

Global assessment of molecularly identified *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae) in their teleost intermediate hosts

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Abstract: Here, we present the ITS ribosomal DNA (rDNA) sequence data on 330 larvae of nematodes of the genus *Anisakis* Dujardin, 1845 collected from 26 different bony fish species from 21 sampling locations and different climatic zones. New host records are provided for *Anisakis simplex* (Rudolphi, 1809) sensu stricto (s.s.) and *A. pegreffii* Campana-Rouget et Biocca, 1955 from *Anoplopoma fimbria* (Pallas) (Santa Barbara, East Pacific), *A. typica* (Diesing, 1860) from *Caesio cuning* (Bloch), *Lepturacanthus savala* (Cuvier) and *Katsuwonus pelamis* (Linnaeus) (Indonesia, West Pacific), *A. simplex* s.s. from *Cololabis saira* (Brevoort) (Hawaii, Central Pacific), *A. simplex* C of Nascetti et al. (1986) from *Sebastolobus alascanus* Bean (Santa Barbara, East Pacific) and *A. physeteris* Baylis, 1923 from *Synaphobranchus kaupii* Johnson (Namibia, East Atlantic). Comparison with host records from 60 previous molecular studies of *Anisakis* species reveals the teleost host range so far recorded for the genus. Perciform (57 species) and gadiform (21) fishes were the most frequently infected orders, followed by pleuronectiforms (15) and scorpaeniforms (15). Most commonly infected fish families were Scombridae (12), Gadidae (10), Carangidae (8) and Clupeidae (7), with *Merluccius merluccius* (Linnaeus) alone harbouring eight *Anisakis* species. Different intermediate host compositions implicate differing life cycles for the so far molecularly identified *Anisakis* sibling species.

Keywords: Internal transcribed spacer (ITS), life cycle ecology, toothed/baleen whales, anisakiasis, paratenic host, sibling species, host range

Anisakid nematodes have a worldwide distribution and are known as causative agents of the human anisakidosis, a painful inflammation of the gastrointestinal tract caused by the ingestion of the third-stage larvae (L₃). Infections in humans can be acquired by ingestion of raw or insufficiently cooked fish and fisheries products (Sakanari and McKerrrow 1989, Nadler et al. 2005, Audicana and Kennedy 2008). Due to the high prevalence in commercially important fish species such as *Clupea harengus* Linnaeus or species of *Oncorhynchus* Suckley, members of the genus *Anisakis* Dujardin, 1845 can be considered the most common causative agents of this zoonosis (disease: anisakiasis) (Mattiucci and Nascetti 2008, Hochberg and Hamer 2010, Karl et al. 2011). Besides the major clinical symptoms such as vomiting, fever or epigastric pain, the incidence of *Anisakis*-related severe allergic disorders is a frequently documented problem and reflects the necessity of continuous research efforts in this field (Daschner and Pascual 2005, Audicana and Kennedy 2008, Daschner et al. 2011).

Anisakis has an indirect, heteroxenous life cycle including four larval stages. Typical definitive hosts are toothed and baleen whales (Cetacea) of the families Delphinidae, Ziphiidae, Physeteridae and Kogiidae, but species of the Balaenopteridae, Pontoporidae, Monodontidae, Phocoenidae, Neobalaenidae and even Otariidae are known to become infected (e.g. Klimpel et al. 2004, 2010a, Nadler et al. 2005, Mattiucci and Nascetti 2008, Kuhn et al. 2011). The eggs are shed into to the surrounding water column with the faeces of the definitive hosts. The larvae hatch and are subsequently ingested by small crustaceans (Køie et al. 1995, Marcogliese 1995, Køie 2001, Busch et al. 2012). They are then transferred within the food chain into larger macroinvertebrates (e.g. amphipods, cephalopods), where they grow in length and become infective to fishes and definitive hosts (Klumpel et al. 2004, 2008, McClelland 2005, Mattiucci and Nascetti 2008, Busch et al. 2012). Macroinvertebrates as well as small schooling fish are thought to be important intermediate/paratenic

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hosts. *Anisakis* is also capable of infecting larger piscivorous hosts (e.g. *Gadus morhua* Linnaeus, *Merluccius merluccius* [Linnaeus]) without further moult. This leads to the accumulation of enormous numbers of larvae and enhances the transmission probability to their respective definitive hosts (Lile 1998, Hammerschmidt et al. 2009, Klimpel and Palm 2011, Busch et al. 2012).

Until molecular techniques became routine diagnostic tools, the identification of larval anisakid nematodes was difficult and largely based on slight differences in the arrangements and morphometrics of the internal structures such as the alimentary tract, the orientation of the excretory pore and the shape of the tail (Mattiucci and Nascetti 2008, Klimpel and Palm 2011). In the case of the morphological almost indistinguishable species *Anisakis* (cryptic species), a correct assignment to their respective species was not feasible, which often led to erroneous identifications (Klimpel and Palm 2011). Population-based multilocus enzyme-electrophoresis (MAE) as well as PCR-based approaches such as PCR-RFLP (Restriction Fragment Length Polymorphism), SSCP (Single-Strand Conformation Polymorphism) or DNA sequencing of genetic markers have remarkably increased our knowledge of the actual species diversity and species composition (e.g. Anderson et al. 1998, Klimpel et al. 2008). Application of these methods to taxonomic studies of *Anisakis* spp. has revealed the existence of nine distinct species with different host preferences, ecology and zoogeography (e.g. Klimpel et al. 2004, 2008, Valentini et al. 2006, Mattiucci and Nascetti 2008).

The genus comprises two major clades; the first clade includes the *A. simplex*-complex (*A. simplex* [Rudolphi, 1809] sensu stricto [s.s.], *A. pegreffii* Campana-Rouget et Biocca, 1955, *A. simplex* C of Nascetti, Paggi, Orecchia, Smith, Mattiucci et Bullini, 1986) as well as *A. typica* (Diesing, 1860) and two sister-species *A. nascettii* Mattiucci, Paoletti et Webb, 2009 and *A. ziphidarum* Paggi, Nascetti, Webb, Mattiucci, Cianchi et Bullini, 1998. The second clade consists exclusively of the *A. physeteris*-complex (*A. brevispiculata* Dollfus, 1966, *A. paggiae* Mattiucci, Nascetti, Dailey, Webb, Barros, Cianchi et Bullini, 2005, *A. physeteris* [Baylis, 1923]) (Mattiucci et al. 2009, Klimpel et al. 2011, Kuhn et al. 2011).

Because of the abundance of anisakids in commercially important fishes and its implication for human health, an enormous number of host records of *Anisakis* has accumulated in the literature, however, often resulting only from morphological identification. Klimpel and Palm (2011) suggested that not all *Anisakis* species are dangerous to humans, and thus a closer look to the real host range of the different species is necessary. So far, no comprehensive overview has illustrated the host and distribution patterns of *Anisakis* spp., making it difficult to uncover the life cycle ecology and zoogeographical distribution patterns.

The present study summarizes a large sample size of *Anisakis* individuals from different teleost intermediate hosts, geographical regions and climate zones, using direct sequencing of the ITS molecular marker. The obtained data set is combined with already existing parasite-host records based on molecular identification, demonstrating the different hosts and zoogeographical distribution patterns for the species. Implications for the life cycles of nine different *Anisakis* species are discussed.

MATERIALS AND METHODS

Sample collection

A total of 330 *Anisakis* third-stage larvae were isolated from 26 teleost species from 21 localities around the world (Table 1; in part Kuhn et al. 2011). Specimens were extensively washed in 0.9% saline solution and identified morphologically to the genus level according to Anderson (2000). All samples were preserved in EtOH (100%) prior to molecular examination.

Molecular analysis

Genomic DNA was isolated and purified from individual larvae using a genomic DNA extraction kit (Peqlab Biotechnology GmbH, Erlangen) 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 primers NC2 (5'-TTA-GTT-TCT-TTT-CCT-CCG-CT-3') and TK1 (5'-GGC-AAA-AGT-CGT-AAC-AAG-GT-3') (Zhu et al. 2000, Kuhn et al. 2011). PCR-reaction (50 µl) included 25 µl Master-Mix (Peqlab Biotechnology GmbH, Erlangen) containing dNTP, MgCl₂ Buffer and Taq-Polymerase, 3 µl of each primer (10 pmol/µl), 14 ddH₂O and 5 µl genomic DNA extract.

PCR reactions were performed in an Advanced Primus 96 thermocycler (Peqlab) under the following conditions: initial denaturation at 95 °C for 1 min; 40 cycles of 94 °C for 45 sec (denaturation), 55 °C for 45 sec (annealing), 72 °C for 45 sec (extension); final extension at 72 °C for 10 min. Negative controls (reactions without DNA) were included in each PCR run. PCR products were examined on 1% agarose gels and purified with Cycle-Pure Kit (Peqlab Biotechnology GmbH, Erlangen) and a total volume of 7 µl, including 2 µl primer (100 pmol/µl) and 5 µl of the PCR product (250 ng/µl) was sequenced by Seqlab (Goettingen GmbH). Both spacers and the 5.8S gene from each PCR product were sequenced using the primer TK1.

Sequence analyses

For sequence analyses, electropherograms were checked manually and sequences were aligned using CLUSTALX (v.2.0.1.0) (Larkin et al. 2007) to visualize genetic polymorphisms. For species identification, sequence data of each reaction were compared with previously published data in GenBank by using the BLASTn algorithm (Altschul et al. 1990).

RESULTS AND DISCUSSION

The ITS ribosomal DNA (rDNA) sequence data of 330 larvae of *Anisakis* spp. from 26 different bony fishes and 21 localities revealed the presence of five out of nine currently recognized *Anisakis* species, namely *A. simplex* s.s., *A. pegreffii*, *A. simplex* C, *A. typica*, *A. physeteris*, and two genotypes (*Anisakis* sp. SAN2004, *Ani-*

Table 1. Information on the nematodes identified by ITS sequence analyses. Sampling localities, host and *Anisakis* species are provided for 330 nematodes.

Sample location	Host									Total	Accession-No.
		Asimss	Apeg	AsimC	Atyp	Aphy	AspHC05	Asp-SAN04	Aindet		
Alaska (Bay Nushagak)	<i>Oncorhynchus keta</i>	12	-	-	-	-	-	-	-	12	JN968655–JN968666
	<i>Oncorhynchus nerka</i>	11	-	-	-	-	-	-	-	11	JN968667–JN968677
Alaska (Cordova)	<i>Oncorhynchus keta</i>	12	-	-	-	-	-	-	-	12	JN968678–JN968689
	<i>Oncorhynchus nerka</i>	12	-	-	-	-	-	-	-	12	JN968690–JN968701
Antarctic (South Shetland Islands)	<i>Gymnoscopelus nicholsi</i>	-	-	14	-	-	-	-	-	14	JN968638–JN968651
Baltic Sea (Kattegat)	<i>Scomber scombrus</i>	1	-	-	-	-	-	-	-	1	JN968759
	<i>Clupea harengus</i>	11	1	-	-	-	-	-	-	12	JN968748–JN968759
Baltic Sea (off Kiel)	<i>Clupea harengus</i>	9	-	-	-	-	-	-	-	9	JN968760–JN968768
Baltic Sea (off Rügen)	<i>Clupea harengus</i>	-	-	-	-	-	-	-	1	1	KC121366
Barents Sea (Bellsund Banks)	<i>Melanogrammus aeglefinus</i>	13	-	-	-	-	-	-	-	13	JN968770–JN968782
	<i>Micromesistius poutassou</i>	11	-	-	-	-	-	-	1	12	JN968783–JN968793; KC121367
Barents Sea (Björnöya)	<i>Gadus morhua</i>	2	-	-	-	-	-	-	-	2	JN968794–JN968795
Barents Sea (Forland Banks)	<i>Micromesistius poutassou</i>	9	-	-	-	-	-	-	-	9	JN968796–JN968804
Barents Sea (Sjubre Banks)	<i>Melanogrammus aeglefinus</i>	12	-	-	-	-	-	-	-	12	JN968805–JN968816
China	<i>Scomberomorus niphonius</i>	-	3	-	-	-	-	-	-	3	JN968607–JN968609
	<i>Cleisthenes herzensteini</i>	-	7	-	-	-	-	-	-	7	JN968594–JN968600
	<i>Gadus macrocephalus</i>	-	6	-	-	-	-	-	-	6	JN968601–JN968606
	<i>Trichiurus lepturus</i>	-	11	-	-	-	-	-	-	11	JN968610–JN968620
Hawaii	<i>Cololabis saira</i>	5	-	-	-	-	-	-	-	5	JN968817–JN968821
	<i>Selar crumenoptalmus</i>	-	-	-	6	-	-	-	-	6	JN968907–JN968912
	<i>Katsuwonus pelamis</i>	-	-	-	1	-	-	-	-	1	JN968906
Indonesia	<i>Trichiurus lepturus</i>	-	-	-	26	-	-	-	-	26	JN968936–JN968961
	<i>Auxis thazard thazard</i>	-	-	-	5	-	-	-	-	5	JN968927–JN968931
	<i>Auxis rochei rochei</i>	-	-	-	14	-	-	-	-	14	JN968913–JN968926
	<i>Lepturacanthus savala</i>	-	-	-	2	-	1	-	-	3	JN968934; JN968935; KC121379
	<i>Caesio cuning</i>	-	-	-	2	-	-	-	-	2	JN968932, JN968933
Italy (Ionian Sea)	<i>Merluccius merluccius</i>	-	10	-	-	-	-	-	-	10	JN968621–JN968630
Italy (Tyrrhenian Sea)	<i>Scomber japonicus</i>	-	3	-	-	-	-	-	-	3	JN968631–JN968633
Madeira	<i>Aphanopus carbo</i>	16	-	-	-	-	-	-	-	16	JN968822–JN968837
Namibia	<i>Synaphobranchus kaupii</i>	-	-	-	-	1	-	-	-	1	JN968637
Moorea	<i>Katsuwonus pelamis</i>	-	-	-	3	-	-	-	-	3	JN968962–JN968964
	<i>Selar crumenoptalmus</i>	-	-	-	1	-	-	-	-	1	JN968965
North Sea (off Scheveningen)	<i>Trachurus trachurus</i>	8	-	-	-	-	-	-	-	8	JN968898–JN968905
	<i>Scomber scombrus</i>	20	-	-	-	-	-	-	-	20	JN968878–JN968897
North Sea (off German Coast)	<i>Clupea harengus</i>	40	-	-	-	-	-	-	2	42	JN968838–JN968877; KC121368; KC121369
Santa Barbara	<i>Anoplopoma fimbria</i>	1	-	2	-	-	-	-	-	3	JN968769; JN968652; JN968653
	<i>Sebastolobus alascanus</i>	-	-	1	-	-	-	1	-	2	JN968654; KC121371
	Total	205	41	17	60	1	1	1	4	330	

Asimss – *Anisakis simplex* s.s.; Apeg – *A. pegreffii*; AsimC – *A. simplex* C; Atyp – *A. typica*; AspHC05 – *Anisakis* sp. HC2005; AspSAN04 – *Anisakis* sp. SAN 2004; Aindet – *Anisakis* indetermined.

sakis sp. HC2005) previously recorded by Nadler et al. (2005) and Kijewska et al. (2009), respectively (Table 1). Findings of *A. simplex* s.s. and *A. pegreffii* in *Anoplopoma fimbria* (Pallas) from Santa Barbara, *A. typica* in *Caesio cuning* (Bloch), *Lepturacanthus savala* (Cuvier) and *Katsuwonus pelamis* (Linnaeus) from Indonesia and Moorea, *A. simplex* s.s. in *Cololabis saira* (Brevoort) from Hawaii, *A. simplex* C in *Sebastolobus alascanus* Bean from Santa Barbara as well as *A. physeteris* in *Synaphobranchus kaupii* Johnson from Namibia represent new host records (Table 1).

Most abundant was *A. simplex* s.s. with a total of 205 records, followed by *A. typica* (60), *A. pegreffii* (41), *A. simplex* C (17) and *A. physeteris* (1) (Table 1). In addition, four sequences that cannot be unambiguously classified by sequence analyses were identified (*Anisakis* indet_NSG_Char_39.5 and *Anisakis* indet_NSG_Char_40.8 in *Clupea harengus* from Scheveningen, North Sea, *Anisakis* indet_BalRu_Char_121.1 in *C. harengus* from off Rügen, Baltic Sea and *Anisakis* indet_BSBB_Mpou_79_8 in *Micromesistius poutassou* [Risso] from Belsund Bank, Barents Sea). BLAST analysis revealed the same score

(E-value = 0.00) and 99.0% identity to several different sequences, *A. simplex* s.s., *A. pegreffii* and *A. simplex* C, respectively (Table 1).

The sequence Aindet_BalRu_Char_121.1 was heterozygote at positions 281 and 296 of the aligned sequences. These positions are known as diagnostic sites of the ITS marker between the sister species *A. simplex* s.s. and *A. pegreffii*. Abollo et al. (2003) identified hybrids between both species in a sympatric area along the Galician coast of Spain (Atlantic Ocean). The authors eliminated the regular polymorphisms as well as incomplete concerted evolution within a multicopy gene as possible reasons and cited the exchange of genetic material between both species as the most likely cause for such hybridization events. Despite limited sample size, this is further corroborated by the fact that both presumably admixed genotypes were encountered in populations where both pure species occur.

It is not known whether hybridization in *Anisakis* enables adaptation to particular environmental conditions or whether it is a consequence of incomplete barriers to hybrid mating, but it could reflect the radiation within the genus *Anisakis*. However, since DNA turnover mechanisms, including slippage during DNA replication, gene conversion, unequal crossing-over or transposition events or simply intraspecific variations cannot be excluded as cause for the polymorphisms, the hybrid hypothesis within the genus *Anisakis* needs to be rigorously tested and critically evaluated by analyzing natural populations and large sample sizes, and more genomic loci (Hailer et al. 2012).

The obtained sequence data were combined with the host records from 60 previous molecular studies to evaluate the role of the teleost intermediate and paratenic hosts in the life cycle of these parasites. A total of 155 teleost fish species from 71 families and 19 orders have been reported in the literature (Table 2). Perciform (57 species) and gadiform (21) fishes were the most frequently infected orders, followed by pleuronectiforms (15) and scorpaeniforms (15). The Scombridae (12), Gadidae (10), Carangidae (8), Clupeidae (7), Pleuronectidae (6) and Salmonidae (5) were the most frequently represented families, with *Merluccius merluccius* alone harbouring eight different *Anisakis* species (Table 2).

The vast majority are food fishes of high commercial interest, reflecting the extensive scientific effort that has dealt with the role of *Anisakis* as causative agent of the food-borne parasitic zoonosis called anisakiasis. *Anisakis* is usually associated with the traditions of consuming raw or insufficiently cooked fish (e.g. salted herring, sushi, sashimi) and the increasingly popular habit not to overcook food (Audicana and Kennedy 2008).

The fish hosts recorded were classified into three habitats (pelagic, benthopelagic, demersal), according to the definitions provided by Froese and Pauly (2012). Pelagic

fish species live and feed in the open sea and are usually associated with the surface or the middle depths of a water body. Benthopelagic fishes are living near the bottom as well as in midwaters, where they feed on benthic and/or free swimming organisms. In contrast, demersal fishes live and feed on or near to the sea floor (Froese and Pauly 2012). Our data demonstrate that 71 of the 155 reported hosts live in pelagic and benthopelagic environments.

Nearly the same number (72) is demersal and another 12 are known to be reef-associated (Froese and Pauly 2012) (Fig. 1). Although the majority of fishes have been classified demersal, many of them are known to extend their feeding range into the benthopelagic and feed on both benthic and (bentho-)pelagic organisms (e.g. species of *Merluccius*, *Gadus* Linnaeus, *Pollachius* Nilsson, *Hippoglossus* Cuvier and *Conger* Bosc). Taking into account the large number of hosts of each species that live in pelagic, demersal and benthopelagic habitats, it becomes apparent that the life cycle of *Anisakis* includes food chains throughout the water column with a bias towards (bentho-)pelagic environments (see also Fig. 1).

Anisakis pegreffii (101 records) was the most frequently reported species in the literature, followed by *A. simplex* s.s. (63) (Table 2). This is very likely due to the fact that the former species is widely distributed in western Pacific waters around Japan, where most food-borne diseases like anisakiasis occur (e.g. Klimpel and Palm 2011, Kuhn et al 2011). However, there was no consensus whether *A. pegreffii* and *A. simplex* s.s. complete life cycles mainly in pelagic or in benthic habitats (e.g. Mattiucci et al. 1997, Abollo et al. 2001, Mattiucci and Nascetti 2008). Abollo et al. (2001) demonstrated that 80% of the fish hosts of *A. simplex* s.s. are nektobenthic or demersal, whereas no infection with *A. pegreffii* was found in large pelagic cephalopods. Our data demonstrate that both parasite species as well as *A. simplex* C have similar proportions of fish hosts that live in pelagic, benthopelagic and demersal habitats suggesting similar life cycle strategies (Fig. 1).

These data are congruent with the so far known spectrum of definitive hosts reported for these species. The very closely related *A. simplex* s.s., *A. pegreffii* and *A. simplex* C mature mainly in oceanic dolphins, which are known to feed offshore on large pelagic and benthopelagic fishes (Højgaard 1998, Jefferson et al. 2008, Klimpel et al. 2011, Kuhn et al. 2011). This would suggest a mainly (bentho-) pelagic life cycle for these species. According to Klimpel et al. (2004, 2007), it seems that at least *A. simplex* s.s. can perform a pelagic life cycle in the Norwegian Deep off the continental shelf.

With 26 records of *A. typica*, 16 of *A. physeteris*, 7 of *A. ziphidarum*, 6 of *A. nascettii* and *A. paggiae* and 5 of *A. brevispiculata*, respectively, these species were far less frequently reported from fish intermediate hosts. However, considering the feeding behaviour of the reported cetacean definitive hosts, e.g. *Ziphius cavirostris* Cuvier, *Phy-*

Table 2. Teleost intermediate/paratenic hosts for *Anisakis* spp. so far detected by molecular analyses. Order, family, host, habitat, ocean and references are given for each *Anisakis* species.

Order	Family	Host species	Habitat	Asimss	Apeg	AsimC	Atyp	Anas	Azip	Aphy	Apag	Abre	Reference			
Anguilliformes	Congridae	<i>Conger conger</i>	D	EA	CM, EA, YS								2, 32, 33			
		<i>Conger myriaster</i>	D		CM, YS								9, 32, 59, 60			
	Muraenesocidae	<i>Muraenesox cinereus</i>	D		YS								9, 32			
	Muraenidae	<i>Muraena helena</i>	Ra		CM, EA								12			
Aulopiformes	Synbranchiidae	<i>Synbranchichthys kaupii</i>	D							SEA			25			
	Synodontidae	<i>Saurida elongata</i>	D		YS								9			
Beloniformes	Belonidae	<i>Belone belone</i>	P	BS, EA	EA								2, 30, 32, 55			
		<i>Cheilopogon agoo</i>	P		YS								9			
	Scomberesocidae	<i>Cololabis</i> sp.	P	CP										25		
		<i>Scomberesox saurus saurus</i>	P	NWA, WA										30, 32, 33		
Beryciformes	Anoplogastridae	<i>Anoplogaster cornuta</i>	P										24			
	Berycidae	<i>Beryx splendens</i>	Bp							WP	WP	WP	42			
	Trachichthyidae	<i>Hoplostethus atlanticus</i>	Bp			IO/SP								30, 33		
		<i>Hoplostethus cadenati</i>	Bp				EA		EA	EA		EA		17		
Clupeiformes	Clupeidae	<i>Clupea harengus</i>	Bp	BS, EP, NEA, NEP, NS	BS								8, 17, 25, 30, 31, 32, 33, 45, 55, 60			
		<i>Clupea pallasii pallasii</i>	P		YS									9, 59		
		<i>Etrumeus whiteheadi</i>	P		SA									30, 32, 33		
		<i>Konosirus punctatus</i>	P		YS									9		
		<i>Sardina pilchardus</i>	P		CM, EM									6, 41		
		<i>Sardinella zunasi</i>	P		YS									9		
		<i>Sardinops sagax</i>	P	EP	EP	EP								4		
		Engraulidae	<i>Engraulis encrasicolus</i>	P		CM, WM									6, 30, 32, 33, 41	
		<i>Engraulis japonicus</i>	P		YS										9	
		Gadiformes	Gadidae	<i>Boreogadus saida</i>	D	NA, EA									10, 30, 32, 33	
				<i>Gadus macrocephalus</i>	D		WP, YS									9, 25, 49, 52
				<i>Gadus morhua</i>	Bp	BS, EA, NA, NEA, NWA										17, 25, 30, 32, 45, 55
				<i>Gadus ogac</i>	D	NEA										17
	<i>Melanogrammus aeglefinus</i>			D	BS, NEA										25, 55	
<i>Merlangius merlangus</i>	Bp			NS										45		
<i>Micromesistius poutassou</i>	Bp			EA, NEA, NS, WM, WP	CM, EA, EM, WM					CM				2, 3, 6, 12, 25, 30, 32, 41, 44, 45		
<i>Pollachius virens</i>	D			BS, NS											45, 55	
<i>Theragra chalcogramma</i>	Bp			EP, NEA, NWA	WP								WP		30, 32, 33, 45, 49, 50, 52, 55, 57	
Lotidae	<i>Trisopterus luscus</i>			Bp	EA										2, 30, 32	
	<i>Brosme brosme</i>	D	EA, NEA	N/A									30, 31, 32, 33			
Macrouridae	<i>Molva dipterygia</i>	D	EA										2, 30, 32			
	<i>Macrourus berglax</i>	Bp	NA, NWA										16, 19			
Merlucciidae	<i>Trachyrincus scabriscus</i>	D		EA					EA				17			
	<i>Merluccius capensis</i>	D		SA, SEA									30, 31, 32, 33			

(continued)

Table 2. Continued.

Order	Family	Host species	Habitat	Asimss	Apeg	AsimC	Atyp	Anas	Azip	Aphy	Apag	Abre	Reference
		<i>Merluccius hubbsi</i>	Bp		SWA								30, 31, 32, 33
		<i>Merluccius merluccius</i>	D	BS, EA, EM, NEA, SM, WM	CM, EA, EM, NEA, SM, WM		CM, EA, EM, WM	EA	EA, EM	CM, EA, SM, WM	NEA	EA, EM, SM	2, 6, 11, 12, 25, 30, 31, 32, 33, 34, 35, 36, 37, 38, 41, 45, 55
		<i>Merluccius polli</i>	D		EA		EA	EA		EA			17
	Moridae	<i>Pseudophycis bachus</i>	D		SP	SP							30, 31, 32, 33
	Phycidae	<i>Phycis blennoides</i>	Bp		CM					CM			12, 32
		<i>Phycis phycis</i>	Bp		CM, EA		CM, EA			CM, EA			12, 32
Lophiiformes	Lophiidae	<i>Lophius litulon</i>	D		YS								9
		<i>Lophius piscatorius</i>	D	BS, EA	CM, EA, SM								59
		<i>Lophius vomerinus</i>	D		SA								30, 32, 33
Mugiliformes	Mugilidae	<i>Mugil cephalus</i>	Bp		YS								59
Myctophiformes	Myctophidae	<i>Electrona carlsbergi</i>	P			SA							32
		<i>Gymnoscopelus nicholsi</i>	P		SA	SA							22, 25
		<i>Myctophum punctatum</i>	P	CA	CA								21, 23
		<i>Notoscopelus kroyeri</i>	P	CA									21
Ophidiiformes	Ophidiidae	<i>Genypterus capensis</i>	D		SA								30, 32, 33
Osmeriformes	Argentinidae	<i>Argentina silus</i>	D	NA									19
	Bathylagidae	<i>Bathylagus euryops</i>	P	NA									19, 30, 32
	Osmeridae	<i>Hypomesus japonicus</i>	D	WP									32, 57
Perciformes	Bramidae	<i>Brama brama</i>	P		SA								30, 32, 33
	Caesionidae	<i>Caesio cuning</i>	Ra				WP						25
	Carangidae	<i>Decapterus maruadsi</i>	Ra		YS								9
		<i>Selar crumenophthalmus</i>	Ra				CP, WP						25, 32, 60
		<i>Seriola dumerili</i>	Ra		WP								49
		<i>Seriola lalandi</i>	Bp		YS								9
		<i>Trachurus capensis</i>	P		SA								30, 32, 33
		<i>Trachurus mediterraneus</i>	P	EA	CM, EA								11, 30, 32, 33, 41
		<i>Trachurus picturatus</i>	Bp	EA	EA		EA						30, 32, 33, 35, 41, 48
		<i>Trachurus trachurus</i>	P	EA, EM, CM, NEA, NS, WM	CM, EA, EM, NEA, SWP, WM		EA, EM	EA		CM	EA		2, 3, 6, 11, 17, 25, 27, 30, 31, 32, 33, 38, 39, 41, 44, 45
	Centracanthidae	<i>Spicara smaris</i>	D		EM								6
	Coryphaenidae	<i>Coryphaena hippurus</i>	P		YS		IO, WP						9, 32, 35, 47, 59
	Emmelichthyidae	<i>Emmelichthys nitidus nitidus</i>	D		SA								30, 32, 33
	Gempylidae	<i>Thyrsites atun</i>	Bp		SA, SEA	SA, SEA							30, 31, 32, 33
	Lateolabracidae	<i>Lateolabrax japonicus</i>	Ra		YS								9
	Lutjanidae	<i>Lutjanus erythropterus</i>	Ra		YS								9
	Moronidae	<i>Dicentrarchus labrax</i>	D	NEA									5
	Mullidae	<i>Mullus barbatus barbatus</i>	D	EM									6
		<i>Mullus surmuletus</i>	D		CM								41
	Nemipteridae	<i>Nemipterus bathybius</i>	D				WP						32, 60
		<i>Nemipterus virgatus</i>	D				WP						32, 60
	Nototheniidae	<i>Notothenia coriiceps</i>	D			SEA							9
		<i>Notothenia rossii</i>	D			SEA							9
	Pholidae	<i>Pholis nebulosa</i>	D		YS								9
	Pinguipedidae	<i>Parapercis colias</i>	D		SP	SP							30, 31, 32, 33

(continued)

Table 2. Continued.

Order	Family	Host species	Habitat	Asimss	Apeg	AsimC	Atyp	Anas	Azip	Aphy	Apag	Abre	Reference
	Rachycentridae	<i>Rachycentron canadum</i>	Ra		YS								9
	Sciaenidae	<i>Collichthys niveatus</i>	D		YS								9
		<i>Larimichthys polyactis</i>	Bp		YS								9
		<i>Nibea albiflora</i>	Bp		YS								9
		<i>Pennahia argentata</i>	Bp		YS								9
	Scombridae	<i>Auxis rochei rochei</i>	P				WP						25, 47
		<i>Auxis thazard thazard</i>	P		YS		WA, WP, YS			WA			9, 14, 25, 35
		<i>Euthynnus affinis</i>	P				IO						35
		<i>Euthynnus alletteratus</i>	Ra	EA	EA								11, 30, 32, 33, 41
		<i>Katsuwonus pelamis</i>	P				CP						25
		<i>Scomber australasicus</i>	P	WP	WP		WP						7, 49, 52
		<i>Scomber colias</i>	P		EM								6, 33
		<i>Scomber japonicus</i>	P	EA, WM, WP	CM, EA, WM, WP, YS		EA, IO	EA	EA	EA	EA, WM		1, 3, 9, 11, 12, 17, 25, 26, 30, 32, 45, 48, 49, 56, 57, 58, 59
		<i>Scomber scombrus</i>	P	BS, EA, EM, CM, NEA, NS, WM	BS, CM, EA, EM, NEA, WM		CM, EA	EA	EA	EA	CM, EA		2, 3, 6, 11, 12, 25, 30, 32, 33, 41, 45, 55
		<i>Scomberomorus commerson</i>	P				IO						35
		<i>Scomberomorus niphonius</i>	P		YS								9, 25, 49, 59
		<i>Thunnus thynnus</i>	P	WP	CM, WA		WA						12, 30, 31, 32, 33, 35
	Sillaginidae	<i>Sillago sihama</i>	Ra		YS								9
	Sparidae	<i>Boops boops</i>	D		CM								45
		<i>Diplodus annularis</i>	Bp		EM								6
		<i>Pagellus bogaraveo</i>	Bp	EA	EA		EA		EA	EA			13
		<i>Spondylisoma cantharus</i>	Bp	EA									2, 30
	Stromateidae	<i>Pampus argenteus</i>	P		YS								9
	Trachinidae	<i>Aphanopus carbo</i>	Bp	EA	EA			EA	EA	EA	EA		25, 37, 40, 48
		<i>Echiichthys vipera</i>	D		CM								12
		<i>Lepidopus caudatus</i>	Bp		CM, SEA								30, 31, 32, 33, 45
		<i>Lepturacanthus savala</i>	Bp				WP						25
		<i>Trachinus draco</i>	D		CM								12
		<i>Trichiurus lepturus</i>	Bp	WP	CM, WP, YS		WP						9, 12, 25, 26, 54
	Trichodontidae	<i>Arctoscopus japonicus</i>	D		WP								52
	Xiphiidae	<i>Xiphias gladius</i>	P	CM	CM, EA		EA		CM, EA, WM	CM, WM	CA	CA, EA	30, 32, 33
	Zoarceidae	<i>Zoarces elongatus</i>	D		YS								9
Pleuronectiformes	Bothidae	<i>Arnoglossus imperialis</i>	D	EA	EA								28, 29, 32
	Citharidae	<i>Citharus linguatula</i>	D	EA, NEA	EA, NEA								28, 29, 32
	Paralichthyidae	<i>Paralichthys olivaceus</i>	D	EXP	EXP								51
	Pleuronectidae	<i>Cleisthenes herzensteini</i>	D		YS								9, 25
		<i>Hippoglossoides dubius</i>	D		WP								52
		<i>Hippoglossus hippoglossus</i>	D	BE									30, 32
		<i>Platichthys flesus</i>	D				EA						28, 29, 32
		<i>Pseudopleuronectes yokohamae</i>	D		YS								9

(continued)

Table 2. Continued.

Order	Family	Host species	Habitat	Asimss	Apeg	AsimC	Atyp	Anas	Azip	Aphy	Apag	Abre	Reference	
Salmoniformes	Scophthalmidae	<i>Reinhardtius hippoglossoides</i>	Bp	NEA, NWA									17, 32, 33	
		<i>Lepidorhombus boscii</i>	D	EA	EA								2, 28, 29, 30, 32	
		<i>Lepidorhombus whiffiagonis</i>	D	EA									29	
	Soleidae	<i>Zeugopterus regius</i>	D	EA										29
		<i>Dicologlossa cuneata</i>	D	EA	EA									28, 29
		<i>Solea senegalensis</i>	D	EA										28, 29
		<i>Synapturichthys kleinii</i>	D	EA										28
	Salmonidae	<i>Oncorhynchus gorboscha</i>	D	NEP, NP										12, 30, 32
		<i>Oncorhynchus keta</i>	Bp	NEP, WP										15, 25, 30, 32, 49
		<i>Oncorhynchus mykiss</i>	Bp	EXP	EXP									51
<i>Oncorhynchus nerka</i>		Bp	NEP										15, 17, 25	
<i>Salmo salar</i>		Bp	NEA, NS, NWA, WA										30, 31, 32, 33, 43, 45, 46	
Scorpaeniformes	Anoplopomatidae	<i>Anoplopoma fimbria</i>	D	EP		EP							25	
		Hexagrammidae	<i>Hexagrammos agrammus</i>	D			YS							9
	<i>Hexagrammos otakii</i>		D			YS							9	
	<i>Pleurogrammus azonus</i>		D	NEA, WP									32, 57, 58	
	Platycephalidae	<i>Pleurogrammus monopterygius</i>	D	BE										17
		<i>Cociella crocodilus</i>	Ra			YS								9
	Scorpaenidae	<i>Platycephalus richardsoni</i>	D			IO/SP								53
		<i>Hoplosebastes armatus</i>	D			YS								9
	Sebastidae	<i>Scorpaena scrofa</i>	D	EA	EA									2, 30, 32
		<i>Helicolenus dactylopterus</i>	D		CM, SEA									30, 33
Synanceiidae	<i>Sebastiscus marmoratus</i>	D			YS								59	
	<i>Sebastolobus alascanus</i>	D				EP							25	
	<i>Inimicus japonicus</i>	D			YS								9	
	Triglidae	<i>Chelidonichthys kumu</i>	D			YS		YS						9
<i>Eutrigla gurnardus</i>		D	EA										2	
Stomiiformes	Sternoptychidae	<i>Maurollicus muelleri</i>	P	CA, NEA									18, 20	
		<i>Borostomias antarcticus</i>	D	NA										19
Tetraodontiformes	Monacanthidae	<i>Thamnaconus modestus</i>	Ra				YS						9	
		<i>Takifugu niphobles</i>	D				YS							9
Zeiformes	Zeidae	<i>Takifugu poecilonotus</i>	D				WP						52	
		<i>Zeus faber</i>	Bp			CM, YS							9, 41	

Asimss – *A. simplex* s.s.; Apeg – *A. pegreffii*; AsimC – *A. simplex* C; Atyp – *A. typica*; Anas – *A. nascettii*; Azip – *A. ziphidarum*; Aphy – *A. physeteris*; Apag – *A. paggiae*; Abre – *A. brevispiculata*. Bp – benthopelagic; D – demersal; P – pelagic; Ra – reef-associated. BE – Bering Sea; BS – Baltic Sea; CA – Central Atlantic; CM – Central Mediterranean Sea; CP – Central Pacific; EA – East Atlantic; EM – East Mediterranean Sea; EP – East Pacific; EXP – experimental infection; IO – Indian Ocean; NA – North Atlantic; N/A – Not Available; NEA – North East Atlantic; NEP – North East Pacific; NP – North Pacific; NS – North Sea; NWA – North West Atlantic; SA – South Atlantic; SEA – South East Atlantic; SM – South Mediterranean Sea; SP – South Pacific; SWA – South West Atlantic; SWP – South West Pacific; WA – West Atlantic; WM – West Mediterranean Sea; WP – West Pacific; YS – Yellow Sea. 1 – Abattouy et al. 2011; 2 – Abollo et al. 2001; 3 – Abollo et al. 2003; 4 – Baldwin et al. 2011; 5 – Bernadi et al. 2011; 6 – Chaligiannis et al. 2012; 7 – Chou et al. 2011; 8 – Cross et al. 2007; 9 – Du et al. 2010; 10 – Dzido et al. 2009; 11 – Farjallah et al. 2008a; 12 – Farjallah et al. 2008b; 13 – Hermida et al. 2012; 14 – Iñiguez et al. 2009; 15 – Karl et al. 2011; 16 – Kellermanns et al. 2007; 17 – Kijewska et al. 2009; 18 – Klimpel et al. 2004; 19 – Klimpel et al. 2006; 20 – Klimpel et al. 2007; 21 – Klimpel et al. 2008; 22 – Klimpel et al. 2010a; 23 – Klimpel et al. 2010b; 24 – Klimpel et al. 2011; 25 – Kuhn et al. 2011; 26 – Lee et al. 2009; 27 – MacKenzie et al. 2002; 28 – Marques et al. 2006; 29 – Marques et al. 2009; 30 – Mattiucci and Nascetti 2006; 31 – Mattiucci and Nascetti 2007; 32 – Mattiucci and Nascetti 2008; 33 – Mattiucci et al. 1997; 34 – Mattiucci et al. 2001; 35 – Mattiucci et al. 2002; 36 – Mattiucci et al. 2004; 37 – Mattiucci et al. 2005; 38 – Mattiucci et al. 2007; 39 – Mattiucci et al. 2008; 40 – Mattiucci et al. 2009; 41 – Meloni et al. 2011; 42 – Murata et al. 2011; 43 – Murphy et al. 2010; 44 – Nadler et al. 2005; 45 – Nascetti et al. 1986; 46 – Noguera et al. 2009; 47 – Palm et al. 2008; 48 – Pontes et al. 2005; 49 – Quiazon et al. 2008; 50 – Quiazon et al. 2009; 51 – Quiazon et al. 2011a; 52 – Quiazon et al. 2011b; 53 – Shamsi et al. 2011; 54 – Shih 2004; 55 – Skov et al. 2010; 56 – Suzuki et al. 2010; 57 – Umehara et al. 2006; 58 – Umehara et al. 2008; 59 – Zhang et al. 2007; 60 – Zhu et al. 2007.

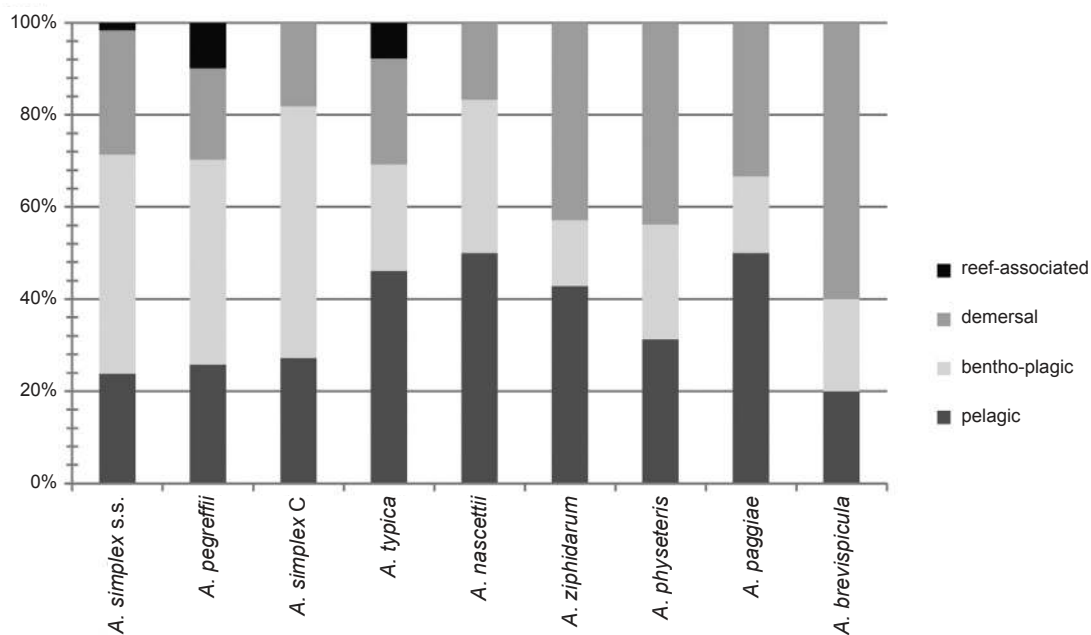


Fig. 1. Proportions of *Anisakis* teleost fish hosts in demersal, benthopelagic, pelagic and reef-associated habitats.

seter macrocephalus Linnaeus, *Mesoplodon grayi* von Haast, *Kogia breviceps* (Blainville) and *K. sima* (Owen), which are known to feed especially in the pelagic realm, the life cycle of these nematodes has to shift at some point from (benthopelagic) habitats into the free water column (Table 2, Fig. 1). Palm et al. (2008) recorded 21 mainly pelagic or reef associated fish species as intermediate hosts for *Anisakis* spp., four of them for *A. typica* and two closely related genotypes. Klimpel et al. (2011) identified *A. paggiae* in the common fangtooth *Anoplogaster cornuta* (Valenciennes) in the Irminger Sea and extended the distribution range of their definitive hosts (*Kogia breviceps* and *K. sima*) reported so far.

Mesopelagic fishes such as *A. cornuta* transfer parasites into larger paratenic hosts (e.g. *Xiphias gladius* Linnaeus) and may therefore play a crucial role within the life cycle of this anisakid nematode. Furthermore, the authors suggested similar life cycles for *A. paggiae* and the other species of the *A. physeteris* species complex (*A. brevispiculata*, *A. physeteris*) as well as for both sister species *A. nascettii* and *A. ziphidarum* in meso/bathypelagic waters, in accordance with the most common feeding ecology and depth range of their reported intermediate fish and definitive hosts (Klumpel et al. 2011). The latter four *Anisakis* species mainly use the same (meso-)pelagic, piscivorous fish species such as *Aphanopus carbo* Lowe, *Beryx splendens* Lowe, *Hoplostethus cadenati* Quéro, *Merluccius merluccius* and *M. polli* (Cadenat) as well as *Pagellus bogaraveo* (Brünnich) in their life cycles (Table 2).

Our data confirm that *Anisakis* species are less host-specific regarding the intermediate teleost hosts. Busch

et al. (2012) demonstrated that *Anisakis* larvae can be found in crustaceans from pelagic, benthopelagic and benthic habitats. Euphausiids are considered important crustacean hosts that transmit larvae directly to baleen whales, which feed on them extensively (Marcogliese 1995). By utilizing intermediate hosts from different trophic levels and habitats within the marine food webs, *Anisakis* larvae are abundant in virtually every depth horizon, in both the pelagic and benthic zone. However, taking into account the distribution, habitats and feeding behavior of toothed- and baleen whales, transmission to their respective definitive hosts has to be associated with the free water column.

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