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MOLECULAR SYSTEMATICS, PHYLOGENY AND ECOLOGY OF ANISAKID NEMATODES OF THE GENUS *ANISAKIS* DUJARDIN, 1845: AN UPDATE

MATTIUCCI S.* & NASCETTI G.**

Summary:

Advances in the taxonomy and ecological aspects concerning geographical distribution and hosts of the so far genetically recognised nine taxa of the nematodes belonging to genus *Anisakis* (i.e. *A. pegreffii, A. simplex s.s., A. simplex* C, *A. typica, A. ziphidarum, Anisakis* sp., *A. physeteris, A. brevispiculata* and *A. paggiae*) are here summarized. Genetic differentiation and phylogenetic relationships inferred from allozyme (20 enzyme-loci) and mitochondrial (sequences of *cox-2* gene) markers, are revised and compared. The two genetic analyses are congruent in depicting their phylogenetic relationships. Two main clusters are showed to exist in the obtained trees, one encompassing the species *A. pegreffii, A. simplex s.s., A. simplex* C, *A. typica, A. ziphidarum* and *Anisakis* sp.; while, the second including *A. physeteris, A. brevispiculata* and *A. paggiae*. The existence of two clades is also supported by their morphological differentiation in adult and larval morphology. Comparison of phylogenetic relationships among *Anisakis* spp. with those currently available for their cetacean definitive hosts suggests parallelism between host and parasite phylogenetic tree topologies. Preliminary data for reconstruction of a possible co-evolutionary scenario between cetacean hosts and their *Anisakis* endoparasites suggests that cospeciation and host-switching events may have accompanied the evolution of this group of parasites. Finally, genetic/molecular markers for the identification of the so far genetically recognized taxa of *Anisakis* at any life-stage and both sexes were given also in relation to human anisakiosis is discussed.

KEY WORDS : *Anisakis*, allozyme markers, mtDNA *cox-2*, genetic relationships, intermediate/paratenic hosts, cetaceans, coevolution, anisakiosis.

Résumé : TAXONOMIE, PHYLOGÉNIE ET ÉCOLOGIE DES NÉMATODES DU GENRE *ANISAKIS* DUJARDIN, 1845 : MISE AU POINT

On présente les connaissances actuelles sur la taxonomie et l'écologie qui concerne la distribution géographique et les hôtes des neuf espèces du genre Anisakis *génétiquement identifiées à ce jour (i.e.* A. pegreffii, A. simplex s.s., A. simplex C, A. typica, A. ziphidarum, Anisakis *sp.,* A. physeteris, A. brevispiculata *et* A. paggiae*). On montre et compare la différentiation génétique et les relations phylogénétiques par l'analyse des divers marqueurs génétiques moléculaires (20 loci isoenzymatiques, et la succession des 629 paires de bases de* cox-2 *de l'ADN mitochondrial). Les résultats des deux analyses concordent : elles démontrent les relations phylogénétiques des espèces d'*Anisakis *étudiées jusqu'à présent. Les deux analyses ont mis en évidence deux principaux clusters, l'un qui comprend* A. pegreffii, A. simplex s.s., A. simplex C, A. typica, A ziphidarum, Anisakis *sp., et l'autre qui comprend* A. physeteris, A. brevispiculata *et* A. paggiae*. L'existence de deux clusters est démontrée grâce aussi à la différentiation morphologique à un stade larvaire et adulte. La comparaison des relations phylogénétiques entre les espèces d'*Anisakis *par rapport à celles de leurs hôtes définitifs (cétacés), connues jusqu'à présent, montre un parallélisme entre les topologies des relations phylogénétiques des hôtes et des parasites. Ces données préliminaires montrent, en effet, que des phénomènes co-évolutifs ont accompagné l'histoire évolutive de ce groupe de parasites. Enfin, on fournit des marqueurs génétiques moléculaires pour l'identification des espèces d'*Anisakis *à n'importe quel stade de développement des deux sexes ; cela sert aussi pour l'identification génétique des larves d'*Anisakis *qui sont la cause de l'anisakidose humaine.*

MOTS CLÉS : Anisakis, *isoenzyme, mtADN* cox-2, *relations phylogénétiques, hôte intermédiaire, cétacés, phénomènes co-évolutifs, anisakidose.*

INTRODUCTION

The nematodes of the genus *Anisakis* Dujardin, 1845 are parasites found in the stomach of marine mammals, especially cetaceans. These become infected by preying on paratenic and intermediate hosts, such as squids and fish, or directly on crustaceans (Smith & Wootten, 1978), that harbour larval stages of the parasite.

Of the 20 or so nominal species that have been described as parasites of cetaceans and pinnipeds from arctic, tropical, sub-antarctic and temperate regions of the world, Davey in his revision (1971) considered three species, only, as valid: *A. simplex, A. typica* and *A. physeteris*. Thus, several of the previously described species were considered by the author synonyms of the three above mentioned species, and the status of some remains unresolved, such as the case of *A. alexandri, A. schupakovi* and *A. dussumierii*. The systematics and the taxonomic status of the species belon-

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* Department of Public Health Sciences (DSSP), Section of Parasitology, University of Rome "La Sapienza", Ple Aldo Moro, 5, 00185 Rome, Italy.
** Department of Ecology and Sustainable Economic Development (DECOS), Tuscia University, Via S. Giovanni Decollato, 01100 Viterbo, Italy.
Correspondence: Simonetta Mattiucci.
Tel.: ++39 06 49914894 – Fax: ++39 06 49914644.
Email: simonetta.mattiucci@uniroma1.it

ging to the genus *Anisakis* thus became the subject of our past and ongoing studies using both genetic/molecular and morphological approaches. Indeed, the taxonomy of *Anisakis* species has traditionally relied on adult (According to Davey's revision, these included length and shape of ventriculus, length and shape of male spicules, and arrangements of male caudal papillae), and larval morphology (length of ventriculus, presence/absence of caudal spine) (Berland, 1961). In the past 18 years, our knowledge of the epidemiology of the parasites of the genus *Anisakis* has grown substantially with the development of the genetic/molecular techniques. These methodologies, in some cases combined with morphological studies, have demonstrated that the revision by Davey (1971) failed to recognize "true" species within the genus *Anisakis,* demonstrating that some of the morphospecies, considered in the past as cosmopolitan and euriecious species are, on the contrary, a complex of morphologically sibling, but reproductively isolated gene pools, thus corresponding to biological species. A prime example is *A. simplex* which, so far, contains three sibling species, *A. simplex s.s.* Nascetti *et al.*, 1986, *A. pegreffii* Campana-Rouget et Biocca, 1980 (Nascetti *et al.*, 1986), and *A. simplex* C (Mattiucci *et al.*, 1997). These members are genetically characterized at nuclear level by allozyme markers (Nascetti *et al.*, 1986; Mattiucci *et al.*, 1997), by RFLP of ITS-DNA (D'Amelio *et al.*, 2000; Nadler *et al.*, 2006), and at mitochondrial level, by mtDNA *cox-2* sequence analyses (Valentini *et al.,* 2006). The same genetic (allozyme) markers have been very useful in the disclosing of new species, such as *A. ziphidarum* Paggi *et al.*, 1998a and *A. paggiae* Mattiucci *et al.*, 2005, and in genetically characterizing *A. typica* from various hosts and geographic areas (Mattiucci *et al.*, 2002). The discovery of a complex of sibling species within *A. physeteris* Baylis, 1920 are analogous results. Adult specimens morphologically recognized as *A. physeteris* were genetically characterized on the base of several allozyme markers (Mattiucci *et al.*, 1986) and reproductive isolation from *A. brevispiculata* Dollfus, 1968 sympatrycally detected in the same definitive host, the pigmy sperm whale, was demonstrated (Mattiucci *et al.,* 2001), thus it was possible to define the taxonomic status of *A. brevispiculata,* a species considered by Davey (1971) as a synonym of *A. physeteris*. In addition, the new species, *A. paggiae* (Mattiucci *et al.*, 2005) was found to belong to the same complex of species (Valentini *et al.*, 2006). The same markers (allozymes) have demonstrated that *A. simplex s.s., A. pegreffii, A. simplex* C, *A. typica* and *A. ziphidarum* are Type I larval morphotype, while *A. physeteris, A. brevispiculata* and *A. paggiae* are Type II (Orecchia *et al.,* 1986; Mattiucci *et al.*, 2002, 2005).

Herein, we present the current understanding of the taxonomy and molecular systematics of this group and review information on their ecology. Indeed, genetic (nuclear and mitochondrial markers) and ecological data on the species of *Anisakis* so far recognized, are reported and summarized. The relevance of some morphological characters at both adult and larval stages, after the recognition of biological species by genetic markers within the genus *Anisakis* is discussed, and larval identifications are provided. Hosts (definitive and intermediate) of the species of *Anisakis* genetically recognised are listed, as are the geographic ranges so far recorded. Host-parasite association coevolutionary aspects between *Anisakis* spp. and their cetacean hosts are detected.

MOLECULAR SYSTEMATICS OF *ANISAKIS* SPP.

The inconsistency in morphological characters of *Anisakis* species impeded development of a credible scheme of their phylogeny. This prompted the need to classify these nematodes by genetic and/or biochemical methods. Thus, beginning in the late 1980s, researchers started to evaluate their taxonomy, and genetic differentiation and relationships between taxa of this genus (Nascetti *et al.*, 1986; Mattiucci *et al.*, 1986; Nadler *et al.*, 1990, 1995).

Today, the existence of two main clades is clearly shown, by genetic studies, in the genus *Anisakis*: one encompassing the species showing the larval stage indicated as *Anisakis* Type I (*sensu* Berland, 1961), and a second sharing the larval morphology *Anisakis* Type II (*sensu* Berland, 1961). The first clade includes the species of *A. simplex* complex (i.e. *A. simplex s.s., A. pegreffii, A. simplex* C), *A. typica*, *A. ziphidarum* and *Anisakis* sp. The second includes the species *A. physeteris, A. brevispiculata* and *A. paggiae* (Mattiucci *et al.*, 2005; Valentini *et al.*, 2006).

GENETIC DIFFERENTIATION AND PHYLOGENETIC RELATIONSHIPS OF THE SPECIES OF *ANISAKIS*

At allozyme level, the values of Nei's (1972) standard genetic distance (D_{Nei}) based on allele frequencies are given in Table I. At the interspecific level, among the nine genetically characterized species of *Anisakis*, the highest genetic identity was observed between the three sibling species of the *A. simplex* complex (average I_{Nei} = 0.68, range 0.66-0.70). Values of I_{Nei} ranging from 0.001 to 0.034 were found when comparing *A. physeteris* and *A. brevispiculata* with either the *A. simplex* complex (average I_{Nei} = 0.003, range 0.001-0.012) or *A. typica* (average I_{Nei} = 0.019, range 0.008-0.034). *A. paggiae* showed the lowest level of genetic differentiation with respect to *A. brevispiculata* D_{Nei} = 0.79, rather than to *A. physeteris* D_{Nei} = 1.06; while *Ani-*

sakis sp. was found genetically correlated to *A. ziphidarum* D_{Nei} = 0.68, however, well distinct from it, with which its was detected in sympatry in the same definitive hosts (Table I).

At mtDNA level, genetic divergence among the considered taxa, estimated by *p-distance*, inferred from sequence analysis (629 bp) of the *cox2* gene ranged from p = 0.055 between sibling species of the *A. simplex* complex, to p = 0.12, between morphologically differentiated species, i.e. *A. ziphidarum* and *A. typica* (Table I). The highest level was detected when comparing *A. physeteris, A. brevispiculata,* and *A. paggiae versus A. simplex* complex (on average p = 0.13) or versus *A. typica* (on average p = 0.14). Sequence data from the newly identified *Anisakis* sp. poorly aligned with other *Anisakis* species but was most similar to *A. ziphidarum* (p = 0.08) (Valentini *et al.*, 2006) (Table I).

To date, most work performed on nematode phylogenetic studies within this genus, has been done using unweighted pair group method using arithmetic averages (UPGMA-based methods). Among the first extensive dendrograms, were those generated by Mattiucci *et al*, 1997 and Paggi *et al.*, 1998a. Recently (Mattiucci *et al.*, 2005), distance methods based on Neighbour-Joining (NJ) analysis of the allozyme data, provided trees showing the same topology to those generated by UPGMA. The phenetic clustering (UPGMA) inferred from Genetic Identity (I_{Nei}, Nei 1972) values from allozyme data (20 enzyme-loci), depicting the genetic relationships among the nine taxa of *Anisakis,* is reported in Fig. 1A. It showed remarkable congruence in supporting a topology of the species so far included in the genus *Anisakis*, showing the existence of two main clades within the genus, thus demonstrating a strong genetic heterogeneity within the genus.

Recently (Valentini *et al*, 2006), trees produced by methods employing parsimony (MP) analysis (Fig. 1B), Neighbour-Joining (NJ) and Bayesian analysis (BA) as well, inferred from the *mtDNA cox2* gene sequences of the all currently recognised species of *Anisakis*, also showed high congruence with the UPGMA and NJ trees from allozyme data analysis, and bootstrap analysis highly supported the topology obtained. Indeed, mitochondrial and allozyme data analyses showed remarkable congruence in depicting the existence of the two clades with that including *A. physeteris, A. brevispiculata* and *A. paggiae* placed at the base of the tree (Fig. 1). However, they raised questions about the position occupied by *A. typica* within the first clade. In fact, some discrepancies among the phylogenetic analyses resulted in the unresolved placement of *A. typica* within either a subclade with *A. ziphidarum* and *Anisakis* sp., or within a clade containing the *A. simplex* complex (Fig. 1A and B). However, low bootstrap values appeared in all the trees suggesting that further analyses on *A. typica* collected from more individuals, other geographic areas, and perhaps additional hosts are needed to clarify its phylogenetic position with respect to the species of *A. simplex* complex and *A. ziphidarum*. It should be noted, however, that its placement within the clade 1 as the *A. simplex* complex is always supported (Valentini *et al.*, 2006).

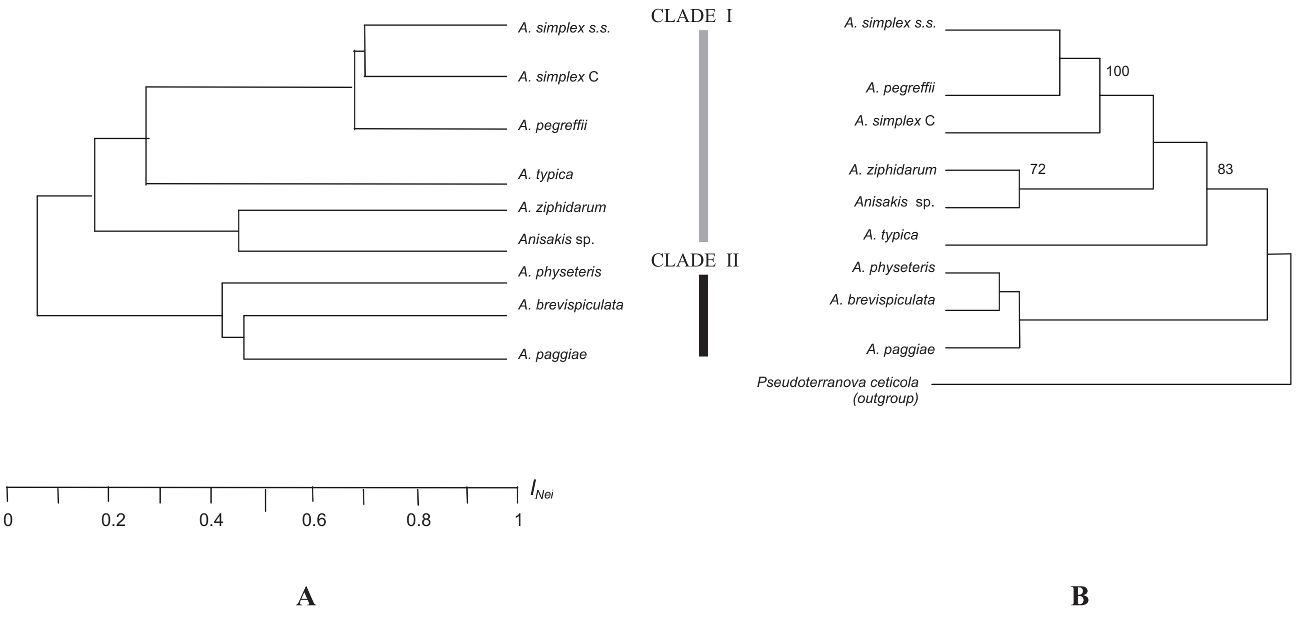

Fig. 1. – Genetic relationships among *Anisakis* spp. depicted by: A) UPGMA tree, based on I_{Nei} (Nei, 1972) values from allozyme data (by BIOSYS-2, Software, Swofford & Selander, 1989); B) Strict consensus tree of Maximum Parsimony (MP) analysis inferred from mtDNA *cox2*, over 71 most parsimonious trees (CI = 0.5725, HI = 0.4275, RI = 0.8842, RC = 0.5062) (187 parsimony-informative characters). Bootstrap values were calculated over 1,000 replicates; percentages ≥ 70 % are shown at the internal nodes.

	A. simplex ss.	**A. pegreffii**	**A. simplex C**	**A. typica**	**A. ziphidarum**	**Anisakis sp.**	**A. physeteris**	**A. brevispiculata**	**A. paggiae**
A. simplex s.s.	*0.010*	0.04	0.06	0.13	0.11	0.12	0.13	0.15	0.13
A. pegreffii	0.40 (0.38-0.42)	*0.001*	0.06	0.12	0.11	0.12	0.13	0.15	0.13
A. simplex C	0.36 (0.360-0.364)	0.37 (0.36-0.37)	–	0.12	0.12	0.12	0.13	0.15	0.13
A typica	1.16 (1.13-1.19)	1.45 (1.44-1.45)	1.14	–	0.12	0.11	0.13	0.16	0.14
A. ziphidarum	1.64 (1.57-1.70)	1.99 (1.98-2.00)	1.62 (1.60-1.69)	1.67	*0.004*	0.08	0.12	0.13	0.10
Anisakis sp.	2.04 (1.98-2.08)	2.63 (2.61-2.64)	1.92 (1.82-2.02)	1.62 (1.58-1.65)	0.68 (0.67-0.68)	*0.002*	0.14	0.14	0.12
A. physeteris	6.90 (6.24-8.13)	8.30 (7.25-9.17)	7.40 (7.37-7.43)	4.77 (4.76-4.79)	∞	∞	*0.002*	0.10	0.11
A. brevispiculata	4.10 (4.39-5.03)	6.11 (5.56-6.43)	5.54 (5.10-5.97)	3.49 (3.39-3.58)	∞	∞	0.95 (0.93-0.96)	*0.014* (0.76-0.83)	0.11
A. paggiae	∞	∞	∞	∞	∞	∞	1.06 (1.08-1.06)	0.79 (0.76-0.83	0.002
A. simplex s.s.	0.02 (± 0.00)	–	–	–	–	–	–	–	–
A. pegreffii	0.04 (± 0.01)	0.01 (± 0.00)	–	–	–	–	–	–	–
A. simplex C	0.06 (± 0.01)	0.06 (± 0.01)	0.01 (± 0.00)	–	–	–	–	–	–
A. typica	0.13 (± 0.01)	0.12 (± 0.01)	0.12 (± 0.01)	0.01 (± 0.00)	–	–	–	–	–
A. ziphidarum	0.11 (± 0.01)	0.11 (± 0.01)	0.12 (± 0.01)	0.12 (± 0.01)	0.01 (± 0.00)	–	–	–	–
Anisakis sp.	0.12 (± 0.01)	0.12 (± 0.01)	0.12 (± 0.01)	0.11 (± 0.01)	0.08 (± 0.01)	0.01 (± 0.00)	–	–	–
A. physeteris	0.13 (± 0.01)	0.13 (± 0.01)	0.13 (± 0.01)	0.13 (± 0.01)	0.12 (± 0.01)	0.14 (± 0.01)	0.01 (± 0.00)	–	–
A. brevispiculata	0.15 (± 0.01)	0.15 (± 0.01)	0.15 (± 0.01)	0.16 (± 0.01)	0.13 (± 0.01)	0.14 (± 0.01)	0.10 (± 0.01)	0.02 (± 0.00)	–
A. paggiae	0.13 (± 0.01)	0.13 (± 0.01)	0.13 (± 0.01)	0.14 (± 0.01)	0.10 (± 0.01)	0.12 (± 0.01)	0.11 (± 0.01)	0.11 (± 0.01)	0.01 (± 0.00)

Table I. – Average and ranges of Nei's (1972) values of standard genetic distance (D_{Nei}, Nei, 1972, below the diagonal) between the species of *Anisakis* so far genetically studied. D_{Nei}, intra-specific values are given along the diagonal (ranges in parenthesis). *p*-distance (above the diagonal) values inferred from 629 bp mtDNA *cox2* performed using MEGA 2.1 program (Kumar *et al.*, 2001). Data from Mattiucci *et al.*, 2005, Valentini *et al.*, 2006, and present paper.

ECOLOGICAL DATA OF *ANISAKIS* SPECIES

ANISAKIS SPP. INCLUDED IN CLADE 1

According to the genetic data, five species (*A. simplex sensu stricto, A. pegreffii, A. simplex* C, *A. typica, A. ziphidarum*) are included in this clade, and one new gene pool recently evidenced, and indicated as *Anisakis* sp. (Valentini *et al.,* 2006 and our data not published). A synopsis of ecological aspects of each species, including host preference and geographical aspects, is presented below.

The *Anisakis simplex* complex

Three species are so far included in the *A. simplex* complex: they are *A. simplex s.s.*, *A. pegreffii*, *A. simplex* C. Their definitive hosts (cetaceans) and intermediate/paratenic (squids and fish) identified to date are listed in Tables II and III.

- *A. simplex s.s.* (Nascetti *et al.,* 1986)

A. simplex s.s. is widespread between 35° N and the Arctic Polar Circle; it is present in both the western and eastern Atlantic and both western and eastern waters of the Pacific Ocean (Mattiucci *et al.,* 1997; 1998; Paggi *et al.*, 1998b; Abollo *et al.*, 2001) (Fig. 2). The southern limit of this species in the north east Atlantic Ocean are the waters around the Gibraltar area. *A. simplex s.s.* is occasionally present also in the western part of the Mediterranean waters due to the migration of pelagic fish species in the far western Mediterranean Sea waters (Alboran Sea) (Mattiucci *et al.*, 2004; and 2006) (Fig. 2). *A. simplex s.s.* has been so far recorded in nine species of cetacean hosts. Four squid and 26 fish species were so far found harbouring larvae of this species along its geographical range. A sympatric area between *A. simplex s.s.* and *A. pegreffii* was identified along the Spanish and Portuguese Atlantic coast (Mattiucci *et al.,* 1997, 2004, 2006; Abollo *et al.*, 2001) and in the Alboran Sea (Mattiucci *et al.*, 2004, 2006). *A. simplex s.s.* also occurs with *A. simplex* C in the eastern Pacific Atlantic Ocean, where it has been identified in some definitive hosts along the East Pacific coast (Mattiucci *et al.*, 1997, 1998; Paggi *et al.*, 1998) (Tables II, III and Fig. 2). Although it has sympatric and syntopic occurrence in mixed infections at both larval and adult stages with other *Anisakis* species (Mattiucci *et al.,* 2004, 2005), reproductive isolation between *A. simplex s.s.* and both *A. pegreffii* and *A. simplex* C was proved by the lack of adult F1 hybrids, and/or backcross genotypes clearly demonstrated at nuclear level (Mattiucci *et al.*, 1997, 2005).

- *A. pegreffii* (Nascetti *et al.*, 1986)

Previously indicated as *A. simplex* A (Nascetti *et al.*, 1986), *A. pegreffii* is the dominant species of the genus *Anisakis* in the Mediterranean Sea, being widespread in all the fish species so far examined. Indeed, it is presently the most important aetiological agent of infection in pelagic and demersal fish of the Mediterranean waters. It appears also widely distributed in the Austral Region between 35° N and 55° S. In the Atlantic waters its upper limit of geographical range is represented by the Iberian coast of the NE Atlantic (Mattiucci *et al.*, 1997, 2004; Abollo *et al.*, 2001). It has been not reported so far from the west part of Atlantic Ocean

	A. simplex s.s.					***A. pegreffii***			***A. simplex C***	
LOCATION CODE	EA	IC	WA	EP	SA	CM	IC	SA	EP	SA
DEFINITIVE HOST SPECIES										
Cetaceans										
Neobalaenidae										
Caperea marginata	--	--	--	--	--	--	--	•	--	--
Balaenopteridae										
Balaenoptera acutorostrata	•	--	--	--	--	--	--	--	--	--
Monodontidae										
Delphinapterus leucas	--	--	•	--	--	--	--	--	--	--
Delphinidae										
Delphinus delphis	--	•	--	--	--	--	•	--	--	--
Globicephala melaena	--	•	--	--	•	--	--	--	--	•
Lagenorhynchus albirostris	•	--	--	--	--	--	--	--	--	--
Lissodelphis borealis	--	--	--	--	--	--	--	--	–	--
Orcinus orca	--	--	--	•	--	--	--	--	--	--
Pseudorca crassidens	--	--	--	•	--	--	--	--	•	--
Stenella coeruleoalba	--	•	--	--	--	--	--	--	--	--
Tursiops truncatus	--	--	--	--	--	•	--	•	--	--
Phocoenidae										
Phocoena phocoena	--	--	--	•	--	--	--	--	--	--

Table II. – Definitive hosts so far evidenced for the species of *A. simplex* complex (data from Nascetti *et al.*, 1986; Mattiucci *et al.*, 1997, 1998, 2005; Paggi *et al.*, 1998c, and present paper). Codes: EA: North-east Atlantic; IC: Iberian Atlantic Coast; WA: West Atlantic; EP: North-east Pacific; CM: Central Mediterranean Sea; SA: South Africa.

	A. simplex s.s.												***A. pegreffii***											***A. simplex* C**		
LOCATION CODE	EA	SI	BS	IC	WA	EP	JA	BE	SA	MA	WM	AZ	CM	EM	WM	IC	EA	FA	SA	MA	NZ	AZ	BR	NZ	TA	SA
INTERMEDIATE HOST SPECIES																										
Cephalopods																										
Sepiidae																										
Sepia officinalis	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ommastrephidae																										
Todaropsis eblanae	--	--	--	•	--	--	--	--	•	--	--	--	--	--	--	•	--	--	•	--	--	--	--	--	--	--
Ommastrephes sagittatus	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ommastrephes angolensis	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Illex coindettii	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fish																										
Pleuronectidae																										
Hippoglossus hippoglossus	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Scophthalmidae																										
Lepidorhombus boscii	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--
Bramidae																										
Brama brama	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Carangidae																										
Trachurus capensis	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Trachurus mediterraneus	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Trachurus picturatus	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	•	--	--	--	--
Trachurus trachurus	•	--	--	•	--	--	--	--	--	•	•	--	•	•	•	•	--	--	--	•	•	--	--	--	--	--
Emmelichthyidae																										
Emmelichthys nitidus nitidus	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Gempylidae																										
Thyrsites atun	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	•
Pinguipedidae																										
Parapercis colias	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	•	--	--
Scombridae																										
Scomber japonicus	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	•	--	--	--	--
Scomber scombrus	•	--	--	•	--	--	--	--	--	--	--	--	•	--	--	•	--	--	--	--	--	--	--	--	--	--
Thunnus thynnus	--	--	--	--	--	--	•	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	•	--	--	--
Sparidae																										
Spondyliosoma cantharus	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Trichiuridae																										
Lepidopus caudatus	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	•	--	--	--	--	--	--	--
Xiphiidae																										
Xiphias gladius	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Scorpaenidae																										
Scorpaena scrofa	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--
Sebastidae																										
Helicolenus dactylopterus	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	•	--	--	--	--	--	--	--
Triglidae																										
Eutrigla gurnardus	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Belonidae																										
Belone belone	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--

(continues)

(continued)

	A. simplex s.s.												**A. pegreffii**											**A. simplex C**		
Location code	EA	SI	BS	IC	WA	EP	JA	BE	SA	MA	WM	AZ	CM	EM	WM	IC	EA	FA	SA	MA	NZ	AZ	BR	NZ	TA	SA
Scomberesocidae																										
Scomberesox saurus	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Gadidae																										
Boreogadus saida	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Gadus morhua	--	--	•	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Micromesistius poutassou	--	--	--	•	--	--	--	--	--	--	--	--	•	--	--	•	--	--	--	--	--	--	--	--	--	--
Theragra chalcogramma	--	--	--	--	--	•	•	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Trisopterus luscus	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Lotidae																										
Molva dypterygia	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Brosme brosme	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Merluccidae																										
Merluccius capensis	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Merluccius hubbsi	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--
Merluccius merluccius	•	--	--	•	--	--	--	--	--	•	--	--	•	•	•	•	•	--	--	•	--	--	--	--	--	--
Moridae																										
Pseudophycis bachus	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	•	--	--
Ophidiidae																										
Genypterus capensis	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Lophiidae																										
Lophius piscatorius	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Lophius vomerinus	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Trachichthyidae																										
Hoplostethus atlanticus	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--
Hoplostethus mediterraneus	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Salmonidae																										
Oncorhynchus gorbuscha	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Oncorhynchus keta	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Salmo salar	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Clupeidae																										
Clupea harengus	•	--	•	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Etrumeus whiteheadi	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Engraulidae																										
Engraulis encrasicolus	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Congridae																										
Conger conger	--	--	--	•	--	--	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--

Data from: Nascetti *et al.*, 1986; Mattiucci *et al.*, 1997; Paggi *et al.*, 1998c; Mattiucci *et al.*, 2004, 2006; and present paper.

Table III. – Intermediate host species so far detected for the species of the *A. simplex* complex. Codes: EA: North-east Atlantic; BS: Baltic Sea; IC: Iberian Atlantic Coast; WM: West Mediterranean; WA: West Atlantic; EP: North-east Pacific; JA: Japan Sea; SI: Sakhalin Islands; BE : Bering Sea; MA: Mauritanian Coast; AZ: Azores Islands; CM: Central Mediterranean Sea; FA: Falkland Islands; SA: South Africa; NZ: New Zealand; EM: East Mediterranean Sea; BR: Brazil Atlantic Coast; TA: Tasman Sea.

(our unpublished data) (Fig. 2). The genetic homogeneity between Mediterranean populations and those from Austral region seems to be maintained by the high levels of gene flow observed in this species (*Nm* = 15.0), allowing the hypothesis of its wide occurrence also in other areas of the southern hemisphere. To date, it has been recorded as parasite at adult stage in three species of oceanic dolphins as definitive hosts, in 28 species of fish, and in two squids (Tables II, III). Among them, two definitive and 11 intermediate were found to be shared with *A. simplex s.s.*, in the contact area of the Iberian Atlantic coast waters (Fig. 2). Whereas, two definitive and five intermediate hosts are shared by *A. pegreffii* and *A. simplex* C, in the austral region of New Zealand waters, the South African coast and the Southern Pacific Chilean coast (Table III and Fig. 2).

- *A. simplex* C Mattiucci *et al.,* 1997

A. simplex C shows, to date, a discontinuous range, including Pacific Canada, Chile, New Zealand waters, and the Atlantic South African coast. *A. simplex* C was identified so far at adult stage from three marine mammals and at larval stages it syntopically occurred with *A. pegreffii* in five fish species (Tables II, III and Fig. 2).

- *A. typica* (Diesing, 1860)

According to the data from *A. typica* genetically studied, its range extends from 30° S to 35° N in warmer temperate and tropical waters (Table IV and Fig. 2) (Mattiucci *et al.*, 2002). In these areas it was found at adult stage in six dolphin species and at larval stages in 10 fish species. *A. typica* was recently identified also in the striped dolphin, *Stenella coeruleoalba* and in the European hake, *Merluccius merluccius* from the eastern Mediterranean Sea (Cyprus). Its presence in these waters could be the result of "lesseptian migration" (through the Suez Channel) (Mattiucci *et al.,* 2004) of its intermediate hosts from the Indian Ocean. Indeed, it is the only species to date responsible for the infections in fish species of these waters (Table IV and Fig. 2).

- *A. ziphidarum* Paggi *et al.*, 1998

A. ziphidarum was detected in the beaked whales, *Mesoplodon layardii* and *Ziphius cavirostris* from the South Atlantic Ocean (South Africa coast). Subsequently,

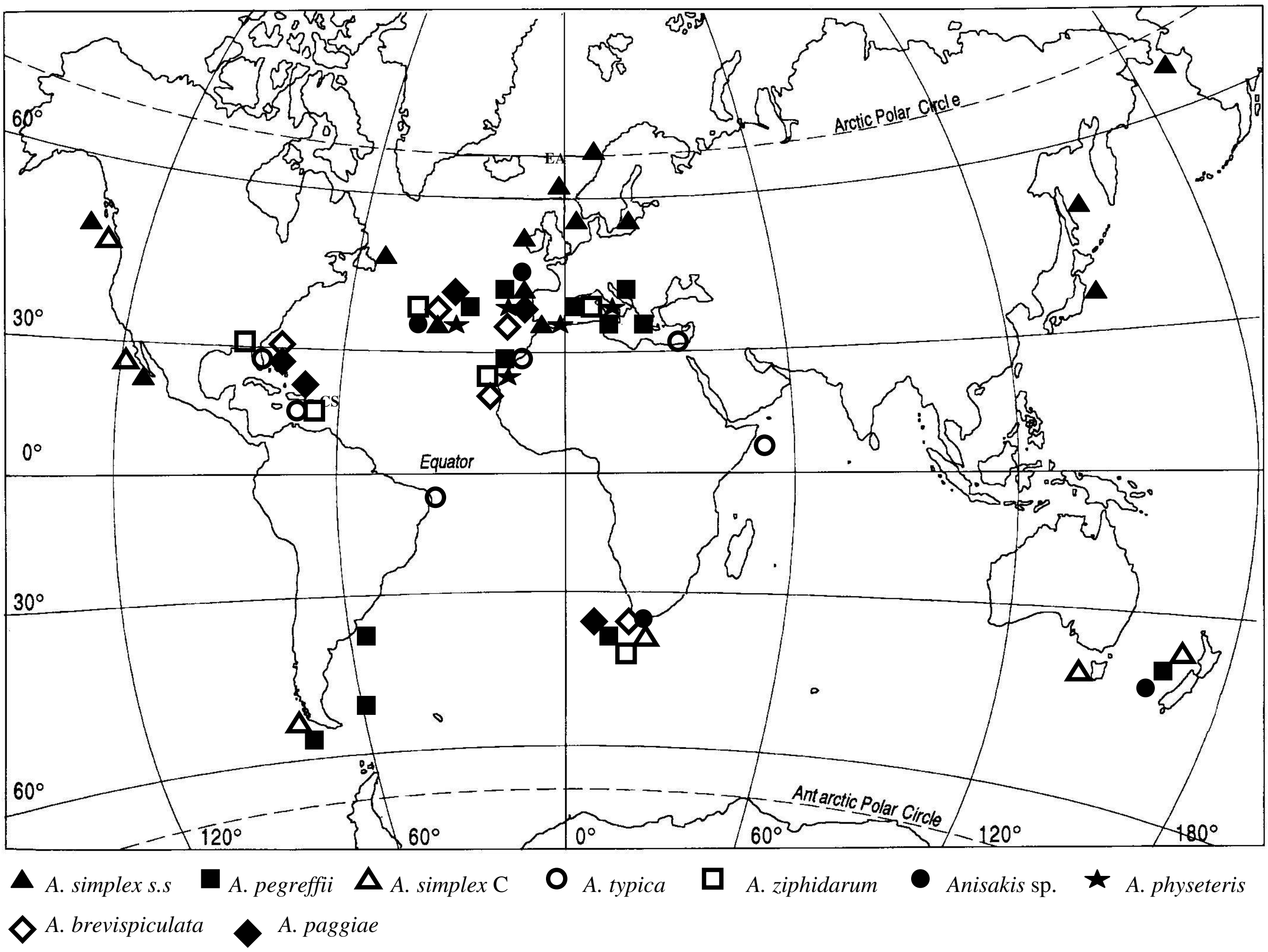

Fig. 2. – Geographical range of *Anisakis* spp. genetically characterised.

	A. typica							***A. ziphidarum***					***Anisakis* sp.**			
LOCATION CODE	BR	AZ	SC	FL	CS	MA	EM	MA	AZ	CM	SA	FL	AZ	SA	NZ	IC
DEFINITIVE HOST SPECIES																
Cetaceans																
Ziphiidae																
Mesoplodon densirostris	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--
Mesoplodon europaeus	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--
Mesoplodon grayi	--	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--
Mesoplodon layardii	--	--	--	--	--	--	--	--	--	--	•	--	--	--	--	--
Mesoplodon mirus	--	--	--	--	--	--	--	--	--	--	--	--	--	•	•	--
Ziphius cavirostris	--	--	--	--	--	--	--	--	--	•	•	--	--	--	--	--
Delphinidae																
Globicephala macrorhynchus	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--
Sotalia fluviatilis	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Stenella attenuata	--	--	--	•	•	--	--	--	--	--	--	--	--	--	--	--
Stenella coeruleoalba	--	--	--	--	--	--	•	--	--	--	--	--	--	--	--	--
Steno bredanensis	--	--	--	--	•	--	--	--	--	--	--	--	--	--	--	--
Tursiops truncatus	--	--	--	•	•	--	--	--	--	--	--	--	--	--	--	--
INTERMEDIATE HOST SPECIES																
Fish																
Carangidae																
Trachurus picturatus	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Trachurus trachurus	--	--	--	--	--	--	•	--	--	--	--	--	--	--	•	--
Coryphaenidae																
Coryphaena hippurus	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Scombridae																
Auxis thazard	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Euthynnus affinis	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Sarda orientalis	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Scomber japonicus	--	•	--	--	--	--	--	--	•	--	--	--	--	--	--	--
Scomberomorus commerson	--	--	•	--	--	--	--	--	--	--	--	--	--	--	--	--
Thunnus thynnus	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Trichiuridae																
Aphanopus carbo	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--	--
Merluccidae																
Merluccius merluccius	--	--	--	--	--	•	•	•	--	--	--	--	--	--	--	•

Table IV. – Definitive and intermediate host species so far detected for *A. typica*, *A. ziphidarum* and *Anisakis* sp. (Data from Mattiucci *et al.*, 2002; Paggi *et al.*, 1998a, and present paper). Codes: IC: Iberian Atlantic Coast; MA: Mauritanian Coast; AZ: Azores Islands; SA: South Africa; EM: East Mediterranean Sea; BR: Brazil Atlantic Coast; SC: Somali Coast; NZ: New Zealand; FL: Florida Coast; CS: Caribbean Sea.

it was also recorded in the Mediterranean Sea, also parasite of *Z. cavirostris*. Since its first morphological description and genetic characterization, it has been recently genetically identified at adult stage also in other species of beaked whale, such as *M. mirus* and *M. grayi* in south Atlantic waters and in *Mesoplodon* sp. and *Ziphius cavirostris* from the Caribbean waters. Thus, its geographical range seems to be wide (Fig. 2) and related to that of its definitive hosts. Scanty data are so far available concerning its infection in fish and/or squid. It is responsible for the low prevalence of infection in some fish species (Mattiucci *et al.,* 2004) that are reported in Table IV.

- *Anisakis* sp. Valentini *et al.*, 2006

Anisakis sp. has been detected only at larval (L4) stage in the beaked whales *Mesoplodon mirus* and *M. grayi* from South African and New Zealand waters (Table IV and Fig. 2). This gene pool has been found reproductively isolated from the sympatric species *A. ziphidarum* occurring in the same hosts and geographic location. It is considered more closely related to *A. ziphidarum* rather than to the other species so far genetically characterized. Although evidenced only at adult stage, the third stage larva of this so far undescribed taxon shares the morphotype Type I, and it was rarely identified in some fish species of North East Atlantic waters (data not published).

ANISAKIS SPP. INCLUDED IN CLADE 2

Three species of *Anisakis* share so far, at larval stage, the morphology known as Type II (*sensu* Berland, 1961). These species represent a complex of sibling species that could be genetically well recognised at both nuclear and mitochondrial level. They are *A. physeteris*, *A. brevispiculata* and *A. paggiae*. A summary of each follows.

- *A. physeteris* (Baylis, 1920)

Its main definitive host is the sperm whale, *Physeter macrocephalus*; no adults genetically identified have been recorded in other cetacean hosts. Type II larvae of A. *physeteris* were genetically identified in very few host species and rarely occurring out of those examined during the study for *Anisakis* spp., thus suggesting that other intermediate hosts (mainly squid) are involved in the life-cycle of this parasite (Mattiucci *et al.*, 2001, 2004).

- *A. brevispiculata* Dollfus, 1966

A. physeteris was found genetically well distinct and reproductively isolated (D_{Nei} = 0.80) from the species *A. brevispiculata* (synonymised by Davey, 1971).

- *A. paggiae* Mattiucci *et al.*, 2005

To the same cluster recently it has been recently demonstrated by both nuclear (Mattiucci *et al.*, 2005) and mitochondrial markers (Valentini *et al.*, 2006) to belong the species *A. paggiae*, whose genetic differentiation with respect to *A. physeteris* and *A. brevispiculata* was respectively, D_{Nei} = 1.0 and D_{Nei} = 0.79. *A. paggiae* was found as parasite, as adults, the pygmy sperm whale, *Kogia breviceps*, and of the dwarf sperm whale, *K. sima* (Table V) from both Florida and the south African Atlantic coast. Scanty data are so far available to identify the intermediate hosts in the life cycle of *A. paggiae* and *A. brevispiculata*. Very few larvae of Type II have been identified as belonging to these species in fish from Atlantic waters (Table V), thus suggesting that other hosts, not yet detected, are involved in the life-cycles of these *Anisakis* species. Some morphological characters of diagnostic value available in male and female adult specimens were found to help in distinguishing *A. paggiae* from *A. physeteris* and *A. brevispiculata* (Mattiucci *et al.*, 2005).

RECONCILIATION OF GENETICS AND MORPHOLOGY

The high genetic heterogeneity of the *Anisakis* spp. is supported by morphology of the species belonging to this genus as well, where two major clades can be delineated as follows: *i)* the ventriculus, at adult stage, is short, never sigmoid and broader than long in the species *A. physeteris, A. brevispiculata* and *A. paggiae* (Mattiucci *et al.*, 2005), and longer than broad and often sigmoid in shape in the species included in clade 1; *ii)* male spicules that are short, stout and of similar length can be observed in *A. physeteris, A. brevispiculata* and *A. paggiae* (Mattiucci *et al.,* 2005), long and often unequal (equal in *A. ziphidarum*, see Paggi *et al.,* 1998) in clade 1; *iii)* Type II larval morphology (*sensu* Berland, 1961) is characteristic of *A. physeteris, A. brevispiculata* and *A. paggiae* (Mattiucci *et al.,* 2001, 2004, 2005) (clade 2), whereas Type I morphology (*sensu* Berland, 1961) can be found in the species of the *A. simplex* complex, *A. typica, A. ziphidarum* and *Anisakis* sp. (clade 1).

The species of the *A. simplex* complex are so far morphologically indistinguishable at both adult and larval

	A. physeteris						***A. brevispiculata***					***A. paggiae***			
Location code	CM	MA	WM	IC	AZ	EM	SA	IC	FL	MA	AZ	SA	FL	IC	AZ
Definitive host species															
Cetaceans															
Physeteridae															
Physeter macrocephalus	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Kogiidae															
Kogia breviceps	--	--	--	--	--	--	•	•	•	--	--	•	•	--	--
Kogia sima	--	--	--	--	--	--	--	--	--	--	--	--	•	--	--
Intermediate host species															
Cephalopods															
Ommastrephidae															
Ommastrephes sagittatus	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fish															
Trichiuridae															
Aphanopus carbo	--	--	--	--	•	--	--	--	--	--	•	--	--	--	•
Xiphiidae															
Xiphias gladius	•	--	--	•	--	--	--	--	--	--	--	--	--	--	--
Carangidae															
Trachurus trachurus	•	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Merluccidae															
Merluccius merluccius	•	•	•	•	--	•	--	--	--	•	--	--	--	•	--

Table V. – Definitive and intermediate hosts species so far detected for *A. physeteris*, *A. brevispiculata* and *A. paggiae.* (Data from Mattiucci *et al.*, 1986, 2001, 2005, and present paper). Codes: CM: Central Mediterranean sea; MA: Mauritanian Coast; WM: west Mediterranean sea; IC: Iberian Atlantic coast; AZ: Azores Island; BR: Brazil Atlantic coast; SA: South African coast; FL: Florida coast; EM: east Mediterranean sea.

stage; consequently, only genetic and molecular methods can be used reliably to identify them at all the developmental stages. On the contrary, both *A. typica* and *A. ziphidarum* are distinguishable at their male adult stage, but not so far at larval stage (Mattiucci *et al.*, 2002; Paggi *et al.*, 1998). A morphological key for the recognition of adult specimens of the species included in clade 2 (i.e. *A. physeteris, A. brevispiculata* and *A. paggiae*) was also given in Mattiucci *et al.*, 2005.

HOST-PARASITE ASSOCIATION AND COEVOLUTION

The presence of two main clusters in the genus *Anisakis* is supported also by ecological data and specific host-parasite relationships. The sperm whales (i.e. *Physeteris catodon*, *Kogia breviceps* and *K. sima*) are hosts so far recorded for *A. physeteris, A. brevispiculata* and *A. paggiae* (Table V) those worms included in the second clade (Fig. 2). These hosts were never found parasitized by other species of *Anisakis* spp. The sole exception so far, is one individual of *P. macrocephalus* from the Mediterranean Sea, found harbouring three adult specimens of *A. pegreffii* out of the 320 specimens of *A. physeteris* genetically identified in syntopy. Oceanic dolphins in the Delphinidae, Arctic dolphins in the Monodontidae, and porpoises in the Phocoenidae are hosts of the species of the *A. simplex* complex and of *A. typica* (Mattiucci *et al.*, 1997, 1998, 2002, 2005).

The beaked whales *Ziphius cavirostris, Mesoplodon layardii, M. mirus* and *M. grayi* are hosts of *A. ziphidarum* (Paggi *et al.*, 1998 and present data) and *Anisakis* sp., both partitioned into the second clade. Moreover, although some *Anisakis* spp., such as *A. ziphidarum* and *A. paggiae* and/or *A. brevispiculata* are found in the same sympatric areas, as the warm temperate tropical water basin, such as the Caribbean and Florida coasts of the Atlantic Ocean, however they were never identified in the same cetacean species (Tables IV, V and Fig. 2)

Phylogenetic relationships proposed elsewhere (Valentini *et al.*, 2006) and reviewed here for species of genus *Anisakis* seem to align with that of their cetacean hosts (Milinkovitch, 1995; Nikaido *et al.,* 2001) (Fig. 3). The phylogeny of cetaceans proposed by Milinkovitch (1995) based on mtDNA (12S, 16S, and *cytb* partial sequences) and myoglobin sequences, and by Nikaido *et al.* (2001) based on retroposon analysis indicate a branching order of the cetacean lineages where the sperm whale and the pygmy sperm whales (Physeteridae and Kogiidae) represent basal taxa, followed by the beaked whales, and freshwater and marine dolphins as the most derived ones.

In accordance with that analysis, the branching order proposed for the *Anisakis* taxa showed that nematodes from the sperm whale and the pygmy sperm whales (*A. physeteris, A. brevispiculata* and *A. paggiae*) always occupy a basal lineage followed by those parasitizing the beaked whales (*A. ziphidarum* and *Anisakis* sp.) (Fig. 3). The species from the "oceanic dolphins" (the definitive hosts of the *A. simplex* complex) consistently appear as the most derived ones, suggesting some level of parallelism or that co-evolutionary events could have accompanied the speciation of these endoparasitic nematodes and their definitive hosts (Fig. 3).

Clearly, a broader dataset is needed to confirm co-speciation and/or host-switching events.

CONCLUDING REMARKS

From the evaluation of almost 20 years of genetic data on these nematodes, several facts have consistently emerged in most *Anisakis* phylogenetic trees, supported so far by allozymes and mitochondrial markers.

First, an overall high congruence was found between the tree topologies obtained from the mitochondrial datasets, and the phenetic clustering gathered from nuclear datasets (allozyme data) (as shown in Fig. 1). Second, that there are ecological, genetic/molecular, and some morphological distinctions between the *Anisakis* species showing larval morphotype I and those having morphotype II, although the monophyly depicted by the tree topology of the first group is not yet solved. However, there is a strong bootstrap support for monophyly among the species of the *Anisakis simplex* complex.

Third, allozyme clustering depicted the species *A. physeteris, A. brevispiculata* and *A. paggiae* as a sister group, highly supported, with respect to the other *Anisakis* taxa, and this was evidenced by the mtDNA-*cox2* sequences as well. Indeed, *A. physeteris*, *A. brevispiculata* and *A. paggiae* are likely candidates to form the basal clade of the group of *Anisakis* species showing Type I derived morphology.

Among the genetic markers so far applied in the study of molecular systematics of *Anisakis* spp. populations, allozymes have been demonstrated the most suitable to prove reproductive isolation between taxa belonging to this genus. This was also largely demonstrated in other anisakid nematodes belonging to other genera, such as *Contracaecum* and *Pseudoterranova*, showing the existence of several sibling species in most of the morphospecies belonging to these genera (Nascetti *et al.*, 1993; Orecchia *et al.,* 2004; Paggi *et al.*, 1991; Mattiucci *et al.*, 2001, 2003).

Alleles at diagnostic enzyme-loci among the distinct *Anisakis* spp. are given for easy and reliable identifi-

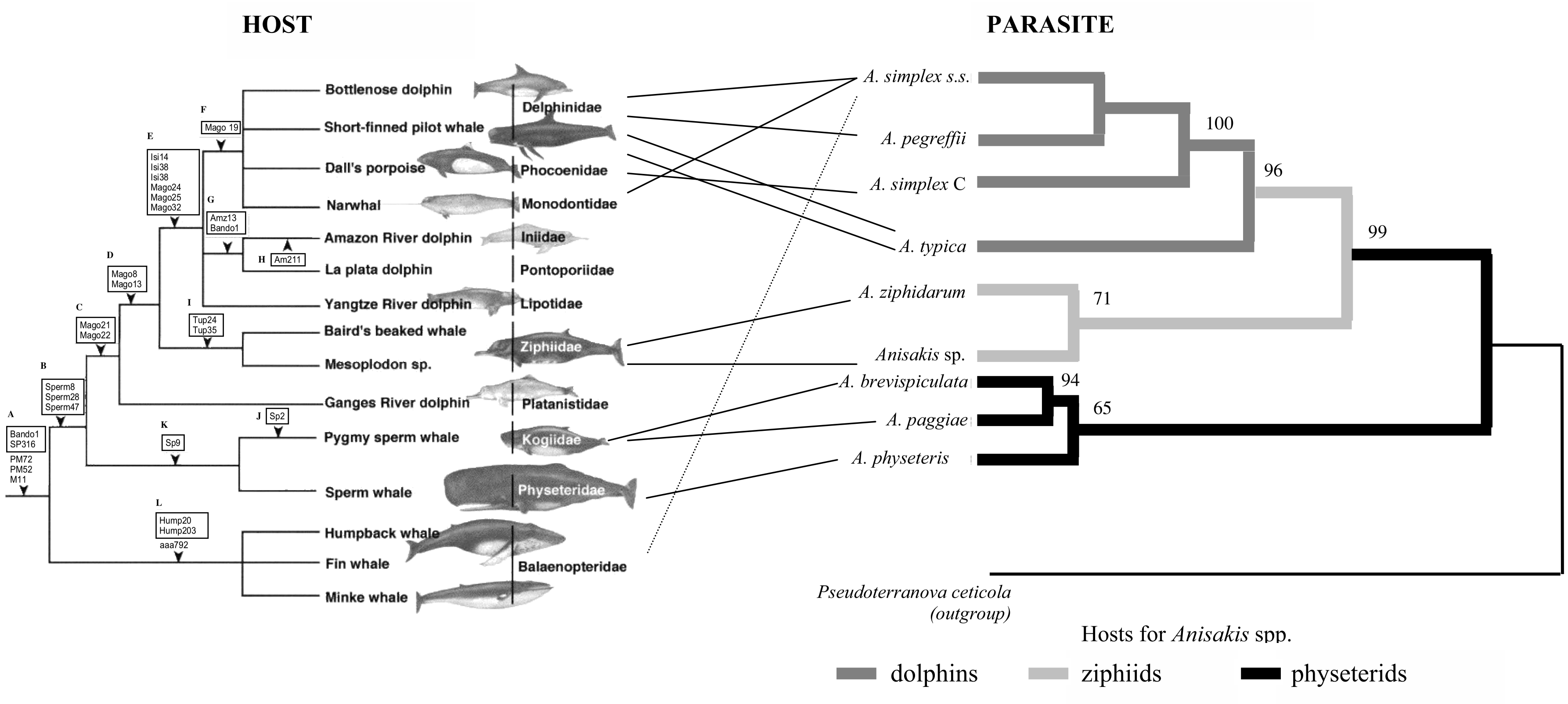

Fig. 3. – The pattern of host and *Anisakis* parasite associations. Phylogenetic analysis for host (cetaceans) (adapted from Nikaido *et al.*, 2001), mapped with the genetic relationships of *Anisakis* spp. Consensus Neighbour Joining (NJ) tree, based on Cavalli-Sforza and Edwards chord distance values from allozyme data, using PHYLIP (Felsestein, 1995 and reference therein). Bootstrap values over 500 replicates are given (modified from Valentini *et al.*, 2006). Lines depict the observed host-parasite associations; the dotted-line indicates possible host-switching events.

Locus	***A. simplex s.s.***	***A. pegreffii***	***A. simplex C***	***A. typica***	***A. ziphidarum***	***Anisakis* sp.**	***A. physeteris***	***A. brevi-spiculata***	***A. paggiae***
Iddh	100	100	90, 100, 105	95	95	105	97, 103	97	97
Mdh-1	100	100	80,90	79	78	78	105	105	105
Icdh	93, 100	93, 100	93, 100	93, 100	93, 100	93	95 ,105	110, 114	105
6Pgdh	97, 100	93, 100	97, 100	86, 93	95	110	76, 86	93, 100	88
Gapdh	100	100	100, 108	120	120	120	115	98	110
Sod-1	92	100	100	98	98	120	97	90	90
Np	100	100	100,110	90	110	100,125	60	70	80
Aat-2	88, 93	100	105, 110	95, 110	120	95, 105	97	85	80
Adk-2	105	100	100	95	90	98	97	103	93
fEst-2	100	100	100	130, 140	125	125	115	90	90
Pep B	70, 80	100	70, 80	70	62, 70	70	75	60	105
Pep C-1	90	100	92	98	108	108	105	95	80
Pep C-2	96	100	92, 96	96	90	90	108	102	105
Mpi	100	100, 103	100	83, 90	105	115	93, 98	80, 88	115
Gpi	100	100	100	88	97	106	90, 98	90, 98	103
Pgm-2	100	100	100	110	113	108	106	106	106

Table VI. – Alleles at diagnostic loci between the species of *Anisakis* so far genetically characterised (data from: Mattiucci *et al*., 1997, 1998, 2001, 2002, 2005, and present paper).

cation of the worms of their sexes and at any stage of their life-history (Table VI). They proved to be very useful in the identification of a large numbers of *Anisakis* larvae, recently collected from some fish species from several localities of their distribution range, showing the detection of significant differences in the distribution of different species of *Anisakis*. This made possible the use of these *Anisakis* as biological tags of different stocks of fish species in European waters (Mattiucci *et al*., 2004, 2006).

On the other hand, the precise and easy identification at species level of *Anisakis* larvae has also a major impact in the diagnosis of human infections by *Anisakis* spp. larvae (Anisakiosis) (Moschella *et al.,* 2004 and citations therein). It acquires importance in the assessment of their relative epidemiological role, especially in those areas where different *Anisakis* species are sympatric and also syntopic in the same host species. This finding becomes important when the fish species are of high commercial value and/or commonly consumed by humans (Mattiucci *et al.,* 2004, 2006).

Despite the numerous observations on the definitive and intermediate hosts were so far collected on the different species of *Anisakis* genetically characterised, their distribution in several geographical areas remain unclear. Specimens of *Anisakis* spp. have not been collected yet from these areas and data in their cetacean hosts is patchy. Nevertheless, the preliminary data for reconstruction of a possible coevolutionary scenario between cetacean hosts and their *Anisakis* endoparasites suggests that cospeciation and host-switching events may have accompanied the evolution of this group of parasites. Indeed, the genetic relationships of the considered *Anisakis* taxa, compared with those so far available for their cetacean definitive hosts first evidenced parallelism between host and parasite phylogenetic tree topologies.

Finally, molecular systematics on further populations of *Anisakis* spp. collected from other hosts and geographic areas and their phylogenetic analysis carried out on other target genes as well, will provide supporting evidences on the evolutionary history of this group of marine ascaridoid nematodes.

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