

CHAPTER 2

Advances and Trends in the Molecular Systematics of Anisakid Nematodes, with Implications for their Evolutionary Ecology and Host–Parasite Co-evolutionary Processes

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Abstract

The application of molecular systematics to the anisakid nematodes of the genera *Anisakis*, *Pseudoterranova* and *Contracaecum*, parasites of aquatic organisms, over the last two decades, has advanced the understanding of their systematics, taxonomy, ecology and phylogeny substantially. Here the results of this effort on this group of species from the early genetic works to the current status of their revised taxonomy, ecology and evolutionary aspects are reviewed for each of three parasitic groups. It has been shown that many anisakid morphospecies of *Anisakis*, *Contracaecum* and *Pseudoterranova* include a certain number of sibling species. Molecular genetic markers provided a rapid, precise means to screen and identify several species that serve as definitive and intermediate and/or paratenic hosts of the so far genetically characterized species. Patterns of differential distribution of anisakid nematodes in various definitive and intermediate hosts are presented. Differences in the life history of related species can be due both to differential host–parasite co-adaptation and co-evolution, and/or to interspecific competition, that can reduce the range of potential hosts in sympatric conditions. Phylogenetic hypotheses attempted for anisakid nematodes and the possible evolutionary scenarios that have been proposed inferred from molecular data, also with respect to the phylogeny of their hosts are presented for the parasite–host associations *Anisakis*–cetaceans and

Contracaecum-pinnipeds, showing that codivergence and host-switching events could have accompanied the evolution of these groups of parasites.

Finally, examples in which anisakid nematodes recognized genetically at the species level in definitive and intermediate/paratenic hosts from various geographical areas of the Boreal and Austral regions and their infection levels have been used as biological indicators of fish stocks and food-web integrity in areas at high versus low levels of habitat disturbance (pollution, overfishing, by-catch) are presented.

1. INTRODUCTION

Adult nematodes of the genera of most species of the anisakid nematodes *Anisakis*, Dujardin, 1845, *Pseudoterranova* Krabbe, 1878 and *Contracaecum* Railliet and Henry, 1913 are parasites of the alimentary tract of aquatic vertebrates. They display indirect life cycles in aquatic ecosystems and involve various hosts at different levels in food webs. Marine mammals (cetaceans and pinnipeds) and fish-eating birds serve as definitive hosts; fish, squids and other invertebrates serve as intermediate or paratenic hosts; and crustaceans serve as first intermediate hosts. In humans, several larval anisakid nematodes cause the zoonotic disease, presently known as ‘anisakidosis’ or ‘anisakiosis’, when consumed in raw or undercooked fish. *Anisakis* is considered as the most important anisakid genus with regard to human infection (Audicana *et al.*, 2002, and references therein), but some species of *Pseudoterranova* also have been implicated in human infections (Adams *et al.*, 1997; Oshima, 1987), and a few species of *Contracaecum* are potentially infective (Vidal-Martinez *et al.*, 1994). This zoonosis has a history starting from the first report (Van Thiel, 1960) of a larval nematode from herring in the gastro-intestinal tract of humans in the Netherlands. Some aspects of human anisakidosis have recently been reviewed by Audicana *et al.* (2002). Because of the existence of several excellent reviews by others authors on the history and clinical aspect of the anisakidosis (Audicana *et al.*, 2002; Chai *et al.*, 2005; Umehara *et al.*, 2007), this introduction will not summarize the major events that mark the history of anisakid nematodes as causative agents of anisakidosis, but instead will highlight major landmarks involved in our understanding of their taxonomic status and ecology based on genetic markers. This field has had a major impact during the last 20 years on our knowledge of these parasites, including their host-specificity, geographical range and the possible identification of human cases of anisakidosis.

Starting from the knowledge summarized in the exhaustive revision by Smith and Wootten (1978), this review treats the explosion in the

literature that has accompanied research activities on these nematodes and resulted in a series of discoveries over the last 25 years on the systematics and ecology of anisakid nematodes using molecular genetic tools applied to their taxonomy. Indeed, based on the advances in our understanding of the basic biology of *Anisakis*, reviewed by [Smith and Wootton \(1978\)](#), this introduction starts from their concluding remarks highlighting knowledge gaps and perspectives for investigations over the next 30 years. One major gap concerned the identification of species. Indeed, in the case of the species belonging to the genus *Anisakis*, they wrote: ‘... despite Davey’s revision (1971), there are still taxonomic problems, including the distinction between *A. simplex* and *A. typica* The generic diagnosis of *Anisakis* should be re-examined *Anisakis larva* (I) from North Atlantic waters has been cultured in vitro and shown to develop into *A. simplex*: There is need to culture the other “larval types” in order to confirm that they do, in fact, represent *Anisakis* and, if so, to determine which species they represent . . .’. In 1978, Smith and Wootton underlined the major gap in our knowledge of nematode biology at that date, as represented by the genus *Anisakis* but also relevant to other anisakid species, that is, the identification of biological species. Indeed, species identification, based on morphological characters only, is difficult for adults, but even more difficult for larval stages.

The need for species identification was especially important for larval stages of *Anisakis* because they have been implicated as causative agents of human anisakidosis. Indeed, [Smith and Wootton \(1978\)](#) concluded their review as follows: ‘... in these circumstances there is need for reliable methods of differential diagnosis. There is much scope for future investigation’. Thus, this ‘future investigation’ started with the application of molecular genetic methodologies in an attempt to address the systematics of these anisakids. The prospect of assessing anisakid nematode biodiversity based on molecular genetic markers as the preferred diagnostic tools seemed promising because the unambiguous identification of specimens causing human disease (anisakidosis) is essential for a proper epidemiological survey.

The power of resolution of molecular genetic methodologies in detecting anisakid species revolutionized the taxonomy of these species during the following 25 years. Most descriptions of parasite species conformed with what can be regarded as the ‘morphological or typological, species concept’. Because genetic speciation is not always accompanied by corresponding morphological change, the actual number of biological species is likely to be greater than the current tally of nominal species, most of which are delineated based on morphological grounds.

Detecting biological species of anisakid nematodes challenged parasitologists via an expected genetic variation and heterogeneity within the nominal species and has led to the definition of anisakid species according to the Mayr’s ([Mayr, 1963](#)) ‘biological species concept’ (BSC).

The aim of this review is to gather together the many and varied aspects concerning the biology and evolutionary ecology of those anisakid species detected genetically and recognized as members of *Anisakis*, *Pseudoterranova* and *Contracaecum*, including (a) species presently accepted as senior synonyms based on the application of different molecular genetic markers; (b) current molecular genetic approaches to identify anisakid species; (c) ecological evidence relating to the geographical distribution of detected species, their host preferences and life cycles; (d) the use of anisakid nematodes as biological tags for fish stock identification in a multidisciplinary approach; (e) correlation between the values of genetic variability and the levels of parasitic infection in definitive and intermediate/paratenic hosts as indicators of the integrity of marine food webs; (f) estimates of genetic divergence at intraspecific and interspecific levels; and (g) estimates of their genetic relationships based on different clustering approaches inferred from different molecular genetic data sets and their use to infer phylogenetic hypothesis and possible co-evolutionary events with their definitive hosts.

2. MOLECULAR SYSTEMATICS OF ANISAKID NEMATODES

The nematode superfamily Ascaridoidea contains ~52 genera; species are mainly parasites of the alimentary tract of vertebrates. Several are of medical and veterinary concern, and some are of economic significance. The classification scheme for members of the Anisakidae proposed by [Hartwich \(1974\)](#) is based largely on features of the ‘excretory system’ and alimentary tract. According to the ‘systematic keys’ of [Hartwich \(1974\)](#), the family Anisakidae contains the subfamilies Anisakinae and Contracaecinae. The Anisakinae includes (cf. [Hartwich, 1974](#)) the genera *Anisakis* and *Pseudoterranova* plus several others. The Contracaecinae includes three genera, *Contracaecum*, *Phocascaris* and *Galeiceps*. Species of *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Phocascaris* have aquatic life cycles and homeothermic final hosts.

Anisakid nematodes have been extensively studied with respect to their alpha-taxonomy ([Berland, 1961, 1964](#); [Bruce and Cannon, 1990](#); [Davey, 1971](#); [Deardoff and Overstreet, 1979](#); [Fagerholm, 1989, 1991](#); [Fagerholm and Gibson, 1987](#); [Gibson, 1983](#); [Osche, 1963](#); [Sprent, 1977, 1978, 1979, 1983](#)) and life cycles ([Huizinga, 1967](#); [Klöser *et al.*, 1992](#); [Køie and Fagerholm, 1995](#)).

Much of the controversy in the systematics of anisakid species, when dealing with identifications based solely on morphological differences, revolved around their confused taxonomic position. Indeed, morphological characters of taxonomic significance in this group are very few (i.e. features of the excretory system, alimentary canal, number and

distribution of male caudal papillae, position of the vulva and length of the spicules) and are applicable to adult specimens only. Furthermore, these are often only relevant to male individuals, making identification difficult for many worms at the species level. In recent decades, molecular genetic techniques have been used to demonstrate that structural features are not always adequate for recognizing 'true' species within *Anisakis*, *Pseudoterranova* and *Contracaecum*. In the last two decades, the diversity of anisakid species has increased due to the detection by genetic markers of several sibling species with reproductively isolated gene pools that are morphologically very similar, and thus correspond to the 'biological species'. There are now morphospecies, or species complexes, based on previously recognized cosmopolitan species (*sensu lato*), that may comprise several recognized species. This solved one of the major problems in the systematics of anisakid nematodes: the occurrence of the parallelism and convergence of morphological features, which confound the systematic value of some morphological criteria and often accompany a high genetic and ecological divergence between the species. The lack of morphological differences in these parasites may be due to factors such as similar selection pressures causing the conservation of morphology; consequently, some morphological characters have little or no taxonomic value because of the evolutionary co-adaptation of these endoparasites to the stable habitat represented by their definitive hosts. Indeed morphospecies may appear to have multiple host species, that is, parasite populations isolated in their hosts have diverged genetically but have conserved morphological features. Moreover, species identification based on morphological characters only makes identification very difficult, especially for larval stages that lack reliable diagnostic features at the species level.

Thus, the inconsistency in morphological characteristics of anisakid nematodes impeded the development of a credible scheme for their taxonomy. This prompted the need to classify these nematodes using genetic and/or biochemical methods. The assessment of anisakid nematodes biodiversity based on molecular genetic markers as preferable tools for specific diagnosis was an important prospect because the unambiguous identification of those anisakids with a zoonotic potential is an essential requirement for a proper epidemiological survey.

Initial attempts to apply population genetics to the study of genetic variation among large samples of anisakids collected from different intermediate/paratenic and definitive hosts in nature employed the use of multilocus allozyme electrophoresis (MAE) (19–24 enzyme loci). These tools revealed the existence of high genetic heterogeneity within certain anisakid morphospecies, such as those of *Anisakis*, *Pseudoterranova* and *Contracaecum* (Bullini *et al.*, 1986; Mattiucci *et al.*, 1986; Nascetti *et al.*, 1986;

Paggi *et al.*, 1991). The species concept (BSC) (Mayr, 1963) was well supported by the application of allozyme markers for several anisakid species. Indeed, after the first such application, the diversity of species belonging to *Anisakis*, *Pseudoterranova* and *Contracaecum* quickly increased after the detection of several sibling species and leads to the discovery and description of several new species. Reproductive isolation and absence of gene flow were demonstrated by these allozymes between sympatric and allopatric sibling species, establishing their specific status (Mattiucci *et al.*, 1997, 2001, 2003, 2005; Nascetti *et al.*, 1993; Paggi *et al.*, 1991). Allozyme markers have allowed us to (a) genetically characterize different species of anisakid nematodes, (b) estimate their genetic differentiation, (c) establish their genetic relationships, and (d) identify their larval stages which lack morphological characters (Arduino *et al.*, 1995; Bullini *et al.*, 1986, 1994, 1997; Mattiucci and Nascetti, 2006; Mattiucci *et al.*, 1986, 1997, 1998, 2001, 2002a, 2003, 2004, 2005, 2006, 2007a, 2008a,b,c; Nascetti *et al.*, 1986, 1993; Orecchia *et al.*, 1986a, b, 1994; Paggi and Bullini, 1994; Paggi *et al.*, 1991, 1998b, 2000, 2001).

The introduction of the polymerase chain reaction (PCR)-derived molecular methodologies later confirmed taxonomic decisions involving anisakid species previously based on allozyme markers. Reference individuals initially characterized by allozymes have been used to develop DNA-based approaches for species identification, such as PCR-RFLP and direct sequencing of ITS rDNA or mitochondrial DNA.

Ascaridoid classifications and inferred patterns of character evolution have been investigated previously, and some evolutionary hypotheses for representative ascaridoids, some of which are anisakids, have been proposed based on phylogenetic methods, including ribosomal DNA sequence data and comparative analysis of morphological and life-history characters (Nadler, 1992, 1995, 2005; Nadler and Hudspeth, 1998; Zhu *et al.*, 2000a). Results from these studies identified some consistent, putative, shared-derived morphological features, strongly suggesting that some morphological features represent ancestral states or highly homoplastic characteristics (Nadler and Hudspeth, 1998). Phylogenetic analysis indeed provided a new perspective for the delimitation of anisakid sibling species, including hierarchical relatedness and relative rates of evolution. An evolutionary perspective provides a conceptual approach to view species as independent evolutionary lineages and provides another approach for delimitating species (Adams, 1998; Nadler, 2002, 2005). Indeed, based on phylogenetic DNA analysis, sibling anisakid species have been confirmed by methods that can test the hypothesis of lineage independence analysing many individual specimens and sometimes detecting new genotypes and species (D'Amelio *et al.*, 2000, 2007; Mattiucci *et al.*, 2008b, c; Nadler *et al.*, 2000; 2005; Valentini *et al.*, 2006).

2.1. Current methods for anisakid identification

Except for some morphological differences between anisakid species (Mattiucci *et al.*, 1998, 2005, 2008c; Paggi *et al.*, 1998b, 2000), all the species genetically characterized are to date indistinguishable at all developmental stages (larvae and adults); consequently, only molecular genetic markers can be used reliably to identify these species. Of all the recently developed tools, and despite the useful PCR-derived molecular markers, allozymes continue to remain the best methodological approach for demonstrating reproductive isolation between anisakids. Starting from the study of genetic variation at several enzyme loci, the main argument in favour of using allozymes is the ability to test (using the Hardy–Weinberg equilibrium) for reproductive isolation of anisakid populations among large numbers of individuals. Accordingly, allozymes provide the best choice for determining molecular genetic markers applied to the systematics of anisakids and for confirming the BSC. Based on allozymes diagnostic for different anisakid taxa, easy and rapid identification of large numbers of individuals can be performed; this method is particularly valuable for identifying larval individuals collected from several intermediate/paratenic hosts and often in mixed infections. Accordingly, such identifications have been demonstrated to be very informative tools for answering epidemiological questions involving geographical range, host preference and life cycles. Moreover, because numerous allozymes (20–24 enzyme loci) have been applied to thousands of anisakid individuals, they have contributed greatly to our knowledge of the genetic diversity of anisakid populations collected from various ecosystems in the Boreal and Austral hemispheres (see also Section 8.2). However, the tool is limited to frozen-preserved or fresh individuals.

This constraint has been resolved by DNA-based diagnostic techniques, which have the advantage of also being able to use alcohol or formalin-preserved specimens. In contrast with allozymes, the DNA-based techniques have increased our ability to study phylogenetic relationships between related anisakids based on the evolutionary lineage concept (Adams, 1998). The PCR-DNA molecular derived methodologies so far applied to the systematics of anisakid nematodes include PCR-restriction fragment length polymorphism (PCR-RFLPs of ITS-DNA) (D'Amelio *et al.*, 2000; Kijewska *et al.*, 2002; Pontes *et al.*, 2005), single-strand conformational polymorphism SSCP-DNA (ITS) of PCR products (Zhu *et al.*, 1998, 2000b, 2007), direct sequencing of PCR-amplified DNA of 28S (LSU) and complete internal transcribed spacer (ITS-1, 5.8S, ITS-2) ribosomal DNA (Hu *et al.*, 2001; Li *et al.*, 2005; Nadler *et al.*, 2000, 2005; Zhu *et al.*, 1998, 2000b, 2001, 2002), mitochondrial cytochromoxidase b (mtDNA *cytb*) (Mattiucci *et al.*, 2003) and mitochondrial cytochromoxidase 2 (mtDNA *cox2*) sequence analyses (Mattiucci and Nascetti, 2006;

Mattiucci *et al.*, 2008b, c; Valentini *et al.*, 2006). PCR-DNA markers have so far confirmed the existence of the sibling species previously detected by allozymes, establishing their taxonomic status. The only exception is represented by the two Antarctic sibling species of the *Contracaecum osculatum* complex (i.e. *C. osculatum* D and *C. osculatum* E), where PCR-DNA markers based on ITS-rDNA (Zhu *et al.*, 2000b) and three mitochondrial DNA genes (i.e. mtDNA *cox1*, *ssrRNA* and *lsrDNA*) (Hu *et al.*, 2001) were unable to identify species previously detected by allozyme markers. In contrast, the existence of the two sibling species, *C. osculatum* D and *C. osculatum* E, first detected by allozymes, was confirmed by sequencing the mtDNA *cox2* gene (Mattiucci *et al.*, 2008b). A scheme of allozymes and PCR-derived methods which can be used routinely to identify a single individual belonging to genetically characterized species and gene pools of anisakids as belonging to the genera *Anisakis*, *Pseudoterranova* and *Contracaecum* is provided in Tables 2.1–2.3, respectively.

3. THE CURRENT TAXONOMY

This section summarizes the current taxonomy of anisakid species of the genera *Anisakis*, *Pseudoterranova* and *Contracaecum* (here considering only those species maturing in pinnipeds) which have been genetically characterized to date. They can be recognized, at any life-history stage, by different molecular genetic approaches, as reported in Tables 2.1–2.3. A synopsis of each recognized anisakid species, including data on both the definitive and intermediate hosts and the geographical range, is also presented.

3.1. The current taxonomy of *Anisakis*

The taxonomy of *Anisakis* species has traditionally relied on adult morphology. According to Davey (1971), the primary characters are the length and shape of the ventriculus, length and shape of the male spicules, and arrangement of the male caudal papillae. According to Berland (1961), larval morphological features (i.e. length of ventriculus and presence/absence of caudal spine) could distinguish *Anisakis* type I and *Anisakis* type II larvae. In the revision by Davey (1971), the generic diagnosis for *Anisakis* states: ‘three lips each bearing a bilobed anterior projection which carries the single dentigerous ridge; interlabia absent; excretory gland with duct opening between ventrolateral lips; oesophagus with anterior muscular portion (proventriculus) and posterior ventriculus, the latter being oblong and sometimes sigmoid or else as broad as long; no oesophageal appendix or intestinal caecum; vulva in middle or first third of the body, spicules of male unequal; preanal papillae numerous; postanal papillae including a group of three or four

TABLE 2.1 Molecular genetic markers for the identification of the species of *Anisakis*

Molecular genetic marker	Method	Identified species ^a	References
22 allozyme loci	MAE	Asstr, Ape	Nascetti <i>et al.</i> , 1986
20 allozyme loci	MAE	Asstr, AsC, Ape	Mattiucci <i>et al.</i> , 1997
25 allozyme loci	MAE	Azi, Ape, AsC, Aph	Paggi <i>et al.</i> , 1998b
20 allozyme loci	MAE	Asstr	Mattiucci <i>et al.</i> , 1998
5.8S rDNA, ITS rDNA	PCR-RFLP; SSCP	Asstr	Zhu <i>et al.</i> , 1998
18S and 5.8S rDNA, ITS rDNA	PCR-RFLP	Ape, AsC, Aty, Azi, Aph, Abr, Asc	D'Amelio <i>et al.</i> , 2000
22 allozyme loci	MAE	Abr, Aph, Azi, Ape, AsC, Aph	Mattiucci <i>et al.</i> , 2001
21 allozyme loci	MAE	Aty, Asstr, Ape, Azi, Ape, AsC, Aph	Mattiucci <i>et al.</i> , 2002a
20 allozyme loci	MAE	Apa, Asstr, Ape, Aty, Az, Aph, Abr	Mattiucci <i>et al.</i> , 2005
18S, 28S and 5.8S rDNA, ITS rDNA	PCR and sequencing	AsC, Ape, Asp	Nadler <i>et al.</i> , 2005
ITS rDNA	PCR-RFLP	Asstr, Ape, Aty, Azi, Aph, AspA	Pontes <i>et al.</i> , 2005

20 allozyme loci	MAE	Asp, Asstr, Ape, AsC, Aty, Azi, Aph, Ab, Apa	Mattiucci and Nascetti, 2006
Mitochondrial cytochrome c-oxidase subunit 2 (mtDNA <i>cox2</i>)	PCR and sequencing	Asstr, Ape, AsC, Aty, Azi, Aph, Abr, Apa, Asp	Valentini <i>et al.</i> , 2006
ITS rDNA, 5.8S rDNA, mitochondrial cytochrome c-oxidase subunit 1 (mtDNA <i>cox1</i>)	PCR-RFLP	Asstr, Ape	Umehara <i>et al.</i> , 2006
ITS rDNA, 5.8S rDNA	PCR-RFLP	Asstr, Ape	Abe <i>et al.</i> , 2006
Mitochondrial cytochrome c-oxidase subunit 1 (mtDNA <i>cox1</i>), NADH dehydrogenase subunit 1	PCR and sequencing	Asstr	Cross <i>et al.</i> , 2007
ITS-2 rDNA	PCR-SSCP	Ape, Aty	Zhu <i>et al.</i> , 2007
ITS rDNA	Multiplex-PCR	Asstr, Ape, Aph	Umehara <i>et al.</i> , 2008

^a Codes for *Anisakis* spp.: Asstr, *A. simplex* (s.s.); Ape, *A. pegreffii*; AsC, *A. simplex* C; Aty, *A. typica*; Azi, *A. ziphidarum*; Aph, *A. physeteris*; Abr, *A. brevispiculata*; Apa, *A. paggiae*; Asp, *Anisakis* sp.; Asc, *A. schupakovi*; AspA, *Anisakis* sp. A.

TABLE 2.2 Molecular genetic markers for the identification of the *Pseudoterranova decipiens* species complex

Molecular genetic marker	Method	Identified species ^a	References
16 allozyme loci	MAE	Pdss, Pkr, Pbu	Paggi <i>et al.</i> , 1991
9 allozyme loci	MAE	Pca	George-Nascimento and Llanos, 1995; George-Nascimento and Urrutia, 2000
16 allozyme loci	MAE	Pdss, Pkr, Paz, Pbu, PdE	Bullini <i>et al.</i> , 1997
20 allozyme loci	MAE	Pbu, Paz, Pdss, Pkr	Mattiucci <i>et al.</i> , 1998
ITS rDNA	PCR-SSCP	Pdss, Pkr, Paz, Pbu, Pca, PdCa1	Zhu <i>et al.</i> , 2002
18S, 28S and 5.8S rDNA, ITS rDNA	PCR and sequencing	Pdss, Pkr, Paz, Pbu, Pca, PdCa1	Nadler <i>et al.</i> , 2005

^a Codes for *Pseudoterranova* spp.: Pdss, *P. decipiens* (s.s.) (= *P. decipiens* B); Pkr, *P. krabbei* (= *P. decipiens* A); Pbu, *P. bulbosa* (= *P. decipiens* C); Paz, *P. azarasi* (= *P. decipiens* D); PdE, *P. decipiens* E; Pca, *P. cattani*; PdCa1, *P. decipiens* from *Chaenocephalus aceratus*.

pairs set close to the tip of the tail on ventral side'. After a critical revision of the 21 included species, Davey (1971) concluded that many of the species had to be considered as junior synonyms of three main species, the only ones accepted in his revision, that is, *A. simplex* (Rudolphi, 1809, det. Krabbe, 1878) with 10 synonyms, *A. typica* (Diesing, 1860) with one synonym and *A. physeteris* (Baylis, 1923) with three synonyms. He also retained four others as species *inquirendae* because of the lack of sufficient data: *A. dussumierii* (van Beneden, 1870) reported from a dolphin in the Indian Ocean, *A. schupakovi* Mozgovoi, 1951 from the Caspian seal, *Pusa caspica* (later redescribed and accepted by Delyamure *et al.*, 1964), *A. alexandri* Hsü and Hoeppli, 1933 from *Sotalia sinensis* and *A. insignis* (Diesing, 1851) from *Inia geoffrensis* in South America rivers (redescribed and accepted by Petter, 1972).

According to Davey's revision, the only morphological characters of systematic value in recognizing the taxonomic status of the species of *Anisakis* were the length and shape of ventriculus, length and shape of the

TABLE 2.3 Molecular genetic markers for the identification of the species of *Contracaecum* and *Phocascaris* from pinnipeds

Molecular genetic marker	Method	Identified species ^a	References
17 allozyme loci	MAE	CoA, CoB, Coss	Nascetti et al., 1993
24 allozyme loci	MAE	CoD, CoE, CoA, CoB, Coss	Orecchia et al., 1994
25 allozyme loci	MAE	Cra, CoA, CoB, Coss, CoD, CoE	Arduino et al., 1995
20 allozyme loci	MAE	CoA, CoB	Mattiucci et al., 1998
28S rDNA	PCR and sequencing	CoA, CoB, Coss, Cba, Crad, Cmir, Pcy, Pph	Nadler et al., 2000
ITS rDNA	PCR-SSCP	CoA, CoB, Coss, Cbai	Zhu et al., 2000
ITS rDNA	PCR-RFLP; PCR-SSCP	Cogm	Zhu et al., 2001
18S and 28S rRNA, mitochondrial cytochrome <i>c</i> -oxidase subunit 1 (mtDNA <i>cox1</i>)	PCR-SSCP	CoA, CoB, Coss, Cbai	Hu et al., 2001
18 allozyme loci	MAE	Cogm, Cmar	Mattiucci et al., 2003

(continued)

TABLE 2.3 (continued)

Molecular genetic marker	Method	Identified species ^a	References
18S, 28S and 5.8S rDNA, ITS rDNA	PCR and sequencing	CoA, CoB, Coss, Crad, Cmir, Cbai, Cmar, Cogm, Pcys, Pph	Nadler <i>et al.</i>, 2005
20 allozyme loci	MAE	CoA, CoB, Coss, CoD, CoE, Cbai, Crad, Cmir, Cmar, Cogm, Pcys	Mattiucci <i>et al.</i>, 2008b
Mitochondrial cytochrome <i>c</i> -oxidase subunit 2 (mtDNA <i>cox2</i>)	PCR and sequencing	CoA, CoB, Coss, CoD, CoE, Cbai, Crad, Cmir, Cmar, Cogm, Pcys	Mattiucci <i>et al.</i>, 2008b

^a Codes for *Contracaecum* spp.: CoA, *C. osculatum* A; CoB, *C. osculatum* B; Coss, *C. osculatum* (s.s.) (= *C. osculatum* C); CoD, *C. osculatum* D; CoE, *C. osculatum* E; Cbai, *C. baicalensis*; Cmar, *C. margolisi*; Crad, *C. radiatum*; Cogm, *C. ogmorhini* (s.s.); Cmir, *C. mirounga*; Pph, *Phocascaris phocae*; Pcys: *Ph. Cystophorae*.

male spicules, arrangement of the male caudal papillae and position of the vulva in females. Morphological features such as the length of the ventriculus and presence/absence of caudal spine were proposed for the recognition of larval stages of *Anisakis*, but these are too inconsistent and inaccurate for use at the specific level (Berland, 1961; Shiraki, 1974).

Despite Davey's valuable discussion, when considering the main morphological characters of the species *Anisakis*, instability of their systematics, including their identification at the species level (especially in the case of females and larvae), remained. The resolution of some of the taxonomic issues, highlighted in this critical revision, has been achieved during the last 20 years facilitated by the adoption of new methodologies, allozymes first and DNA-based molecular methodologies later.

Starting from that critical revision, and based on our growing understanding on the systematic of species *Anisakis*, we can state that Davey's revision failed in considering only three species as valid and several species as synonyms, as some of the latter have been subsequently validated by genetic methodology (Mattiucci *et al.*, 2001). However, this revision anticipated, in some way, a future cladistic analysis by indicating morphological characters likely to be useful for the systematics of the species belonging to *Anisakis* following their genetic identification.

In addition, in his revision, Davey did not consider *Skryabinisakis* Mosgovoï, 1951, proposed for the species *A. physeteris*, as an accepted genus. However, the high level of genetic differentiation of both *A. physeteris*, *A. brevispiculata* and *A. paggiae* (see Mattiucci *et al.*, 2005) from those species of *Anisakis* studied genetically using different nuclear and mitochondrial markers (Mattiucci and Nascetti, 2006; Mattiucci *et al.*, 2001; Valentini *et al.*, 2006) indicates that *Anisakis* is indeed polyphyletic and highly heterogeneous. Today, the existence of two main clades within *Anisakis* is clearly inferred from the phylogenetic analysis based on nuclear data sets from allozymes and mtDNA *cox2* sequence analysis of all the genetically characterized species (Mattiucci and Nascetti, 2006; Mattiucci *et al.*, 2005; Valentini *et al.*, 2006). One clade encompasses species with the larval stage known as *Anisakis* type I (*sensu* Berland, 1961) and the second sharing the larval morphology of *Anisakis* type II (*sensu* Berland, 1961) (see Mattiucci *et al.*, 2005, 2007a; Orecchia *et al.*, 1986a). The first clade includes the species of the *A. simplex* complex [i.e. *A. simplex* (*sensu stricto*), *A. pegreffii* and *A. simplex* C], *A. typica*, *A. ziphidarum* and *Anisakis* sp. The second includes the species *A. physeteris*, *A. brevispiculata* and *A. paggiae* (see Mattiucci *et al.*, 2005; Valentini *et al.*, 2006). The clade including *A. simplex* (s.s.) and *A. simplex* C and *A. pegreffii* is also well supported by a phylogenetic analysis inferred from ITS rDNA sequence data sets (Nadler *et al.*, 2005). This analysis is congruent with both allozymes and mtDNA *cox2* analysis in depicting *A. physeteris* + *A. brevispiculata* as the sister group to the remaining *Anisakis* spp.

3.1.1. *Anisakis* spp. included in clade I

According to the genetic data, six species can currently be included in this clade: three species of the *A. simplex* complex [i.e. *A. simplex* (s.s.), *A. pegreffii* and *A. simplex* C], *A. typica*, *A. ziphidarum* and a new gene pool referred to as *Anisakis* sp. (see [Valentini et al., 2006](#), unpublished data). As far as is known, all of these species have the larval form known as type I (*sensu* [Berland, 1961](#)). A synopsis of the ecological aspects of each species, including their known geographical distribution and both definitive and intermediate/paratenic hosts, is presented below.

A. simplex (Rudolphi, 1809) (*sensu stricto*) (see [Nascetti et al., 1986](#)): *A. simplex* (s.s.) is widespread between 35°N and the Arctic Circle; it is present in both the western and eastern Atlantic and Pacific Oceans ([Abe et al., 2005, 2006](#); [Abollo et al., 2001](#); [Mattiucci et al., 1997, 1998](#); [Nadler et al., 2005](#); [Paggi et al., 1998a](#); [Umehara et al., 2006, 2008](#)) ([Fig. 2.1](#)). The southern limit of this species in the north east Atlantic Ocean is the waters around the Gibraltar area. *A. simplex* (s.s.) is also occasionally present in western Mediterranean waters due to the migration of pelagic fish species into the Alboran Sea from the Atlantic ([Mattiucci and Nascetti, 2006](#); [Mattiucci et al., 2004, 2007a](#)). *A. simplex* (s.s.) has been so far genetically recognized from nine species of cetacean hosts. Several squid and fish species have been found harbouring larvae of this species throughout its geographical range ([Table 2.5](#)). A sympatric area between *A. simplex* (s.s.) and *A. pegreffii* has been identified along the Spanish and Portuguese Atlantic coasts ([Abollo et al., 2001](#); [Marques et al., 2006](#); [Mattiucci et al., 1997, 2004, 2007a](#); [Pontes et al., 2005](#)), in the Alboran Sea ([Mattiucci et al., 2004, 2007a](#)) and recently also in Japanese Sea waters ([Umehara et al., 2006](#)). *A. simplex* (s.s.) also occurs with *A. simplex* C in the eastern Pacific Ocean, where it has been identified in definitive and intermediate/paratenic hosts ([Mattiucci et al., 1997, 1998](#), unpublished data; [Paggi et al., 1998c](#)) ([Tables 2.4 and 2.5](#); [Fig. 2.1](#)). Although it has sympatric and syntopic occurrences in mixed infections at both larval and adult stage with other *Anisakis* species ([Mattiucci et al., 2004, 2005, 2007a](#)), 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 the nuclear level by allozyme markers ([Mattiucci et al., 1997, 2005](#)). Only a few F1 hybrid larval individuals *A. pegreffii*-*A. simplex* (s.s.), of the thousands identified, were detected by allozymes in some fish host from the sympatric area off the Atlantic Iberian coast ([Mattiucci et al., 2004](#)). However, back-crossing of F1 hybrids with parental species has not been detected.

Based on PCR-RFLP of the ITS, [Umehara et al. \(2007\)](#) recognized *A. simplex* (s.s.) as the main source of infections in humans in Japan.

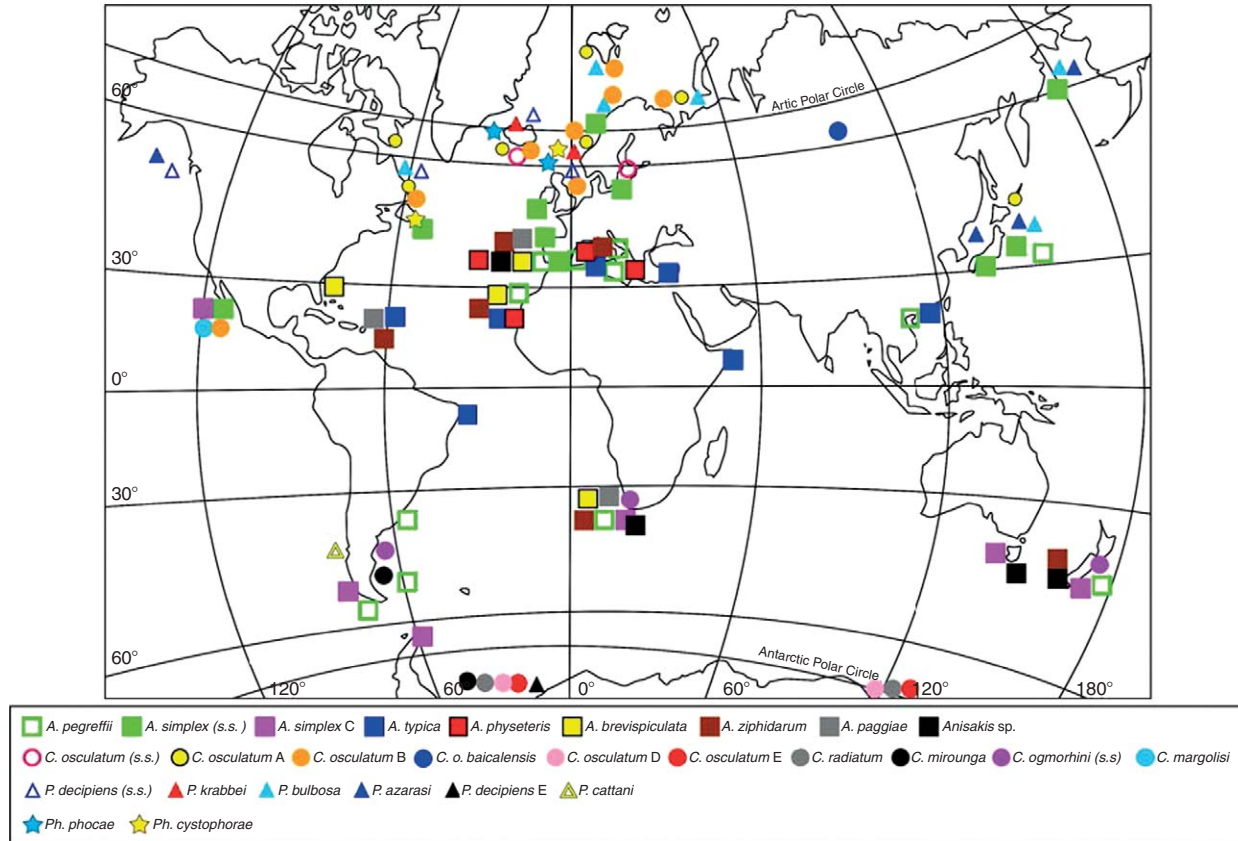


FIGURE 2.1 World map showing the so far known distribution areas of anisakid species of *Anisakis* (□), *Pseudoterranova* (△), *Contracaecum* (○) and *Phocascaris* (*). The geographical areas indicated are related to the sampling localities for their definitive and intermediate hosts.

TABLE 2.4 Definitive hosts so far detected, by molecular genetic markers, for the *Anisakis* spp.

	<i>A. simplex</i> (s.s.)	<i>A. pegreffii</i>	<i>A. simplex</i> C	<i>A. typica</i>	<i>A. ziphidarum</i>	<i>Anisakis</i> sp.	<i>A. physeteris</i>	<i>A. brevispiculata</i>	<i>A. paggiae</i>
Cetaceans									
Balaenopteridae									
<i>Balaenoptera acutorostrata</i>	NEA	–	–	–	–	–	–	–	–
Delphinidae									
<i>Delphinus delphis</i>	IC	IC, WM	–	–	–	–	–	–	–
<i>Globicephala melaena</i>	IC, SA	–	SA	–	–	–	–	–	–
<i>Globicephala macrorhynchus</i>	–	–	–	FL	–	–	–	–	–
<i>Lagenorhynchus albirostris</i>	NEA	–	–	–	–	–	–	–	–
<i>Lissodelphis borealis</i>	–	–	NEP	–	–	–	–	–	–
<i>Orcinus orca</i>	NEP	–	–	–	–	–	–	–	–
<i>Pseudorca crassidens</i>	NEP	–	NEP	–	–	–	–	–	–
<i>Stenella coeruleoalba</i>	IC	WM	–	EM	–	–	–	–	–
<i>Tursiops truncatus</i>	–	CM, SA	–	FL, CS	–	–	–	–	–
<i>Sotalia fluviatilis</i>	–	–	–	BR	–	–	–	–	–
<i>Stenella attenuata</i>	–	–	–	FL, CS	–	–	–	–	–
<i>Stenella longirostris</i>	–	–	–	BR	–	–	–	–	–
<i>Steno bredanensis</i>	–	–	–	CS	–	–	–	–	–

Kogiidae									
<i>Kogia breviceps</i>	–	–	–	–	–	–	–	SA, IC, FL	SA, FL
<i>Kogia sima</i>	–	–	–	–	–	–	–	–	FL
Monodontidae									
<i>Delphinapterus leucas</i>	NWA	–	–	–	–	–	–	–	–
Neobalaenidae									
<i>Caperea marginata</i>	–	SA	–	–	–	–	–	–	–
Phocoenidae									
<i>Phocoena phocoena</i>	NEP	–	–	–	–	–	–	–	–
Physeteridae									
<i>Physeter catodon</i>	–	–	–	–	–	–	CM	–	–
Ziphiidae									
<i>Mesoplodon densirostris</i>	–	–	–	–	SA	–	–	–	–
<i>Mesoplodon europaeus</i>	–	–	–	–	CS	–	–	–	–
<i>Mesoplodon grayi</i>	–	–	–	–	–	SA	–	–	–
<i>Mesoplodon mirus</i>	–	–	–	–	–	SA, NZ	–	–	–
<i>Ziphius cavirostris</i>	–	–	–	–	CM, SA	–	–	–	–

Sampling locality codes: AZ: Azores Islands; BE: Bering Sea; BR: Brazil Atlantic coast; BS: Barents Sea; CM: Central Mediterranean Sea; CS: Caribbean Sea; EM: East Mediterranean Sea; FA: Falkland Islands; FL: Florida coast; IC: Iberian Atlantic Coast; JA: Japan Sea; MA: Mauritanian coast; MD: Madeira Island; NAM: North African Mediterranean coast; NEA: North-East Atlantic; NEP: North-East Pacific; NWA: North-West Atlantic; NZ: New Zealand; PC: Portuguese coast; SA: South Africa coast; SC: Somali coast; SI: off Sakhalin Islands; TA: Tasman Sea; WM: West Mediterranean Sea (data from [Mattiucci and Nascetti, 2006, 2007](#); [Mattiucci et al., 1986, 1997, 2001, 2002, 2004, 2005](#); [Nadler et al., 2005](#); [Nascetti et al., 1986](#); [Paggi et al., 1998a,b,c](#)). Hosts listed by alphabetical order of the family.

TABLE 2.5 Intermediate/paratenic hosts so far detected, by molecular genetic markers, for the *Anisakis* spp.

	<i>A. simplex</i> (s.s.)	<i>A. pegreffii</i>	<i>A. simplex</i> C	<i>A. typica</i>	<i>A. ziphidarum</i>	<i>Anisakis</i> sp.	<i>A. physeteris</i>	<i>A. brevispiculata</i>	<i>A. paggiae</i>
Cephalopods									
Sepiidae									
<i>Sepia officinalis</i>	IC	–	–	–	–	–	–	–	–
Ommastrephidae									
<i>Todaropsis eblanae</i>	IC, SA	IC, SA	–	–	–	–	–	–	–
<i>Todarodes sagittatus</i>	IC	NAM, CM	–	–	–	–	CM	–	–
<i>Todarodes angolensis</i>	–	SA	–	–	–	–	–	–	–
<i>Illex coindettii</i>	IC	–	–	–	–	–	–	–	–
Fishes									
Belonidae									
<i>Belone belone</i>	IC	IC	–	–	–	–	–	–	–
Bothidae									
<i>Arnoglossus laterna</i>	PC	–	–	–	–	–	–	–	–
<i>Arnoglossus imperialis</i>	–	PC	–	–	–	–	–	–	–
Bramidae									
<i>Brama brama</i>	–	SA	–	–	–	–	–	–	–
Carangidae									
<i>Trachurus capensis</i>	–	SA	–	–	–	–	–	–	–
<i>Trachurus mediterraneus</i>	–	CM	–	–	–	–	–	–	–
<i>Trachurus picturatus</i>	AZ, MD	AZ, MD		AZ, MD	–				
<i>Trachurus trachurus</i>	NEA, IC, MA, WM	CM, EM, WM, IC, MA	–	EM	–	AZ	CM	–	–
<i>Selar crumenophthalmus</i>	–	–	–	CHS	–	–	–	–	–

Citharidae										
<i>Citharus linguatula</i>	PC	PC	-	-	-	-	-	-	-	-
Clupeidae										
<i>Clupea harengus</i>	NEA, BS, NEP	-	-	-	-	-	-	-	-	-
<i>Etrumeus whiteheadi</i>	-	SA	-	-	-	-	-	-	-	-
Congridae										
<i>Conger conger</i>	IC	CM	-	-	-	-	-	-	-	-
<i>Astroconger myriaster</i>	-	CHS	-	-	-	-	-	-	-	-
Coryphaenidae										
<i>Coryphaena hippurus</i>	-	-	-	SC	-	-	-	-	-	-
Emmelichthyidae										
<i>Emmelichthys nitidus nitidus</i>	-	SA	-	-	-	-	-	-	-	-
Engraulidae										
<i>Engraulis encrasicolus</i>	-	CM	-	-	-	-	-	-	-	-
Gadidae										
<i>Boreogadus saida</i>	IC	-	-	-	-	-	-	-	-	-
<i>Micromesistius poutassou</i>	IC	CM, IC, NAM	-	-	-	-	-	-	-	-
<i>Gadus morhua</i>	BS, IC	-	-	-	-	-	-	-	-	-
<i>Theragra chalcogramma</i>	NEP, JA, BE	-	-	-	-	-	-	-	-	-
<i>Trisopterus luscus</i>	IC	-	-	-	-	-	-	-	-	-
Gempylidae										
<i>Thyrsites atun</i>	-	-	SA	-	-	-	-	-	-	-

(continued)

TABLE 2.5 (continued)

	<i>A. simplex</i> (s.s.)	<i>A. pegreffii</i>	<i>A. simplex</i> C	<i>A. typica</i>	<i>A. ziphidarum</i>	<i>Anisakis</i> sp.	<i>A. physeteris</i>	<i>A. brevispiculata</i>	<i>A. paggiae</i>
Hexagrammidae									
<i>Pleurogrammus azonus</i>	NEA	—	—	—	—	—	—	—	—
Lophiidae									
<i>Lophius piscatorius</i>	IC	NAM	—	—	—	—	—	—	—
<i>Lophius vomerinus</i>	—	SA	—	—	—	—	—	—	—
Lotidae									
<i>Molva dypterygia</i>	IC	—	—	—	—	—	—	—	—
<i>Brosme brosme</i>	NEA	—	—	—	—	—	—	—	—
Merlucciidae									
<i>Merluccius capensis</i>	—	SA	—	—	—	—	—	—	—
<i>Merluccius hubbsi</i>	—	FA	—	—	—	—	—	—	—
<i>Merluccius merluccius</i>	NEA, IC, MA	CM, EM, WM, IC, NEA, MA	—	MA, EM, NAM	MA	IC	CM, MA, WM, IC, EM, NAM	MA	IC
Muraenidae									
<i>Muraena helena</i>	—	NAM	—	—	—	—	—	—	—
Moridae									
<i>Pseudophycis bachus</i>	—	NZ	NZ	—	—	—	—	—	—
Nemipteridae									
<i>Nemipterus virgatus</i>	—	—	—	CHS	—	—	—	—	—
<i>Nemipterus bathybius</i>	—	—	—	CHS	—	—	—	—	—
Ophidiidae									
<i>Genypterus capensis</i>	—	SA	—	—	—	—	—	—	—

Osmeridae										
<i>Hypomesus pretiosus japonicus</i>	JA	-	-	-	-	-	-	-	-	-
Phycidae										
<i>Phycis phycis</i>	-	NAM	-	NAM	-	-	NAM	-	-	-
<i>Phycis blennoides</i>	-	-	-	-	-	-	NAM	-	-	-
Pinguipedidae										
<i>Parapercis colias</i>	-	NZ	NZ	-	-	-	-	-	-	-
Pleuronectidae										
<i>Hippoglossus hippoglossus</i>	BE	-	-	-	-	-	-	-	-	-
<i>Platichthys flesus</i>	-	-	-	PC	-	-	-	-	-	-
Salmonidae										
<i>Oncorhynchus gorbuscha</i>	SI	-	-	-	-	-	-	-	-	-
<i>Oncorhynchus keta</i>	SI	-	-	-	-	-	-	-	-	-
<i>Salmo salar</i>	NWA	-	-	-	-	-	-	-	-	-
Scophthalmidae										
Scomberesocidae										
<i>Scomberesox saurus</i>	NWA	-	-	-	-	-	-	-	-	-
Scombridae										
<i>Lepidorhombus boscii</i>	IC	IC, NAM	-	-	-	-	-	-	-	-
<i>Scomber japonicus</i>	AZ, MD, JA	AZ, MD, JA	-	AZ, MD	AZ, MD	-	MD	-	-	-
<i>Scomber scombrus</i>	NEA, IC, NAM	CM, IC	-	NAM	-	-	NAM	-	-	-

(continued)

TABLE 2.5 (continued)

	<i>A. simplex</i> (s.s.)	<i>A. pegreffii</i>	<i>A. simplex</i> C	<i>A. typica</i>	<i>A. ziphidarum</i>	<i>Anisakis</i> sp.	<i>A. physeteris</i>	<i>A. brevispiculata</i>	<i>A. paggiae</i>
<i>Thunnus thynnus</i>	JA	CM, BR	–	BR	–	–	–	–	–
<i>Auxis thazard</i>	–	–	–	BR	–	–	–	–	–
<i>Euthynnus affinis</i>	–	–	–	SC	–	–	–	–	–
<i>Sarda orientalis</i>	–	–	–	SC	–	–	–	–	–
<i>Scomberomorus</i> <i>commerson</i>	–	–	–	SC	–	–	–	–	–
Scorpaenidae									
<i>Scorpaena scrofa</i>	IC	IC	–	–	–	–	–	–	–
Sebastidae									
<i>Helicolenus</i> <i>dactylopterus</i>	–	CM, SA	–	–	–	–	–	–	–
Soleidae									
<i>Dicologlossa cuneata</i>	–	PC	–	–	–	–	–	–	–
<i>Solea senegalensis</i>	PC	–	–	–	–	–	–	–	–
Sparidae									
<i>Spondyllosoma</i> <i>cantharus</i>	IC	–	–	–	–	–	–	–	–
Sternoptychidae									
<i>Maurollicus muelleri</i>	NEA	–	–	–	–	–	–	–	–
Trachichthyidae									
<i>Hoplostethus</i> <i>atlanticus</i>	–	–	TA	–	–	–	–	–	–
<i>Hoplostethus</i> <i>mediterraneus</i>	–	CM	–	–	–	–	–	–	–
Trachinidae									
<i>Echiichthys vipera</i>	–	NAM	–	–	–	–	–	–	–
Trichiuridae									
<i>Lepidopus caudatus</i>	–	CM, SA	–	–	–	–	–	–	–

<i>Aphanopus carbo</i>	MD	MD	–	–	MD	AZ	AZ	AZ	AZ
<i>Trichiurus lepturus</i>	–	NAM							
Triglidae									
<i>Eutrigla gurnardus</i>	IC	–	–	–	–	–	–	–	–
Xiphiidae									
<i>Xiphias gladius</i>	NEA	CM	–	CA	CA	–	CM, IC, NEA, CA, EM	AZ, CA	AZ

Sampling locality codes: AZ: Azores Islands; BE: Bering Sea; BR: Brazil Atlantic coast; BS: Barents Sea; CHS: China Sea; CA: Central Atlantic Ocean; CM: Central Mediterranean Sea; CS: Caribbean Sea; EM: East Mediterranean Sea; FA: Falkland Islands; FL: Florida coast; IC: Iberian Atlantic Coast; JA: Japan Sea; MA: Mauritanian coast; MD: Madeira Island; NAM: North African Mediterranean coast; NEA: North-East Atlantic; NEP: North-East Pacific; NWA: North-West Atlantic; NZ: New Zealand; PC: Portuguese coast; SA: South Africa coast; SC: Somali coast; SI: off Sakhalin Islands; TA: Tasman Sea; WM: West Mediterranean Sea (data from: [Abollo et al., 2001](#); [Farjallah et al., 2008](#); [Klimpel et al., 2007](#); [Marques et al., 2006](#); [Mattiucci and Nascetti, 2006, 2007](#); [Mattiucci et al., 1986, 1997, 2001, 2002a, 2004, 2005, 2007a](#); [Nascetti et al., 1986](#); [Orecchia et al., 1986a](#); [Paggi et al., 1998a, b, c](#); [Pontes et al., 2005](#); [Umehara et al., 2006, 2008](#); [Zhu et al., 2007](#)). Hosts listed by alphabetical order of the family.

A. pegreffii Campana-Rouget and Biocca, 1955: Previously indicated as *A. simplex* A (see [Nascetti et al., 1986](#)), *A. pegreffii* is the dominant species of *Anisakis* in the Mediterranean Sea, being widespread in all the fish species. Indeed, it is presently the most important anisakid nematode in several pelagic and demersal fish from Mediterranean waters ([Farjallah et al., 2008](#); [Mattiucci et al., 1997, 2007a](#); [Paggi et al., 1998a](#)). It is also widely distributed at both adult and larval stage in the Austral Region between 30°N and 55°S ([Mattiucci et al., 1997](#)). In Atlantic waters, the northerly limit of its geographical range is represented by the Iberian coast ([Abollo et al., 2001](#); [Marquez et al., 2006](#); [Mattiucci et al., 1997, 2004, 2007a](#); [Pontes et al., 2005](#)), and has not so far been reported from the western Atlantic (our unpublished data). It has been detected, using the PCR-RFLP of the ITS region, at the larval stage in some fish hosts from Japanese waters ([Abe et al., 2006](#); [Umehara et al., 2006, 2008](#)) (Table 2.5; Fig. 2.1). SSCP-based identification of *A. pegreffii* larvae in fish from China waters, using genetic markers in the ITS-2 rDNA was also reported ([Zhu et al., 2007](#)) (Table 2.5; Fig. 2.1).

To date, it has been recorded as an adult in three species of dolphins, belonging to the family Delphinidae, and in several species of fish and three squids as a larva (Tables 2.4 and 2.5). Among these, two definitive and sixteen intermediate/paratenic hosts were found to be shared with *A. simplex* (s.s.) in the contact area between the two species [Iberian Atlantic coast, western Mediterranean Sea (Alboran Sea) and Japan Sea waters] (Fig. 2.1). Whereas, two definitive and few intermediate/paratenic hosts are shared by *A. pegreffii* and *A. simplex* C in the Austral waters off New Zealand, the South African coast and the southern Chilean coast (Table 2.5; Fig. 2.1). Based on PCR-RFLP of the ITS, [D'Amelio et al. \(1999\)](#) and direct sequencing of the mtDNA *cox2* ([Mattiucci et al., 2007b](#)), *Anisakis* larvae removed by endoscopy from humans in Italy were recognized as belonging to *A. pegreffii*.

A. simplex C of [Mattiucci et al. \(1997\)](#): *A. simplex* C currently exhibits a discontinuous range, including the Canadian and Chilean Pacific coasts, New Zealand waters and the South African Atlantic coast ([Mattiucci et al., 1997](#), unpublished data; [Nadler et al., 2005](#)). This species has been identified at the adult stage in cetaceans and as a larva it occurs syntopically with *A. pegreffii* in some fish species (Tables 2.4 and 2.5; Fig. 2.1). It has been occasionally identified also in *Mirounga leonina* from sub-Antarctic area (our unpublished data) and in *M. angustirostris* from North-East Pacific Ocean ([Nadler et al., 2005](#)).

A. typica (Diesing, 1860): According to the data from the *A. typica* populations so far detected genetically, its range extends from 30°S to 35°N in warmer temperate and tropical waters (Tables 2.4 and 2.5; Fig. 2.1) ([Mattiucci et al., 2002a](#)). In these areas it was found by genetic markers as an adult in dolphin species and as a larva in several fish

species (Tables 2.4 and 2.5). *A. typica* has also been identified in the striped dolphin, *Stenella coeruleoalba*, and in the European hake, *Merluccius merluccius*, from the eastern Mediterranean Sea (off Cyprus). Its presence in these waters could be the result of the ‘Lessepsian migration’ (through the Suez Canal) (Mattiucci *et al.*, 2004) of its intermediate/paratenic hosts from the Indian Ocean. It was recently recognized using RFLP-PCR of the ITS rDNA in the flatfish *Platichthys flesus* captured in central Portuguese waters of the NE Atlantic Ocean (Marques *et al.*, 2006), and only rarely in some fish caught along the North African coast of the Mediterranean Sea (Farjallah *et al.*, 2008). SSCP-based identification of *Anisakis* spp. larvae, using genetic markers in the ITS-2 rDNA, detected *A. typica* larvae in fish from China waters (Zhu *et al.*, 2007) (Table 2.5; Fig. 2.1).

Anisakis sp. 1: Recently, a new gene pool, referred to as *Anisakis* sp. 1 was genetically detected by both allozyme and mtDNA analysis, at the larval stage, as a parasite of the fish *Nemipterus japonicus* caught off the Malaysian coast (Berland and Mattiucci, unpublished data). This new taxon is genetically distinct from all the known species of *Anisakis* but most closely related to *A. typica* from central Atlantic waters. Although known only at the larval stage, the third-stage larva of this undescribed taxon is a type I larva (*sensu* Berland, 1961). The preliminary results appear to indicate that this taxon may be a sibling species of *A. typica* occurring in central Pacific waters (Berland and Mattiucci, unpublished data).

A. ziphidarum Paggi, Nascetti, Webb, Mattiucci, Cianchi and Bullini, 1998: *A. ziphidarum* was first described, both genetically and morphologically, as an adult in the beaked whales *Mesoplodon layardii* and *Ziphius cavirostris* from the South Atlantic Ocean (off the South African coast). Subsequently, it has also been recorded in the Mediterranean Sea, also in *Z. cavirostris*. Since its first morphological description and genetic characterization (Paggi *et al.*, 1998b), it has recently been identified genetically as an adult in other species of beaked whale, such as *M. mirus* and *M. grayi*, in South Atlantic waters and in *Mesoplodon* sp. and *Z. cavirostris* in Caribbean waters (Mattiucci and Nascetti, 2006). Thus, its geographical range appears to be wide (Fig. 2.1) and related to that of its definitive hosts. Only scanty data are available concerning its infection in fish and/or squid, but it is responsible for a low prevalence of infection in some fish species, such as in *Merluccius merluccius* (see Mattiucci *et al.*, 2004) and *Aphanopus carbo* (see Pontes *et al.*, 2005; Saraiva *et al.*, 2007) in central Atlantic waters. However, it seems that this species may involve other intermediate hosts, such as squid, rather than fish in its life cycle, as these represent the main food source of beaked whales.

Anisakis sp. of Valentini *et al.* (2006): This species has been detected only at a larval stage (L4) in the beaked whales *Mesoplodon mirus* and *M. grayi* from South African and New Zealand waters (Mattiucci and

Nascetti, 2006) (Fig. 2.1; Table 2.4). The gene pool was found to be reproductively isolated from the sympatric species *A. ziphidarum* occurring in the same hosts and geographical region. It is genetically very distinct from the other species of *Anisakis*, but is most closely related to *A. ziphidarum* (see Section 5.1; Table 2.8). The third-stage larva of this undescribed taxon is apparently of type I and has on rare occasions been identified in the fishes (see Mattiucci *et al.*, 2007a; Saraiva *et al.*, 2007) caught in North East Atlantic waters. Conversely, this species has been genetically identified, at the larval stage, heavily infecting the squid *Moroteuthis ingens* in Tasman Sea waters (our unpublished data). This appears to support the hypothesis that this species involves squid rather than fish in its life cycle. Recently, Pontes *et al.* (2005) detected the occurrence of a new taxon at larval stage in *Aphanopus carbo* and *Scomber japonicus* from Madeira waters (Atlantic Ocean) indicated as *Anisakis* sp. A, genetically closely related to *A. ziphidarum*. However, the exact correspondence between this taxon and *Anisakis* sp. of Valentini *et al.* (2006) has not yet been assessed.

3.1.2. *Anisakis* spp. included in clade II

Three species and one new gene pool of *Anisakis* so far comprises this clade, as clearly demonstrated by allozymes (Mattiucci *et al.*, 2005) and mtDNA *cox2* sequence analysis (Valentini *et al.*, 2006). They are *A. physeteris*, *A. brevispiculata* and *A. paggiae*, which represent a complex of sibling species readily recognized genetically at both nuclear and mitochondrial levels. The existence of *A. physeteris* clustering with *A. brevispiculata* in the same clade, as a sister group to the other species included in the clade I, was also supported by rDNA ITS sequence phylogenetic analysis (Nadler *et al.*, 2005) (see also Section 4.1). As far as is known, all these species share at the third larval stage the morphology known as type II (*sensu* Berland, 1961). A summary of each follows, including a synopsis of some ecological aspects of each species in relation to their known geographical distribution and both definitive and intermediate/paratenic hosts (Tables 2.4 and 2.5).

A. physeteris (Baylis, 1920): A population of this species was first genetically characterized in its main definitive host, the sperm whale, *Physeter macrocephalus*, from Mediterranean waters (Mattiucci *et al.*, 1986); no genetically identified adults have been recorded in other cetacean hosts. Type II larvae of *A. physeteris* have been genetically identified, occurring rarely in only a very few fish host species (Mattiucci *et al.*, 1986, 2001, 2004), except for the swordfish, *Xiphias gladius*, from Mediterranean and Atlantic waters in which it represents the main *Anisakis* species (Mattiucci *et al.*, 2007a) (Tables 2.4 and 2.5; Fig. 2.1). Despite the fact that the swordfish might only represent an accidental host in the life cycle of

Anisakis spp., acquiring the infection by preying on infected invertebrates or squid (main prey of the swordfish), suggests that other intermediate hosts, mainly squids rather than fish are involved in the life cycle of this parasite.

A. brevispiculata Dollfus, 1966: A population of *A. brevispiculata* was initially characterized genetically using allozymes based on material from a pygmy sperm whale, *Kogia breviceps*, in South African and North East Atlantic waters (Iberian coast) (Mattiucci *et al.*, 2001) (Table 2.4; Fig. 2.1). Reproductive isolation from the morphologically closely related *A. physeteris* was demonstrated, establishing the validity of *A. brevispiculata* (see Mattiucci *et al.*, 2001), which has been synonymized with *A. physeteris* by Davey (1971). Later, the same species was sequenced at the mtDNA *cox2* gene and its genetic relationships with respect to the other *Anisakis* spp. was established, confirming that *A. brevispiculata* clusters well with those *Anisakis* species forming the second clade, as also indicated by nuclear markers (allozymes). A congruent result was inferred from the ITS rDNA sequence analysis (Nadler *et al.*, 2005) (see Section 4.1). *Anisakis* larvae of type II corresponding to *A. brevispiculata* were recognized by allozyme markers as a rare parasites of the fish *Merluccius merluccius* (see Mattiucci *et al.*, 2004, 2007a) and the swordfish *Xiphias gladius* in Atlantic waters (Mattiucci *et al.*, 2007a).

A. paggiae Mattiucci *et al.* (2005): In the cluster formed by *A. physeteris* and *A. brevispiculata*, this third species has been recently demonstrated by both allozymes (Mattiucci *et al.*, 2005) and mtDNA *cox2* sequence analysis (Valentini *et al.*, 2006) (Fig. 2.1). *A. paggiae* was first genetically characterized and described morphologically as an adult parasite of the pygmy sperm whale, *Kogia breviceps*, and the dwarf sperm whale, *K. sima*, from off both Florida and the South African Atlantic coast (Mattiucci *et al.*, 2005). Scanty data are so far available regarding the identification of the intermediate hosts in the life cycle of *A. paggiae*. A very few larvae of type II have been identified as belonging to these species in fish from Atlantic waters (i.e. *M. merluccius* and *X. gladius*) (see Mattiucci *et al.*, 2007a, unpublished data), thus suggesting that other hosts, not yet detected, are involved in the life cycle of this *Anisakis* species.

Anisakis sp. 2: A further gene pool, referred to as *Anisakis* sp. 2, has been detected genetically by means of allozyme markers and mtDNA *cox2* sequence analysis based on larvae of type II from the swordfish *X. gladius* in the equatorial area (Mattiucci *et al.*, 2007a, unpublished data). The new taxon was shown to be genetically distinct from all the other *Anisakis* species, and that it is mostly closely related to *A. physeteris*. Preliminary phylogenetic analysis showed that it clusters with the clade formed by *A. physeteris*, *A. brevispiculata* and *A. paggiae*, thus suggesting that this new gene pool might represent an undescribed sibling species belonging to this same complex.

The high genetic heterogeneity of the *Anisakis* spp. is now also supported by some differential morphological features detected in the species belonging to this genus, where the two major clades can be delineated as follows: (i) the ventriculus, in the adult stage, is short, never sigmoid and broader than long in *A. physeteris*, *A. brevispiculata* and *A. paggiae* (see Mattiucci *et al.*, 2005), and longer than broad and often sigmoid in shape in those species included in clade I (the species of the *A. simplex* complex, *A. typica* and *A. ziphidarum*) (see Mattiucci *et al.*, 2005; Paggi *et al.*, 1998b); (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) but are long and often unequal (equal in *A. ziphidarum*; see Paggi *et al.*, 1998b) in species of clade I; and (iii) type II larval morphology (*sensu* Berland, 1961) is characteristic of *A. physeteris*, *A. brevispiculata*, *A. paggiae* and *Anisakis* sp. 2 (Mattiucci *et al.*, 2001, 2004, 2005, 2007a) (clade II), whereas a type I morphology (*sensu* Berland, 1961), can be found in all the species of the *A. simplex* complex, *A. typica*, *A. ziphidarum* and *Anisakis* sp. and *Anisakis* sp. 1 (clade I).

While no morphological characters are so far known which help in distinguishing the sibling species of the *A. simplex* complex, some morphological features, of diagnostic value, available in male and female adult specimens, were used to help in distinguishing *A. paggiae* from *A. physeteris* and *A. brevispiculata* (see Mattiucci *et al.*, 2005). Indeed a morphological key to the recognized adults of *Anisakis* spp. so far included in clade II was provided by Mattiucci *et al.* (2005).

3.2. The current taxonomy of *Pseudoterranova decipiens* (*sensu lato*)

The species *decipiens* was first described as *Ascaris decipiens* by Krabbe (1878). *A. decipiens* was later linked with *Terranova* Leiper and Atkinson, 1914 (a genus erected for parasites of elasmobranchs) by Baylis (1916), because of the presence of an intestinal caecum. Subsequently, Baylis linked this species within *Porrocaecum* Railliet and Henry, 1912 (a group of ascarids now mainly restricted to terrestrial birds), because he considered it as a senior synonym of *Terranova*. *Terranova* was resurrected at full generic level by Johnston and Mawson (1945) and as a subgenus by Karokhin (1946), the latter being the first to use the combination *T. decipiens*. *T. decipiens* was accepted by Mozgovoi (1951, 1953), Hartwich (1957) and Yamaguti (1961). However, because *Terranova* included species maturing in different definitive hosts (elasmobranchs, reptiles, marine mammals), Myers (1959) erected *Phocanema* (with *decipiens* as the type and only species) for those species included in *Terranova* which were parasites of marine mammals on the base of some morphological features at the cephalic end (structure of the labia) and the male caudal end.

Accordingly, the species *decipiens* and *Terranova kogiae* Johnston and Mawson, 1945 (described from the pygmy sperm whale, *Kogia breviceps*) were included in *Phocanema*. Later, Gibson (1983) considered *Phocanema* as a synonym of *Pseudoterranova*, previously erected by Mozgovoi (1951; listed in Mozgovoi, without indication) for parasites from sperm whales, and *decipiens* was transferred to this genus (combination first used in Gibson and Colin, 1982). According to the same authors, the group of species of *Pseudoterranova* from marine mammals included *P. kogiae* (Johnston and Mawson, 1945), *P. ceticola* (Deardoff and Overstreet, 1981) and *P. decipiens*.

This is the *status quo* which has been widely accepted. Although several nominal species related to *Pseudoterranova decipiens* (i.e. occurring in seals and possessing only an intestinal caecum) having been described, these species have tended to be considered as synonyms of *P. decipiens*. This species appeared to have a low host specificity, with up to 18 seal species been recorded as definitive hosts (e.g. Hartwich, 1975; King, 1983; Myers, 1959), and exhibited some morphological variation between specimens collected from the bearded seal, *Erignathus barbatus*, in Canadian waters (Baylis, 1916).

Population genetic analysis, first performed by allozyme markers, on specimens of *P. decipiens* (s.l.) recovered from 10 fish species, and from four seal species, collected at several locations of the North Atlantic Ocean from the Canadian Atlantic eastwards to the Barents Sea, demonstrated the existence of a remarkable genetic heterogeneity with striking variation in allele frequencies among the samples (Paggi *et al.*, 1991). Indeed, the observed genotype frequencies at various loci showed significant deviations from those expected using the Hardy–Weinberg equilibrium, with a complete absence or marked deficiency of various heterozygous classes in material recovered from several sites and also between worms recovered from one host individual. The only possible explanation was that three distinct biological species occurred sympatrically in the samples of *P. decipiens* (s.l.) collected in seals hosts from those geographical areas, with no gene flow between them: the three taxa genetically recognized were thus provisionally designated as *P. decipiens* A, *P. decipiens* B and *P. decipiens* C (see Paggi *et al.*, 1991). Morphological analysis carried out on male specimens identified by allozyme markers as *P. decipiens* A and B allowed the detection of significant differences in a number of characters between these two members; on the basis of such differences the nomenclature designation for *P. decipiens* A and *P. decipiens* B was proposed (see Paggi *et al.*, 2000). The names *Pseudoterranova krabbei* Paggi *et al.*, 2000 and *P. decipiens* (s.s.) were proposed, respectively, for species A and B, and a formal description of the two taxa was provided (see Paggi *et al.*, 2000).

The name of *P. bulbosa* (Cobb, 1888) was proposed for the taxon *P. decipiens* C (see Mattiucci *et al.*, 1998), as the latter taxon was demonstrated

to correspond morphologically with *Ascaris bulbosa* described by Cobb (1888) from the collection of Dr. Kukenthal collected from the bearded seal, *Erignathus barbatus*, at Spitzbergen (NE Atlantic Ocean). Furthermore, a sample of *P. decipiens* C from that same locality was studied both genetically and morphologically by [Paggi et al. \(1991, 2000\)](#). Previously, *Ascaris bulbosa* had been considered a synonym of *Phocanema decipiens* [later included in *Pseudoterranova* by [Gibson and Colin \(1982\)](#)] by [Myers \(1959\)](#). Therefore, [Mattiucci et al. \(1998\)](#) proposed the name *P. bulbosa* (Cobb, 1888) n. comb. for *P. decipiens* C.

A further taxon, provisionally designated as *P. decipiens* D (see [Mattiucci et al., 1998](#)), was later included in the *P. decipiens* complex; this was detected by exhibiting several fixed differences at some enzyme loci with respect to *P. decipiens* A, *P. decipiens* B and *P. decipiens* C. It was found to occur sympatrically with *P. bulbosa* in the same geographical areas (Japanese waters) and occasionally in the same definitive host, the bearded seal *Erignathus barbatus*, from which it was demonstrated to be reproductively isolated ([Mattiucci et al., 1998](#)). *P. decipiens* D was found to correspond to the measurements and tail drawing of *Porrocaecum azarasi* [Yamaguti and Arima, 1942](#), a species described by [Yamaguti and Arima \(1942\)](#) based on specimens recovered in the ribbon seal *Phoca* (= *Histriophoca*) *fasciata* on the islands of Sakhalin and Hokkaido. This taxon was synonymized by [Margolis \(1956\)](#) with '*Phocanema decipiens*'. Therefore, [Mattiucci et al. \(1998\)](#) proposed the name *Pseudoterranova azarasi* ([Yamaguti and Arima, 1942](#)) n. comb. for species *P. decipiens* D.

Using allozyme markers on larval and adult population of *P. decipiens* (s.l.) collected from four fish species and the Austral fur seal, *Otaria byronia*, in the SE Pacific Ocean, a further member of the *P. decipiens* complex has been shown to exist ([George-Nascimento and Llanos, 1995](#)). In the formal description, this taxon was named *P. cattani* [George-Nascimento and Urrutia, 2000](#).

A summary of the sibling species *P. decipiens* (s.l.) recognized by using several molecular genetic methodologies are reported below with ecological data on their host preference ([Table 2.5](#)) and geographical range ([Fig. 2.1](#)).

P. decipiens (Krabbe, 1868) (*sensu stricto*) (= *P. decipiens* B): This species was initially recognized as *P. decipiens* B by [Paggi et al. \(1991\)](#); its formal description was later given based on morphological features considered to be of diagnostic value with respect to the other members included in the *P. decipiens* complex (see [Paggi et al., 2000](#)). Its geographical range appears to be wide and mainly in the Arctic and sub-Arctic regions, including the North East Atlantic (comprising Scottish, Faroes, southern Icelandic and Norwegian waters), the Canadian Atlantic (including Newfoundland waters and the Gulf of S. Lawrence) ([Bratney and Stenson, 1993](#); [Paggi et al., 1991](#)) and the Canadian Pacific waters ([Mattiucci et al.,](#)

1998; Nadler *et al.*, 2005; Paggi *et al.*, 1998c). Areas of sympatry between *P. decipiens* (s.s.) and other species of the *P. decipiens* complex have been detected; in the North Eastern Atlantic waters in mixed infections with both *P. krabbei* and *P. bulbosa* and in Canadian Atlantic waters with *P. bulbosa* (see Paggi *et al.*, 1991) (Fig. 2.1). *P. decipiens* (s.s.) was recognized, using genetic markers, as a parasite at the adult stage in the common seal, *Phoca vitulina*, grey seal, *Halichoerus grypus*, in both North and West Atlantic waters, and in *Phoca vitulina richardsii* and *Zalophus californianus* in northern Pacific waters (Paggi *et al.*, 1998c). Its larval forms have been identified in some gadoid fish species of the North Atlantic Ocean (Mattiucci *et al.*, 1998; Paggi *et al.*, 1991) (Table 2.6).

P. krabbei Paggi, Mattiucci *et al.*, 2000: This species was previously referred to as *P. decipiens* A (Paggi *et al.*, 1991). To date it has been found only in the North-East Atlantic (including off Scotland, the Faroes, southern Iceland and Norway) (Fig. 2.1). It is mainly an adult parasite of the grey seal, *Halichoerus grypus*, and the common seal, *Phoca vitulina*, often in mixed infections in the same individual host with *P. decipiens* (s.s.) (Table 2.6). However, in the eastern Atlantic waters, when detected in sympatry with *P. decipiens* (s.s.), *P. krabbei* prevails in the grey seal rather than in the common seal. The ecological significance of this finding is discussed in Section 7. *P. krabbei* has been recognized genetically at the larval stage as a parasite of *Gadus morhua*, *Melanogrammus aeglefinus* and *Pollachius virens* (see Paggi *et al.*, 1991) (Table 2.6; Fig. 2.1). *P. krabbei* exhibits, in male specimens with respect to *P. decipiens* (s.s.), the following diagnostic morphological features: shorter spicules; a proximal papilla (nomenclature according to Fagerhom, 1989) smaller than d1 versus the same papilla size in *P. decipiens* (s.s.); distal papillae 1, 2 and 4 closer to each other; and caudal plates of a similar width and narrower than in *P. decipiens* (s.s.) where plates (wp) 1 (wp1) and 2 (wp2) are of a similar width but plate 3 (wp3) is narrower (Paggi *et al.*, 2000).

P. bulbosa (Cobb, 1888): Previously referred to as *P. decipiens* C (Paggi *et al.*, 1991) (see Mattiucci *et al.*, 1998), this species has been recorded from the Barents and Norwegian Seas, the Canadian Atlantic and the Sea of Japan, between 40°N and 80°N (Bratley and Stenson, 1993; Mattiucci *et al.*, 1998; Paggi *et al.*, 1991). Its main definitive host so far detected is the bearded seal, *Erignathus barbatus*. In this phocid host from the Otaru Sea (Sea of Japan), *P. bulbosa* has been identified as occurring in mixed infections with *P. azarasi* (see Mattiucci *et al.*, 1998). The benthic flatfishes *Hippoglossoides platessoides* and *Reinhardtius hippoglossoides* are reported as its intermediate hosts (Paggi *et al.*, 1991) (Table 2.6; Fig. 2.1).

P. bulbosa differs from the other members of the *P. decipiens* complex in having the following morphological features in adult male specimens: longer spicules; caudal plates (wp) of unequal width, with plate 2 (wp2)

TABLE 2.6 Definitive and intermediate/paratenic hosts so far detected, by molecular genetic markers, for the *Pseudoterranova decipiens* species complex

	<i>P. krabbei</i>	<i>P. decipiens</i> (s.s.)	<i>P. bulbosa</i>	<i>P. azarasi</i>	<i>P. decipiens</i> E	<i>P. cattani</i>
Pinnipeds						
Otariidae						
<i>Eumetopias jubatus</i>	—	—	—	NWP, JA	—	—
<i>Otaria byronia</i>	—	—	—	—	—	SEP
<i>Zalophus californianus</i>	—	NEP	—	NWA	—	—
Phocidae						
<i>Phoca vitulina richardsii</i>	—	NEP	—	NWA	—	—
<i>Phoca vitulina</i>	NEA	NEA, NWA, NEP	—	—	—	—
<i>Erignathus barbatus</i>	—	—	NEP, NEA, LS	NWP, JA	—	—
<i>Halichoerus grypus</i>	NEA	NEA, NWA	—	—	—	—
<i>Cystophora cristata</i>	—	NWA, NEA	—	—	—	—
<i>Mirounga angustirostris</i>	—	NEP	—	—	—	—
<i>Leptonychotes weddellii</i>	—	—	—	—	AN	—
Fishes						
Channichthyidae						
<i>Chaenocephalus aceratus</i>	—	—	—	—	AN	—

(continued)

TABLE 2.6 (continued)

	<i>P. krabbei</i>	<i>P. decipiens</i> (s.s.)	<i>P. bulbosa</i>	<i>P. azarasi</i>	<i>P. decipiens</i> E	<i>P. cattani</i>
Cottidae						
<i>Myxocephalus scorpius</i>	–	NWA	–	–	–	–
<i>Myxocephalus quadricornis</i>	–	–	NWP	BE, NWP	–	–
Gadidae						
<i>Gadus morhua</i>	–	NWA	NWP	JA, NWP	–	–
<i>macrocephalus</i>						
<i>Gadus morhua</i>	NEA	NEA	–	–	–	–
<i>Gadus ogac</i>	–	NWA	–	–	–	–
<i>Pollachius virens</i>	FI	NWA	–	–	–	–
<i>Melanogrammus aeglefinus</i>	FI	–	–	–	–	–
<i>Boreogadus saida</i>	–	NWA	–	–	–	–
Lotidae						
<i>Brosme brosme</i>	–	NEA	–	–	–	–
Merluccidae						
<i>Merluccius gayi</i>	–	–	–	–	–	SEP
Ophidiidae						
<i>Genypterus maculatus</i>	–	–	–	–	–	SEP
Osmeridae						
<i>Osmerus eperlanus</i>	–	NEA	–	–	–	–

(continued)

TABLE 2.6 (continued)

	<i>P. krabbei</i>	<i>P. decipiens</i> (s.s.)	<i>P. bulbosa</i>	<i>P. azarasi</i>	<i>P. decipiens</i> E	<i>P. cattani</i>
Notothenidae						
<i>Notothenia coriiceps</i>	–	–	–	–	AN	–
<i>Notothenia neglecta</i>	–	–	–	–	AN	–
<i>Trematomus newnesi</i>	–	–	–	–	AN	–
Paralichthyidae						
<i>Paralichthys microps</i>	–	–	–	–	–	SEP
Pleuronectidae						
<i>Hippoglossus hippoglossus</i>	–	–	NWP	BE, NWP	–	–
<i>Hippoglossoides platessoides</i>	NEA	NEA	BS	–	–	–
<i>Reinhardtius hippoglossoides</i>	–	–	NWA	–	–	–
Scophthalmidae						
<i>Psetta maxima</i>	NEA	–	–	–	–	–

Codes: AN: Antarctica; BE: Bering Sea; BS: Barents Sea; FI: Faeroe Islands; JA: Japan Sea; LS: Labrador Sea; NEA: North-East Atlantic; NEP: North-East Pacific; NWA: North-West Atlantic; NWP: North-West Pacific; SEP: South-East Pacific (Chilean coast) (data from [George-Nascimento and Llanos, 1995](#); [George-Nascimento and Urrutia, 2000](#); [Mattiucci et al., 1998](#); [Nadler et al., 2005](#); [Paggi et al., 1991, 1998](#); [Zhu et al., 2002](#)). Hosts listed by alphabetical order of the family.

narrower than 1(wp1) and 3 (wp3); and a different pattern of distal caudal papillae, with d2 far apart from d4 (Mattiucci *et al.*, 1998).

P. azarasi (Yamaguti and Arima, 1942): This species was previously referred to as *P. decipiens* D (see Mattiucci *et al.*, 1998). Its main definitive host has been identified as the Steller's sea lion, *Eumetopias jubatus*. Its geographical distribution appears limited to Japanese and Sakhalinese waters of the North Pacific Ocean (Mattiucci *et al.*, 1998) (Table 2.6; Fig. 2.1).

Both *P. bulbosa* and *P. azarasi* were resurrected, on both allozyme and morphological bases (see Mattiucci *et al.*, 1998), from synonymy with *P. decipiens* (see Margolis, 1956; Myers, 1959). *P. azarasi* differs from the other members of the *P. decipiens* complex in having shorter spicules; caudal plates (wp) of increasing width from 1 to 3; and a different pattern of distal papillae (*sensu* Fagerholm, 1989), with d2 closer to d4 than in *P. bulbosa* but more distant than in *P. krabbei* and *P. decipiens* (s.s.) (see Mattiucci *et al.*, 1998).

P. decipiens E of Bullini *et al.*, 1997. This taxon was genetically detected in the Antarctic Weddell seal, *Leptonychotes weddellii* (see Bullini *et al.*, 1997). Scanty data are so far available in relation to the nature of its intermediate host, although larvae corresponding to this sibling species were found as parasites of benthic fish hosts in subantarctic waters (see Section 7.2) (Table 2.6).

P. cattani George-Nascimento and Urrutia, 2000: As stated above, this species was found as an adult in *Otaria byronia* on the Chilean coast (Table 2.5; Fig. 2.1). Using molecular markers in the internal transcribed spacers of ribosomal DNA (ITS-rDNA), this species was recently shown to cluster with the *P. decipiens* complex (Zhu *et al.*, 2002) (Section 4.2).

3.3. The current taxonomy of *Contracaecum* species from pinnipeds

Contracaecum was originally defined by Railliet and Henry (1912) based on the morphology of the oesophago-intestinal region. *C. spiculigerum* (Rudolphi, 1809) (= *Ascaris spiculigerum*, Rudolphi, 1809) was designated as the type-species. The original type-material was later identified as *C. microcephalum* (Rudolphi, 1809) by Hartwich (1964), and this species was accordingly defined as the new type-species of the genus. Presently, the genus comprises some 50 nominal species, most of which are adults in pinnipeds and fish-eating birds. Hartwich (1975) defined the genus based on morphological details; important features are the presence of interlabia and the position of the excretory pore at the base of the ventral interlabium. However, several later authors suggested that the genus should be divided or amended. Mozgovoi (1951) had previously divided the genus

into three subgenera: *Contracaecum* (*Erschovicaecum*) [clearly representing *Hysterothylacium* (see [Deardoff and Overstreet, 1980](#)) and including species maturing in fish], *Contracaecum* (*Contracecum*) and *Contracaecum* (*Ornitocaecum*). These two new subgenera were considered as synonyms of *Contracaecum* by [Hartwich \(1975\)](#). *Phocascaris* [Höst, 1932](#) included the following definition ‘*interlabia present, reduced or absent . . . parasites of the digestive tract of seals*’, *P. phocae* being the type-species of the genus. According to that definition, [Berland \(1964\)](#), when describing *P. cystophorae* from the seal *Cystophora cristata*, suggested that the species of *Contracaecum* ‘with opposite caeca from seals’ should be transferred to the genus *Phocascaris*. His proposal was to retain within *Contracaecum* species from fish-eating birds. [Berland’s \(1964\)](#) hypothesis was mainly based on the life-cycle patterns of these anisakid nematodes and their definitive hosts, irrespective of their morphological phenotype. However, a taxonomic proposal based on those biological features was not generally accepted by taxonomists, and [Hartwich \(1975\)](#) did not take into account that observation when he produced the ‘Key of Ascaridoidea’, still considering a morphological character (relating to the presence/absence of interlabia) as a valid criterion for distinguishing the species belonging to *Contracaecum* and *Phocascaris*.

Several years later, the same morphological character was shown to be inconsistent and not of useful systematic value for differentiating the two groups of species included in *Contracaecum*. Indeed, based on allozymes (22 enzyme loci), [Orecchia et al. \(1986b\)](#) and [Nascetti et al. \(1990\)](#) used species *Contracaecum* from seals and fish-eating birds to show that of all the species of the *Contracaecum osculatum* complex (see [Section 4.3](#)) are genetically much more closely related to species of *Phocascaris* (i.e. *Phocascaris phocae* and *P. cystophorae*) from seals than to the congeneric species from fish-eating birds, demonstrating high levels of differentiation (*Contracaecum* species from pinnipeds share no alleles in common with those from fish-eating birds). Clustering methods based on allozyme markers showed that *P. phocae* and *P. cystophorae* form a clade with the species of *Contracaecum* from seals ([Nascetti et al., 1990](#)) ([Section 4.3](#)), suggesting an evolutionary hypothesis for the systematic status of these species. [Nadler et al. \(2000\)](#), based on nuclear rDNA sequence data of several taxa of the genera *Contracaecum* and *Phocascaris*, demonstrated the validity of the evolutionary hypothesis previously suggested by allozyme markers. A phylogenetic hypotheses based on different clustering analyses of the ITS-rDNA ([Nadler et al., 2000](#)) and the mtDNA cytochromoxidase-2 (mtDNA *cox2*) ([Mattiucci et al., 2008b](#)) sequences data strongly supported the hypothesis based on allozymes, according to which species of *Phocascaris* are nested within the clade formed by the *Contracaecum* species hosted by phocid seals and are thus closely related to species of the *Contracaecum osculatum* complex. All of the phylogenetic analyses also support the hypothesis

advanced by [Berland \(1964\)](#), based on the biology of these anisakid nematodes, in terms of the monophyly of the species of *Contracaecum* and *Phocascaris* from phocids (see also [Section 4.3](#)).

On the other hand, the molecular genetic approach has been demonstrated to be very fruitful in the detection of sibling species and disclosing new taxa of *Contracaecum* parasites of fish-eating birds ([Bullini et al., 1986](#); [D'Amelio et al., 1990, 2007](#); [Li et al., 2005](#); [Mattiucci et al., 2002b, 2008c](#)), which are not however treated in details in this review.

3.3.1. The *Contracaecum osculatum* species complex

To date, five members of the *C. osculatum* complex have been recognized genetically by the use of allozyme markers. They are the three Arctic sibling species referred to as *C. osculatum* A, *C. osculatum* B and *C. osculatum* (s.s.) (see [Nascetti et al., 1993](#)), and the two Antarctic members, *C. osculatum* D and *C. osculatum* E (see [Orecchia et al., 1994](#)).

C. osculatum A of [Nascetti et al. \(1993\)](#): This species occurs in the Norwegian and Barents Seas, Canadian Atlantic, Icelandic, Canadian Pacific waters and the Sea of Japan, between 40°N and 80°N. Its main definitive host is the bearded seal, *Erignathus barbatus*, in both eastern and western part of the North Atlantic Ocean, but the grey seal, *Halichoerus grypus*, has also been recorded as a host ([Bratley and Stenson, 1993](#); [Nascetti et al., 1993](#)). Subsequently, it has been detected genetically in the Steller's sea lion, *Eumetopias jubatus*, in the Western Pacific Ocean (Sea of Japan) ([Mattiucci et al., 1998](#); [Paggi et al., 1998c](#)). Its larvae have been identified in the gadoid fish *Theragra chalcogramma* from the Sea of Japan ([Mattiucci et al., 1998](#)) ([Table 2.7](#); [Fig. 2.1](#)).

C. osculatum B of [Nascetti et al. \(1993\)](#): This species was first detected genetically as an adult in the phocid seals *Pagophilus groenlandicus*, *Phoca vitulina* and *Halichoerus grypus* from the North-eastern and north-western Atlantic Ocean ([Bratley and Stenson, 1993](#); [Nascetti et al., 1993](#)); reproductive isolation was demonstrated by allozyme markers between this species and the other two Arctic members of the *C. osculatum* complex ([Nascetti et al., 1993](#)). Later, it was identified based on diagnostic allozyme markers as a parasite of the *Phoca vitulina* and *Zalophus californianus* in northern Pacific waters ([Mattiucci et al., 1998](#)) ([Table 2.7](#); [Fig. 2.1](#)).

C. osculatum (Rudolphi, 1802) (*sensu stricto*): This species, named *C. osculatum* C by [Nascetti et al. \(1993\)](#), was genetically characterized from the grey seal, *Halichoerus grypus*; its reproductive isolation from *C. osculatum* B was shown in the sympatric situation of individual seal hosts (i.e. the grey seal) from Iceland waters ([Nascetti et al., 1993](#)). It is the only species of the *C. osculatum* complex present in the Baltic Sea. So far, this species has not been reported from the western part of the Atlantic or from Pacific waters ([Table 2.7](#); [Fig. 2.1](#)).

TABLE 2.7 Definitive and intermediate/paratenic hosts so far detected, by molecular genetic markers, for the *Contracaecum* spp. from pinnipeds

[illegible]

<i>Arctocephalus</i>	–	–	–	–	–	–	–	AR	AR	–
<i>australis</i>										
<i>Arctocephalus</i>	–	–	–	–	–	–	–	–	SA, AU	–
<i>pusillus</i>										
Fishes										
Bathydraconidae										
<i>Gymnodraco</i>	–	–	–	RS	RS	–	–	–	–	–
<i>acuticeps</i>										
<i>Cygnodraco</i>	–	–	–	RS	RS	–	–	–	–	–
<i>mawsonii</i>										
Channichthyidae										
<i>Cryodraco</i>	–	–	–	RS	RS	–	RS	–	–	–
<i>antarcticus</i>										
<i>Chionodraco</i>	–	–	–	RS	RS	–	RS	–	–	–
<i>hamatus</i>										
<i>Pagetopsis</i>	–	–	–	RS	RS	–	–	–	–	–
<i>macropterus</i>										
<i>Chaenodraco</i>	–	–	–	RS	RS	–	–	–	–	–
<i>wilsoni</i>										
Cottidae										
							<i>Myoxocephalus</i>	–	BS	–
							<i>quadricornis</i>			
–	–	–	–	–	–					
Gadidae										
<i>Gadus morhua</i>	BE	–	–	–	–	–	–	–	–	–
<i>macrocephalus</i>										
<i>Theragra</i>	BE	–	–	–	–	–	–	–	–	–
<i>chalcogramma</i>										
Nototothenidae										
	–	–	–	RS	RS	–	–	–	–	–

(continued)

TABLE 2.7 (continued)

	<i>C. oscu- latus</i> A	<i>C. oscu- latus</i> B	<i>C. oscula- tus</i> (s.s.)	<i>C. oscula- tus</i> D	<i>C. oscula- tus</i> E	<i>C. o. baicalensis</i>	<i>C. radiatus</i>	<i>C. mirounga</i>	<i>C. ogmor- hini</i> (s.s.)	<i>C. margolisi</i>
<i>Notothernia neglecta</i>										
<i>Trematomus bernacchii</i>	–	–	–	RS	RS	–	–	–	–	–
<i>Trematomus pennelli</i>	–	–	–	RS	RS	–	–	–	–	–
Pleuronectidae										
<i>Hippoglossus hippoglossus</i>	BE	–	–	–	–	–	–	–	–	–

Sampling locality codes: AN: Antarctica; AR: Argentine coast; AU: Australian coast; BA: Baltic Sea; BE: Bering Sea; BL: Baikal Lake; BS: Barents Sea; JA: Japan Sea; NEA: North-East Atlantic; NEP: North-East Pacific; NWA: North-West Atlantic; NWP: North-West Pacific; RS: Ross Sea; SA: South Atlantic Ocean (off South Africa coast); SEA: South-East Atlantic; SWA: South-West Atlantic; WS: Weddell Sea (data from [Mattiucci and Nascetti, 2007](#); [Mattiucci et al., 1998, 2003, 2008b](#); [Nadler et al., 2000, 2005](#); [Nascetti et al., 1993](#); [Orecchia et al., 1994](#); [Paggi et al., 1998c](#)). Hosts listed by alphabetical order of the family.

C. osculatum D and *C. osculatum* E of [Orecchia et al. \(1994\)](#): The Antarctic members of the *C. osculatum* complex, *C. osculatum* D and *C. osculatum* E were demonstrated to be reproductively isolated by allozyme markers. They occur sympatrically in the same definitive host, the Weddell seal, *Leptonychotes weddellii*, and have so far been reported from both the Weddell and the Ross Seas (Antarctica) ([Orecchia et al., 1994](#)). The larval stages of the two sibling species have been identified by allozyme markers from several fish species belonging to the families Channichthyidae, Bathydraconidae and Nototheniidae, in which a differential distribution of the two sibling species is reported ([Mattiucci and Nascetti, 2007](#); see also Section 7.2) ([Table 2.7](#); [Fig. 2.1](#)).

3.3.2. The *Contracaecum ogmorhini* species complex

The pinniped parasite *Contracaecum ogmorhini* [Johnston and Mawson, 1941](#), first described from the leopard seal, *Hydrurga leptonyx*, in South Australian waters, was later synonymized with *C. osculatum* (see [Johnston and Mawson, 1945](#)). However, it was considered valid by [Fagerholm and Gibson \(1987\)](#). The species was found to be genetically heterogenous using allozyme markers (18 enzyme loci), indicating the existence of two reproductively isolated taxa (sibling species) included within the morphospecies. A formal description of the two taxa was given by [Mattiucci et al. \(2003\)](#), and they were named *C. ogmorhini* [Johnston and Mawson \(1941\)](#) (*sensu stricto*) and *C. margolisi* [Mattiucci et al. \(2003\)](#). A morphological description of *C. ogmorhini* (s.s.) from *Arctocephalus australis* was given by [Timi et al. \(2003\)](#). *C. ogmorhini* (s.s.) has been detected as an adult in the otariid seals *Arctocephalus pusillus pusillus*, *A. pusillus doriferus* and *A. australis* in the Austral region, while *C. margolisi* was detected in the otariid *Zalophus californianus* in the Boreal region ([Table 2.6](#); [Fig. 2.1](#)) (see also [Section 4.3](#)). To date, the allopatric distribution of these two sibling species appears to be related to that of their definitive hosts, ranging from 20 to 55°N in Boreal Pacific waters, but from 20 to 50°S in South Atlantic and South Pacific waters.

3.3.3. Validity of species of *Contracaecum*, parasitic in seals, using molecular markers

C. osculatum baicalensis Moszgovoi and Ryzhykov, 1950: The specific status of *C. osculatum baicalensis* was established genetically using allozyme markers (22 enzyme loci) with respect to the species of the *C. osculatum* complex listed above ([D'Amelio et al., 1995](#)). It was found to be genetically well distinct from the other members of this complex, as well as from the other congeneric taxa ([Mattiucci et al., 2008b](#)). *C. osculatum baicalensis* is an adult parasite of the freshwater Baikal seal, *Phoca sibirica*, endemic to Lake Baikal. The genetic relationships between *C. osculatum baicalensis* and other congeneric taxa were inferred from

LSU rDNA sequences (Nadler *et al.*, 2000) and from the mtDNA *cox2* sequence (Mattiucci *et al.*, 2008b) analyses. Both phylogenetic analyses were congruent with the allozyme clustering in showing *C. osculatum baicalensis* nested within the clade formed by the species of the *C. osculatum* complex (see Section 4.3). The genetic relationships found between *C. osculatum baicalensis* and the members of the *C. osculatum* complex suggest that the evolutionary divergence of this taxon likely took place following its host's isolation during a Pliocene-Pleistocene refuge (Deméré *et al.*, 2003). At a larval stage, *C. osculatum baicalensis* was recognized by allozymes in the endemic fish species *Cottomephorus grewingki*, *C. inermis*, *Comephorus baicalensis*, *Coregonus lavaretus*, *C. autumnalis migratorius* and *Thymallus arcticus*, which represent prey items of *Phoca sibirica* in Lake Baikal (our unpublished data).

C. radiatum (v. Linstow, 1907) Baylis, 1920: The taxonomic status of this species was confirmed genetically by Arduino *et al.* (1995) on the basis of 24 enzyme loci. Several allozymes were found to be diagnostic between *C. radiatum* and the other taxa so far characterized as belonging to *Contracaecum* species from seals (Arduino *et al.*, 1995; Mattiucci *et al.*, 2008b, unpublished data). Reproductive isolation from the two Antarctic members of the *C. osculatum* complex (i.e. *C. osculatum* D and *C. osculatum* E) occurring sympatrically in the same definitive hosts (the Weddell seal) was proved by the lack of F1 hybrids and recombinant or introgressed individuals between the Antarctic taxa in the sympatric areas of the Weddell and Ross Seas (Arduino *et al.*, 1995). The genetic relationships between *C. radiatum* and other congeneric taxa were later inferred from LSU rDNA sequences (Nadler *et al.*, 2000) and mtDNA *cox2* sequence analyses (Mattiucci *et al.*, 2008b). Morphological distinction between *C. radiatum* and *C. osculatum* (s.l.) was given by Klöser and Plötz (1992). *C. radiatum* has been genetically identified as an adult in *Leptonychotes weddellii* and as a larva in the pelagic channichthyid fishes *Chionodraco hamatus* and *Criodraco antarcticus* (see Arduino *et al.*, 1995). This finding supports a previous report by Klöser *et al.* (1992), according to which *C. radiatum* has become adapted to a pelagic food web. Other definitive hosts recorded for this species in Antarctic waters are the leopard seal, *Hydrurga leptonyx*, and the Ross seal, *Ommatophoca rossi* (see Baylis, 1937; Dailey, 1975). Genetic investigations on this parasite of Antarctic seals are needed in order to determine any host preference of this species.

C. mirounga Nikolskii, 1974: The taxonomic status of the species was confirmed genetically by allozyme markers (20 enzyme loci) (Mattiucci *et al.*, 2008b, unpublished data). It was detected genetically as an adult in *Mirounga leonina* from the Antarctic and sub-Antarctic area (Mattiucci *et al.*, 2008b) and also in the otariid *Arctocephalus australis* (see Mattiucci *et al.*, 2003). Several allozymes were found to be diagnostic between *C. mirounga* and other *Contracaecum* species from seals (Mattiucci *et al.*, 2008b,

unpublished data). Reproductive isolation from the two Antarctic members of the *C. osculatum* complex (i.e. *C. osculatum* D and *C. osculatum* E) occurring sympatrically in the same definitive host (the Weddell seal), and from *C. ogmorhini* (s.s.) which occurs syntopically in the same host (the fur seal, *Arctocephalus australis*), was demonstrated by the lack of F1 hybrids and recombinant or introgressed individuals. The genetic relationships between *C. mirounga* and other congeneric taxa were later inferred from LSU rDNA sequences (Nadler *et al.*, 2000) and from the mtDNA *cox2* sequences analyses (Mattiucci *et al.*, 2008b). No data on the larvae of this species which have been identified genetically are available.

4. PHYLOGENETIC SYSTEMATICS OF ANISAKID NEMATODES

Allozyme data, typically consisting of allele frequencies obtained from starch gel electrophoresis of proteins, are an important source of characters for understanding the genetic structure of a population of anisakid nematodes and, consequently, for reconstructing phylogenies among conspecific populations and closely related species. Despite the increasing use of DNA sequence data in phylogenetics, allozyme data remain widely used in systematic and evolutionary studies of anisakid nematodes and have some advantages. For example, allozyme data consist of multiple unlinked nuclear loci, with each locus providing independent estimate of the species differentiation and phylogeny. Therefore, in contrast to results of nuclear and mitochondrial DNA data sets, allozyme data are less likely to be systematically missed by mismatches between gene trees and species trees. Furthermore, it is relatively cheap and easy to survey allozyme variation for a large number of individual anisakid nematodes. A problematic aspect using allozyme data, however, is that there is a long-standing and continuing controversy as to the preferred method for their phylogenetic analysis. Doubt remains as to the validity of allozyme data as a basis for inference of genetic relationship among related taxa.

Although this issue is still debated in the literature, aspects could be addressed with empirical data recently obtained for anisakid nematodes. Genetic relationships inferred from allozyme markers have been largely obtained, in this group of parasites, by different methods such as phenograms from genetic distance methods, Neighbour Joining (NJ) from genetic matrix distances, spatial representation [Multidimensional Scale Ordination (MDS), Principal Component Analysis (PCA)] based on distance values or allele frequencies data, between several conspecific anisakid nematodes.

From a phylogenetic point of view, anisakid species have been recognized based upon evidence of independent evolutionary lineages

reflected in the form of character states unique to the same individuals of a species. Indeed, genetically defined clades, corresponding to distinct evolutionary lineages, are consistent with their recognition as separate species. Congruence studies so far carried out on anisakid nematodes provided, in general, that clades supported by many different lines of evidence (allozymes, DNA sequences) can effectively be considered to be 'known' clades (as determined and supported by [Felsenstein, 1985](#)). As it has been presented in previous paragraphs, several molecular systematic studies of anisakid nematodes have employed more than one gene to define the taxonomic status of the species so far known. Indeed, generally, concordant evidence from independent gene analysis inspires much more confidence than the patterns that reflect separate lineage (and, thus, species existence) inferred from data obtained from a single gene.

Congruence and incongruence evidences between allozyme data sets and not-allozyme data (i.e. DNA sequence analysis at both nuclear and mitochondrial genes) in anisakid nematode phylogenies are here reviewed. The opportunity to compare independent data sets supporting a given clade could affirm the existence of the clade with greater authority. In other words, shared hypothetical phylogenetic history seems to be the most likely explanation when congruence between diverse genetic data sets is observed. In this sense we are revising the phylogenetic relationships so far attempted for each group of anisakid nematodes belonging to the genera *Anisakis*, *Pseudoterranova* and *Contracaecum*. Indeed, phylogenetic analysis of partial 28S (LSU) sequences carried out by [Nadler et al. \(2005\)](#) of *Anisakis*, *Pseudoterranova* and *Contracaecum* taxa revealed strong support for the monophyly of the Anisakinae, *Contracaecum* plus *Phocascaris*, *Pseudoterranova* and *Anisakis*. Parsimony and ML analyses indicated that the Raphidascarididae, *Contracaecum* plus *Phocascaris*, and the Anisakinae (here considering only *Pseudoterranova* and *Anisakis*) are each monophyletic, the latter two groups with consistently strong bootstrap support at MP and ML analyses ([Nadler et al., 2005](#)).

4.1. Genetic relationships between *Anisakis* spp.

Work performed on the phylogenetic studies within this genus was done initially using the unweighted pair group method using arithmetic averages (UPGMA phenograms) on the studied populations and species of *Anisakis* based on distance values inferred from D_{Nei} ([Nei, 1972](#)) and D_c (chord distance by [Cavalli-Sforza and Edwards, 1967](#)). Among the first extensive UPGMA phenograms were those generated by [Mattiucci et al. \(1997, 2001\)](#) and [Paggi et al. \(1998b\)](#) showing the genetic relationships between the species of *Anisakis*. This suggested that *Anisakis* is polyphyletic and highly heterogeneous, with a high genetic differentiation of *A. physeteris* and *A. brevispiculata* from the rest of the *Anisakis* taxa

recognized genetically. Other elaborations to infer genetic relationships between seven taxa of *Anisakis* (i.e. the *A. simplex* complex, *A. typica*, *A. ziphidarum*, *A. brevispiculata* and *A. physeteris*) included the MDS (Guttman, 1968) from chord-distance values (*Dc*, Cavalli-Sforza and Edwards, 1967), demonstrating that *A. physeteris* and *A. brevispiculata* are forming a well-distinct clade with respect to that formed by *A. simplex* complex, as well as from both *A. typica* and *A. ziphidarum* (see Mattiucci *et al.*, 2002a). A consensus NJ tree was later inferred from Cavalli-Sforza and Edwards chord-distance values estimated at 19 enzyme loci between eight *Anisakis* taxa, including the new species *A. paggiae* (see Mattiucci *et al.*, 2005). This showed consistently the existence of two distinct clades supported by high bootstrap values, including, respectively: (1) the three members of *A. simplex* complex, *A. typica* and *A. ziphidarum* within clade I and (2) *A. physeteris*, *A. brevispiculata* and *A. paggiae* within clade II (Fig. 2.2). Both *A. ziphidarum* and *A. typica* form two distinct subclades, closely related to the *A. simplex* species complex (Mattiucci *et al.*, 2005).

Maximum parsimony (MP) analysis of the ITS data set of 724 characters in seven taxa out of those genetically recognized in *Anisakis*, was performed by Nadler *et al.* (2005). This produced a strict consensus of the MP trees which also depicted *A. physeteris* plus *A. brevispiculata* as the sister group to the remaining *Anisakis* taxa. This clade was well supported in the MP bootstrap tree. According to this analysis, the remaining ingroup *Anisakis* were monophyletic in maximum likelihood (ML) and MP consensus trees, with 100% bootstrap support (Nadler *et al.*, 2005).

Later, Valentini *et al.* (2006) provided a phylogenetic hypothesis for all the nine taxa currently recognized species in *Anisakis*, using MP, NJ and Bayesian analysis (BA) inferred from mtDNA *cox2* sequences data (629 bp). Phylogenetic trees generated show high congruence with the UPGMA and NJ trees obtained from allozyme data sets performed on the same species of *Anisakis*. An overall high congruence was indeed found between the tree topologies obtained from consensus NJ inferred from mtDNA *cox2* sequence data and consensus NJ tree generated from allozyme data sets of the same taxa (Fig. 2.2). Both depicted the species *A. physeteris*, *A. brevispiculata* and *A. paggiae* as a sister group, highly supported, with respect to the other *Anisakis* taxa. In addition, *A. paggiae* appears to share a common ancestor with *A. brevispiculata*, and this is well supported by the NJ allozyme data. In addition, allozyme tree topology clearly demonstrated that *Anisakis* sp. formed a monophyletic group with *A. ziphidarum*; this was supported by the MP, BI and NJ of mtDNA *cox2* sequences (Valentini *et al.*, 2006) (Fig. 2.2). Finally, consistent tree topologies were observed between nuclear gene products and mitochondrial genes for the position occupied by *A. typica* as forming a separate clade—as depicted by mtDNA *cox2*-derived NJ (Valentini *et al.*, 2006),

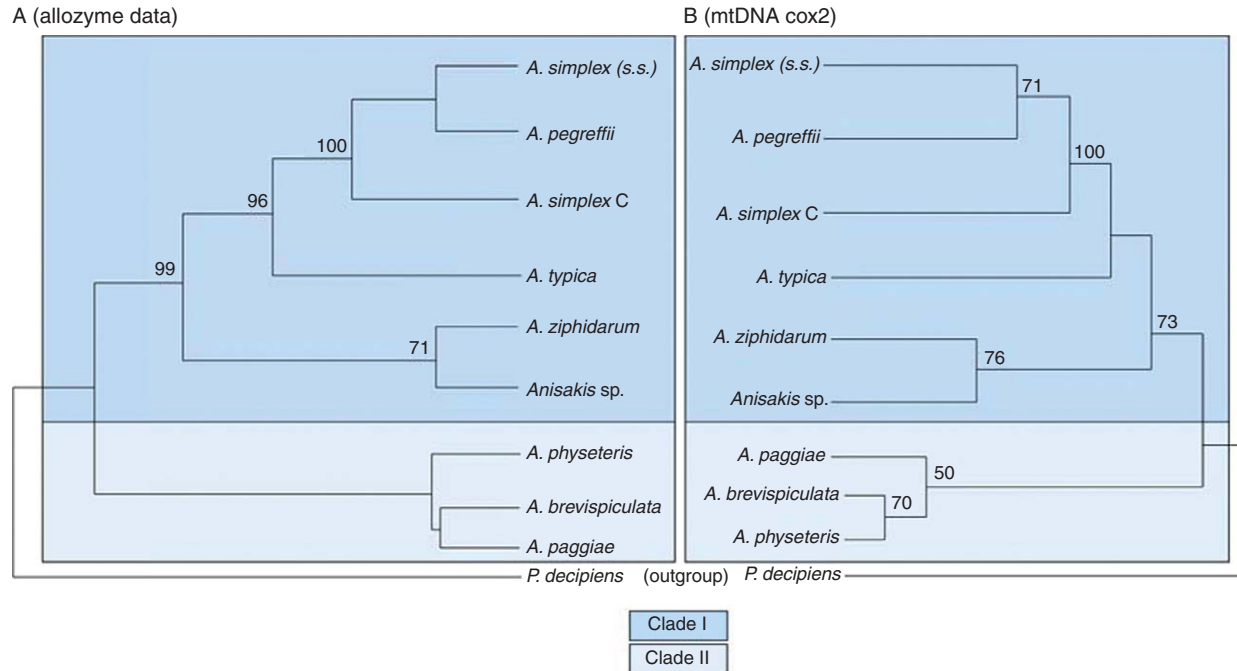


FIGURE 2.2 Genetic relationships among *Anisakis* spp. depicted by (A) neighbour-joining (NJ) tree inferred from chord-distance values (Dc, Cavalli-Sforza and Edwards, 1967) from allozyme data; (B) NJ inferred from K2P distance values obtained by mtDNA *cox2* sequences analysis (data from Valentini *et al.*, 2006). Bootstrap values ≥ 60 are shown at the internal nodes. *Pseudoterranova ceticola* as outgroup. The two data sets are congruent in depicting the existence of two main clades: (I) includes the *A. simplex* species complex [*A. pegreffii*, *A. simplex* (s.s.), *A. simplex* C], *A. typica*, *A. ziphidarum* and *Anisakis* sp.; (II) comprises the species *A. physeteris*, *A. brevispiculata* and *A. paggiae*. The species included in the two main clusters show a different larval morphology: type I (*sensu* Berland, 1961) in the first group and type II in the second one.

allozyme data, and inferred from ITS rDNA sequence analysis (Nadler *et al.*, 2005) (Fig. 2.2).

Therefore, phylogenies inferred from different sets of nuclear and mitochondrial data (i.e. allozymes, ITS rDNA sequences, mtDNA *cox2*) (Mattiucci *et al.*, 2005; Nadler *et al.*, 2005; Valentini *et al.*, 2006) supported the group of species formed by *A. physeteris*, *A. brevispiculata* and *A. paggiae* as sister group to the remaining *Anisakis* taxa. Finally, the clade formed by the sibling species of the *A. simplex* complex received strong support in all the phylogenetic elaborations from allozymes (NJ), ITS-rDNA (MP) and mtDNA *cox2* sequences (MP, NJ, BI). They were concordant to show a sister-group relationship for *A. simplex* (s.s.) and *A. pegreffii*, with *A. simplex* C as a sister to this clade (Mattiucci and Nascetti, 2006; Nadler *et al.*, 2005; Valentini *et al.*, 2006) (Fig. 2.2).

4.2. Genetic relationships between *Pseudoterranova* spp.

Genetic relationships between *Pseudoterranova* species have included analyses based on allozyme genetic distance methods (Bullini *et al.*, 1997, Paggi *et al.*, 1991, 1998c). The allozyme analysis inferred from *Dc* chord-distance values between the five sibling species of the *P. decipiens* complex indicated the following topology: [*P. decipiens* (s.s.), *P. azarasi*, *P. krabbei*, *P. bulbosa*, *P. decipiens* E], with a close genetic relationship between *P. decipiens* (s.s.) and *P. azarasi* (Fig. 2.3).

An UPGMA phenogram of uncorrected ITS rDNA distances (Zhu *et al.*, 2002) was also produced for individual specimens, previously characterized by allozymes, belonging to the taxa: *P. decipiens* (s.s.),

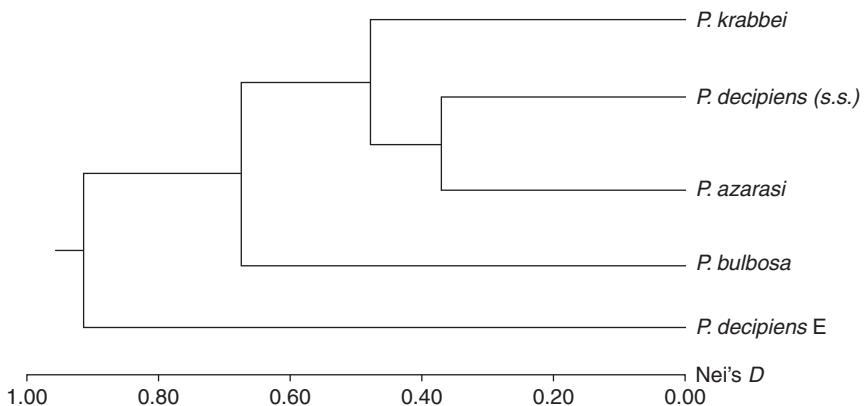


FIGURE 2.3 Genetic relationships among *Pseudoterranova* spp. depicted by neighbour-joining (NJ) tree inferred from chord-distance values (*Dc*, Cavalli-Sforza and Edwards, 1967) from allozyme data (redrawn from Bullini *et al.*, 1997).

P. krabbei, *P. bulbosa*, *P. azarasi*, *P. cattani*, and a taxon indicated as *P. decipiens* Ca1. This analysis showed the following relationship: [*P. decipiens* (s.s.), *P. azarasi*, *P. bulbosa*, *P. cattani*, *P. krabbei*, *P. decipiens* Ca1]. [Zhu et al. \(2002\)](#) also suggested that, because of the geographical origin, the *P. decipiens* Ca1 from *Chaenocephalus aceratus*, it is likely to correspond to *P. decipiens* E, but this correspondence has not been verified.

An overall congruence was found between the topology produced by the allozyme phenogram (except for the absence of *P. cattani* from the allozyme analysis) produced by [Bullini et al. \(1997\)](#) and that obtained by both MP and ML analyses of ITS rDNA sequences by [Nadler et al. \(2005\)](#) performed on all the *P. decipiens* species complex. These topologies were congruent in depicting the close relationship between *P. decipiens* (s.s.) and *P. azarasi*. [Nadler et al. \(2005\)](#) showed that *P. cattani* and *P. decipiens* Ca1 are not closely related and they do not represent sister taxa with respect to the other taxa. According to those authors, this finding suggests a more complex evolutionary scenario than might be explained by a simple biogeographical scenario of their definitive hosts.

4.3. Genetic relationships between *Contracaecum* spp.

A first phylogenetic hypothesis of the species included in the genus *Contracaecum* was that attempted using allozyme data on several species of *Contracaecum* from pinnipeds and fish-eating birds as definitive hosts, plus *Phocascaris phocae* and *P. cystophorae* (see [Nascetti et al., 1990](#); [Orecchia et al., 1986b](#)). It was shown that *Contracaecum* species from seals were genetically most similar to each other rather than to the avian *Contracaecum*, with no alleles found in common between the two groups of species. In addition, it was clearly demonstrated that species of *Phocascaris* (i.e. *P. phocae* and *P. cystophorae*), despite their morphological characters (interlabia absent and/or reduced), were clustering within the clade formed by the species of the *C. osculatum* complex (interlabia present), which now includes five sibling species [*C. osculatum* A, *C. osculatum* B, *C. osculatum* (s.s.), *C. osculatum* D and *C. osculatum* E] ([Nascetti et al., 1993](#); [Orecchia et al., 1994](#)) (see also [Section 3.3.](#))

[Nadler et al. \(2000\)](#) supported this first phylogenetic hypothesis using the nuclear-encoded large subunit ribosomal DNA sequences (LSUrDNA) for several taxa of *Contracaecum* (including seven species from pinnipeds and seven from fish-eating birds) and *Phocascaris* (two taxa), previously identified by allozyme markers. These data provided high support for the monophyly of all *Contracaecum* and *Phocascaris* of phocid seals. This finding is consistent with [Berland's hypothesis \(1964\)](#) that such species form a group sharing the same life-cycle pathway. However, Berland's proposal to recognize all species in phocid seals as *Phocascaris*, with all the species from birds as *Contracaecum*, was not congruent, according to

Nadler *et al.* (2000) with the inferred 28S rDNA trees produced, because it would result in a paraphyletic *Contracaecum*. Moreover, that phylogenetic elaboration, with respect to Berland's hypothesis, would not depict avian parasites as monophyletic; some of them, for instance *C. rudolphii* species A and B, cluster in the same clade with the otariid parasite *C. ogmorhini* (see Nadler *et al.*, 2000, 2005).

A phenetic clustering (UPGMA), inferred from the allozyme data set (22 enzyme loci) only in *Contracaecum* spp. using pinnipeds as definitive hosts, showing their genetic relationship was produced by Nascetti *et al.* (1993). Later Arduino *et al.* (1995) and Bullini *et al.* (1997) produced a phenetic tree (UPGMA) showing the genetic relationships among six taxa, including the species of the *C. osculatum* complex [*C. osculatum* A, *C. osculatum* B, *C. osculatum* (s.s.), *C. osculatum* D, *C. osculatum* E] and *C. radiatum*. This depicted *C. radiatum* as the basal species of the first group of *Contracaecum* plus *Phocascaris*. Other graphical representations of the genetic relationships among the same group of *Contracaecum* taxa were those produced by MDS ordination obtained from *Dc* values in Orecchia *et al.* (1994) and the PCA analysis in Arduino *et al.* (1995), also including *C. radiatum*.

A phenogram depicting the genetic differences observed in the entire ITS rDNA among specimens genetically identified previously by allozyme markers as belonging to the five taxa of the *C. osculatum* complex [i.e. *C. osculatum* A, *C. osculatum* B, *C. osculatum baicalensis*, *C. osculatum* (s.s.), *C. osculatum* D and *C. osculatum* E] was produced by Zhu *et al.* (2000b). This cluster analysis showed that *C. osculatum* A, *C. osculatum* D, *C. osculatum* E and *C. osculatum baicalensis* were genetically more similar to *C. osculatum* B than each was to *C. osculatum* (s.s.). Moreover, Zhu *et al.* (2000b) found *C. osculatum* (s.s.) the most genetically distinct taxon from the other members of the complex. This latter finding was concordant with that previously presented in Nascetti *et al.* (1993) and Orecchia *et al.* (1994). However, in the same genetic studies and phylogenetic representation, Zhu *et al.* (2000b) did not find any genetic differentiation in the ITS rDNA sequence analysis between *C. osculatum* D and *C. osculatum* E, previously detected by allozymes (Orecchia *et al.*, 1994). Similarly, the lack of unequivocal nucleotide differences in any of three mtDNA sequences analysed (mtDNA *cox1*, *ssrRNA* and *lsrrRNA*) between *C. osculatum* D and *C. osculatum* E was also shown by Hu *et al.* (2001). These findings are inconsistent with allozyme data analysis (MAE) which, on the contrary, clearly demonstrated the reproductive isolation between *C. osculatum* D and *C. osculatum* in sympatry, with several allozyme loci fixed for alternative alleles in the two species. Moreover, the specific status of *C. osculatum* D and *C. osculatum* E was confirmed by the phylogenetic analysis, inferred from mtDNA *cox2* sequences, which depicted the two taxa as two separate lineages (phylogenetic units) (Mattiucci *et al.*, 2008b) (Fig. 2.4).

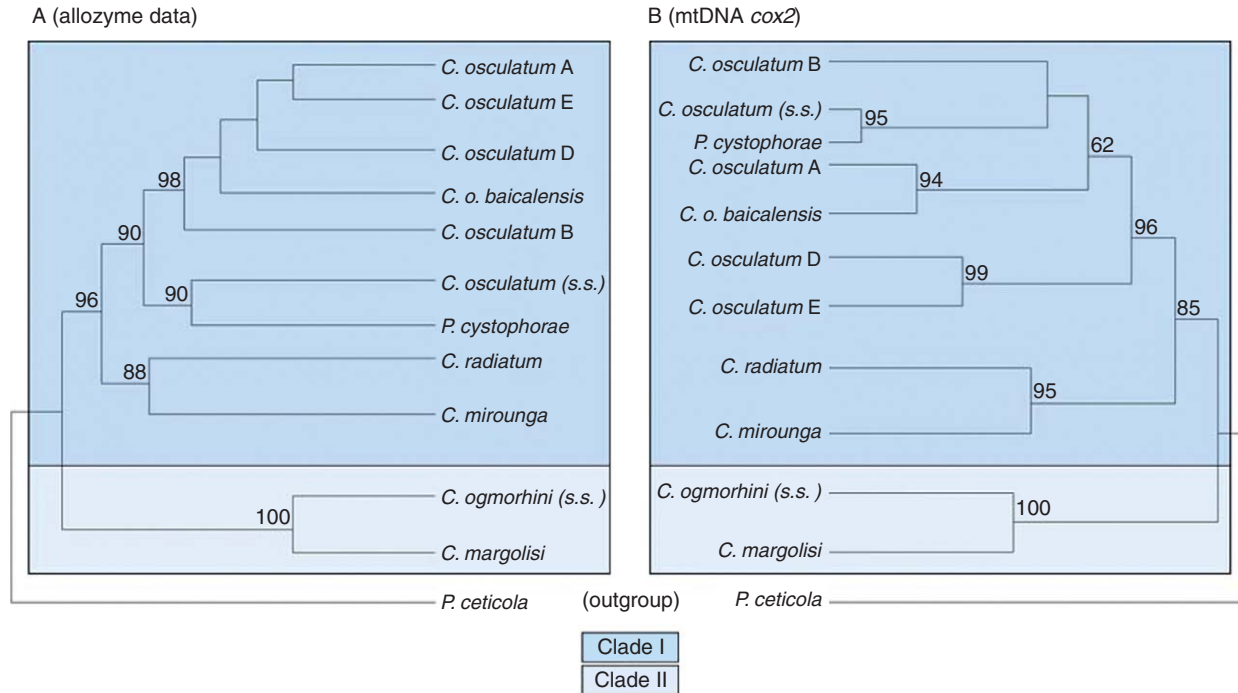


FIGURE 2.4 Genetic relationships between species of *Contracaecum* and *Phocascaris* from pinnipeds depicted by (A) NJ tree inferred from chord-distance values (D_c , Cavalli-Sforza and Edwards, 1967) from allozyme data and (B) NJ inferred from mtDNA *cox2* sequences analysis. Bootstrap values ≥ 60 are shown at the internal nodes. *Pseudoterranova ceticola* as outgroup. The two data sets are congruent in depicting the existence of two main clades: (I) includes the species of *C. osculatum* complex, *Phocascaris cystophorae*, *C. radiatum*, *C. mirounga*; (II) comprises *C. ogmorhini* (s.s.) and *C. margolisi*. The two phylogenetic trees show *P. cystophorae* nested in the subclade formed by the species of *C. osculatum* complex.

The overall concordant results with the allozyme data sets have been found in the tree topology inferred from the mtDNA *cox2* sequences data (519 bp) ascertained for all the *Contracaecum* plus *Phocascaris* taxa so far recognized by allozyme markers [i.e. *C. osculatum* A, *C. osculatum* B, *C. osculatum* (s.s.), *C. osculatum* D, *C. osculatum* E, *C. osculatum baicalensis*, *C. radiatum*, *C. mirounga*, *C. ogmorhini* (s.s.), *C. margolisi*, *Phocascaris cystophorae*] (see [Mattiucci et al., 2008b](#)) ([Fig. 2.4](#)). A high congruence in the topology of the trees generated by a consensus NJ tree inferred from mtDNA *cox2* sequences and that produced by a NJ based on *Dc* chord distance from allozyme markers was observed ([Fig. 2.4](#)). They are highly consistent in showing the existence of two well-supported clades: (1) one including the species of the *C. osculatum* complex, *Phocascaris cystophorae*, plus *C. radiatum* and *C. mirounga* (clade I), with the last two taxa being genetically closely related and forming a supported subclade; (2) the second clustering of the species *C. ogmorhini* (s.s.) and *C. margolisi* (clade II). Moreover, both elaborations from different data sets showed that the species *P. cystophorae* is nested in the subclade of the species of the *C. osculatum* complex. This is despite its morphological differences from the species of the *C. osculatum* complex. Allozyme NJ elaboration clearly depicted the species *C. ogmorhini* (s.s.) and *C. margolisi* as sister taxa with respect to the other *Contracaecum* taxa, and this was also shown by the mtDNA *cox2* data. In addition, the Antarctic taxon *C. osculatum* D forms a monophyletic group with the other Antarctic member *C. osculatum* E, from which it was also found to be clearly genetically distinct ([Table 2.10](#) and [Fig. 2.4](#); see also [Section 6](#)).

An overall congruence of this phylogenetic analysis was with that produced by [Nadler et al. \(2000\)](#) based on the LSU rDNA sequences analysis of seven *Contracaecum* species from pinnipeds [i.e. *C. osculatum* A, *C. osculatum* B, *C. osculatum* (s.s.), *C. osculatum baicalensis*, *C. radiatum* and *C. mirounga*]. Indeed, both NJ tree inferred from the full alignment data set using log-determinant distances and MP produced by [Nadler et al. \(2000\)](#) demonstrated the monophyly of *Contracaecum* plus *Phocascaris* from phocids. A strong support in all bootstrap trees also indicated for the subclades formed by *C. radiatum* and *C. mirounga* and that formed by *C. osculatum* A and *C. osculatum baicalensis* (see [Nadler et al., 2005](#)). These subclades were shown to be highly supported at MP and NJ analyses inferred from mtDNA *cox2* sequences analysis ([Mattiucci et al., 2006, 2008b](#)) ([Fig. 2.4](#)). Furthermore, the trees inferred by all the methods, were congruent in demonstrating that *Phocascaris* spp. are clustering with the *C. osculatum* complex group of species and are well nested in the clade including the *Contracaecum* from phocids (see [Mattiucci et al., 2008b](#); [Nadler et al., 2000, 2005](#)) (see also [Section 6](#)). Meanwhile, *C. ogmorhini* (s.s.) and *C. margolisi* cluster in a separate clade ([Mattiucci et al., 2008b](#)).

5. GENETIC DIFFERENTIATION IN ANISAKIDS

Although the level of genetic differentiation is not a suitable parameter or a measurement for establishing species delimitation, values of genetic differentiation between taxa of anisakid nematodes inferred from nuclear loci analysis (19–22 enzyme loci) were found to be of a similar order. In sympatric or partially sympatric sibling species of anisakid nematodes, a significant deficiency or complete lack of some heterozygote classes at polymorphic loci strongly suggests that the sample being dealt with comprises distinct gene pools. This was widely demonstrated in samples of *A. simplex* (s.l.), *P. decipiens* (s.l.), and *C. osculatum* (s.l.) (see [Mattiucci et al., 1997](#); [Nascetti et al., 1986, 1993](#); [Paggi et al., 1991](#)). In allopatric anisakid nematodes, allozymes have provided reliable information on their specific status, with clear evidence of alternative and unique allozymes existing in allopatric populations. As the most common mode of speciation of endoparasites is the *peripatric model* (*sensu* [Mayr, 1963, 1976](#)), the build up of genetic divergence is generally a better predictor of whether two allopatric populations will interbreed upon recontact, than is conventional morphology. Indeed, according to the *peripatric model of speciation*, this involves geographical isolation of small populations whose genetic structure begins to differ from that of the parental population since the beginning of the process by genetic drift phenomena. In the *Anisakis simplex* complex and *Contracaecum osculatum* complex of species, genetic data strongly support the notion that adaptation to different hosts and speciation is strictly related to the geographical isolation of the hosts (see also Section 6.1).

At least, in the case of anisakid nematodes, when [Nei's \(1972\)](#) genetic distance between populations reaches values of ~ 0.2 – 0.3 , gene exchange is interrupted by intrinsic reproductive isolating mechanisms (RIMs). On the other hand, in anisakid nematodes, conspecific populations generally show similar allele frequencies, even when located thousands of kilometers apart. Accordingly, their D_{Nei} values are quite low (0.0001 – 0.002). Average genetic distance values inferred from allozymes within and among species of the genera are given in [Tables 2.8–2.10](#).

5.1. Genetic differentiation at interspecific level

Between sibling species of anisakid nematodes (sympatric and allopatric), the genetic differentiation, estimated at the nuclear level (allozymes), ranges on average, from values of [Nei's \(1972\)](#) standard genetic distance (D_{Nei}) $D_{Nei} \approx 0.20$ (as observed between the Arctic taxon *C. osculatum* A and the Antarctic species *C. osculatum* E) ([Table 2.10](#)) to $D_{Nei} \approx 0.90$ (as found between the Antarctic taxon *P. decipiens* E *vs* the other species of

TABLE 2.8 Average of standard genetic distance by [Nei \(1972, \$D_{Nei}\$](#) , below the diagonal) inferred from 20 enzyme-loci, and by *Kimura-2-parameter* ([Kimura, 1980, K2P](#), above the diagonal) inferred from 629bp of mtDNA *cox2* between the species of *Anisakis* so far genetically detected

Species	<i>A. simplex</i> (s.s.)	<i>A.</i> <i>pegreffii</i>	<i>A. simplex</i> C	<i>A.</i> <i>typica</i>	<i>A.</i> <i>ziphidarum</i>	<i>Anisakis</i> sp.	<i>A.</i> <i>physeteris</i>	<i>A.</i> <i>brevispiculata</i>	<i>A.</i> <i>paggiae</i>
<i>A. simplex</i> (s.s.)		0.05	0.06	0.14	0.12	0.13	0.14	0.17	0.14
<i>A. pegreffii</i>	0.40		0.06	0.14	0.12	0.13	0.14	0.17	0.14
<i>A. simplex</i> C	0.36	0.37		0.14	0.13	0.14	0.14	0.17	0.15
<i>A. typica</i>	1.16	1.45	1.14		0.13	0.12	0.15	0.18	0.15
<i>A. ziphidarum</i>	1.64	1.99	1.62	1.67		0.09	0.13	0.14	0.12
<i>Anisakis</i> sp.	2.04	2.63	1.92	1.62	0.68		0.15	0.16	0.13
<i>A. physeteris</i>	6.90	8.30	7.40	4.77	∞	∞		0.11	0.12
<i>A. brevispiculata</i>	4.10	6.11	5.54	3.49	∞	∞	0.95		0.13
<i>A. paggiae</i>	∞	∞	∞	∞	∞	∞	1.06	0.79	

(Data from: [Mattiucci et al., \(2001, 2002a, 2005\)](#), [Mattiucci and Nascetti, 2006](#); [Paggi et al., 1998b](#), and [Valentini et al., 2006](#)).

TABLE 2.9 Average values of genetic distance, D_{Nei} (1972), between species of the *Pseudoterranova decipiens* complex

Species	<i>P. krabbei</i>	<i>P. decipiens</i> (s.s.)	<i>P. bulbosa</i>	<i>P. azarasi</i>
<i>P. decipiens</i> (s.s.)	0.51			
<i>P. bulbosa</i>	0.93	0.60		
<i>P. azarasi</i>	0.56	0.38	0.64	
<i>P. decipiens</i> E	0.91	0.96	0.97	0.93

Data from Bullini *et al.* (1997), Mattiucci *et al.* (1998, unpublished data), Paggi *et al.* (1991, 1998c).

P. decipiens complex), or $D_{Nei} = 0.93$ (as between *P. krabbei* and *P. bulbosa*) (Table 2.9). Similar high values were those observed in the comparison of Arctic members of the *C. osculatum* complex [i.e. *C. osculatum* D vs *C. osculatum* (s.s.), $D_{Nei} = 0.70$] and between members of the *A. physeteris* complex (i.e. *A. physeteris* vs *A. paggiae* and *A. brevispiculata*) ($D_{Nei} \approx 0.90$) (see Mattiucci *et al.*, 2005) (Table 2.8).

The highest interspecific differentiation is that found at the allozyme level in the comparison of anisakid species that are morphologically distinct, such as the *A. simplex* complex and *A. ziphidarum* (see Paggi *et al.*, 1998b) or the *A. physeteris* complex (Mattiucci *et al.*, 2005). In the last group of species, spicule length of male specimens and the ventriculus are significantly shorter than those of the members of *A. simplex* complex. Moreover, in *A. paggiae*, the spicule length and ventriculus shape were demonstrated to be of diagnostic level not only from the members of *A. simplex* complex, but also with respect to genetically closely related species, that is, *A. physeteris* and *A. brevispiculata* (see Mattiucci *et al.*, 2005).

Analogously, a high genetic divergence at the allozyme level was also found between members of the *C. osculatum* complex with respect to *C. mirounga* (on average, $D_{Nei} \approx 1.05$) (Mattiucci *et al.*, 2008b) and to the *C. ogmorhini* species complex (on average, $D_{Nei} \approx 1.30$) (Mattiucci *et al.*, 2003, 2008b), which are morphologically distinct. The highest level of differentiation at allozyme level was found between the *A. simplex* species complex with respect to the *A. physeteris* species complex (Mattiucci *et al.*, 2005) (Table 2.8).

Nuclear and mitochondrial DNA polymorphisms among species of anisakid nematodes demonstrate significantly high genetic variation among sibling species and morphospecies of those anisakid nematodes previously characterized genetically by allozyme markers. Indeed, it has recently been shown that most of the genetic diversity is strongly structured between species rather than within species. Pairwise comparisons were estimated of the level of sequence differences at the ITS rDNA between 14 *Anisakis* samples by Nadler *et al.* (2005). Sequence differences

TABLE 2.10 Average of standard genetic distance by [Nei \(1972, \$D_{Nei}\$](#) , below the diagonal) inferred from 20 enzyme-loci, and by *Kimura-2-parameter* ([Kimura, 1980](#), K2P, above the diagonal) inferred from 519 bp of mtDNA *cox-2* between *Contracaecum* spp. from pinnipeds.

Species	COSA	COSB	COSS	COSD	COSE	CBAI	PCYS	CRAD	CMIR	COGM	CMAR
<i>C. osculatum</i> A (COSA)		0.09	0.10	0.09	0.09	0.06	0.09	0.14	0.13	0.16	0.15
<i>C. osculatum</i> B (COSB)	0.41		0.09	0.09	0.10	0.08	0.10	0.14	0.13	0.15	0.15
<i>C. osculatum</i> (s.s.) (COSS)	0.57	0.80		0.11	0.10	0.10	0.07	0.16	0.15	0.14	0.15
<i>C. osculatum</i> D (COSD)	0.23	0.38	0.64		0.05	0.09	0.11	0.13	0.13	0.16	0.16
<i>C. osculatum</i> E (COSE)	0.20	0.36	0.53	0.25		0.09	0.11	0.15	0.13	0.14	0.15
<i>C. baicalensis</i> (CBAI)	0.30	0.27	0.41	0.60	0.50		0.09	0.16	0.12	0.15	0.16
<i>P. cystophorae</i> (PCYS)	0.45	0.83	0.46	0.68	0.51	0.64		0.15	0.15	0.16	0.16
<i>C. radiatum</i> (CRAD)	0.72	0.76	1.30	0.78	0.67	0.79	0.96		0.09	0.14	0.15
<i>C. mirounga</i> (CMIR)	1.00	1.06	1.13	1.15	0.86	1.20	1.07	0.95		0.14	0.15
<i>C. ogmorhini</i> (s.s.) (COGM)	1.30	1.35	1.37	1.40	1.48	1.70	1.90	1.60	1.81		0.05
<i>C. margolisi</i> (CMAR)	1.35	1.40	1.42	1.39	1.45	1.70	1.89	1.75	1.80	0.30	

(Data from: [Nascetti et al., 1993](#); [Orecchia et al., 1994](#); [Mattiucci et al., 2008b](#) and unpublished).

(absolute differences) of 0–7 existed among individuals belonging to the same species, whereas it ranged from 37 to 149 between individuals belonging to distinct species (Nadler *et al.*, 2005). At the mtDNA level, the genetic divergence estimation among the nine *Anisakis* taxa, previously characterized on the basis of allozyme data, was calculated at Kimura-2-parameters distance values (K2P) inferred from sequence analysis (629 bp) of the mtDNA *cox2* gene by Valentini *et al.* (2006) (Table 2.8). The mtDNA *cox2* fragment in all the *Anisakis* was found to be A+T rich (60.7%, 64.9% and 74.7% at the first, second and third positions, respectively). The lowest level of interspecific genetic distance was found between sibling species of the *Anisakis simplex* complex (on average, K2P $\approx$ 0.05). Whereas, values of K2P = 0.14 and 0.13 were observed when comparing the *A. simplex* complex, on average, with *A. typica* and *A. ziphidarum*. Similar values (on average K2P $\approx$ 0.13) were obtained when *A. physeteris*, *A. brevispiculata* and *A. paggiae* are compared to *A. ziphidarum* (see Valentini *et al.*, 2006) (Table 2.8). At the amino acid level, a total of 31 variable positions were identified in the mtDNA *cox2* gene for *Anisakis* spp. The average variation ranged from 2.4% between the sibling species of *A. simplex* complex to as high as 7.7% between morphologically distinct species (i.e. *A. physeteris*, *A. brevispiculata* and *A. paggiae* vs the *A. simplex* complex (Valentini *et al.*, 2006)).

Single strand conformation polymorphism (SSCP) analysis of the ITS rDNA, performed by Zhu *et al.* (2002), has revealed that, while no variation in single stranded ITS profiles existed within each of the five sibling species of *P. decipiens* complex (with the exception of a slight microheterogeneity evidenced in *P. bulbosa* and *P. cattani*), SSCP analysis of the ITS-2 amplicons allowed significant differentiation between them (Zhu *et al.*, 2002). Pairwise comparison of the ITS sequences revealed nucleotide differences ranging from 0 to 6.8%, which were within the range observed in other complex members (0–2.3% in the *C. osculatum* complex) (see Zhu *et al.*, 2000b).

Sequence variation at the same internal transcribed spacers of ribosomal DNA (ITS-rDNA) within and among members of the *Contracaecum osculatum* complex and SSCP was used to screen the ITS-1 and ITS-2 amplicons separately for sequence variation within and among individuals (Zhu *et al.*, 2000b). The G+C contents of the sequences obtained were 45.7% (ITS-1) and 42.0–43.5% (ITS-2). However, no variation in single-strand profiles was detected between/among samples representing a taxon by Zhu *et al.* (2000b), except for slight nucleotide polymorphism detected in the ITS for *C. osculatum* A and *C. osculatum* B. Indeed, there was no variation in the length of the ITS-1 (499 bp) or ITS-2 (262 bp) sequence for any of the individuals examined, and no-intraspecific variation in the sequence was observed. Whereas, significant inter-taxon

differences in SSCP profiles were detected between *C. osculatum* A, *C. osculatum* B, *C. osculatum* C and *C. osculatum baicalensis*. This revealed a reliable genetic differentiation of these taxa from one another, except in the case of *C. osculatum* D versus *C. osculatum* E, which exhibited identical sequences (Zhu *et al.*, 2000b). Each of the other four taxa had distinct sequences with interspecific differences ranging from 0.3 to 2.3% (Zhu *et al.*, 2000b). *C. osculatum* (s.s.) was the most distinct taxon with respect to all the other members. Comparison among the taxa revealed 22 variable nucleotide positions in the ITS sequence. Each of the taxa could be identified by the presence of 1–10 fixed nucleotide differences, except in the case of *C. osculatum* D and *C. osculatum* E, which were found at the ITS rDNA gene to show the same nucleotide sequence.

At the mitochondrial level, the genetic divergence estimated from sequence analysis (519 bp) of the *cox2* gene was given for the five members of the *C. osculatum* complex [i.e. *C. osculatum* A, *C. osculatum* B, *C. osculatum* (s.s.), *C. osculatum* D and *C. osculatum* E] with respect to *C. osculatum baicalensis*, *C. radiatum*, *C. mirounga*, *Phocascaris cystophorae* and to the otariid parasites, *C. ogmorhini* (s.s.) and *C. margolisi* (see Mattiucci *et al.*, 2008b). Genetic divergence has been shown to range from K2P = 0.05 (*C. osculatum* D vs *C. osculatum* E) to K2P = 0.11 [*C. osculatum* (s.s.) vs *C. osculatum* D] between sibling species of the *C. osculatum* complex. Higher values of K2P were those estimated between morphologically differentiated species such as in the comparison of *C. radiatum* and the species of *C. osculatum* complex (on average K2P $\approx$ 0.14), or *C. osculatum* complex versus *C. ogmorhini* complex species (on average K2P $\approx$ 0.15) (Mattiucci *et al.*, 2008b) (Table 2.10). However, despite morphologically distinct characters between *Phocascaris* spp. and the species so far included in the *C. osculatum* complex, *P. cystophorae* is genetically very closely related to this group of species (on average K2P $\approx$ 0.10) (Mattiucci *et al.*, 2008b) (Table 2.10) (see also Section 4.3).

The *Contracaecum* mtDNA *cox2* gene was found to be A+T rich. This, however, is consistent with that found in other anisakid nematodes, such as *Contracaecum* parasites of fish-eating birds (Mattiucci *et al.*, 2008c). Similar distance values were those estimated at the same mitochondrial gene (mtDNA *cox2*) among species of *Contracaecum* parasitic in fish-eating birds. Indeed K2P ranged from 0.08 between the two sibling species, *C. rudolphii* A versus *C. rudolphii* B, to $\approx$ 0.13 between the two sibling species and species morphologically well differentiated (i.e. the *C. multipapillatum* complex) (Mattiucci *et al.*, 2008c, unpublished data).

The genetic divergence among the anisakid taxa so far evaluated at the mtDNA *cox2* locus is of the same order as found between other nematode species (Anderson *et al.*, 1998; Blouin *et al.*, 1998; La Rosa *et al.*, 2001; Pozio and Murrel, 2006; Zarlenga *et al.*, 1998).

5.2. Genetic differentiation at the intraspecific level and gene flow

In *Anisakis* spp., most of the genetic diversity has been found within populations rather than among populations (see also [Section 8.2](#)). Indeed, for instance, at the intraspecific level, the values of D_{Nei} found between populations of *A. simplex* (s.s.) ranged from 0.003 to 0.0038. Such a pattern of genetic distance is related to that of geographical distance (isolation by distance). Average D_{Nei} values is ≈ 0.007 among Atlantic populations, $D_{Nei} \approx 0.015$ among Pacific ones, and $D_{Nei} \approx 0.025$ between the two groups. The highest value ($D_{Nei} \approx 0.038$) was found between the most distant populations, from the Baltic Sea and off Hokkaido Island, Japan. This finding is mainly a consequence of the differences in allele frequencies at the loci *Sod-2*, *fEst-2*, *Lap-2* and *Pep B* in populations of *A. simplex* (s.s.), compared with those from the other localities ([Mattiucci et al., 1997, 1998](#)). At the interpopulation level, *A. pegreffii* showed the lowest values of *Gst* (average 0.02, [Mattiucci et al., 1997](#)) and D_{Nei} is, on average ≈ 0.005 . This finding indicates remarkable genetic homogeneity of *A. pegreffii* throughout its wide range, from the Austral region to the Mediterranean Sea. A more consistent intraspecific distance (D_{Nei} on average, ≈ 0.045 ; *Gst* = 0.10) was found in *A. simplex* C between the population from the Canadian Pacific and those from Austral region (South Africa, New Zealand, Tasman Sea and sub-Antarctic area) ([Table 2.8](#)). This is mainly revealed by the locus *Sod-1*, which markedly differentiated the sample from the Canadian Pacific and those from the Austral region ([Mattiucci et al., 1997](#)).

Also in the case of *A. typica* (s.l.), remarkable genetic homogeneity was observed in larval and adult samples despite being geographically quite distant. Interpopulational genetic distances values detected among populations from Somali, Mediterranean and Brazilian waters were low, ranging from $D_{Nei} \approx 0.004$ (eastern Mediterranean vs Somali waters) to $D_{Nei} \approx 0.010$ (Brazilian vs Somali). The average value of *Fst* among all the populations was ≈ 0.04 ([Mattiucci et al., 2002a](#)). Similar low levels of population structuring were those found among Boreal and Austral populations of other taxa, such as $D_{Nei} \approx 0.002$ within *A. ziphidarum* (see [Paggi et al., 1998b](#)), $D_{Nei} \approx 0.008$ and $D_{Nei} \approx 0.009$, respectively, in *A. brevispiculata* and *A. physeteris* ([Mattiucci et al., 2001](#)) and, finally $D_{Nei} \approx 0.005$ in *A. paggiae*, despite the geographical distance between the samples, indicating high levels of gene flow in all of these *Anisakis* taxa ([Table 2.8](#)).

Similar values, at the intraspecific level, were found in the comparison of populations belonging to the sibling species of the *P. decipiens* complex. The four members of this complex from the Arctic Boreal region showed D_{Nei} among populations ranging from, on average 0.001 (in *P. krabbei*) to $D_{Nei} \approx 0.005$ [in *P. decipiens* (s.s.)]. Indeed, the lowest genetic heterogeneity was found within *P. bulbosa*, despite the geographical isolation of the

populations (NE and NW Atlantic, Pacific) with an average of 0.004. The highest intraspecific differentiation was detected within *P. azarasi*, for example, the sample from Anadyr (Bering Sea) showed a value of $D_{Nei} = 0.026$ with respect to conspecific Japanese samples (Mattiucci *et al.*, 1998; Paggi *et al.*, 1998c). Both *P. decipiens* (s.s.) and *P. azarasi* showed a strict correlation between geographical and genetic distance, whereas higher levels of gene exchange were detected within *P. bulbosa*.

Analogous average values and ranges of genetic diversity between conspecific populations were reported for the members of the *C. osculatum* complex, from both Arctic and Antarctic regions. At the intraspecific level, the values of D_{Nei} within *C. osculatum* A ranged from 0.002 to 0.017, with an average of $D_{Nei} \approx 0.009$. Average D_{Nei} values were 0.004 within Northern Atlantic populations, while they were 0.008 among the conspecific populations and 0.018 between the two groups (Mattiucci *et al.*, 1998; Paggi *et al.*, 1998c). This demonstrates a geographical pattern in the genetic variation, although less marked than that observed in *A. simplex* (s.s.). Among conspecific populations of *C. osculatum* B, a lower level of differentiation was found $D_{Nei} \approx 0.005$ on average, with a range of values from 0.001 to 0.014, the last value being reported among Pacific and Atlantic populations (Paggi *et al.*, 1998c). A lower degree of interpopulational genetic differentiation was found between populations of the two Antarctic taxa, *C. osculatum* D and *C. osculatum* E, detected in definitive and fish hosts from the Weddell and Ross Seas, on average, $D_{Nei} \approx 0.003$ and $D_{Nei} \approx 0.002$, respectively.

The highest levels of interpopulational distance between anisakid populations were those found within the otariid parasite *C. ogmorhini* (s.s.), ranging from $D_{Nei} = 0.021$ to $D_{Nei} = 0.029$ between samples from Argentina, South African and New Zealand waters (on average, $D_{Nei} = 0.024$) (Mattiucci *et al.*, 2003). Similar values are generally found among races in different organisms (Ayala, 1975; Bullini and Sbordoni, 1980).

Allozyme data sets have indicated that genetic variation of anisakids is generally not structured in geographical races or subspecies. Such low values of Nei's D between populations located thousands of kilometres apart are likely to be caused by the homogenizing effects of gene flow, enhanced by the high dispersal capacity of intermediate/paratenic and definitive hosts. Indeed, levels of interpopulational gene flow, indirectly estimated from allele frequencies and F_{st} values, have been reported in these marine ascaridoid nematodes to be at high levels (Table 2.11). Despite the geographical distance between sampling locations, high rates of gene flow were detected within species belonging to three genera. The highest values were those observed in the species included in the *Anisakis simplex* complex. As we have illustrated above (Section 3.1), these worms mature in several species of 'oceanic dolphins' whose large geographical distribution could maintain the high level of gene flow observed in these anisakid nematodes (Table 2.11). Similarly, the high

TABLE 2.11 Values of intraspecific gene flow, estimated in anisakids from the standardized variance of allele frequencies, F_{st} (Crow and Aoki, 1984; Slatkin and Barton, 1989); m is the fraction of immigrant individuals in a population of effective size N

Species	Gene flow (Nm)	References
<i>A. simplex</i> (s.s.)	8.00	Mattiucci <i>et al.</i> , 1997
<i>A. pegreffii</i>	15.00	Mattiucci <i>et al.</i> , 1997
<i>A. simplex</i> C	9.00	Mattiucci <i>et al.</i> , 1997
<i>A. typica</i>	6.00	Mattiucci <i>et al.</i> , 2002
<i>A. physeteris</i>	5.10	Unpublished data
<i>A. brevispiculata</i>	6.20	Unpublished data
<i>P. krabbei</i>	4.38	Mattiucci <i>et al.</i> , 1998; Paggi <i>et al.</i> , 1991
<i>P. decipiens</i> (s.s.)	3.66	Mattiucci <i>et al.</i> , 1998; Paggi <i>et al.</i> , 1991
<i>P. bulbosa</i>	9.36	Mattiucci <i>et al.</i> , 1998; Paggi <i>et al.</i> , 1991
<i>C. osculatum</i> A	5.20	Mattiucci <i>et al.</i> , 1998; Nascetti <i>et al.</i> , 1993
<i>C. osculatum</i> B	4.90	Nascetti <i>et al.</i> , 1993
<i>C. osculatum</i> (s.s.)	3.90	Nascetti <i>et al.</i> , 1993
<i>C. osculatum</i> D	4.60	Orecchia <i>et al.</i> , 1994, unpublished data
<i>C. osculatum</i> E	6.10	Orecchia <i>et al.</i> , 1994, unpublished data
<i>C. ogmorhini</i> (s.s.)	4.10	Mattiucci <i>et al.</i> , 2003

mobility and dispersal capacity of their intermediate/paratenic hosts (fish) could enhance the high degree of gene exchange. Such figures are similar to those found for the Arctic-Boreal members belonging to the *C. osculatum* and *P. decipiens* complexes maturing in pinnipeds (Table 2.11).

These findings confirm that the complex life cycle of these nematodes does not limit gene exchange, but enhances it through the high mobility of the different hosts, such as fish and marine mammals (see also Section 7). On the other hand, the possible correlation between gene exchange and host mobility has also been suggested for other nematodes with different life cycles, as inferred by other genetic markers (Anderson *et al.*, 1998; Blouin *et al.*, 1995, 1998; Criscione *et al.*, 2005).

6. HOST-PARASITE COPHYLOGENY

Uncovering the phylogenetic history of anisakid parasites and their hosts represents a steadily advancing part of their systematics and ecology.

Thus, *cophylogeny mapping* has become a recent development in the study of the relationships among and between ecologically related anisakid nematodes with respect to their hosts. Generally, in cophylogeny studies, a dependent phylogeny, such as that of a taxonomic group of

parasites, is mapped onto an independent one, such as that of their hosts. Co-evolutionary studies mostly rely on morphological or molecular data which are now becoming increasingly available in both parasites and their hosts, allowing detailed comparison of parasite phylogenies with those proposed for their hosts. Results of such studies have demonstrated how the phylogeny of the parasites comes to ‘mirror’ that of their hosts (*sensu* Eichler, 1948; Fahrenholz, 1913). In *cophylogeny mapping*, the map is constructed to provide the best possible explanation for the phylogenies and thus determine whether the parasites have co-diverged with their hosts, or whether they have undergone host switching, duplication by independent speciation or even whether parasites had extinction events (Charleston, 2003; Desdevises, 2007). Another method of uncovering ancient associations between dependent and independent taxonomic groups is the ‘biogeographical method’ also known as Brooks’ Parsimony Analysis (Brooks, 1990; Brooks and Hoberg, 1981). In this model, hosts are considered as ‘geographical regions’ and their parasites are their ‘endemic species’, undergoing codivergence in vicariant speciation, loss by extinction, ‘missing the boat’ (*sensu* Paterson *et al.*, 1993), sympatric speciation and horizontal transfer by migration.

Similar investigations have been made into host–parasite associations between anisakid nematodes and their definitive hosts. Phylogenetic trees, mostly inferred from molecular genetic data sets, are now available for these parasites and for some taxonomic groups of their definitive hosts. Comparison of these trees has allowed identification of cophylogenetic events.

Mattiucci and Nascetti (2006) have recently provided evidence for such cophylogenetic events in the host–parasite systems of *Anisakis* spp. and cetaceans (their main definitive hosts). The presence of the two main clades, as presented in the phylogenetic relationships between *Anisakis* spp. (Section 4.1), is supported also by ecological data and specific host–parasite relationships. Phylogenetic relationships proposed elsewhere and here revised for species of *Anisakis* ‘mirror’, in several host–parasite associations, that of their definitive hosts (Arnason *et al.*, 2004; Cassens *et al.*, 2000; Milinkovitch, 1995; Nikaido *et al.*, 2001). The sperm whales, *Physeter catodon*, *Kogia breviceps* and *K. sima* are the main definitive hosts for *A. physeteris*, *A. brevispiculata* and *A. paggiae*, respectively (Mattiucci and Nascetti, 2006; Mattiucci *et al.*, 2001, 2005) (Figs. 2.2 and 2.5). Several oceanic dolphins in the Delphinidae, Arctic dolphins in the Monodontidae and porpoises in the Phocoenidae (Table 2.4) are hosts of the species of the *A. simplex* complex and of *A. typica* (see Mattiucci *et al.*, 1997, 2002a, 2005). The beaked whales *Ziphius cavirostris*, *Mesoplodon layardii*, *M. mirus* and *M. grayi* are hosts of *A. ziphidarum* (see Paggi *et al.*, 1998b) and *Anisakis* sp. (see Valentini *et al.*, 2006) that are partitioned in clade I in the parasite phylogenetic tree (Fig. 2.3). The phylogeny of cetaceans

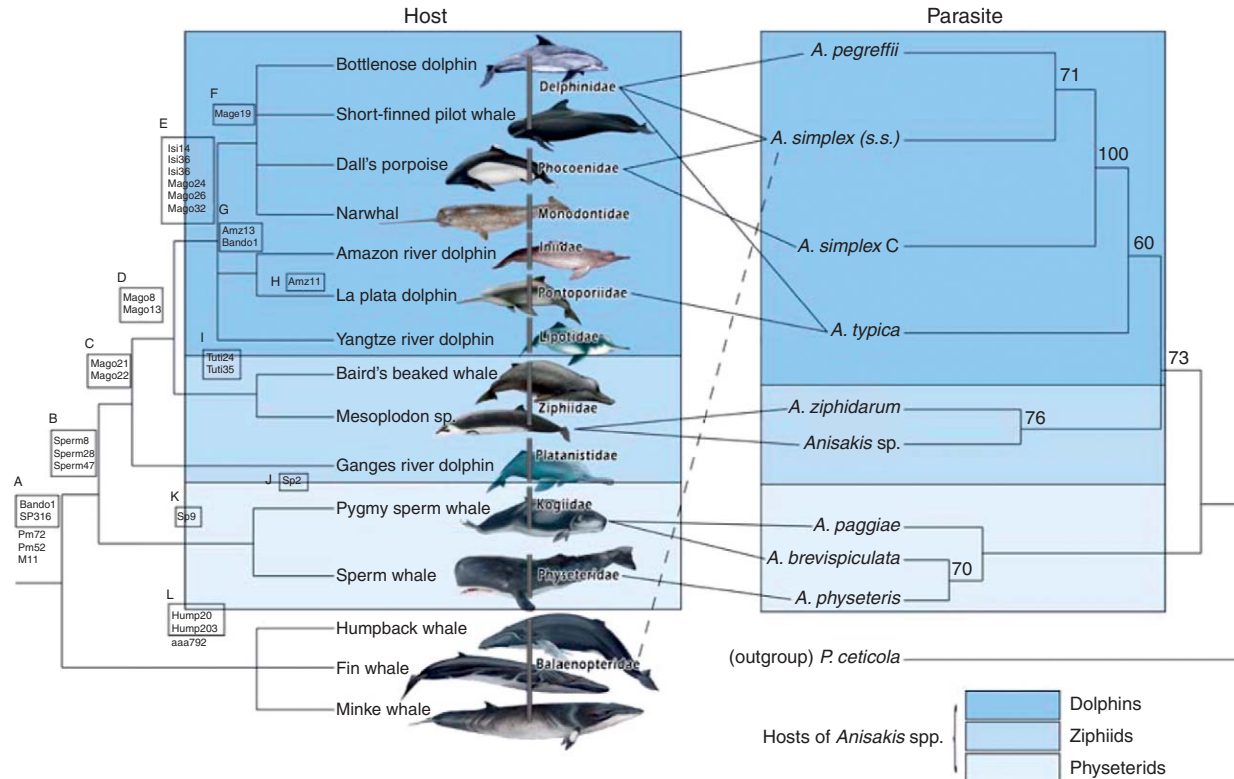


FIGURE 2.5 Tanglegram of phylogenies of *Anisakis* spp. and their cetacean hosts. It shows the phylogeny of a group of extant cetaceans (adapted from [Nikaido et al., 2001](#)) mapped into the phylogeny of *Anisakis* spp. inferred from mtDNA *cox2* sequence analysis. Lines depict the observed host–parasite co-speciation events; the dotted line indicates possible host-switching events (redrawn from [Mattiucci and Nascetti, 2006](#)).

representing all extant cetacean families has recently been proposed by Milinkovitch (1995) based on mtDNA genes (12S, 16S and *cytb* partial sequences) and on myoglobin sequences; by Cassens *et al.* (2000) inferred from sequence analyses of 12S, 16S and *cytb*; by Nikaido *et al.* (2001) based on retroposon analysis and by Arnason *et al.* (2004) based on analyses of complete mitochondrial genomes. According to the phylogenetic hypothesis proposed by Arnason *et al.* (2004), the Cetacea group splits into monophyletic Mysticeti (baleen whales) and monophyletic Odontoceti (toothed whales). The Odontoceti diverged into the four extant lineages, Physeteridae (sperm whales: represented by the sperm and pygmy sperm whales), Ziphiidae (beaked whales), Platanistidae (Indian river dolphins) and Delphinoidea (encompassing the families Iniidae, Monodontidae, Phocoenidae and Delphinidae). Phylogenetic trees provided by Nikaido *et al.* (2001) and Arnason *et al.* (2004) were congruent in depicting the branching order of the extant cetacean lineages, where the sperm whale and the pygmy sperm whales (belonging to the families Physeteridae and Kogiidae) represent basal taxa, followed by the beaked whales (belonging to the family Ziphiidae) and the freshwater and marine dolphins as the most derived (Fig. 2.5). In accordance with those analyses, the branching order so far proposed for the *Anisakis* taxa showed that nematodes from the sperm whale and pygmy sperm whales (i.e. *A. physeteris*, *A. brevispiculata* and *A. paggiae*) always occupy a basal lineage, always well supported, followed by those parasitizing the beaked whales (*A. ziphidarum* and *Anisakis* sp.). The species of the *A. simplex* complex and *A. typica*, parasites of delphinoids, are the most derived (Mattiucci and Nascetti, 2006) (Fig. 2.5). Elaboration of these empirical results to assess the global congruence between host and parasite trees gained by ParaFit (Legendre *et al.*, 2002) was statistically significant ($P < 0.05$). Individual host–parasite associations which contributed more to the cophylogenetic cetacean–*Anisakis* spp. mapping were represented by those between *A. physeteris*–*Physeter catodon*, *A. brevispiculata*–*Kogia breviceps* and *A. ziphidarum*–*Mesoplodon* spp., suggesting host–parasite co-speciation events. Whereas, a lower significant contribute to the total test was that formed by the host–parasite association *A. simplex* (s.s.)–*Balenoptera acutorostrata*, suggesting a possible host-switching event (our unpublished data).

Such parallelism between host and parasite phylogenies is also attempted for the group of *Contracaecum* taxa and their definitive hosts, the pinnipeds of the Families Phocidae and Otariidae. The presence of the two main clades, as presented in the phylogenetic relationships among *Contracaecum* spp. (Fig. 2.4), is also supported by the ecological data concerning host preference (see also Section 7.1) and specific host–parasite relationships. Phylogenetic relationships so far proposed and here reviewed for species of *Contracaecum* parallel that recently reported for their definitive hosts based on molecular data (Arnason *et al.*, 1995;

Deméré *et al.*, 2003). Several phocid seals (true seals) in the Phocinae are hosts of the species of the *C. osculatum* complex and *Phocascaris* spp. in the Arctic Boreal region. Moreover, seals in the Monachinae are hosts for *C. mirounga* and *C. radiatum* in the Antarctic and sub-Antarctic region. These anisakids are included in clade I in the parasite phylogenetic tree (Mattiucci *et al.*, 2008b) (Figs. 2.4 and 2.6). Whereas, the otariid species in the Otariinae (sea lions) *Zalophus californianus*, and in the Arctocephalinae (fur seals) *Arctocephalus* spp. are hosts of the *C. ogmorhini* species complex [*C. margolisi* and *C. ogmorhini* (s.s.)]. These anisakids are included in clade II in the parasite phylogenetic tree (Mattiucci *et al.*, 2008b) (Figs. 2.4 and 2.6). Although a complete species-level phylogeny for pinnipeds including fossil and extant taxa is as yet, unavailable, a molecular assessment of pinniped relationships was performed by Arnason *et al.* (1995) using the complete sequences of the mitochondrial cytochrome *b* gene (mtDNA *cytb*) of the Phocidae, Odobenidae and Otariidae. Later, Deméré *et al.* (2003) used a composite tree inferred from the basic topology of generic level, morphological and molecular data, fossil taxa and consensus phylogeny of the phocid subfamilies to propose an integrated hypothesis for pinniped evolutionary biogeography. According to that data elaboration, the Pinnipedia includes three major monophyletic clades: (1) the Otariidae (fur seals and sea lions), (2) the Odobenidae, and (3) the Phocidae (true seals), *plus* the extinct desmatophocids. In this combined tree, the fur seals and sea lions comprising the Otariinae (*Zalophus californianus*) and the Arctocephalinae (*Arctocephalus* spp. from the southern hemisphere), are represented as well-supported basal groups (Arnason *et al.*, 1995; Deméré *et al.*, 2003). In accordance with that analysis, the branching order so far proposed for the *Contracaecum* taxa showed that nematodes from the Otariidae [i.e. *C. ogmorhini* (s.s.) from *Arctocephalus* spp. and *C. margolisi* from *Zalophus californianus*] always occupy a basal lineage of the parasite phylogenetic tree, with the species of the *C. osculatum* complex from the Phocinae (true seals) as the most derived (Fig. 2.6).

Speciation of the members of *C. osculatum* complex is apparently related to their geographical isolation, through that of their hosts, as well as to a rapid host-parasite adaptation and co-evolution. Such processes apparently occurred in different times during the Plio-Pleistocene, when extreme climatic variation took place. The genetic relationships found between the members of the *C. osculatum* complex suggest that the evolutionary divergence of the most differentiated species [*C. osculatum* (s.s.)] started more than 3 million years ago, in a Pleistocene refuge (the Baltic Sea). As to the other *C. osculatum* species, their evolutionary divergence probably took place during the Pleistocene, when the complex achieved a distribution over both polar regions. This process involved two distinct colonizations of the marine Antarctic region by ancestors of the northern hemisphere, giving rise to *C. osculatum* D and *C. osculatum* E,

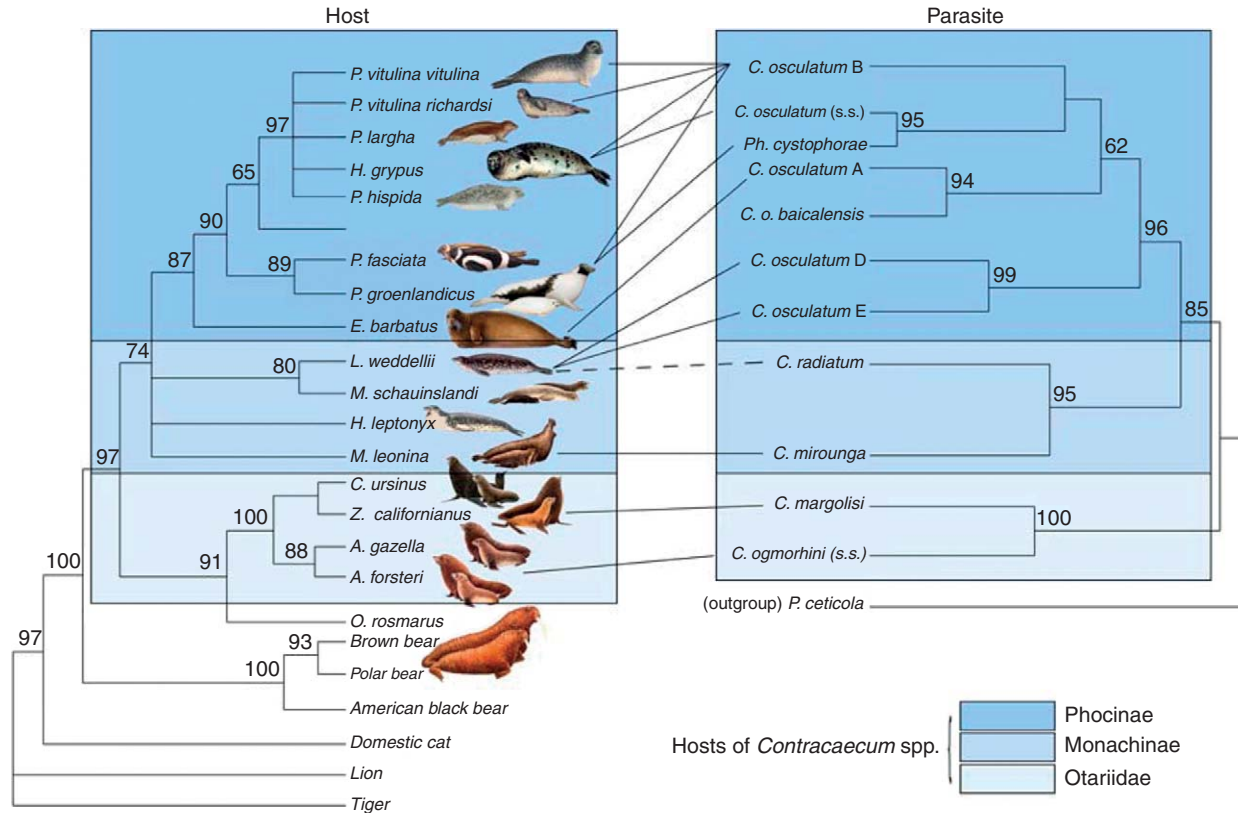


FIGURE 2.6 Tanglegram of phylogenies of *Contracaecum* spp. and their pinnipeds hosts. It shows the phylogeny of a group of extant pinnipeds (adapted from [Arnason et al., 1995](#)) mapped into the phylogeny of *Contracaecum* spp. inferred from mtDNA *cox2* sequence analysis (adapted from [Mattiucci et al., 2008b](#)). Lines depict possible host–parasite co-speciation events; the dotted line indicates possible host-switching events.

both parasites of *Leptonychotes weddellii* (see Bullini *et al.*, 1997). This hypothesis seems to fit with the evolutionary biogeography of a pinnipedimorph hypothesis based on both dispersal and vicariant events in the context of a species-level phylogenetic framework proposed by Deméré *et al.* (2003). This hypothesis supports an eastern North Pacific origin during the late Oligocene coincident with start of glaciation in Antarctica. During the late Miocene, pinnipedimorphs remained restricted to the eastern North Pacific, where they began to diversify. Fur seals remained restricted to the North Pacific until the late Pliocene, with a dispersal and rapid speciation in the Southern Ocean during the Pleistocene. The phocine seal diversification took place in the Arctic and North Atlantic during the late Miocene with a subsequent dispersal into the Paratethys and Pacific during the Pleistocene. Finally, the monachine seals, including *Mirounga leonina* and *Leptonychotes weddellii*, seem to have the southern hemisphere as the centre of diversification (Deméré *et al.*, 2003).

The mode of speciation which apparently fits well with the anisakid nematodes is the *peripatric model* proposed by Mayr (1963, 1976) (see also Section 5). This involves the geographical isolation of small populations whose genetic structure begins to differ from the parental one by different genetic mechanisms. In the case of the *Anisakis* spp., and the *C. osculatum* and *P. decipiens* species complexes, molecular genetic data strongly suggest that adaptation to different hosts and speciation is related to the geographical isolation of the hosts. Such processes apparently occurred in different times from the lower Miocene to Pliocene, and Pleistocene, when extreme climatic variation took place. During glacial maxima (a period also of lowest sea level), smaller populations of hosts and their endoparasites would have remained isolated in marine refuges, promoting genetic divergence and co-adaptation. Then during interglacial periods, geographical ranges might have expanded, favouring host range expansion (Bullini *et al.*, 1997). Similar co-evolutionary processes have been proposed by Hoberg (1992, 2005) for other host-parasite interactions, involving Holarctic cestodes and their definitive hosts (fish-eating birds and marine mammals).

7. HOST PREFERENCE, ECOLOGICAL NICHE AND COMPETITION

Molecular genetic markers of the so far genetically characterized species of anisakid nematodes provide a rapid, precise means to screen and identify several marine mammals, fish and squids that serve as their definitive and intermediate and or/paratenic hosts. These data give important information regarding the life-history traits and epizootiological aspects of these parasites.

Marked differences in definitive and intermediate host preferences were detected among the species belonging to *Anisakis*, members of *Pseudoterranova decipiens* complex and species of *Contracaecum* from pin-nipeds. They have shown distinct host preferences, with respect to their definitive and intermediate hosts, often exhibiting differential distribution in various host species and/or with different ecology. Patterns of differential distribution are the product of ecological processes and therefore interactions between parasite populations could promote and even enhance the complexity of multi-species assemblages observed in a given host (definitive and intermediate). Such differences in the life history and host preference between related species of anisakid nematodes appear to be due to differential host–parasite co-adaptation and co-evolution as well as to interspecific competition. This acts either in reducing the range of potential hosts or promoting a differential use of resources from a single individual host in sympatric conditions. In other words, the realized niche in some anisakid species could be more restricted than the fundamental niche. Some examples of such ecological processes have been found in anisakids of the *P. decipiens* complex and *C. osculatum* complex and are described below.

In the North Atlantic and Pacific Oceans (Fig. 2.7), *P. bulbosa* is the only species present in the bearded seal, *Erignathus barbatus*, both in the eastern and western parts of the Atlantic Ocean, and also in the Pacific (Table 2.5), whereas in the North Eastern Atlantic waters *P. decipiens* (s.s.) and *P. krabbei* occur sympatrically as adults and often also syntopically in the same individual host in the common seal, *Phoca vitulina*, and in the grey seal, *Halichoerus grypus* (see Mattiucci *et al.*, 1998; Paggi *et al.*, 1991). However, statistically significant differences in relative numbers have been observed between *P. decipiens* (s.s.) and *P. krabbei* identified by genetic markers (allozymes) in those hosts, where *P. decipiens* (s.s.) predominates about tenfold in the common seal, while *P. krabbei* is the main prevalent species in the grey seal. On the other hand, in the western Atlantic, where *P. krabbei* is absent, *P. decipiens* (s.s.) occurs in equal proportions in both grey and common seals (Nascetti, 1992; Paggi and Bullini, 1994) (Fig. 2.7). This finding was also observed by Bratney and Stenson (1993), using the same genetic markers (diagnostic allozymes), to identify to species level anisakid nematodes in seal hosts from Newfoundland and Labrador. The bearded seal was found to be parasitized by *P. bulbosa* (under its former name, *P. decipiens* C, by Bratney and Stenson, 1993), while both the grey and common seal were found heavily infected by *P. decipiens* (s.s.) (under its former name, *P. decipiens* B, by Bratney and Stenson, 1993). This finding could reflect differential adaptation of the two parasites, which have evolved allopatrically. After their secondary contact and, consequently, their sympatric occurrence in the North East Atlantic seal hosts, interspecific competition between the two

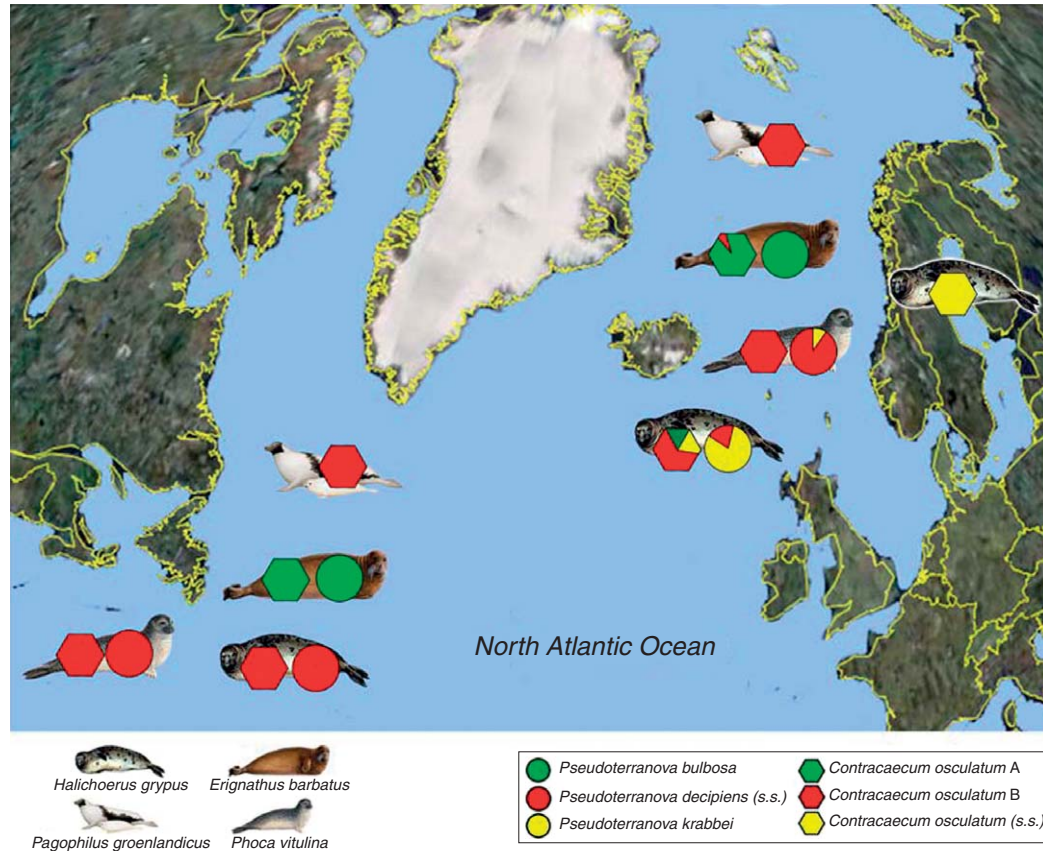


FIGURE 2.7 Relative proportions of *Pseudoterranova decipiens* and *Contracaecum osculatum* species complexes in definitive hosts (seals) from the North Atlantic Ocean. It shows differences in their definitive host preferences.

Pseudoterranova species could have led to a niche subdivision, evolving by character displacement (Bullini *et al.*, 1997; Nascetti, 1992).

Another example of niche subdivision is represented by the two Antarctic members of the *C. osculatum* complex, that is, *C. osculatum* D and *C. osculatum* E. The two sibling species share the same definitive host, the Weddell seal, *Leptonychotes weddellii*, in both the Weddell and Ross Seas, of Antarctica. However, the two anisakid species appear to be adapted in this sympatric area to pelagic and benthic food webs, respectively. Larval individuals ($n = 1306$) of *C. osculatum* D and *C. osculatum* E, genetically identified to the species level by allozymes, were found to be heavily infecting fish species in the Ross Sea (Mattiucci and Nascetti, 2007, unpublished data). Statistically significant differences were detected in the relative proportions of the two Antarctic species of the *C. osculatum* complex in benthic and mesopelagic fish intermediate host species. Indeed, *C. osculatum* D outnumbers *C. osculatum* E in channichthyid fish (i.e. *Chionodraco hamatus*, *Pagetopsis macropterus*) and in bathydraconids (i.e. *Gymnodraco acuticeps*, *Cygnodraco mawsoni*); while, higher relative proportions of the infection by *C. osculatum* E were observed in nototheniid fish (*Trematomus bernacchii*, *T. pennellii*, *T. newnesi* and *Notothenia neglecta*) (Fig. 2.8). The last group of nototheniid fish are bottom living and feed on benthic invertebrates, such as benthic amphipods (Daniels, 1982; Foster and Montgomery, 1993; Vacchi *et al.*, 1994), whereas channichthyid and bathydraconid fish have a pelagic/mesopelagic mode of life (Daniels, 1982). In the sibling species *C. osculatum* D and E, competition in the intermediate hosts could be avoided by spatial separation in the pelagic and benthic components of food webs in the Antarctic marine ecosystem.

On the other hand, Klöser *et al.* (1992) suggested that *C. radiatum* in Antarctic waters uses a pelagic food web. This was also confirmed by the genetic identification of larval stages of *C. radiatum*, at a high prevalence, in pelagic channichthyid fish (i.e. *Chionodraco hamatus*) (Arduino *et al.*, 1995).

Life-history differences in anisakid nematodes genetically recognized at the species level were also reported in the case of the *P. decipiens* species complex. In the northern hemisphere, *P. bulbosa* is a parasite at adult stage of the bearded seal both in the North Atlantic and Pacific Oceans, and at larval stage it occurs in the flatfishes *Hippoglossoides platessoides*, *Reinhardtius hippoglossoides* and *Hippoglossus hippoglossus*, in *Myoxocephalus quadricornis*, and, rarely, in *Gadus morhua macrocephalus* (see Brattey and Davidson, 1996; Bristow and Berland, 1992; Mattiucci *et al.*, 1998). The hosts so far detected for *P. bulbosa* are specialized benthic species, with the exception of *G. morhua* which is benthopelagic (Paggi *et al.*, 1991, 1998c). This supports the hypothesis that *P. decipiens* (s.l.) in the northern hemisphere has a benthic life cycle (Bristow and Berland, 1992). A similar benthic life-history pathway can be suggested for *P. azarasi* in Pacific

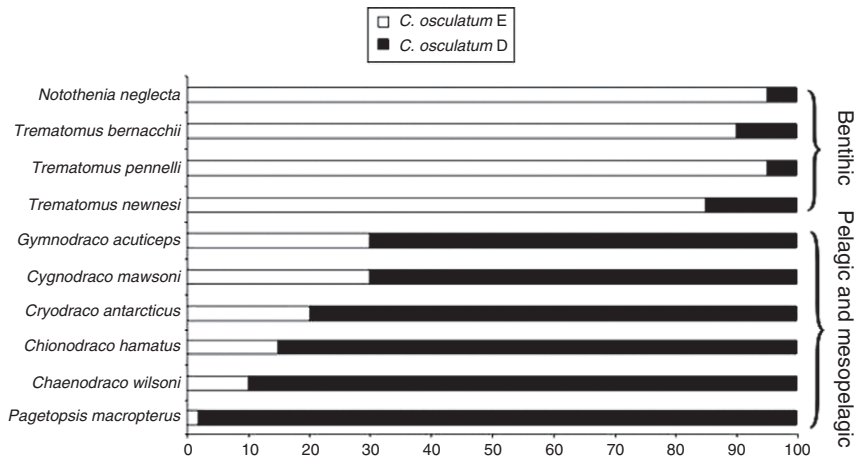


FIGURE 2.8 Relative proportions of larval specimens of the Antarctic anisakids *Contracaecum osculatum* D and *C. osculatum* E, genetically identified from fish hosts. It depicts differential distribution of the two species in pelagic, mesopelagic and benthic fish hosts respectively, in Antarctica.

waters. It was detected genetically at the adult stage in the benthic feeding Steller's sea lion, *Eumetopias jubatus*, and also in mixed infections with *P. bulbosa* in the bearded seal; and at larval stage in the flatfish *Hippoglossus hippoglossus* (see Mattiucci *et al.*, 1998; Paggi *et al.*, 1998c).

Benthic and benthopelagic fish species (i.e. *Chaenocephalus aceratus*, *Notothenia coriiceps*, *N. neglecta*, *Trematomus newnesi*) are found infected by the Antarctic taxon *P. decipiens* E (our unpublished data), confirming the suggestion of Palm *et al.* (1994), according to which *P. decipiens* (s.l.) completes its life cycles in the Weddell Sea environment in organisms of the benthic food web.

Interactions in seals between anisakid species of different genera are likely to exist, since they often co-occur in the same host individual. As anisakine nematodes become numerous in seal stomachs, they may be increasingly prone to the consequences of intra- and inter-specific competition. These effects could provoke high mortality rates in one species, retard its maturity or result in lower uterine egg counts in female worms. McClelland *et al.* (1985) and Burt (1994) speculated that *P. decipiens* (s.l.) abundance in seal stomachs may be limited as a consequence of 'competitive exclusion' by the related nematode *C. osculatum* (s.l.). McClelland *et al.* (1985) suggested that *C. osculatum* (s.l.) interfered with the proliferation of *P. decipiens* (s.l.), since seasonal and geographical reductions in the numbers of *P. decipiens* (s.l.) in grey seals often coincided with increases in the abundance of *C. osculatum* (s.l.). This negative correlation between the abundance of the two anisakid nematodes was not supported by the data

of Bratney and Stenson (1993) according to which, these changes could be equally well due to differences in diet among grey seals, as these tend to acquire infection with larval *P. decipiens* (s.l.) by preying more on benthic fish (McClelland *et al.*, 1990); whereas others feed more on pelagic fish species, such as capelin, *Mallotus villosus*, or cod, *Gadus morhua*, that harbour larvae of *C. osculatum* (s.l.) (see Pålsson and Beverly-Burton, 1984). Marcogliese (1997), remarking on the relatively small size and low uterine egg counts of female *P. decipiens* (s.l.) in grey seals from Anticosti Island, suggested that *P. decipiens* may be suppressed as a consequence of competitive pressure from *C. osculatum*. McClelland (2002) suggested, however, that significant negative correlation between the abundance of the two nematode species, *P. decipiens* and *C. osculatum*, could result when samples taken in a given area also include migrant seals acquiring infections in other areas. However, when members of the *P. decipiens* and *C. osculatum* complexes infest the same seal host, a differential use of resources could take place, as the former occupy the seal intestine when the stomach (which is probably the suitable microhabitat for both anisakids) is highly parasitized by *C. osculatum* (s.l.) (see Berland, 1964; Klöser *et al.*, 1992; Paggi and Bullini, 1994; Mattiucci and Nascetti, unpublished data).

Berland (1964) speculated that *C. osculatum* competes with and displaces *Phocascaris* spp. from the stomach of harp *Pagophilus groenlandicus*, and hooded seal *Cystophora cristata*. This result was later confirmed by genetic identification (allozymes) of several individuals of *C. osculatum* B and *Phocascaris* spp. It is likely that *C. osculatum* B is a better competitor than *Phocascaris phocae* or *P. cystophorae* during syntopic occurrence, at the adult stage, in the same individual seal host. Marked differences were indeed observed in their relative proportions when they occur syntopically in the harp and common seals. In these cases, the stomach is mostly occupied by *C. osculatum* B, while *P. phocae* and *P. cystophorae* are displaced to the upper intestine. In contrast, the hooded seal has always been found parasitized solely by *P. cystophorae*/*P. phocae* in both sites (stomach and intestine). In this seal, *Phocascaris* spp., in the absence of a better competitor, usually occupies the stomach. These phenomena of niche subdivision, promoting character displacement, could reduce the inter-specific competition between two closely related anisakid species (i.e. *C. osculatum* B and *P. phocae*/*P. cystophorae*) for a trophic resource (Nascetti, 1992; Paggi and Bullini, 1994).

8. ANISAKIDS AS BIOLOGICAL INDICATORS

In recent years, marine parasites have been widely used in biological and ecological surveys of marine ecosystems as biological indicators of food chain structure (Thompson *et al.*, 2005), pollution (Sures, 2004), global

climate changes (Brooks and Hoberg, 2007; Poulin, 2006), anthropogenic impacts and environmental stresses (Marcogliese, 2005), fish stock assessment (MacKenzie, 2002) and of general 'ecosystem health' (Marcogliese, 2005).

Anisakid parasites are an integral part of aquatic ecosystems. They are indeed important components of any ecosystem. Not only do they play key roles in population dynamics and community structure, they can provide important information about the general biodiversity of the ecosystem.

Recent data on the possible use of anisakid nematodes are presented in this section as biological indicators of (i) the definition of fish stocks within a multidisciplinary approach, (ii) integrity and stability of trophic webs and (iii) habitat disturbance.

8.1. *Anisakis* spp. larvae as biological tags of fish stocks

One of the major goals of the last decade in the study of the parasite fauna of aquatic organisms has been, among the others, the assessment of fish stocks, their movement and recruitment. Indeed, the use of parasites to discriminate fish host populations has been one of the most useful approaches in a multidisciplinary study of fish stock detection and characterization. Nowadays, the modern concept of 'fish stock' integrates all the information gathered from a broad spectrum of techniques, ranging from morphology (morphometrics, meristic, otolith microchemistry) to biology (life-history characteristics, mark-recapture, parasites) and genetic structure (allozymes, microsatellites, DNA sequences) of the fish hosts throughout their geographical range. In this 'holistic approach' to the definition of fish stock (Begg and Waldman, 1999), among the biological approaches, the use of parasites as 'biological tags' has become a useful tool, mainly concerning species with a high commercial value in fisheries. Trends in the use of parasites as biological tags in population studies of marine fish species was recently reviewed (MacKenzie, 2002). Among the parasite species that have been used in fish stock definition, the larval anisakid nematodes of the genus *Anisakis* represent one of the best biological tags (MacKenzie, 2002). Allozyme markers were first used to differentiate populations of herring, *Clupea harengus* L., in the North Atlantic, on the basis of different allele frequencies observed at one enzymatic locus in *Anisakis* larvae (Beverly-Burton *et al.*, 1977). The application of diagnostic genetic markers in the identification of larval *Anisakis* to species level, and the biogeographical aspects of *Anisakis* spp., have, in recent years, allowed the fish stock identification of several demersal and pelagic species (Mattiucci *et al.*, 2007a). A parasitological survey carried out on several fish specimens of the European hake, *Merluccius merluccius*, from 14 localities in the North East Atlantic and

Mediterranean Sea included in the range of distribution of the fish species, has allowed detection, by allozyme markers, of the occurrence of different biological species of larval *Anisakis* ($n = 1950$). Indeed, seven species of *Anisakis* were genetically recognized [*A. pegreffii*, *A. simplex* (s.s.), *A. typica*, *A. ziphidarum*, *A. physeteris*, *A. brevispiculata* and *A. paggiae*] showing statistically significant differences in their distribution in the hake sampled from Mediterranean and Atlantic waters (Mattiucci *et al.*, 2004) (Fig. 2.9). This indicated that (i) two stocks of *M. merluccius* from Mediterranean and Atlantic waters exist; (ii) in the North Atlantic area, at least two different population units of European hake are present; one northern and another from off the Atlantic coast of Morocco; and (iii) some sub-structuring in the Mediterranean population was also recognized (Mattiucci *et al.*, 2004). These findings are in accordance with genetic results obtained from allozyme markers on the same fish host samples, demonstrating that Atlantic and Mediterranean populations of *M. merluccius* belong to two different stocks. These two populations are separated at the Almeria-Oran front (Cimmaruta *et al.*, 2005), as this front has been proved to be the boundary between Atlantic and Mediterranean stocks of several marine organisms (Pannacciulli *et al.*, 1997; Rios *et al.*, 2002). In this area a strong inflow transports surface Atlantic waters into the Alborán Sea, creating an Atlantic domain within the Mediterranean, and a partial isolation of the Alborán with respect to the other Mediterranean water masses. It was also demonstrated that salinity and temperature are responsible for maintaining the genetic differentiation between the two fish population groups through selective processes (Cimmaruta *et al.*, 2005).

In the case of horse mackerel, *Trachurus trachurus*, different populations can be distinguished according to the spatial distribution of the two most dominant parasite species, genetically recognized as *A. simplex* (s.s.) and *A. pegreffii*. Indeed, the significant differences indicated by their relative proportions between the samples from the Mediterranean Sea (excluding the Alborán Sea) in comparison with the Atlantic ones seem to indicate the discrete sub-structuring of populations of *T. trachurus* in Mediterranean and Atlantic waters. Moreover, in Atlantic waters, the differences found in the proportion of *A. pegreffii* between the southern horse mackerel samples and those remaining seem to suggest a possible existence of a ‘southern’ sub-population of *T. trachurus* readily distinguished from a ‘northern’ one. The samples of *T. trachurus* from the Alborán Sea were closer to the southern populations of the Atlantic than to the other samples from the Mediterranean Sea. This finding, and particularly the presence of *A. simplex* (s.s.) in some individual hosts from the Alborán Sea, suggests that these populations could be the result of a migration of this small pelagic fish species from Atlantic waters into this extreme western area of the Mediterranean Sea, and also possibly

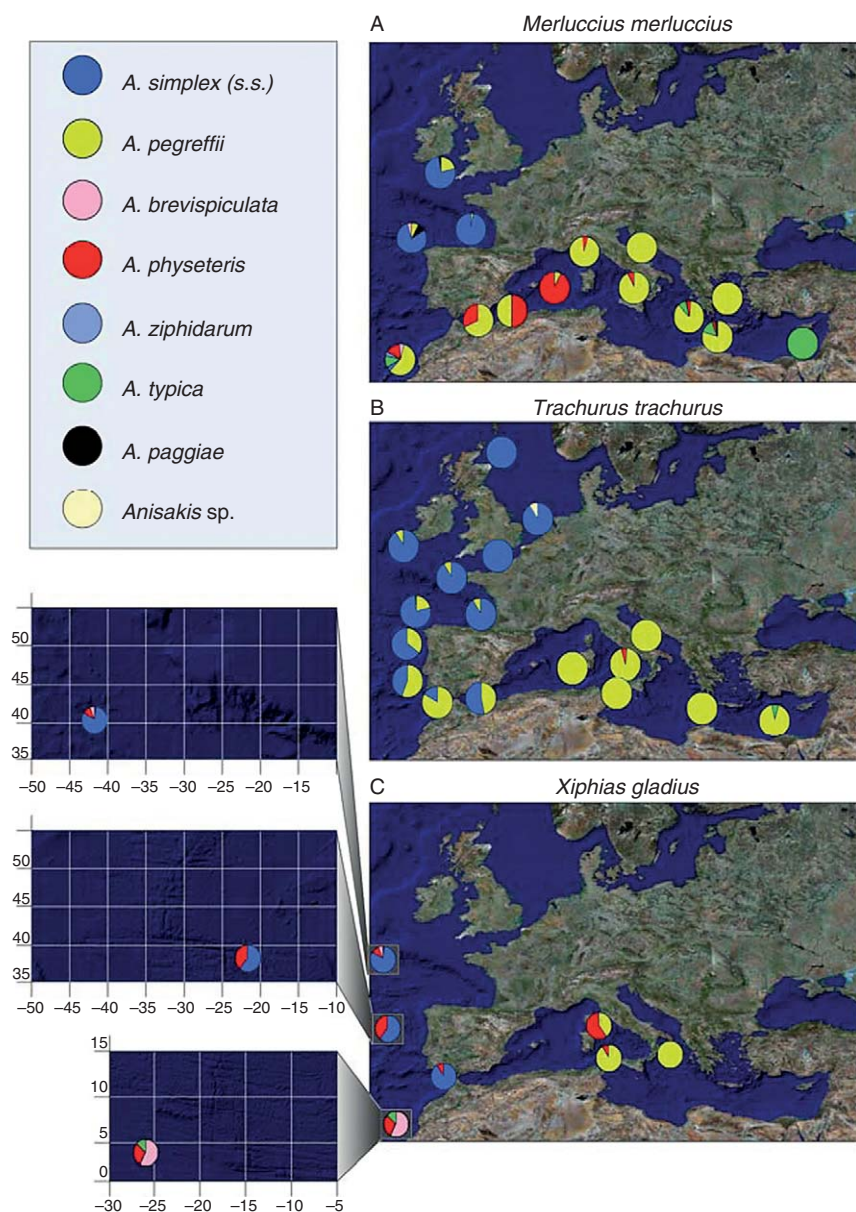


FIGURE 2.9 Relative proportions of larval specimens of *Anisakis* spp. from different fish hosts: (A) *Merluccius merluccius*; (B) *Trachurus trachurus*; (C) *Xiphias gladius* throughout their range of distribution. It shows the use of biogeographical aspects of *Anisakis* spp., genetically identified to species level, as biological tags for their fish stocks definition in European waters.

mixing with Mediterranean populations. This, in turn, is related to the possible Almeria–Alborán boundary in Mediterranean waters. Furthermore, the high percentage of fish exhibiting infection by *A. simplex* (s.s.) from this area was in accordance with the high dispersal capacity of this small pelagic fish (Mattiucci *et al.*, 2008a) (Fig. 2.9). This is in agreement with the high genetic variability values shown for *T. trachurus*, at the nuclear level (allozymes), in the same fish samples (Cimmaruta *et al.*, 2008). However, in contrast with the parasitological findings, a substantial genetic homogeneity, inferred from allozyme markers on the same fish samples, was found between the Atlantic and Mediterranean populations of horse-mackerel; a partial sub-structuring of *T. trachurus* observed between the far eastern and western Mediterranean Sea has been reported (Cimmaruta *et al.*, 2008).

As for the swordfish, *Xiphias gladius*, it has been recently demonstrated, using different molecular genetic markers and biological data, that the Mediterranean population is separated from the Atlantic one (Cimmaruta *et al.*, 2006; Kotoulas *et al.*, 2006; Lu *et al.*, 2006; Reeb and Block, 2006). The existence of a further separation between northern and southern populations of swordfish in the Atlantic has been also suggested (Alvarado-Bremer *et al.*, 2006). The occurrence of different species of *Anisakis* spp. larvae in Mediterranean and Atlantic swordfish populations was recently shown (Mattiucci *et al.*, 2007a). In particular, the absence of *A. simplex* (s.s.) in the Mediterranean swordfish samples seems to support the idea that the Mediterranean population of *X. gladius* is separated from the Atlantic one. Moreover, in Atlantic waters, the possible existence of two populations, one northern and one southern, has been suggested (Mattiucci *et al.*, 2007a) (Fig. 2.9). This is also supported by population genetics data obtained from the swordfish sampled in this area, indicating a possible boundary of the two stocks between 0°N and 5°S in Atlantic waters (Alvarado-Bremer *et al.*, 2006).

Recently, it has been shown that the polymorphism observed in mtDNA *cox1* gene sequences, analysed for several samples of *A. simplex* (s.s.) collected between and within spawning seasons of its fish host (the herring, *Clupea harengus*), exhibited a high degree of temporal stability (Cross *et al.*, 2007). This underlines the potential use of this species as a biological tag, and mtDNA *cox1* as a suitable genetic marker for future investigation in fish host stock structure definition (Cross *et al.*, 2007).

8.2. Anisakids as indicators of trophic web stability and habitat disturbance of marine ecosystems

Food webs are networks of trophic relationships which map the location of energy flow in a community. Despite the increasing details that have been incorporated into marine food webs, some functional groups remain

neglected. Among these are the parasites and host–parasite interactions that are known to affect, among others, community structure and trophic relationships (Marcogliese, 2001, 2005; Poulin and Morand, 2004). The transmission pathways of parasites with indirect life cycles are fully included in food webs of aquatic ecosystems. In other words, just as food webs have exerted strong selective pressure on the evolution of parasite transmission strategies, parasites are now shaping some of the ecological properties of existing food webs.

The transmission routes of anisakid nematodes follow closely the trophic relationships among their successive hosts, and thus they are parasites embedded in food webs. As a consequence, the completion of life cycles as complicated as those of anisakid nematodes requires a stable trophic web. As a result, the life cycle of anisakid nematodes in marine ecosystems with various degrees of habitat disturbance could be affected by changes in host population size. Indeed, when the population size of the hosts participating in the life cycle of these parasites is reduced due to different causes (pollution, by-catch of marine mammals, viral diseases of marine mammals, overfishing, etc.) (Fig. 2.10), the population size of their anisakid endo-parasites could also be reduced. This would result in a higher probability of genetic drift in the parasite gene pools and, consequently, a decrease in their genetic variability values.

It has recently been shown (Mattiucci and Nascetti, 2007) that the distribution of the genetic variability of anisakid nematode populations in geographical areas with different levels of environmental stress is likely to reflect the influence of a range of factors that could promote their genetic diversity. These include a large effective parasite population size, the wide range, availability, and population size of their hosts, and the stability of marine trophic webs. The values of the genetic variability [estimated at the parameters of percentage of polymorphic loci (P), mean number of alleles per locus (A) and expected heterozygosity per locus (He)], obtained at 19 allozyme loci, were compared among 53 populations of anisakid nematodes belonging to 20 species of *Anisakis*, *Pseudoterranova* and *Contracaecum* from several hosts in the Boreal and Austral regions (Mattiucci and Nascetti, 2007). Austral populations of species belonging to these three genera exhibited significantly higher genetic variability values than those from the Boreal regions [expected mean of heterozygosity per locus, $He = 0.19$ (in Austral populations) and $He = 0.09$ (in Boreal populations) ($P < 0.01$)] (Fig. 2.11). A more remarkable difference in their genetic variability values was observed when only Antarctic and sub-Antarctic populations were compared directly with Arctic and sub-Arctic populations [$He = 0.23$ and $He = 0.07$ ($P < 0.001$), respectively] (Mattiucci and Nascetti, 2007). One conclusion is that the observed values of genetic variability could be related to extreme latitudes, a parameter often considered as relevant (Nevo *et al.*, 1984). However, the data suggested that a

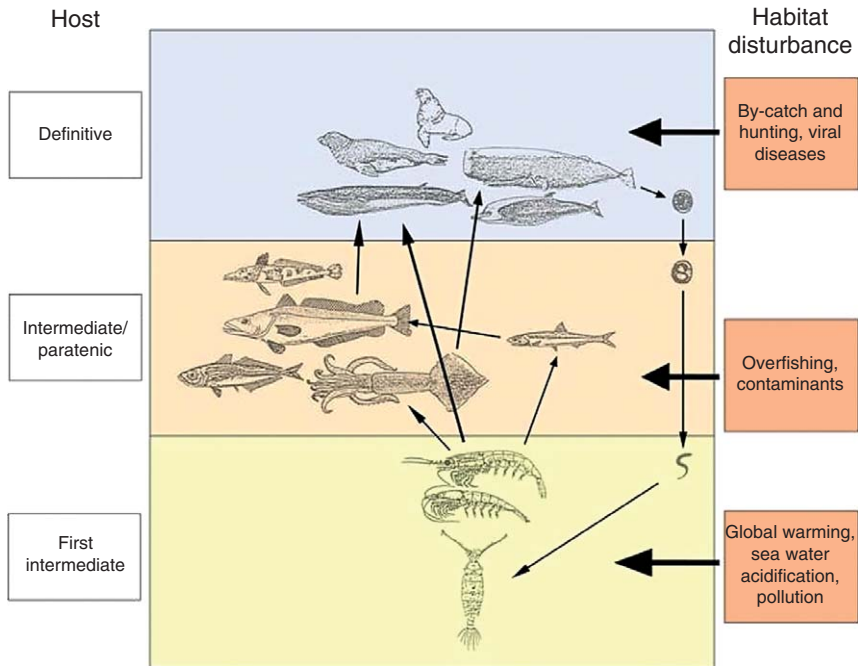


FIGURE 2.10 Relationship between habitat disturbance and host population size. It depicts causes of habitat disturbance (right) that can affect the population size of definitive and intermediate hosts (at different trophic levels of a marine food web) involved in speculative life-cycle pathways of anisakid nematodes of the genera *Anisakis*, *Pseudoterranova* and *Contracaecum* (includes only species from pinnipeds). (Modified from [Mattiucci and Nascetti, 2007](#), with permission from Elsevier.)

significantly higher level of genetic variability found in the Antarctic members considered (i.e. *C. osculatum* D, *C. osculatum* E, *C. radiatum*, *P. decipiens* E, *A. simplex* C and *A. pegreffii* populations from sub-Antarctic regions) coincide with a lower degree of habitat disturbance (e.g. overfishing, by-catch of cetaceans, hunting and diseases mortality of seals, sea water pollution and acidification). This would allow host species to reach higher population sizes, resulting in higher anisakid population sizes, with a reduced probability of genetic drift phenomena in the parasite gene pools. Consequently, a higher level of genetic diversity in the Antarctic populations of these nematodes was observed.

Likewise, a much higher abundance and intensity of infection was observed in the Antarctic and sub-Antarctic populations and species of anisakid nematodes, where more than 10^5 individual worms were typically collected from a single host, compared to $<10^2$ from Arctic and sub-Arctic hosts ([Beverly-Burton, 1971](#); [Brattey and Stenson, 1993](#);

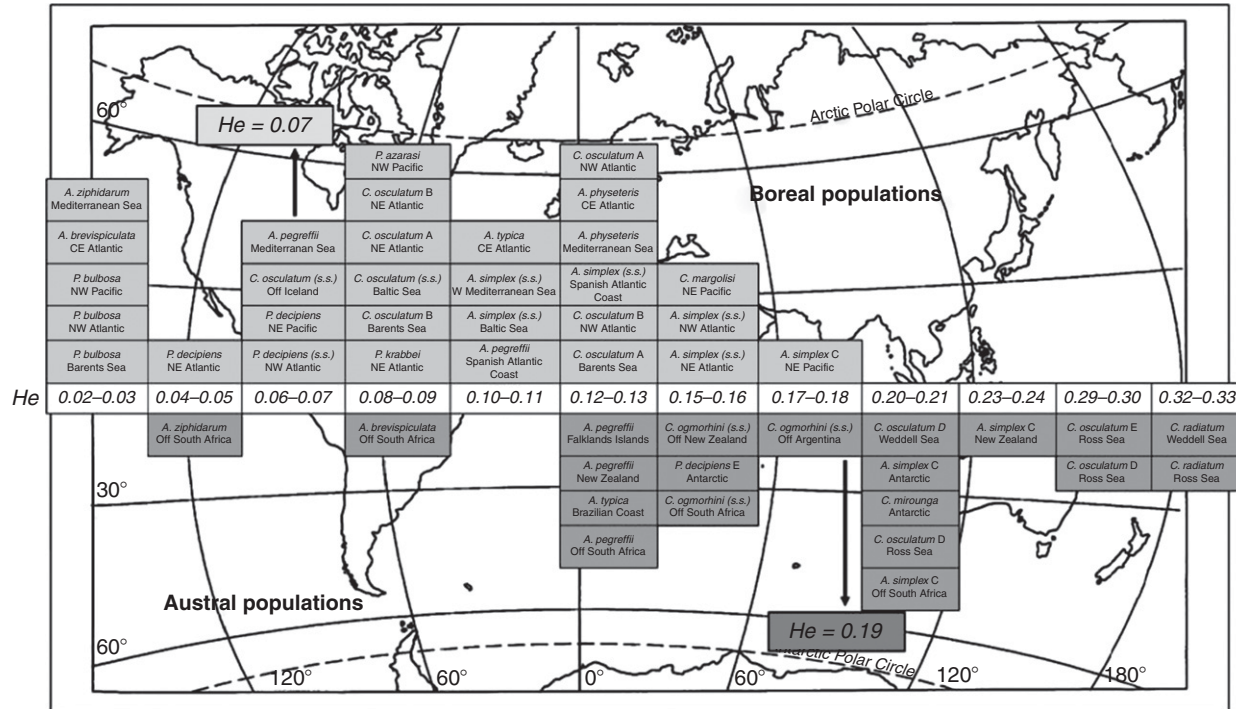


FIGURE 2.11 Distribution of the genetic diversity (expected mean heterozygosity per locus, He) (in white coloured cells) among anisakid populations from Boreal (in light grey coloured cells) and Austral regions (in dark grey coloured cells). The arrows represent the mean values of He in Boreal and Austral populations. The genetic variability of anisakid populations is higher in the Antarctic region than in other geographical areas, whether they be similar or ecologically differentiated. Such difference can be explained by the lower habitat disturbance of the Antarctic region, which permits the maintenance of more stable trophic webs in this ecosystem (redrawn from Mattiucci and Nascetti, 2007).

Klöser *et al.*, 1992; Mattiucci and Nascetti, 2007; McClelland *et al.*, 1983) (Tables 2.12 and 2.13). The data are consistent with biotic factors, such as host density of those suitable definitive and intermediate hosts for the anisakid nematodes in the Antarctic and sub-Antarctic areas, maintaining the high genetic diversity in the anisakid gene pools. In support of this hypothesis, a high density of marine mammal populations has been documented in these geographical areas (Wickens, 1995), relative to populations from the Arctic-Boreal regions (Read *et al.*, 2006). For example, in Antarctic waters, the Weddell seal (main definitive host of *C. osculatum* D and *C. osculatum* E, see Section 3.3) has not suffered a reduction in its population numbers in recent years (Kendall *et al.*, 2003). This is contrary to the phocids of the Arctic–Boreal region, whose population size has decreased due to different causes of habitat disturbance (hunting, by-catch, viral diseases, etc.) (Andersen *et al.*, 2006; Barron *et al.*, 2003; Di Guardo *et al.*, 2005; Pastor *et al.*, 2004). Similarly, the Antarctic fish species examined here and utilized as intermediate hosts by these nematodes have not been subjected to the population reduction from over-fishing that has occurred in the host fish species from the Boreal region (i.e. *Gadus morhua*) (Anon, 1997; Cook *et al.*, 1997). Various causes of habitat disturbance (pollution, capture, overfishing, global climate change) possibly place undue stress on the trophic webs involving different species of marine mammals, fish and invertebrates (suitable as definitive and intermediate hosts of anisakid nematodes). This in turn could adversely affect the population size of their endoparasitic anisakid nematodes and, consequently, their genetic variability. Small anisakid nematode burdens tend to have a lower genetic diversity, as smaller populations lose diversity more quickly than large populations, due to genetic drift. Large populations of anisakid nematodes, such as those from the Antarctic, show higher levels of genetic diversity. These data fit well into the pattern which has recently been demonstrated for a range of other parasitic nematode species, showing that low levels of genetic diversity are often positively associated with low levels of infection (Webster *et al.*, 2007).

It is likely that elevated host density in the Antarctic and sub-Antarctic areas will lead to an increase in anisakid parasite prevalence and abundance in both suitable definitive and intermediate hosts. The high levels of parasitic infection reported for the *C. osculatum* (s.l.) complex in Antarctic fish species, which are prey for the Weddell seal, are consistent with the high integrity and stability of the food webs in this marine ecosystem. This, in turn, facilitates the completion of the life cycles of Antarctic and sub-Antarctic anisakid nematodes (Mattiucci and Nascetti, 2007). These results support the hypothesis that parasite infections give indications about the hosts that are available to them (Hudson *et al.*, 2006; Poulin, 2006).

TABLE 2.12 Prevalence (*P*, %), Intensity (*I*) and Abundance (*A*) of the infection with the species *Contracaecum osculatum* (*s.l.*) in intermediate/paratenic (fish) and definitive (pinnipeds) hosts from the Boreal and Antarctic regions

Host species/ geographic origin	Parasite	<i>P</i>	<i>I</i> (min–max)	<i>A</i>
Intermediate host				
Boreal region				
<i>Gadus morhua</i> (NE Atlantic)	<i>C. osculatum</i> B	50	(4–20)	4.0
<i>Hippoglossoides platessoides</i> (NE Atlantic)	<i>C. osculatum</i> A	26.0	(2–10)	1.2
Antarctic region				
<i>Gymnodraco acuticeps</i> (Ross Sea)	<i>C. osculatum</i> D	100.0	(53–554)	308.7
<i>Cygnodraco mawsoni</i> (Ross Sea)	<i>C. osculatum</i> D	100.0	(60–520)	210.0
<i>Cryodraco antarcticus</i> (Ross Sea)	<i>C. osculatum</i> D	100.0	(86–619)	342.8
<i>Chionodraco hamatus</i> (Ross Sea)	<i>C. osculatum</i> D	100.0	(30–209)	105.1
<i>Trematomus bernacchii</i> (Ross Sea)	<i>C. osculatum</i> E	100.0	(20–350)	110.2
Definitive host				
Boreal region				
<i>Pagophilus groenlandicus</i> (NE Atlantic)	<i>C. osculatum</i> B	97.7	(13–2351)	321.9
<i>Halichoerus grypus</i> (NE Atlantic)	<i>C. osculatum</i> B	80.7	(44–2783)	528.5
<i>Phoca vitulina</i> (NE Atlantic)	<i>C. osculatum</i> B	100.0	(1–543)	76.8
<i>Erignathus barbatus</i> (NE Atlantic)	<i>C. osculatum</i> A	100.0	(48–1223)	313.7
Antarctic region				
<i>Leptonychotes weddellii</i> (Weddell Sea)	<i>C. osculatum</i> (<i>s.l.</i>)	100.0	(20671– 130200)	42270.2
<i>Leptonychotes weddellii</i> (Ross sea)	<i>C. osculatum</i> (<i>s.l.</i>)	100.0	(6300– 32500)	25310.1

Modified from [Mattiucci and Nascetti \(2007\)](#), with permission from Elsevier).

TABLE 2.13 Prevalence (*P*,%), intensity (*I*) and abundance (*A*) of parasitic infection levels by *Anisakis pegreffii* in different definitive and intermediate hosts from the Boreal and Austral regions

Host species/geographic origin		<i>P</i>	<i>I</i> (min–max)	<i>A</i>
Definitive host				
Boreal region				
<i>Tursiops truncatus</i>	Mediterranean Sea	10.0	(1–15)	10.5
<i>Stenella coeruleoalba</i>	Mediterranean Sea	2.0	(1–30)	15.0
<i>Delphinus delphis</i>	Mediterranean Sea	2.0	(1–22)	20.0
Austral region				
<i>Caperea marginata</i>	SE Atlantic Ocean (South Africa coast)	–	–	100.5
<i>Cephalorhynchus haevinsedi</i>	SE Atlantic Ocean (South Africa coast)	–	–	120.4
<i>Tursiops truncatus</i>	SE Atlantic Ocean (South Africa coast)	–	–	250.9
Intermediate host				
Boreal region				
<i>Trachurus trachurus</i>	Mediterranean Sea	44.4	(1–50)	10.5
<i>Merluccius merluccius</i>	Mediterranean Sea	85.0	(1–130)	25.0
<i>Lepidopus caudatus</i>	Mediterranean Sea	80.0	(10–120)	20.0
<i>Engraulis encrasicolus</i>	Mediterranean Sea	17.7	(1–10)	1.8
<i>Micromesistius poutassou</i>	Mediterranean Sea	48.5	(1–12)	5.1
Austral region				
<i>Merluccius capensis</i>	SE Atlantic Ocean (South Africa coast)	98.0	(10–130)	90.5
<i>Lepidopus caudatus</i>	SE Atlantic Ocean (South Africa coast)	100	(30–250)	212.0

(continued)

TABLE 2.13 (continued)

Host species/geographic origin		<i>P</i>	<i>I</i> (min–max)	<i>A</i>
<i>Etrumeus whiteheadi</i>	SE Atlantic Ocean (South Africa coast)	100	(20–120)	80.8
<i>Thyrssites atun</i>	SE Atlantic Ocean (South Africa coast)	100	(20–360)	205.1
<i>Merluccius hubbsi</i>	SW Atlantic Ocean (Falklands Islands)	95.0	(30–170)	90.5

Modified from [Mattiucci and Nascetti \(2007\)](#), with permission from Elsevier).

The Antarctic ecosystem is generally considered ‘pristine’ ([Battaglia et al., 1997](#)). Existing results on the genetic variability of anisakid nematodes have shown that, despite the ‘extreme’ ecological conditions of marine Antarctic ecosystems, the genetic diversity of the host–parasite system formed by ascaridoid worms in fish and marine mammals is higher in the Antarctic region than in other geographical areas whether they be similar or ecologically differentiated. Such difference can be explained by the lower habitat disturbance of the Antarctic region, which permits the maintenance of more stable trophic webs in this ecosystem ([Mattiucci and Nascetti, 2007](#)).

9. CONCLUSIONS AND IDENTIFICATION OF GAPS IN OUR KNOWLEDGE OF ANISAKIDS TO BE FILLED BY FUTURE RESEARCH

The objective of this review was to compile current knowledge on the anisakid species of *Anisakis*, *Pseudoterranova* and *Contracaecum* acquired since the introduction of molecular markers as an aid to their systematics. Thus, we have attempted to detail the advances in their taxonomy, epidemiology, geographical distribution, population genetics and phylogenetic relationships, highlighting ecological implications and co-evolutionary processes. New insights into our knowledge of anisakids have been developed, but several questions still need to be addressed. This section thus highlights some of the main gaps which, in our opinion, will present new perspectives and opportunities for future research on anisakid nematodes.

9.1. Molecular systematics

From a taxonomic point of view, we have collected examples of, and evidence for, criteria useful for the species diagnosis of anisakid nematodes.

Describing reproductive isolation is crucial for speciation and the use of allozyme markers, and (by MAE) is particularly useful for this aim because (1) they are co-dominant markers (homozygotes are distinguishable from heterozygotes), (2) the polymorphisms which they can reveal are representative of the genetic variation of an anisakid population, and (3) they allow a more complete understanding of Mendelian inheritance of these polymorphisms. Starting from these landmarks we have seen that, in the case of anisakid species, MAE could be the best choice, as a first genetic approach, to define the taxonomic status of a species following the BSC, because they are able to demonstrate the absence of gene flow and, thus, the reproductive isolation between taxa. They are also able to distinguish possible F1 hybrids between related taxa and/or introgressed individuals, where other molecular markers (for instance, RAPDs and/or other not co-dominant markers) may fail to demonstrate them. Moreover, it has been shown that once anisakid species have been designated by allozymes, phylogenetic systematics inferred from other molecular markers (both nuclear and mitochondrial) were congruent and corroborated those results. On the other hand, phylogenetic systematics of anisakid nematodes based on DNA gene sequence analysis has been shown, in most cases and with few exceptions, to be congruent with allozyme data sets establishing their taxonomic status and depicting phylogenetic relationships between related taxa of anisakids. It has also been useful for comparison with phylogenies postulated for their definitive hosts. Among the PCR-DNA methods, the mitochondrial gene of the cytochromoxidase 2 (mtDNA *cox2*) has been demonstrated to be useful both for species identification distinguishing all the anisakid taxa so far detected, and also for their phylogenetic study. This also underlines the utility of being able to infer phylogenetic hypotheses for anisakid nematodes based on data concerning large and varied characters.

Thus, allozymes from MAE and/or DNA-based markers (barcodes) are recommended as rapid and precise tools for the identification of anisakid nematodes and for their cataloguing (barcoding). In the near future, this will allow the comparison of new samples and taxa with deposited DNA material and sequences (obtained from well-established taxa of anisakids) as well as from type-material in museums, to avoid the future synonymy of anisakid taxa.

It is also expected to have, as much as possible, an integrated approach in the study of anisakid nematode systematics, with a reconciliation of genetics with morphology, after the genetic recognition of a biological species. Indeed, in some of the anisakids detected genetically, the formal

description of the species is still lacking (i.e. for the *C. osculatum* species complex). Molecular systematics will in the future clarify the taxonomic status of some species still waiting to be genetically characterized among those considered as *inquirendae* and will help to establish their genetic relationships with related taxa.

9.2. Identification of human infections

The precise and easy identification to species level of anisakid nematodes has a significant implication for the diagnosis of human anisakid infections (anisakidosis) and, therefore, it is an important component of disease surveillance. To date, PCR-DNA methods have shown that human infections in Italy are attributable to *A. pegreffii* (see D'Amelio *et al.*, 1999; Mattiucci *et al.*, 2007b), while in Japan infections are mainly due to *A. simplex* (s.s.), despite the reported occurrence of *A. pegreffii* in fish from the southern Sea of Japan (Umehara *et al.*, 2007). The DNA-based diagnostic techniques have an advantage when an anisakid larva is extracted by endoscopy, because only very small portion of the larva is required for its specific identification and anisakid larvae in any case afford scant features for identification to species level. PCR-DNA markers to recognize larval anisakids in eosinophilic granulomas, after their surgical removal, could be also applied in future for diagnosis of human cases.

The collection of basic information on human infections by anisakids is important in the assessment of their relative epidemiological role, especially where different species are sympatric and even syntopic in the same fish species. This becomes more relevant when the fish are of high commercial value and are used for human consumption. This will in future allow us to show distinct differences between the diseases caused by different anisakid species. For instance, the clinical syndrome in human infections with *Pseudoterranova* larvae is different in Japan from that reported in the USA (Oshima, 1987). While the nature of the pathogenic role is not yet fully understood, Desowitz (1986) has suggested that various clinical aspects of human infections might be caused by different 'strains' of *P. decipiens* in the two different geographical areas. As different sibling species of *P. decipiens* are distributed in fish from these areas (*P. azarasi* and *P. bulbosa* in Japan, but *P. decipiens* (s.s.) and *P. bulbosa* in USA), the correct identification in the future of human infections caused by these anisakid species may be of particular importance.

9.3. Molecular ecology and life cycle

Genetic markers have proved to be an essential tool for basic ecological studies where the ranges of anisakid species overlap. This allows us to investigate patterns of their differential distribution in both definitive and

intermediate/paratenic hosts in order to clarify whether they are the result of ecological processes such as competition and adaptation. In fact, despite the numerous observations on the definitive and intermediate hosts so far collected for the different genetically identified anisakid species, further studies are required to confirm the host range and the geographical distribution of several anisakid species. In addition, studies need to be extended to other areas where anisakids of uncertain taxonomic designation are reported but have not been fully investigated. Anisakid specimens have been recorded from these areas but data concerning their definitive and intermediate hosts are still patchy (for instance, for the species indicated in this review as *Anisakis* sp., *Anisakis* sp.1 and *Anisakis* sp. 2).

Other aspects related to possible life-history pathways speculated for different anisakid species are still scanty and need to be fully demonstrated. This may include, for instance, proof that *A. physeteris* has a life cycle with squid rather than fish as suitable intermediate hosts. This was suggested by [Mattiucci et al. \(2001, 2004\)](#), taking into account the finding that other cephalopods have been reported to be infected by this anisakid species (but not genetically identified) in Pacific waters. These included *Onychoteuthis borealis japonica* (see [Nagasawa and Moravec, 2002](#)) and other squid species (i.e. *Todarodes japonicus*, *Doryteuthis bleekeri* and *Ommastrephes bartrami*) (see [Smith and Wootton, 1978](#)). Similarly, according to the high rates of prevalence found in several pelagic and demersal fish species of *A. simplex* (s.s.) and *A. pegreffii*, it seems that these parasites use pelagic and mesopelagic food chains to complete their life cycles. However, although this was anticipated by several authors ([Klimpel et al., 2007](#); [Mattiucci et al., 1997, 2004](#); [Pontes et al., 2005](#)), further investigations are required to confirm these hypotheses. These would include the molecular identification of anisakid larval stages from invertebrate species adapted to benthic or pelagic food chains and involved as first intermediate hosts in these anisakid life cycles. Indeed, despite several invertebrate species having been recorded as first intermediate hosts for *Anisakis simplex* (s.l.) and *Pseudoterranova decipiens* (s.l.) ([Hurst, 1984](#); [Marcogliese, 1993](#); [Smith and Mooney Snyder, 2005](#)), no data are so far available on the specific identification of the larval anisakids recovered from those hosts.

9.4. Host–parasite co-evolutionary aspects

The long history of host–anisakid associations could have led to reciprocal adaptations in the hosts and their parasites (classical co-adaptation) as well as contemporaneous events in the two lineages (co-speciation). The available host historical biogeography mapped onto the phylogenetic trees postulated for the anisakid nematodes of the genera *Anisakis* and

Contracaecum have made possible reconstructions of co-evolutionary scenarios. However, other genetically unstudied taxa of anisakid nematodes belonging to *Anisakis*, *Pseudoterranova* and *Contracaecum* from cetaceans and pinnipeds should be investigated, and their phylogeny mapped onto that of their definitive hosts as inferred from other molecular markers.

Comparative phylogenetics should also be studied to address the identification of temporal links between host and parasite phylogenies, and thus provide an internal time calibration for comparative studies of rates of evolution in the two groups. Moreover, in a parasite clade that shows evidence for host-switching, further studies will determine whether there are geographical, morphological or ecological correlates of host-switching. Indeed reconstruction of the biogeography history of host-switching events may reveal whether colonization of new hosts by anisakid nematodes was simply 'opportunistic' or whether the parasite is acquiring a particular resource in that host taxon which is not itself correlated with host phylogeny.

Co-adaptation and co-evolution in anisakid nematodes will also be addressed in the future by further examination of the host-parasite assemblages already attempted in the group of *Contracaecum* spp. from fish-eating birds (Mattiucci *et al.*, 2008c).

9.5. Genetic variability of anisakids as an indicator of habitat disturbance

It has recently been shown in several organisms that natural and anthropogenic environmental changes could affect the genetic structure of animal populations causing 'genetic erosion' (loss of number of alleles per locus, decrease in heterozygosity and polymorphism rates) in their gene pools. Different mechanisms such as population fragmentation and size reduction, gene flow interruption and genetic drift can be invoked for this. Thus, we may assume that habitat disturbance of marine ecosystems and food webs can have considerable long-term effects on the genetic and demographic viability of the parasite populations due to a reduction of host population numbers. Indeed, assuming that habitat disturbance and fragmentation reduce the local effective population size (N) or migration rate (m) or both, increased genetic drift should redistribute genetic diversity, such that variation within populations is reduced and variation between populations is increased. In a small and isolated population, the average heterozygosity decreases at a constant rate per generation, so that small populations have a higher probability of losing genetic diversity than larger and continuously distributed populations. The extent to which a species could be affected by habitat disturbance is determined by its degree of specialization and dispersal potential.

Two of the main factors influencing the genetic variability of a population are the amount of gene flow between populations and the population size. In nematode parasites, gene flow is largely determined by the dispersal of host animals (Blouin *et al.*, 1995) and parasite abundance (as a proxy for parasite population size) which are strongly correlated with host density (Arneberg *et al.*, 1998). As a consequence, variation in host dispersal and density could lead to differences in the patterns of genetic diversity distributed among parasite populations (Criscione and Blouin, 2005; May, 1993).

Two complementary strategies have generally been used to examine the genetic effects of habitat disturbance (anthropogenic impact) on the genetic diversity (variability) of a population: (1) comparison of genetic variability of different sets of populations inhabiting disrupted ecosystems (space dimension) and/or (2) comparison of the genetic variability of a particular data set of populations from the same geographical area at different times (time dimension).

As for the first point, it has been shown that the existence of a correlation between genetic variability values and parasitic infection levels of anisakid populations, from different geographical areas of the Boreal and Austral hemispheres, have different levels of habitat disturbance (i.e. Arctic vs Antarctic ecosystems). Could anthropogenic changes such as habitat disturbance provoke the reduction of host population size? In turn this would mean a reduction in habitats for their anisakid nematodes that could lead to a reduction in the genetic variability of the parasites' gene pools, resulting in a loss of genetic polymorphism in some genes. Future efforts could be directed towards testing this hypothesis by monitoring genetic diversity (variability) of anisakid populations over time. Nowadays, the 'genetic monitoring' definition encompasses the quantification of temporal changes in population genetic data generated using suitable molecular genetic markers such as allozymes, microsatellites, single nucleotide polymorphisms (SNPs), amplified fragment length polymorphism (AFLP) and single-strand polymorphisms (SSCPs) (Schwartz *et al.*, 2006).

Preliminary data on the 'genetic monitoring' of anisakid populations through time seem to indicate that the possible phenomenon of the loss of polymorphism could have affected their gene pools. This has been observed in populations of *Anisakis pegreffii*, a sibling species of the *A. simplex* complex widespread in the Mediterranean Sea but also occurring in the Austral region. Loss of observed heterozygosity at some polymorphic allozyme loci has been found in the Mediterranean populations of *A. pegreffii* collected from fish hosts in recent years, as compared with that estimated in samples collected earlier in the genetic studies on these parasites (Fig. 2.12). In contrast, the Austral populations of *A. pegreffii* have maintained the same level of heterozygosity at the same

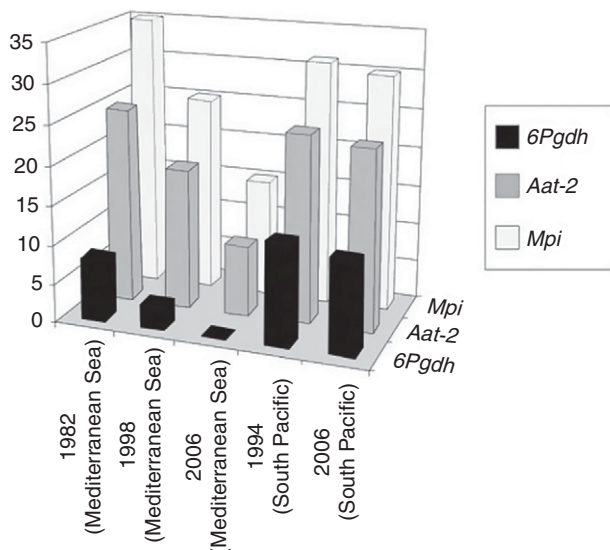


FIGURE 2.12 Temporal variation in the distribution of the genetic diversity (expected heterozygosity, h_e) at three allozyme loci (*6Pgdh*, *Aat-2* and *Gpi*) in populations of *Anisakis pegreffii* from the Mediterranean Sea and South Pacific Ocean. Loss of observed heterozygosity (*genetic erosion*) at some polymorphic allozyme loci has been found in recent Mediterranean populations of *A. pegreffii* with respect to those estimated from the same populations in previous years and from the Austral region.

gene loci through time, despite the high level of gene flow observed. It is possible that this finding in Mediterranean waters could be related to a reduced population size in *A. pegreffii*. This in turn could correlate to habitat disturbance-mediated reductions in population sizes of their definitive and intermediate hosts. Clearly, further comparative genetic analyses of these parasite populations and their levels of abundance over time need to be undertaken to investigate this possibility.

Molecular genetic markers, other than allozymes, could provide estimates of intraspecific genetic variation of these populations over time. Among these, those which have been demonstrated to show high genetic nucleotide variation in nematode populations (such as mitochondrial genes) (Blouin, 2002; Blouin *et al.*, 1998; Hu and Gasser, 2006; Hu *et al.*, 2004; Kim *et al.*, 2006) could be promising for gathering data from earlier anisakid populations in museum collections.

Thus, monitoring of the genetic diversity of anisakid nematodes and their abundance levels in hosts from different geographical areas, over time and space, could be used in the future as a tool for monitoring trophic web stability and for assessing the general biodiversity of marine ecosystems. Given that many of the anisakid nematodes are host specific, an ecosystem rich in their hosts should also be the one rich in anisakid parasites.

We could thus conclude with a ‘paradox of anisakid’: A healthy marine ecosystem is one with high level of infections by anisakid nematodes!

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REFERENCES

- Abe, N., Ohya, N., and Yanagiguchi, R. (2005). Molecular characterization of *Anisakis pegreffii* larvae in Pacific cod in Japan. *J. Helminthol.* **79**, 303–306.
- Abe, N., Tominaga, K., and Kimata, I. (2006). Usefulness of PCR-restriction fragments length polymorphism analysis of the internal transcribed spacer region of rDNA for identification of *Anisakis simplex* complex. *Jpn. J. Infect. Dis.* **59**, 60–62.
- Abollo, E., Gestal, C., and Pascal, S. (2001). *Anisakis* infestation in marine fish and cephalopods from Galician waters: An updated perspective. *Parasitol. Res.* **87**, 492–499.
- Adams, B. J. (1998). Species concepts and the evolutionary paradigm in modern nematology. *J. Nematol.* **30**, 1–21.
- Adams, A. M., Murrell, K. D., and Cross, J. H. (1997). Parasites of fish and risks to public health. *Rev. Sci. Technol.* **16**, 652–660.
- Alvarado-Bremer, J. R., Mejuto, J., Gómez-Márquez, J., Pla-Zanuy, C., Viñas, J., Marques, C., Hazin, F., Griffiths, M., Ely, B., Sánchez, B., and Greig, T. W. (2006). Genetic population structure of Atlantic swordfish: Current status, controversies, and future directions. Report of the 2006 ICCAT Swordfish Stock Structure Workshop, Crete (Greece), 13–14 March 2006. SCRS/2006/041.
- Andersen, M., Gwynn, J. P., Dowdall, M., Kovacs, K. M., and Lydersen, C. (2006). Radio-caesium (137Cs) in marine mammals from Svalbard, the Barents Sea and the North Greenland Sea. *Sci. Total Environ.* **363**, 87–94.
- Anderson, T. J. C., Blouin, M. S., and Beech, R. N. (1998). Population biology of parasitic nematodes: Application of genetic markers. *Adv. Parasitol.* **41**, 219–283.
- Anon, R. N. (1997). Fishing by numbers reveals its limits. *Nature* **387**, 110.
- Arduino, P., Nascetti, G., Cianchi, R., Plötz, J., Mattiucci, S., D’Amelio, S., Paggi, L., Orecchia, P., and Bullini, L. (1995). Isozyme variation and taxonomic rank of *Contracaecum radiatum* (v. Linstow, 1907) from the Antarctic Ocean (Nematoda, Ascaridoidea). *Syst. Parasitol.* **30**, 1–9.
- Arnason, U., Bodin, K., Gullberg, A., Ledje, C., and Mouchaty, S. (1995). A molecular view of pinniped relationships with particular emphasis on the true seals. *J. Mol. Evol.* **40**, 78–85.
- Arnason, U., Gullberg, A., and Janke, A. (2004). Mitogenomic analyses provide new insight into cetacean origin and evolution. *Gene* **333**, 27–34.
- Arneberg, P., Skorpning, A., Grenfell, B., and Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proc. R. Soc. Lond., B: Biol. Sci.* **265**, 1283–1289.

- Audicana, M. T., Ansotegui, I. J., de Corres, L. F., and Kennedy, M. W. (2002). *Anisakis simplex*: Dangerous—Dead and alive? *Trends Parasitol.* **18**, 20–25.
- Ayala, F. J. (1975). Genetic differentiation during the speciation process. *Evol. Biol.* **8**, 1–78.
- Barron, M. G., Heintz, R., and Krahn, M. M. (2003). Contaminant exposure and effects in pinnipeds: Implications for sea lion declines in Alaska. *Sci. Total Environ.* **311**, 111–133.
- Battaglia, B., Valencia, J., and Walton, D. W. H. (1997). In “Antarctic Communities: Species, Structure and Survival,” p. 464. Cambridge University Press.
- Baylis, H. A. (1916). Some ascarids in the British Museum (Nat. Hist.). *Parasitology* **8**, 360–378.
- Baylis, H. A. (1937). On the ascarids parasitic in seals, with special reference to the genus *Contracaecum*. *Parasitology* **24**, 121–130.
- Begg, G. A., and Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fish. Res.* **43**, 35–44.
- Berland, B. (1961). Nematodes from some Norwegian marine fishes. *Sarsia* **2**, 1–50.
- Berland, B. (1964). *Phocascaris cystophorae* sp. nov. (Nematoda) from the hooded seal, with an emendation of the genus. *Årbok for Universitetet i Bergen, Mat.-naturv., Series 1963* **17**, 1–21.
- Beverly-Burton, M. (1971). Helminths from the Weddell Sea, *Leptonychotes weddellii* (Lesson, 1826) in the Antarctic. *Can. J. Zool.* **49**, 75–83.
- Beverly-Burton, M., Nyman, O. L., and Pippy, J. H. C. (1977). The morphology, and some observation on the population genetics of *Anisakis simplex* larvae (Nematoda: Ascaridoidea) from fishes of the North Atlantic. *J. Fish. Res. Board Can.* **34**, 105–112.
- Blouin, M. S. (2002). Molecular prospecting for cryptic species of nematodes: Mitochondrial DNA versus internal transcribed spacer. *Int. J. Parasitol.* **32**, 527–531.
- Blouin, M. S., Yowell, C. A., Courtney, C. H., and Dame, J. B. (1995). Host movement and the genetic structure of populations of parasitic nematodes. *Genetics* **141**, 1007–1014.
- Blouin, M. S., Yowell, C. A., Courtney, C. H., and Dame, J. B. (1998). Substitution bias, rapid saturation, and the use of mtDNA for nematode systematics. *Mol. Biol. Evol.* **15**, 1719–1727.
- Bratley, J., and Davidson, W. S. (1996). Genetic variation within *Pseudoterranova decipiens* (Nematoda: Ascaridoidea) from Canadian Atlantic marine fishes and seals: Characterization by RFLP analysis of genomic DNA. *Can. J. Fish. Aquat. Sci.* **53**, 333–341.
- Bratley, J., and Stenson, G. B. (1993). Host specificity and abundance of parasitic nematodes (Ascaridoidea) from the stomachs of five phocid species from Newfoundland and Labrador. *Can. J. Zool.* **71**, 2156–2166.
- Bristow, G. A., and Berland, B. (1992). On the ecology and distribution of *Pseudoterranova decipiens* (Nematoda: Anisakidae) in an intermediate host, *Hippoglossoides platessoides*, in northern Norwegian waters. *Int. J. Parasitol.* **22**, 203–208.
- Brooks, D. R. (1990). Parsimony analysis in historical biogeography and coevolution: Methodological and theoretical update. *Syst. Zool.* **39**, 14–30.
- Brooks, D. R., and Hoberg, E. P. (1981). Hennig's parasitological method: A proposed solution. *Syst. Zool.* **30**, 229–249.
- Brooks, D. R., and Hoberg, E. P. (2007). How will global climate change affect parasite-host assemblages? *Trends Parasitol.* **23**, 571–574.
- Bruce, N. L., and Cannon, L. R. G. (1990). Ascaridoid nematodes from sharks from Australia and the Solomon Islands, Southwestern Pacific Ocean. *Invertebr. Taxon.* **4**, 763–783.
- Bullini, L., and Sbordoni, V. (1980). Electrophoretic studies of gene-enzyme systems: Micro-evolutionary processes and phylogenetic inference. *Boll. Zool.* **47**, 95–112.
- Bullini, L., Nascetti, G., Paggi, L., Orecchia, P., Mattiucci, S., and Berland, B. (1986). Genetic variation of ascaridoid worms with different life cycles. *Evolution* **40**, 437–440.
- Bullini, L., Arduino, P., Cianchi, R., Nascetti, G., D'Amelio, S., Mattiucci, S., Paggi, L., and Orecchia, P. (1994). Genetic and ecological studies on nematode endoparasites of the genera *Contracaecum* and *Pseudoterranova* in the Antarctic and Arctic-Boreal regions.

- In "Proceedings of the 2nd Meeting 'Biology in Antarctica', Padova" (A. Martello, ed.), pp. 131–146. Edizioni Universitarie Patavine, Padova.
- Bullini, L., Arduino, P., Cianchi, R., Nascetti, G., D'Amelio, S., Mattiucci, S., Paggi, L., Orecchia, P., Plotz, J., Smith, J. H., and Brattey, J. (1997). Genetic and ecological research on anisakid endoparasites of fish and marine mammals in the Antarctic and Arctic-Boreal regions. In "Antarctic Communities: Species, Structure and Survival" (B. Battaglia, J. Valencia, and D. W. H. Walton, eds.), pp. 39–44. Cambridge University Press, Cambridge.
- Burt, M. D. B. (1994). The sealworm situation. In "Parasitic and Infectious Diseases" (M. E. Scott and G. Smith, eds.), pp. 347–362. Academic Press, New York.
- Cassens, I., Vicario, S., Waddell, V. G., Balchowsky, H., Van Belle, D., Ding, W., Chen, F., Mohan, R. S. L., Simoes-Lopes, P. C., Bastida, R., Meyer, A., Stanhope, M. J., *et al.* (2000). Independent adaptation to riverine habitats allowed survival of ancient cetacean lineages. *Proc. Natl. Acad. Sci. USA* **97**, 11343–11347.
- Cavalli-Sforza, L. L., and Edwards, A. W. F. (1967). Phylogenetic analysis: Models and estimation procedures. *Am. J. Hum. Genet.* **19**, 233–257.
- Chai, Y. Y., Murrell, D. K., and Lymbery, A. (2005). Fish-borne parasitic zoonoses: Status and issues. *Int. J. Parasitol.* **35**, 1233–1254.
- Charleston, M. A. (2003). Recent results in cophylogeny mapping. *Adv. Parasitol.* **54**, 303–330.
- Cimmaruta, R., Bondanelli, P., and Nascetti, G. (2005). Genetic structure and environmental heterogeneity in the European hake (*Merluccius merluccius*). *Mol. Ecol.* **14**, 2577–2591.
- Cimmaruta, R., Paoletti, M., Bondanelli, P., Santos, M. N., Garcia, A., and Nascetti, G. (2006). Genetic structure of Mediterranean and Atlantic swordfish investigated using allozymes and RFLPs. Report of the 2006 ICCAT swordfish stock structure workshop Crete (Greece) 13–14 March 2006.
- Cimmaruta, R., Bondanelli, P., Ruggi, A., and Nascetti, G. (2008). Genetic structure and temporal stability in horse mackerel (*Trachurus trachurus*). *Fish. Res.* **89**, 114–121.
- Cook, R. M., Sinclair, A., and Stefansson, G. (1997). Potential collapse of North Sea cod stocks. *Nature* **385**, 521–522.
- Criscione, C. D., and Blouin, M. S. (2005). Effective size of macroparasite populations: A conceptual model. *Trends Parasitol.* **21**, 212–217.
- Criscione, C. D., Poulin, R., and Blouin, M. S. (2005). Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. *Mol. Ecol.* **14**, 2247–2257.
- Cross, M. A., Collins, C., Campbell, N., Watts, P. C., Chubb, J. C., Cunningham, C. O., Hatfield, E. M. C., and MacKenzie, K. (2007). Levels of intra-host and temporal sequence variation in a large CO1 sub-units from *Anisakis simplex sensu stricto* (Rudolphi 1809) (Nematoda: Anisakidae): Implications for fisheries management. *Mar. Biol.* **151**, 695–702.
- Crow, J. F., and Aoki, K. (1984). Group selection for a polygenic behavioural trait: Estimating the degree of population subdivision. *Proc. Natl. Acad. Sci. USA* **81**, 6073–6077.
- D'Amelio, S., Nascetti, G., Mattiucci, S., Cianchi, R., Orecchia, P., Paggi, L., Berland, B., and Bullini, L. (1990). Ricerche elettroforetiche su alcune species del genere *Contracaecum*, parassiti di uccelli ittiofagi (Ascaridida: Anisakidae). *Parassitologia* **32**(suppl. 1), 77.
- D'Amelio, S., Mattiucci, S., Paggi, L., Koie, M., Podvyaznaya, I., Pugachev, O., Rusinek, O., Timoskhkin, O., and Nascetti, G. (1995). Taxonomic rank and origin of *Contracaecum osculatum baicalensis* Mozgovoi and Ryjikov, 1950, parasite of *Phoca sibirica* from Lake Baikal, with data on its occurrence in fish hosts. "Abstracts, IVth International Symposium on Fish Parasitology, Munich, October 3–7 2005," 27.
- D'Amelio, S., Mathiopoulos, K. D., Brandonisio, O., Lucarelli, G., Doronzo, F., and Paggi, L. (1999). Diagnosis of a case of gastric anisakidosis by PCR-based restriction fragment length polymorphism analysis. *Parassitologia* **41**, 591–593.
- D'Amelio, S., Mathiopoulos, K., Santos, C. P., Pugachev, O. N., Webb, S. C., Picanco, M., and Paggi, L. (2000). Genetic markers in ribosomal DNA for the identification of members

- of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase chain reaction-based restriction fragment length polymorphism. *Int. J. Parasitol.* **30**, 223–226.
- D'Amelio, S., Barros, N. B., Ingrosso, S., Fauquier, D. A., Russo, R., and Paggi, L. (2007). Genetic characterization of members of the genus *Contracaecum* (Nematoda: Anisakidae) from fish-eating birds from west-central Florida, USA, with evidence of new species. *Parasitology* **134**, 1041–1051.
- Dailey, M. D. (1975). The distribution and intraspecific variation of helminth parasites in pinnipeds. *Rapport et Proces-Verbaux des Reunions-Conseil International pour l'Exploration de la Mer* **169**, 338–352.
- Daniels, R. (1982). Feeding ecology of some fishes of the Antarctic Peninsula. *Fish. Bull.* **80**, 575–588.
- Davey, J. T. (1971). A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridata). *J. Helminthol.* **45**, 51–72.
- Deardoff, T. L., and Overstreet, R. M. (1979). *Contracaecum multipapillatum* (= *C. robustum*) from fishes and birds in the northern Gulf of Mexico. *J. Parasitol.* **66**, 853–856.
- Deardoff, T. L., and Overstreet, R. M. (1980). Review of *Hysterothylacium* and *Iheringascaris* (both previously = *Thynnascaris*) (Nematoda: Anisakidae) from the northern Gulf of Mexico. *Proc. Biol. Soc. Wash.* **93**, 1035–1079.
- Deardorff, T. L., and Overstreet, R. M. (1981). *Terranova ceticola* n. sp. (Nematoda: Anisakidae) from the dwarf sperm whale; *Kogia simus* (Owen), in the Gulf of Mexico. *Syst. Parasitol.* **3**, 25–28.
- Delyamure, S. L., Kurochkin, Y. V., and Skryabin, A. S. (1964). Contributions to the helminth fauna of the Caspian seal (*Phoca caspica* Gm.). *Trudy Astrakanskogo Zapovednika* **9**, 105–118 (In Russian).
- Deméré, T. A., Berta, A., and Adam, P. J. (2003). Pinnipedimorphs evolutionary biogeography. *Bull. Am. Mus. Nat. Hist.* **279**, 32–76 (Chapter 3).
- Desdevises, Y. (2007). Cophylogeny: Insights from fish-parasite systems. *Parassitologia* **49**, 125–128.
- Desowitz, R. S. (1986). Human and experimental anisakiasis in the United States. *Hokkaido J. Med. Sci.* **61**, 358–351.
- Di Guardo, G., Marruchella, G., Agrini, U., and Kennedy, S. (2005). Morbillivirus infections in aquatic mammals: A brief overview. *J. Vet. Sci.* **52**, 88–93.
- Dollfus, R. (1966). Helminthofauna de *Kogia breviceps* (de Blainville, 1838) cetacea odeontocete. *Annls Soc. Sci. Nat. Charente-Marit.* **4**, 3–6.
- Eichler, W. (1948). Some rules in ectoparasitism. *Ann. Mag. Natl. Hist.* **12**, 588–598.
- Fagerholm, H. P. (1989). Intra-specific variability of the morphology in a single population of the seal parasite *Contracaecum osculatum* (Rudolphi) (Nematoda: Ascaridoidea), with a redescription of the species. *Zool. Scripta* **18**, 33–41.
- Fagerholm, H. P. (1991). Systematic implications of male caudal morphology in ascaridoid nematode parasites. *Syst. Parasitol.* **19**, 215–228.
- Fagerholm, H. P., and Gibson, D. I. (1987). A redescription of the pinniped parasite *Contracaecum ogmorhini* (Nematoda, Ascaridoidea), with an assessment of its antiBoreal circum-polar distribution. *Zool. Scripta* **16**, 19–24.
- Fahrenholz, H. (1913). Ectoparasitism und Abstammungslehre. *Zool. Anz. Leipzig* **41**, 371–374.
- Farjallah, S., Slimane, B. B., Busi, M., Paggi, L., Amor, N., Blél, H., Said, K., and D'Amelio, S. (2008). Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of Mediterranean Sea. *Parasitol. Res.* **102**, 371–379.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791.
- Foster, B. A., and Montgomery, C. (1993). Planktivory in benthic nototheniid fish in McMurdo Sound, Antarctica. *Environ. Biol. Fish.* **36**, 313–318.

- George-Nascimento, M., and Llanos, A. (1995). Micro-evolutionary implications of allozymic and morphometric variations in sealworms *Pseudoterranova* sp. (Ascaridoidea: Anisakidae) among sympatric hosts from the southeastern Pacific Ocean. *Int. J. Parasitol.* **25**, 1163–1171.
- George-Nascimento, M., and Urrutia, X. (2000). *Pseudoterranova cattani* sp. nov. (Ascaridoidea: Anisakidae), a parasite of the South American sea lion *Otaria byronia* De Blainville from Chile. *Revta Chil. Hist. Nat.* **73**, 93–98.
- Gibson, D. I. (1983). The systematics of ascaridoid nematodes: A current assessment. In “Concepts in Nematode Systematics” (A. R. Stone, H. M. Platt, and L. F. Khalil, eds.), pp. 321–338. Academic Press, New York.
- Gibson, D. I., and Colin, J. A. (1982). The *Terranova* enigma. *Parasitology* **85**, XXXVI–XXXVII.
- Guttman, L. A. (1968). A general nonmetric technique for finding the smallest coordinate space for a configuration of points. *Psychometric* **33**, 469–506.
- Hartwich, G. (1957). Zur Systematik der Nematoden-Superfamilie Ascaridoidea. *Zool. Jb., Abteilung für Systematik, Jena* **85**, 211–252.
- Hartwich, G. (1964). Revision der Vogelparasitischen Nematoden Mitteleuropas II. Die Gattung *Contracaecum* Railliet and Henry, 1912 (Ascaridoidea). *Mitt. Zool. Mus. Berl.* **40**, 15–53.
- Hartwich, G. (1974). Keys to genera of the Ascaridoidea. In “CIH Keys to the Nematode Parasites of Vertebrates” (R. C. Anderson, A. G. Chabaud, and S. Wilmott, eds.), p. 153. Farnham Royal, Commonwealth Agriculture Bureau, Richmond.
- Hartwich, G. (1975). Rhabditida und Ascaridida. *Tierwelt Dtl.* **62**, 1–256.
- Hoberg, E. P. (1992). Congruent and synchronic patterns in biogeography and speciation among seabirds, pinnipeds, and cestodes. *J. Parasitol.* **78**, 601–615.
- Hoberg, E. P. (2005). Coevolution in marine systems. In “Marine Parasitology” (K. Rodhe, ed.), pp. 327–339. CBI Publishing, Oxon.
- Höst, P. (1932). *Phocascaris phocae* n.gen.n.sp., eine neue Askaridenart aus *Phoca groenlandica*. *Fabr. Zbl. Bakt. 1Ab. Orig.* **125**, 335–340.
- Hu, M., and Gasser, R. B. (2006). Mitochondrial genomes of parasitic nematodes: Progress and perspectives. *Trends Parasitol.* **22**, 78–84.
- Hu, M., D’Amelio, S., Zhu, X. Q., Paggi, L., and Gasser, R. (2001). Mutation scanning for sequence variation in three mitochondrial DNA regions for members of the *Contracaecum osculatum* (Nematoda: Ascaridoidea) complex. *Electrophoresis* **22**, 1069–1075.
- Hu, M., Chilton, N. B., and Gasser, R. B. (2004). The mitochondrial genomics of parasitic nematodes of socio-economic importance: Recent progress and implications for population genetics and systematic. *Adv. Parasitol.* **56**, 133–212.
- Hudson, P. J., Dobson, A. P., and Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends Ecol. Evol.* **21**, 381–385.
- Huizinga, H. W. (1967). Studies on the life cycle and development of *Contracaecum spiculigerum* (Rudolphi, 1809) (Ascaridoidea: Heterocheilidae) from marine piscivorous birds. *J. Elisa Mitchell Sci. Soc.* **82**, 180–195.
- Hurst, R. J. (1984). Marine invertebrate hosts of New Zealand Anisakidae (Nematoda). *N. Z. J. Mar. Freshwater Res.* **18**, 187–196.
- Johnston, T. H., and Mawson, P. M. (1941). Nematodes from Australian marine mammals. *Rec. S. Aust. Mus.* **6**, 429–434.
- Johnston, T. H., and Mawson, P. M. (1945). Parasitic nematodes. *Rep. Bri. NZ. Austral. Antarct. Res. Exped. Ser. B* **5**, 74–159.
- Karokhin, V. I. (1946). Two new species of *Porrocaecum* from Siberian birds of prey. In “Helminthological collection, Nauka, Moscow” (V. P. Pod’yanolskaya, ed.), pp. 132–141. In Russian.

- Kendall, K. A., Ruhl, H. A., and Wilson, R. C. (2003). Distribution and abundance of marine bird and pinniped populations within Port Foster, Deception Islands, Antarctica. *Deep-Sea Res. Part II: Top. Stud. Oceanogr.* **50**, 1873–1888.
- Kijewska, A., Rokicki, J., Sitko, J., and Wegrzyn, G. (2002). Ascaridoidea: A simple DNA assay for identification of 11 species infecting marine and freshwater fish, mammals and fish-eating birds. *Exp. Parasitol.* **101**, 35–39.
- Kim, K. H., Eom, K. S., and Park, J. K. (2006). The complete mitochondrial genome of *Anisakis simplex* (Ascaridida: Nematoda) and phylogenetic implications. *Int. J. Parasitol.* **36**, 319–328.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**, 111–120.
- King, J. E. (1983). "Seals of the World" (British Museum Natural History) 2nd edn. p. 240. London and Oxford University Press, Oxford.
- Klimpel, S., Kellermanns, E., Palm, H. W., and Moravec, F. (2007). Zoogeography of fish parasites of the pearlside (*Maurolicus muelleri*), with genetic evidence of *Anisakis simplex* (s.s.) from the Mid-Atlantic Ridge. *Mar. Biol.* **152**, 725–732.
- Klöser, H., Plötz, J., Palm, H., Bartsch, A., and Hubold, G. (1992). Adjustment of anisakid nematode life cycles to the high Antarctic food web as shown by *Contracaecum radiatum* and *C. osculatum* in the Weddell sea. *Antarct. Sci.* **4**, 171–178.
- Koie, M., and Fagerholm, H. P. (1995). The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) *sensu stricto* (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections. *Parasitol. Res.* **81**, 481–489.
- Kotoulas, G., Mejuto, J., Antoniou, A., Tserpes, G., Piccinetti, C., Peristeraki, P., Kasapidis, P., Garcia-Cortes, B., Oikonomaki, K., De la Serna, J. M., and Magoulas, A. (2006). Genetic structure of the swordfish *Xiphias gladius* at a global scale using microsatellite markers. "Report of the 2006 ICCAT swordfish stock structure workshop, Crete (Greece), 13–15 March, 2006." SCRS/2006/035.
- La Rosa, G., Marucci, G., Zarlenga, D. S., and Pozio, E. (2001). *Trichinella pseudospiralis* populations of the PaleArctic region and their relationship with populations of the NeArctic and Australian regions. *Int. J. Parasitol.* **31**, 297–305.
- Legendre, P., Desdevises, Y., and Bazin, E. (2002). A statistical test for host-parasite coevolution. *Syst. Biol.* **51**, 217–234.
- Li, A., D'Amelio, S., Paggi, L., He, F., Gasser, R. B., Lun, Z., Abollo, E., Turchetto, M., and Zhu, X. (2005). Genetic evidence for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) and the validity of *Contracaecum septentrionale* (Kreis, 1955) (Nematoda: Anisakidae). *Parasitol. Res.* **96**, 361–366.
- Lu, C. P., Chen, C. A., Hui, C. F., Tzeng, T. D., and Yeh, S. Y. (2006). Population genetic structure of the swordfish, *Xiphias gladius* (Linnaeus, 1758), in the Indian Ocean and West Pacific inferred from the complete DNA sequence of the mitochondrial control region. *Zool. Stud.* **45**, 269–279.
- MacKenzie, K. (2002). Parasites as biological tags in population studies of marine organisms: An update. *Parasitology* **124**, 153–163.
- Marcogliese, D. J. (1993). Larval nematodes infecting *Amphiporeia virginiana* (Amphipoda: Pontoporeioidea) on Sable Island, Nova Scotia. *J. Parasitol.* **79**, 959–962.
- Marcogliese, D. J. (1997). Fecundity of sealworm (*Pseudoterranova decipiens*) infecting grey seals (*Halichoerus grypus*) in the Gulf of St. Lawrence, Canada: Lack of density-dependent effects. *Int. J. Parasitol.* **27**, 1401–1409.
- Marcogliese, D. J. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Can. J. Zool.* **79**, 1331–1352.
- Marcogliese, D. J. (2005). Parasites of the superorganism: Are they indicators of ecosystem health? *Int. J. Parasitol.* **35**, 705–716.

- Margolis, L. (1956). Parasitic helminths and arthropods from pinnipedia of the Canadian pacific coast. *J. Fish. Res. Board Can.* **13**, 489–505.
- Marques, J. F., Cabral, H. N., Busi, M., and D'Amelio, S. (2006). Molecular identification of *Anisakis* species from Pleuronectiformes off the Portuguese coast. *J. Helminthol.* **80**, 47–51.
- Mattiucci, S., and Nascetti, G. (2006). Molecular systematics, phylogeny and ecology of anisakid nematodes of the genus *Anisakis* Dujardin, 1845: An update. *Parasité* **13**, 99–113.
- Mattiucci, S., and Nascetti, G. (2007). Genetic diversity and infection levels of anisakid nematodes parasitic in fish and marine mammals from Boreal and Austral hemispheres. *Vet. Parasitol.* **148**, 43–57.
- Mattiucci, S., Nascetti, G., Bullini, L., Orecchia, P., and Paggi, L. (1986). Genetic stucture of *Anisakis physeteris* and its differentiation from the *Anisakis simplex* complex (Ascaridida: Anisakidae). *Parasitology* **93**, 383–387.
- Mattiucci, S., Nascetti, G., Cianchi, R., Paggi, L., Arduino, P., Margolis, L., Bratney, J., Webb, S. C., D'Amelio, S., Orecchia, P., and Bullini, L. (1997). Genetic and ecological data on the *Anisakis simplex* complex with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). *J. Parasitol.* **83**, 401–416.
- Mattiucci, S., Paggi, L., Nascetti, G., Ishikura, H., Kikuchi, K., Sato, N., Cianchi, R., and Bullini, L. (1998). Allozyme and morphological identification of *Anisakis*, *Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda: Ascaridoidea). *Syst. Parasitol.* **40**, 81–92.
- Mattiucci, S., Paggi, L., Nascetti, G., Abollo, E., Webb, S. C., Pascual, S., Cianchi, R., and Bullini, L. (2001). Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae). *Int. J. Parasitol.* **31**, 9–14.
- Mattiucci, S., Paggi, L., Nascetti, G., Portes Santos, C., Costa, G., Di Benedetto, A. P., Ramos, R., Argyrou, M., Cianchi, R., and Bullini, L. (2002a). Genetic markers in the study of *Anisakis typica* (Diesing, 1860): Larval identification and genetic relationships with other species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae). *Syst. Parasitol.* **51**, 159–170.
- Mattiucci, S., Turchetto, M., Brigantini, F., and Nascetti, G. (2002b). On the occurrence of the sibling species of *Contracaecum rudolphii* complex (Nematoda: Anisakidae) in cormorants (*Phalacrocorax carbo sinensis*) from Venice and Caorle lagoons: Genetic markers and ecological studies. *Parassitologia* **44**, 105.
- Mattiucci, S., Cianchi, R., Nascetti, G., Paggi, L., Sardella, N., Timi, J., Webb, S. C., Bastida, R., Rodriguez, D., and Bullini, L. (2003). Genetic evidence for two sibling species within *Contracaecum ogmorhini* Johnston and Mawson (1941) (Nematoda: Anisakidae) from otariid seals of Boreal and Austral regions. *Syst. Parasitol.* **54**, 13–23.
- Mattiucci, S., Abaunza, P., Ramadori, L., and Nascetti, G. (2004). Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. *J. Fish Biol.* **65**, 495–510.
- Mattiucci, S., Nascetti, G., Dailey, M., Webb, S. C., Barros, N., Cianchi, R., and Bullini, L. (2005). Evidence for a new species of *Anisakis* Dujardin, 1845: Morphological description and genetic relationships between congeners (Nematoda: Anisakidae). *Syst. Parasitol.* **61**, 157–171.
- Mattiucci, S., Contadini, R., Paoletti, M., and Nascetti, G. (2006). Relazioni genetiche tra specie del genere *Contracaecum* (Railliet et Henry, 1912), parassiti di pinnipedi, ed aspetti coevolutivi ospite-parassita. *Abstracts XVI Congresso Società Italiana di Ecologia* 98.
- Mattiucci, S., Abaunza, P., Damiano, S., Garcia, A., Santos, M. N., and Nascetti, G. (2007a). Distribution of *Anisakis* larvae identified by genetic markers and their use for stock characterization of demersal and pelagic fish from European waters: An update. *J. Helminthol.* **81**, 117–127.
- Mattiucci, S., Paoletti, M., De Angelis, M., Sereno, S., and Cancrini, G. (2007b). Human Anisakidosis in Italy: Molecular and histological identification of two new cases. *Abstracts of the VII International Symposium on Fish Parasites: Parassitologia* **49**(suppl. 2), 226.

- Mattiucci, S., Farina, V., Campbell, N., Mackenzie, K., Ramos, P., Pinto, A. L., Abaunza, P., and Nascetti, G. (2008a). *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock identification. *Fish. Res.* **89**, 146–171.
- Mattiucci, S., Paoletti, M., Webb, S. C., Sardella, N., Timi, J. T., Berland, B., and Nascetti, G. (2008b). Genetic relationships among species of *Contracaecum* Railliet and Henry, 1912 and *Phocascaris* Höst, 1932 (Nematoda: Anisakidae) from pinnipeds based on mitochondrial *cox2* sequences, and congruence with allozyme data. *Parasite* **15**(3).
- Mattiucci, S., Paoletti, M., Olivero-Verbel, J., Baldiris, R., Arroyo-Salgado, B., Garbin, L., Navone, G., and Nascetti, G. (2008c). *Contracaecum biocai* n. sp. from the brown pelican *Pelecanus occidentalis* (L.) in Colombia (Nematoda: Anisakidae): Morphology, molecular evidence and its genetic relationship with congeners from fish-eating birds. *Syst. Parasitol.* **69**, 101–121.
- May, R. M. (1993). Agriculture: Resisting resistance. *Nature* **361**, 593–594.
- Mayr, E. (1963). "Animal Species and Evolution." Belknap Press, Harvard University Press, Cambridge, Massachusetts.
- Mayr, E. (1976). "Evolution and the Diversity of Life." Belknap Press, Harvard University Press, Cambridge, Massachusetts.
- McClelland, G. (2002). The trouble with sealworms (*Pseudoterranova decipiens* species complex, Nematoda): A review. *Parasitology* **124**, 183–203.
- McClelland, G., Misra, R. K., and Marcogliese, D. J. (1983). Variation in abundance of larval anisakines, sealworm (*Phocanema decipiens*) and related species in cod and flatfish from the Southern Gulf of St. Lawrence (4T) and the Breton shelf (4Vn). *Can. Tech. Rep. Fish. Aquat. Sci.* No. 27, p. 1201.
- McClelland, G., Misra, R. K., and Martell, D. J. (1985). Variations in abundance of larval anisakines, sealworm (*Pseudoterranova decipiens*) and related species, in eastern Canadian cod and flatfish. *Can. Tech. Rep. Fish. Aquat. Sci.* No. 1392, p. 57.
- McClelland, G., Misra, G. R. K., and Martell, D. J. (1990). Larval anisakine nematodes in various fish species from Sable Island Bank and vicinity. *Can. Bull. Fish. Aquat. Sci.* **222**, 83–113.
- Milinkovitch, M. C. (1995). Molecular phylogeny of cetaceans prompts revision of morphological transformations. *Trends Ecol. Evol.* **10**, 328–334.
- Mozgovoi, A. A. (1951). Ascarids of mammals of the U.S.S.R. (Anisakidae). *Trudy gel'mint. Lab., Akademiya Nauk SSR* **5**, 12–22.
- Mozgovoi, A. A. (1953). Ascaridata of animals and man, and the diseases caused by them. *Osn. Nemat. II. Izdatielstvo AN SSSR, Moskva*, 616.
- Myers, B. J. (1959). *Phocanema*, a new genus for the anisakid nematode of seals. *Can. J. Zool.* **37**, 459–465.
- Nadler, S. A. (1992). Phylogeny of some ascaridoid nematodes, inferred from comparison of 18s and 28s rRNA sequences. *Mol. Biol. Evol.* **9**, 932–944.
- Nadler, S. A. (1995). Microevolution and genetic structure of parasite populations. *J. Parasitol.* **81**, 395–403.
- Nadler, S. A. (2002). Species delimitation and nematode biodiversity: Phylogenies rule. *Nematology* **4**, 615–625.
- Nadler, S. A. (2005). Speciation and species delimitation. In "Marine Parasitology" (K. Rohde, ed.), pp. 339–345. CABI Publishing, Oxon.
- Nadler, S. A., and Hudspeth, D. S. S. (1998). Ribosomal DNA and phylogeny of the Ascaridoidea (Nematoda: Secernentea): Implications for morphological evolution and classification. *Mol. Phylogenet. Evol.* **10**, 221–236.
- Nadler, S. A., D'Amelio, S., Fagerholm, H. P., Berland, B., and Paggi, L. (2000). Phylogenetic relationships among species of *Contracaecum* Railliet and Henry, 1912 and *Phocascaris*

- Höst, 1932 (Nematoda: Ascaridoidea) based on nuclear rDNA sequence data. *Parasitology* **121**, 455–463.
- Nadler, S. A., D'Amelio, S., Dailey, M. D., Paggi, L., Siu, S., and Sakanari, J. A. (2005). Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from Northern Pacific marine mammals. *J. Parasitol.* **91**, 1413–1429.
- Nagasawa, K., and Moravec, F. (2002). Larval anisakid nematodes from four species of squid (Cephalopoda: Teuthoidea) from the central and western North Pacific Ocean. *J. Nat. Hist.* **36**, 883–891.
- Nascetti, G. (1992). Studio e riconoscimento di specie gemelle in nematodi endoparassiti: Analisi della variazione genetica, dell'isolamento riproduttivo, della nicchia ecologica e della competizione. *Atti della Società Italiana di Ecologia* **15**, 287–312.
- Nascetti, G., Paggi, L., Orecchia, P., Smith, J. W., Mattiucci, S., and Bullini, L. (1986). Electrophoretic studies on *Anisakis simplex* complex (Ascaridida: Anisakidae) from the Mediterranean and North East Atlantic. *Int. J. Parasitol.* **16**, 633–640.
- Nascetti, G., Bullini, L., Cianchi, R., Paggi, L., Orecchia, P., Mattiucci, S., D'Amelio, S., and Berland, B. (1990). Genetic relationships among anisakid species belonging to the genera *Contracaecum* and *Phocascaris*. *Bull. Soc. fr. Parasitol.* **8**, 261.
- Nascetti, G., Cianchi, R., Mattiucci, S., D'Amelio, S., Orecchia, P., Paggi, L., Bratney, J., Berland, B., Smith, J. W., and Bullini, L. (1993). Three sibling species within *Contracaecum osculatum* (Nematoda, Ascaridida, Ascaridoidea) from the Atlantic Arctic-Boreal region: Reproductive isolation and host preferences. *Int. J. Parasitol.* **23**, 105–120.
- Nei, M. (1972). Genetic distance between populations. *Am. Nat.* **106**, 283–292.
- Nevo, E., Beiles, A., and Ben-Shlomo, R. (1984). The evolutionary significance of genetic diversity: Ecological, demographic and life history correlates. *Evol. Dyn. Genet. Divers.* **53**, 14–213.
- Nikaido, M., Matsumo, F., Hamilton, H., Brownell, R. L., Cao, Y., Ding, W., Zuoyan, Z., Shedlock, A. M., Fordyce, R. E., Hasegawa, M., and Okada, N. (2001). Retroposon analysis of major cetaceans lineages: The monophyly of toothed whales and the paraphyly of river dolphins. *Proc. Natl. Acad. Sci. USA* **98**, 7384–7389.
- Orecchia, P., Paggi, L., Mattiucci, S., Nascetti, G., Smith, J. W., and Bullini, L. (1986a). Electrophoretic identification of larvae and adults of *Anisakis* (Ascaridida: Anisakidae). *J. Helminthol.* **60**, 331–339.
- Orecchia, P., Berland, B., Paggi, L., Nascetti, G., Mattiucci, S., and Bullini, L. (1986b). Divergenza genetica di specie dei generi *Contracaecum* e *Phocascaris* (Ascaridida, Anisakidae) con diverso ciclo biologico. *Ann. Ist. sup. Sanità* **22**, 345–348.
- Orecchia, P., Mattiucci, S., D'Amelio, S., Paggi, L., Plotz, J., Cianchi, R., Nascetti, G., Arduino, P., and Bullini, L. (1994). Two new members in the *Contracaecum osculatum* complex (Nematoda, Ascaridoidea) from the Antarctic. *Int. J. Parasitol.* **24**, 367–377.
- Osche, G. (1963). Morphological, biological, and ecological considerations in the phylogeny of parasitic nematodes. In "The Lower Metazoa, Comparative Biology and Phylogeny" (E. C. Dougherty, ed.), pp. 283–302. California University Press, Berkeley.
- Oshima, T. (1987). Anisakiasis is the Sushi bar guilty? *Parasitol. Today* **3**, 44–48.
- Paggi, L., and Bullini, L. (1994). Molecular taxonomy in anisakids. *Bull. Scand. Soc. Parasitol.* **4**, 25–39.
- Paggi, L., Nascetti, G., Cianchi, R., Orecchia, P., Mattiucci, S., D'Amelio, S., Berland, B., Bratney, J., Smith, J. W., and Bullini, L. (1991). Genetic evidence for three species within *Pseudoterranova decipiens* (Nematoda, Ascaridida, Ascaridoidea) in the North Atlantic and Norwegian and Barents Seas. *Int. J. Parasitol.* **21**, 195–212.
- Paggi, L., Mattiucci, S., D'Amelio, S., and Nascetti, G. (1998a). Nematodi del genere *Anisakis* in pesci, cefalopodi e cetacei del Mar Mediterraneo e dell'Oceano Atlantico e Pacifico. *Biol. Mar. Mediterr.* **5**, 1585–1592.

- Paggi, L., Nascetti, G., Webb, S. C., Mattiucci, S., Cianchi, R., and Bullini, L. (1998b). A new species of *Anisakis* Dujardin, 1845 (Nematoda: Anisakidae) from beaked whale (Ziphiidae): Allozyme and morphological evidence. *Syst. Parasitol.* **40**, 161–174.
- Paggi, L., Mattiucci, S., Ishikura, H., Kikuchi, K., Sato, N., Nascetti, G., Cianchi, R., and Bullini, L. (1998c). Molecular genetics in anisakid nematodes from the Pacific Boreal Region. In "Host Response to International Parasitic Zoonoses" (H. Ishikura, M. Aikawa, H. Itakura, and K. Kikuchi, eds.), pp. 83–107. Springer-Verlag, Tokyo.
- Paggi, L., Mattiucci, S., Gibson, D. I., Berland, B., Nascetti, G., Cianchi, R., and Bullini, L. (2000). *Pseudoterranova decipiens* species A and B (Nematoda: Ascaridoidea): Nomenclatural designation, morphological diagnostic characters and genetic markers. *Syst. Parasitol.* **45**, 185–197.
- Paggi, L., Mattiucci, S., and D'Amelio, S. (2001). Allozyme and PCR-RFLP markers in anisakid nematodes, aetiological agents of human anisakidosis. *Parassitologia* **43**, 21–27.
- Palm, H., Anderson, K., Klöser, H., and Plötz, J. (1994). Occurrence of *Pseudoterranova decipiens* (Nematoda) in fish from the southeastern Weddell Sea (Antarctic). *Polar Biol.* **14**, 539–544.
- Pålsson, J., and Beverly-Burton, M. (1984). Helminth parasites of capelin, *Mallotus villosus* (Pisces: Osmeridae) of the north Atlantic. *Proc. Helminthol. Soc. Wash.* **51**, 248–254.
- Pannacciulli, F., Bishop, J. D. D., and Hawkins, S. J. (1997). Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. *Mar. Biol.* **128**, 73–82.
- Pastor, T., Garza, J. C., Allen, P., Amos, W., and Aguilar, A. (2004). Low genetic variability in the highly endangered Mediterranean monk seal. *J. Hered.* **95**, 291–300.
- Paterson, A. M., Gray, R. D., and Wallis, G. P. (1993). Parasites, petrels and penguins: Does louse phylogeny reflect seabird phylogeny? *Int. J. Parasitol.* **23**, 515–526.
- Petter, A. J. (1972). Redescription of *Anisakis insignis* Diesing (Ascaridoidea), parasite of the Amazon river dolphin, *Inia geoffrensis*. In "Investigation on Cetacea" (G. Pilleri, ed.), Vol. IV, pp. 93–99. Brain Anatomy Institute, University of Berne, Switzerland.
- Pontes, T., D'Amelio, S., Costa, G., and Paggi, L. (2005). Molecular characterization of larval anisakid nematodes from marine fishes of Madeira by a PCR-based approach, with evidence for a new species. *J. Parasitol.* **91**, 1430–1434.
- Poulin, R. (2006). Variation in infection parameters among populations within parasite species: Intrinsic properties *versus* local factors. *Int. J. Parasitol.* **36**, 877–885.
- Poulin, R., and Morand, S. (2004). "The Parasite Biodiversity." Smithsonian Institution Press, S. Smithsonian Books, Washington.
- Pozio, E., and Murrel, K. D. (2006). Systematics and epidemiology of *Trichinella*. *Adv. Parasitol.* **63**, 367–439.
- Railliet, A., and Henry, A. (1912). Quelques nematodes parasites des reptiles. *Bull. Soc. Path. exot.* **5**, 251–259.
- Read, A. J., Drinker, P., and Northridge, S. (2006). By-catch of marine mammals in U.S. and global fisheries. *Conserv. Biol.* **20**(1), 163–169.
- Reeb, C. A., and Block, B. (2006). Ten microsatellite loci show that Mediterranean swordfish are genetically unique from other populations worldwide. "Report of the 2006 ICCAT swordfish stock structure workshop, Crete (Greece), 13–15 March 2006.", SCRS/2006/02.
- Rios, C., Sanz, S., Saavedra, C., and Pena, J. B. (2002). Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia: Pectinidae), across the Almeria-Oran front. *J. Exp. Mar. Biol. Ecol.* **267**, 223–244.
- Saraiva, A., Faranda, A., Damiano, S., Hermida, M., Santos, M. J., Ventura, C. H., Soares, J. P., Cruz, C., Eiras, J. C., and Mattiucci, S. (2007). Six species of *Anisakis* (Nematoda: Anisakidae) parasites of the black scabbardfish, *Aphanopus carbo* from NE Atlantic waters: Genetic markers and fish biology. *Abstracts of VII International Symposium on Fish Parasites, Parassitologia* **49**(suppl. 2), 229.

- Schwartz, M. K., Luikart, G., and Waples, R. S. (2006). Genetic monitoring as a promising tool for conservation and management. *Trends Ecol. Evol.* **22**, 25–33.
- Shiraki, T. (1974). Larval nematodes of family Anisakidae (Nematoda) in the Northern Sea of Japan—As a causative agent of eosinophilic phlegmone or granuloma in the human gastro-intestinal tract. *Acta Med. Biol.* **22**, 57–98.
- Slatkin, M., and Barton, N. H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**, 1349–1368.
- Smith, J. W., and Mooney Snyder, J. (2005). New locality for third-stage larvae of *Anisaks simplex* (sensu lato) (Nematoda: Ascaridoidea) in euphausiids *Euphasia pacifica* and *Thysanoessa raschii* from Prince William Sound, Alaska. *Parasitol. Res.* **97**, 539–542.
- Smith, J. W., and Wootten, R. (1978). *Anisakis* and anisakiasis. *Adv. Parasitol.* **16**, 93–163.
- Sprent, J. F. A. (1977). Ascaridoid nematodes of amphibians and reptiles: *Dujardinascaris*. *J. Helminthol.* **51**, 253–287.
- Sprent, J. F. A. (1978). Ascaridoid nematodes of amphibians and reptiles: *Gedolstascaris* n. g. and *Ortleppascaris* n. g. *J. Helminthol.* **52**, 261–282.
- Sprent, J. F. A. (1979). Ascaridoid nematodes of amphibians and reptiles: *Multicaecum* and *Brevimulticaecum*. *J. Helminthol.* **53**, 91–116.
- Sprent, J. F. A. (1983). Ascaridoid nematodes of amphibians and reptiles: *Typhlophorus*, *Hartwichia* and *Trispiculascaris*. *J. Helminthol.* **57**, 179–189.
- Sures, B. (2004). Environmental parasitology: Relevancy of parasites in monitoring environmental pollution. *Trends Parasitol.* **20**, 170–177.
- Thompson, R. M., Mouritsen, K. N., and Poulin, R. (2005). Importance of parasites and their life cycle characteristics in determining the structure of a large marine food web. *J. Anim. Ecol.* **74**, 77–85.
- Timi, J. T., Sardella, N. H., and Mattiucci, S. (2003). *Contracaecum ogmorhini* s.s. Johnston and Mawson, 1941 (Nematoda: Anisakidae), parasite of *Arctocephalus australis* (Zimmermann, 1783) off the Argentinian coast. *Helminthologia* **40**, 27–31.
- Umehara, A., Kawakami, Y., Matsui, T., Araki, J., and Uchida, A. (2006). Molecular identification of *Anisakis simplex sensu stricto* and *Anisakis pegreffii* (Nematoda: Anisakidae) from fish and cetacean in Japanese waters. *Parasitol. Int.* **55**, 267–271.
- Umehara, A., Kawakami, Y., Araki, J., and Uchida, A. (2007). Molecular identification of the etiological agent of the human anisakiasis in Japan. *Parasitol. Int.* **56**, 211–215.
- Umehara, A., Kawakami, Y., Araki, J., and Uchida, A. (2008). Multiplex PCR for the identification of *Anisakis simplex sensu stricto*, *Anisakis pegreffii* and the other anisakid nematodes. *Parasitol. Int.* **57**, 49–53.
- Vacchi, M., La Mesa, M., and Castelli, A. (1994). Diet of two coastal nototheniid fish from Terra Nova Bay, Ross Sea. *Antarct. Sci.* **6**, 61–65.
- Valentini, A., Mattiucci, S., Bondanelli, P., Webb, S. C., Mignucci-Giannone, A., Colom-Llavina, M. M., and Nascetti, G. (2006). Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *cox2* sequences, and comparison with allozyme data. *J. Parasitol.* **92**, 156–166.
- Van Thiel, P. H. (1960). Anisakiasis. *Parasitology* **52**, 16–17.
- Vidal-Martinez, V. M., Osorio-Saraiba, D., and Overstreet, R. M. (1994). Experimental infection of *Contracaecum multipapillatum* (Nematoda: Anisakinae) from Mexico in the domestic cat. *J. Parasitol.* **80**, 576–579.
- Webster, L. M. I., Johnson, P. C. D., Adam, A., Mable, B. K., and Keller, L. F. (2007). Macrogeographic population structure in parasitic nematode with avian host. *Vet. Parasitol.* **144**, 156–166.
- Wickens, P. A. (1995). A review of operational interactions between pinnipeds and fisheries. *FAO Fisheries Technical Paper* 346.
- Yamaguti, S. (1961). “Systema Helminthum: The Nematodes of Vertebrates,” 679 pp. Interscience Publishers.

- Yamaguti, S., and Arima, S. (1942). *Porrocaecum azarasi* n. sp. (Nematoda) from the Japanese seal. *Trans. Sapporo Nat. Hist. Soc.* **17**, 113–116.
- Zarlenga, D. S., Hoberg, E. P., Stringfellow, F., and Lichtenfield, J. R. (1998). Comparison of two polymorphic species of *Ostertagia* and phylogenetic relationships with the *Ostertaginiinae* (Nematoda: Tricoststrongiloidea) inferred from DNA repeat and mitochondrial DNA sequences. *J. Parasitol.* **84**, 806–812.
- Zhu, X. Q., Gasser, R. B., Podolska, M., and Chilton, N. B. (1998). Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. *Int. J. Parasitol.* **28**, 1911–1921.
- Zhu, X. Q., Gasser, R. B., Jacobs, D. E., Hung, G. C., and Chilton, N. B. (2000a). Relationships among some ascaridoid nematodes based on ribosomal DNA sequence data. *Parasitol. Res.* **86**, 738–744.
- Zhu, X. Q., D'Amelio, S., Paggi, L., and Gasser, R. B. (2000b). Assessing sequence variation in the internal transcribed spacers of ribosomal DNA within and among members of the *Contraecum osculatum* complex (Nematoda: Ascaridoidea: Anisakidae). *Parasitol. Res.* **86**, 677–683.
- Zhu, X., D'Amelio, S., Hu, M., Paggi, L., and Gasser, R. B. (2001). Electrophoretic detection of population variation within *Contraecum ogmorhini* (Nematoda: Ascaridoidea: Anisakidae). *Electrophoresis* **22**, 1930–1934.
- Zhu, X. Q., D'Amelio, S., Palm, H. W., Paggi, L., George-Nascimento, M., and Gasser, R. B. (2002). SSCP-based identification of members within the *Pseudoterranova decipiens* complex (Nematoda: Ascaridoidea: Anisakidae) using genetic markers in the internal transcribed spacers of ribosomal DNA. *Parasitology* **124**, 615–623.
- Zhu, X. Q., Podolska, M., Liu, J. S., Yu, H. Q., Chen, H. H., Lin, Z. X., Luo, C. B., Song, H. Q., and Lin, R. Q. (2007). Identification of anisakid nematodes with zoonotic potential from Europe and China by single-strand conformation polymorphism analysis of nuclear ribosomal DNA. *Parasitol. Res.* **101**, 1703–1707.