*), two from each of the investigated areas, the Mid-Atlantic Ridge and the Norwegian Deep (AsMAR1-AsMAR15, AsND1-AsND15), were used for molecular identification. Genomic DNA was isolated and purified from individual larvae by 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, 5.8 S, ITS-2 and flanking sequences (=ITS+) was amplified by 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). PCR-reactions (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 was performed in a thermocycler (Biometra, Germany) under the following conditions: after initial denaturation at 95°C for 15 min, 30 cycles at 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 checked on 1% agarose gels. A 100-bp ladder marker (Peqlab Biotechnology GmbH, 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 5.8 S gene were sequenced in both directions from each PCR product, 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 the *Anisakis simplex* complex, using CLUSTAL W (1.83) Multiple Sequence Alignments (Thompson et al. 1994).

Analysis of the stomach contents

Subsamples of 40 fish specimens from the MAR and 50 specimens from the ND were analyzed for stomach contents. In order to determine the relative importance of food items, the frequency of occurrence of each prey item *i* ($\%F_i$) and its percentage by number ($\%N$) was calculated (Hyslop 1980). $\%F_i$ was calculated as the number of stomachs with prey item *i* compared to all non-empty stomachs; $\%N$ was calculated as the number of prey item *i* compared to the total number of all prey items (Hyslop 1980).

Results

Parasite fauna of *M. muelleri*

Mid-Atlantic Ridge (MAR)

The parasite fauna of *M. muelleri* with total lengths between 4.1 and 6.3 cm (mean 5.6 cm) and total weights ranging from 0.494 to 2.630 g (mean 1.337 g) consisted of two different parasite species (Table 1). The third-stage larvae (L3) of the anisakid nematode *Anisakis simplex* (*s.s.*) (molecular analysis see below) were found in or on the organs of the body cavity. Larval tetraphyllidean cestodes (*Scolex pleuronectis*) were found in the lumen of the intestine. These tetraphyllideans are distributed circumpolar and have been found in various fish species (e.g. Klimpel et al. 2001). However, further identification is not possible without strobila characters or knowledge of the life-cycle.

Table 1 Prevalence (*P*), intensity (*I*), and mean intensity (mI ± SD) of infestation of *M. muelleri* from the Mid-Atlantic Ridge (MAR) and the Norwegian Deep (ND)

Geographical region parasite species	Adult/larva	Mid-Atlantic Ridge (MAR)			Norwegian Deep (ND)		
		P (%)	*I*	mI (±SD)	*P* (%)	*I*	mI (±SD)
Digenea							
Brachyphallus crenatus	a				4.0	1	1.0(±0.20)
Lecithaster confuses	a				12.0	1–2	1.1(±0.36)
Cestoda							
Tetraphyllidea indet. (*Scolex pleuronectis*)	l	24.0	1–4	1.7(±0.69)	20.0	1–4	1.4(±0.67)
Nematoda							
Anisakis simplex (*s.s.*)	l	19.0	1	1.0(±0.42)	24.0	1–3	1.3(±0.61)
Hysterothylacium aduncum	l				100.0	1–7	5.2(±1.63)

a adult, *l* larva

Table 2 Average length (in bp) and G + C contents (in %) of the ITS-1 (partial sequence), 5.8S and ITS-2 (complete sequence) rDNA sequences of the third stage larvae of *Anisakis simplex* (*s.s.*) isolated from *M. muelleri* from the Mid-Atlantic Ridge (MAR) and the Norwegian Deep (ND)

		ITS-1		5.8S		ITS-2	
Parasite species	Geographical origin	Length	G + C	Length	G + C	Length	G + C
Anisakis simplex (*s.s.*)	MAR	362	47.1	157	50.9–51.6	326	36.3–41.4
Anisakis simplex (*s.s.*)	ND	362	46.8–47.1	157	51.6	331	42.1–42.7

Norwegian Deep (ND)

Five different parasite species were found in *M. muelleri* with total lengths between 5.0 and 6.4 cm (mean 5.9 cm) and total weights between 0.989 and 2.693 g (mean 1.803 g) (Table 1). Both Digenea, *Brachyphallus crenatus* and *Lecithaster confuses*, were adult and were located either in the stomach or in the intestine, respectively. The intestinal lumen of *M. muelleri* was infested with unidentified tetraphyllidean larvae (*S. pleuronectis*), that were the predominant parasites together with the nematodes. Nematoda consisted of the anisakid species *A. simplex* (*s.s.*) (molecular analysis see below) and the raphidascarid species *H. aduncum*. The third-stage larvae (L3) of *A. simplex* (*s.s.*) were found in or on the organs of the body cavity. Third-/fourth-stage larvae (L3/L4) of *H. aduncum* specimens were isolated exclusively from the organs of the body cavity.

Molecular analysis of the *Anisakis simplex* complex

The ITS-1, 5.8 S and ITS-2 rDNA sequences were determined for 15 anisakid nematodes from MAR (AsMAR1-AsMAR15) and 15 from the ND (AsND1-AsND15). For the three rDNA regions no size variation was detected on the agarose gels among any of the 30 samples. The identification via GenBank showed that all 30 samples belong to *A. simplex* (*s.s.*). Pairwaise comparison with other species of the *A. simplex* complex showed more differences than with *A. simplex* (*s.s.*). Table 2 shows the characteristics for each individual. The G + C contents for the three regions of rDNA of all individuals ranged from 36.3 to 51.6%. The length of the ITS-1 sequence was 362 bp for both sampling areas whereas the ITS-2 sequences ranged from 254 to 331 bp, depending on the sampling area. The length of the 5.8 S sequence of the samples was 157 bp, respectively. Sequence differences between the ITS-2 (MAR 0.6%, ND 0.3%) were greater than for the ITS-1 (0.0%) and 5.8 S gene (0.0%). The polymorphism in the ITS-2 is negligibly low and the alignment of the ITS-2 consensus sequence of one example of *A. simplex* (*s.s.*) from the MAR and from the ND are given in Fig. 2. *A. simplex* (*s.s.*) was found with similar infestation rates in *M. muelleri* from both areas (see Table 1).

Stomach contents of *M. muelleri*

A total of 25 *M. muelleri* specimens from the MAR had empty stomachs, while 15 had food items in the stomach.

Fig. 2 Alignment of the ITS-2 (complete sequence) consensus sequence for third stage larva of *Anisakis simplex* (*s.s.*) (As) from the Mid-Atlantic Ridge (MAR), Norwegian Deep (ND) and reference (Ref) of Abe et al. (2005). The *numbers* refer to the alignment position and the *asterisk* indicates differences among the two localities

The diet consisted exclusively of crustaceans, belonging to the Copepoda and Crustacea indet (Table 3). The frequency of occurrence (F_i%) and numerically (N%) was highest for the Crustacea indet., followed by the Copepoda.

A total of 16 *M. muelleri* specimens from the ND had empty stomachs, while 34 had one or more food items in their stomachs. All prey items belonged to the Crustacea, mainly Copepoda and Euphausiacea (Table 3). Numerically (N%), the Copepoda *Calanus finmarchicus* was dominant, followed by the copepod *Paraeuchaeta (Euchaeta) norvegica* and the euphausiid *Meganyctiphanes norvegica*. The frequency of occurrence (F_i%) was highest for the Euphausiacea, followed by Copepoda, and Crustacea indet.

Discussion

Maurolicus muelleri from the Mid-Atlantic Ridge (MAR) as well as from the Norwegian Deep (ND) were both infected with the anisakid nematode *Anisakis simplex* based on morphological characters. The identification and differentiation of the larval stages within the genus *Anisakis* on morphology alone, however, is neither easy nor always possible. Molecular techniques have provided alternative methods for easier parasite identification (e.g. McManus and Bowles 1996; Abollo et al. 2003; Marques et al. 2006). Genetic analyses of the *A. simplex* complex classify at least three sibling species in the Atlantic Ocean: *A. simplex* (*s.s.*) (formerly *A. simplex* B), *A. pegreffii* (formerly *A. simplex* A) and *A. simplex* C (Nascetti et al. 1986). Within the present study we amplified the ITS-1, 5.8 S, and ITS-2 rDNA regions with earlier described primers (Zhu et al. 1998, 2000). The length of the PCR products of *A. simplex* (*s.s.*) from MAR and ND including the ITS-1, 5.8 S and ITS-2 were 899–918 bp long, respectively, thus corresponding to the results by Zhu et al. (1998). Sequence differences between the ITS-2 (MAR 0.6%, ND 0.3%) regions were greater than for the ITS-1 (0.0%) and 5.8 S gene (0.0%), similarly than recorded from other specimens from *A. simplex* (*s.s.*). These sequence differences in the ITS-2 between the *A. simplex* (*s.s.*) of both studied area are within the range of that established among members of anisakid nematodes (Zhu et al. 2002). Polymorphism has been shown to exist in the ITS of other parasitic nematodes and appears to be a consequence of DNA turnover mechanisms e.g. gene conversion and transposition (Zhu et al. 1998).

Solely two positions in the ITS-2 sequence of AsMAR2 (pos. 267 and 300) differ from the reference (Ref) sequence (Abe et al. 2005). Position 267 differed from any of the currently described sibling species within the *A. simplex* complex. The only difference in the sequence of AsND2 from Ref-sequence was on pos. 300 and is the same with pos. 267 of Ref-sequence (Fig. 2). The sequences of *A. simplex* from both areas corresponded (MAR 98.0% identities; ND 99.0% identities) to previously described sequences of *A. simplex* (*s.s.*) (Abe et al. 2005).

In contrast to Marques et al. (2006) and Abe et al. (2005), no hybrids or other sibling species, such as *A. pegreffii*, could be detected in the 30 samples from *M. muelleri* from both localities. The former authors could demonstrate that hybrids of the *A. simplex* complex only occur if *A. simplex* (*s.s.*) and sibling species occur in the same fish species and the same area. The absence of hybrids associated with the absence of sibling species within the present study might be explained by the presence of similar suitable final hosts for *A. simplex* (*s.s.*) at both localities. The MAR as well as the ND are typical pelagic systems with a temporal occurrence of the cetacean final hosts during their extensive long-range migrations. Main definitive hosts, such as *Phocoena phocoena* (Harbour porpoise), *Globicephala melas* (Long-finned pilot whale), *Grampus griseus* (Risso's dolphin), *Pseudorca crassidens* (False killer whale), *Orcinus orca* (Killer whale) and *Balaenoptera acutorostrata* (Minke whale) are abundant at the MAR and the ND (Reid et al. 2003), and are heavily infested with *A. simplex* (*s.s.*) (Mattiucci et al. 1997; Gibson et al. 1998).

Table 3 Frequency of occurrence (F_i) and numerical percentage of prey *i* (N) of the food items identified from the stomach contents of *M. muelleri* from the Mid-Atlantic Ridge (MAR) and the Norwegian Deep (ND)

Geographical region	Mid-Atlantic Ridge (MAR)		Norwegian Deep (ND)	
prey item	F_i (%)	N (%)	F_i (%)	N (%)
Copepoda				
Calanus finmarchicus			73.53	87.38
Paraeuchaeta (*Euchaeta*) *norvegica*			17.65	8.68
Copepoda (Calanoida)	6.67	43.32		
Euphausiacea				
Meganyctiphanes norvegica			17.65	2.96
Crustacea indet.	93.33	56.68	5.88	0.99

Mattiucci et al. (1997) suggested and Abollo et al. (2001) supported that *A. simplex* (*s.s.*) has a benthic or demersal life-cycle, while *A. pegreffii* mainly follows a pelagic life-cycle. *A. pegreffii* has been recorded from the Mediterranean Sea and was suggested to occur from the North Atlantic to the Australian Sea, thus also being able to occur at the MAR. The studied *M. muelleri* is a strictly mesopelagic fish species that migrates into the shallower parts of the mesopelagial and into the epipelagial, but does not live demersal. It feeds only on pelagic invertebrates that can serve as parasite transmitters. Thus, the fish ecology would favor infestation with *A. pegreffii* instead of *A. simplex* (*s.s.*) at the studied locality at the MAR. However, we could only isolate *A. simplex* (*s.s.*) with relatively high infestation rates. Therefore we cannot support a benthic or demersal life-cycle for *A. simplex* (*s.s.*) within the studied oceanic environments. A recent publication on the diet composition and the trophic level of marine mammals (whales and seals) demonstrated that the final hosts of *A. simplex* (*s.s.*) mainly feed on pelagic invertebrates and vertebrates such as larger zooplankton, squids and pelagic fish (Pauly et al. 1998). Klimpel et al. (2004) proposed a pelagic life-cycle for *A. simplex* in the ND, involving the pelagic copepode *Paraeuchaeta (Euchaeta) norvegica* as first intermediate and the mesopelagic *M. muelleri* as second intermediate host. These nematodes were identified as *A. simplex* (*s.s.*) within the present study and we propose a similar pelagic life-cycle for these species at the MAR.

The mesopelagic *M. muelleri* is distributed in the North and Central Atlantic Ocean and occurs down to a water depth of 1,500 m (Froese and Pauly 2007), but rather between 100 and 400 m by day with a dusk migration into the upper 100 m (Badcock 1984). The distribution in the ND is relatively shallower, with preferred water depths between 150 and 250 m during the day and between 10 and 40 m at night (e.g. Kaartvedt et al. 1998). Previous parasitological and ecological investigations show that *M. muelleri* is heavily infested with different parasite species and that this fish species feeds primarily on copepods and euphausiids (e.g. Hamre and Karslbakk 2002; Klimpel et al. 2001, 2004). Our results from the MAR show that only two parasite species occurred in the sampled fish. *A. simplex* (*s.s.*) as well as the tetraphyllidean cestode larvae (*Scolex pleuronectis*) are also common in the ND within the present and previous studies (Klimpel et al. 2004). The presence of the cestode order Tetraphyllidea in relatively high number in both areas is not surprising, since these cestodes are very common in all kinds of predatory oceanic fish and also in the deep-sea (e.g. Klimpel et al. 2001). Their life-cycle is still unresolved, marine Crustacea (Copepoda) are probably first intermediate and different fish species serve as second intermediate and elasmobranchs as final hosts.

Both fish samples from the MAR and the ND had a similar size range and also harboured similar prey items. As typical zooplankton feeders with small fish size, a parasite accumulation in *M. muelleri* as a paratenic host can be excluded to explain the higher parasite species richness in the ND compared to the MAR. Thus, any difference must be linked to the absence of important intermediate or final hosts in the deep water system around the MAR. Almost all encountered helminth parasites are known or believed to use planktonic invertebrates as intermediate hosts. These include *Brachyphallus crenatus*, *Lecithaster confusus* (both Digenea) and *A. simplex* (*s.s.*). *B. crenatus* is a typical pelagic parasite species of marine fishes with an Arctic-boreal distribution and a depth range from shallow waters down to several hundred meters into the mesopelagic zone (Køie 1992; Bray 2004). The life-cycle includes the gastropod *Retusa obtusa* (Opisthobranchia) as obligatory first intermediate host and pelagic invertebrates as second intermediate hosts (Køie 1992). The geographical distribution of *B. crenatus* in the definitive fish hosts corresponds with the distribution of the gastropod host, because *R. obtusa* occurs from Greenland to Scandinavian waters from the shallow down to 300 m water depth (Thompson 1988; Køie 1992). The occurrence of the suitable first intermediate host can explain the presence of *B. crenatus* in fish from the ND as well as the absence of this parasite in the MAR down to approx. 1,600 m. The life-cycle of *L. confusus* also includes a gastropod as first and smaller pelagic invertebrates as second intermediate hosts. Hunningen and Cable (1943) demonstrated that Copepoda (Calanoida) get infested with free-swimming cercariae and function as intermediate hosts. Because copepods as second intermediate hosts play a pivotal role as food item over the total lifespan of *M. muelleri*, the absence of a suitable first intermediate host in the deep-sea might be considered as the reason for the absence of this trematode species in the MAR.

Both collected nematodes are abundant parasites of invertebrate and vertebrate hosts throughout the entire Atlantic Ocean (Klimpel et al. 2006a, b). The third-stage larvae (L3) of *A. simplex* have been found primarily in small shallow water copepods, in larger deep-sea copepods (*Paraeuchaeta (Euchaeta) norvegica*) and in many euphausiid species (Hays et al. 1998; Klimpel et al. 2004; Smith and Snyder 2005, discussion see above), and occurred at both studied localities. The fish nematode *Hysterothylacium aduncum* uses various inshore pelagic and benthic invertebrates (crustaceans and non-crustaceans) as obligatory intermediate hosts (Køie 1993; Klimpel and Rückert 2005), and was absent in MAR. The diet of *M. muelleri* consists of crustacean zooplankton organisms, with calanoid copepods as the dominant prey item. In particular geographical areas, seasons or stratified waters (e.g. fronts), hyperiids, chaetognaths and euphausiids are included in the

diet and act as intermediate hosts for *H. aduncum* (Hamre and Karslbakk 2002; Klimpel and Rückert 2005). The mature adult stages of *H. aduncum* are commonly found in the digestive tracts of many different marine fish as final hosts. The absence of *H. aduncum* at the MAR can be either explained by the absence of suitable final hosts at the studied locality at the MAR, or by the deep origin of the sampled fish specimens, where the abundance of nematodes is significantly decreasing with water depth (data taken from Klimpel et al. 2001).

Summarizing the above, *M. muelleri* from the MAR has a relatively poor parasite fauna in comparison to the NE Atlantic continental shelf region. This is similar to the results by Reimer (1975) who investigated *M. muelleri* from the Northwest African coast. He recorded the digenean *Lecithaster confusus* and the nematode genus *Contracaecum*, two parasite species from deeper waters between 300 and 550 m. The depth limits add the vertical distribution of the collected pelagic parasite species, though most of them are known for a wide range of zoogeographical distribution. Studies from other regions and different depths along the MAR must further clarify the ability of originally continental or oceanic parasites to invade the deep-sea.

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