Chapter 11
Anisakiasis

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Abstract Anisakiasis refers to the zoonotic disease provoked in humans by the accidental ingestion of larvae of *Anisakis* spp. infecting fish or squid which is consumed raw and/or undercooked. These anisakid nematodes are heteroxenous parasites involving marine mammals (mainly cetaceans) as definitive hosts, while crustaceans (krill), fish and squid act as intermediate/paratenic hosts in their life cycles. This chapter briefly describes the taxonomy of species of *Anisakis*, our present knowledge of the definitive and intermediate/paratenic hosts involved in their life cycle and their geographical distribution. Nine species have so far been detected genetically as belonging to the genus *Anisakis*. Among these, *A. simplex* (*sensu stricto*) and *A. pegreffii* are so far found to play a zoonotic role in humans. The ingestion of infected seafood can provoke gastric anisakiasis (GA), intestinal anisakiasis (IA), gastro-allergic anisakiasis (GAA) or extragastrointestinal anisakiasis. Pathological aspects and the diagnosis of human anisakiasis are also reviewed, including an overview of our current knowledge of the *Anisakis* allergens involved in the human immunological response. Finally, current literature on possible control measures involving the inactivation of *Anisakis* larvae in fish fillets, thus reducing transmission to humans, is reported.

11.1 Introduction

The family Anisakidae includes species of nematodes whose adult stages can be found in fish, fish-eating birds and marine mammals, whereas the third-stage larvae (the infective stage) are commonly present in the body cavity and muscles of numerous fish and squid species. Anisakid nematodes are of both medical and economic concern, due to their public health implications and their associated

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effects on the marketability of fish products, which are often exacerbated by frequent warnings in the media.

Larval forms of anisakid nematodes, in particular those belonging to the genera *Anisakis* and *Pseudoterranova*, are in fact the main causative agents of human 'anisakidosis', a fish-borne parasitic zoonosis caused by the ingestion of raw or undercooked fish or cephalopods, which are infected by these larvae.

This chapter deals with those species of *Anisakis* considered as the major etiological agents of human 'anisakiasis' (the term is related only to *Anisakis* spp. as a causative agent) throughout the world. A recent review considering the pathogenetic aspects and occurrence of zoonoses related to the species of *Pseudoterranova* and *Contracaecum* has been published elsewhere (Mattiucci et al. 2013a).

Interest in *Anisakis* spp. has been growing constantly since human anisakiasis was first reported in the Netherlands in the 1960s and subsequently gained increasing health and economic relevance, particularly in countries where the consumption of raw fish and/or squid is common. Human cases are increasingly reported in Japan, the United States and many European countries (United Kingdom, France, Spain and Italy). This alarm has also raised an interest in gathering information on epizootiological data from anisakid infections in fish and squids, with particular regard to those of commercial value that are commonly part of the human diet worldwide. Official authorities and public bodies have been often involved in assessing a precise scenario for the prevalence and parasite burden infections of *Anisakis* spp. in edible fish, as well as depicting risk maps according to geographical area, season, fish size and other parameters (EFSA 2010).

11.2 The Agent

Morphological characters of taxonomic significance in anisakid nematodes are very few (i.e. features such as the excretory system, the alimentary canal, the number and distribution of male caudal papillae, the position of the vulva and the length of the spicules) and are applicable to adult specimens only. Furthermore, these are often only relevant to male individuals, making the identification of many worms at the species level difficult (Fagerholm 1989; Paggi et al. 1998a; Mattiucci et al. 2005, 2009, 2014a). Indeed, in anisakid nematodes, cladogenetic events have been accompanied by minimal morphological differentiation, as ecological factors have led to a convergence of similar and well-adapted morpho-functional solutions. This has given rise to a large number of morphologically identical but reproductively isolated ('sibling') species. Therefore, morphological traits do not always provide definitive evidence for their identification. The small number of diagnostic characters in adult individuals is even more dramatically marked in larval forms, where the number of structural traits useful for diagnostic purposes is very limited. *Anisakis* spp. larvae can be identified only to the generic level; this is mainly based on the morphology and length of the glandular part of the oesophagus (i.e. the

'ventriculus') and the presence/absence of a caudal spine ('mucron'). Based on these differences, the Type I and the Type II larvae (*sensu* Berland 1961) have been described morphologically. Similarly, at higher taxonomic levels among anisakids, several morphological characters, even if apparently readily differentiating one group from another, can appear to be homoplastic and not always related to the phylogeny of the species or genera within the group.

Thus, the limited taxonomic significance of some morphological characters and the occurrence of speciation processes virtually devoid of morphological differentiation undoubtedly advocate the use of molecular approaches as reliable tools for inferring the systematic relationships and evolution of anisakid nematodes and, consequently, their correct identification at the species level, with obvious implications for their epidemiology.

Accurate epizootiological and epidemiological studies must necessarily rely on the correct identification of the aetiological agent involved. Since the 1980s, pioneer studies on the genetic structure of anisakid nematodes have been carried out using multilocus allozyme electrophoresis (MAE). This tool revealed the existence of a high level of genetic heterogeneity within certain anisakid morphospecies, such as *A. simplex* (e.g. Nascetti et al. 1986). The biological species concept (BSC) (Mayr 1963) was well supported by the application of allozyme markers for several *Anisakis* species. Indeed, the known diversity of species belonging to *Anisakis* quickly increased after the detection of several sibling species (i.e. species which are morphologically very similar but reproductively isolated) and led to the discovery and description of several new species. Reproductive isolation and the absence of gene flow have been demonstrated by allozymes between sympatric and allopatric sibling species, establishing their specific status (Nascetti et al. 1986; Mattiucci et al. 1997, 2001, 2002, 2005, 2009, 2014a; Paggi et al. 1998a).

The introduction of polymerase chain reaction (PCR)-derived molecular methodologies subsequently confirmed the taxonomic assessment of species of *Anisakis* based on allozyme markers. Reference individuals initially characterised by allozymes have been used to develop and establish DNA-based approaches for species identification, such as PCR-RFLP and direct sequencing of ITS rDNA (D'Amelio et al. 2000) or mitochondrial DNA (Valentini et al. 2006).

At present, allozymes (Mattiucci and Nascetti 2006), PCR-RFLP of rDNA (D'Amelio et al. 2000; Cavallero et al. 2011) and DNA sequence analysis of nuclear (ITS region of the rDNA) (Nadler et al. 2005; Cavallero et al. 2011) and mitochondrial genes (mtDNA *cox2* and *rrnS)* (Nadler et al. 2005; Valentini et al. 2006; Mattiucci et al. 2009, 2014a; Cavallero et al. 2011) have demonstrated that the genus *Anisakis* comprises at least nine distinct species. These are the three species included in the *A. simplex* (sensu *lato*) complex, i.e. *Anisakis simplex* (sensu stricto)*, A. pegreffii* (= *A. simplex* A of Nascetti et al. 1986) and *A. berlandi* Mattiucci et al. (2014a) (= *A. simplex* C of Mattiucci et al. 1997); the two closely related taxa *A. ziphidarum* Paggi et al. 1998a and *A. nascettii* Mattiucci et al. 2009; the three closely related species *A. physeteris*, *A. brevispiculata*, and *A. paggiae* Mattiucci et al. 2005; and, finally, *A. typica*.

The existence of two major clades (Clade I and Clade II) has been demonstrated by different phylogenetic inferences (Valentini et al. 2006; Cavallero et al. 2011; Mattiucci et al. 2009, 2014a). Clade I comprises one subclade formed by ((*Anisakis simplex* (*s. s.*)*, A. pegreffii*)*, A. berlandi*) and a second one which encompasses the two species (*Anisakis ziphidarum* and *A. nascettii*), whereas three species currently belong to Clade II, i.e. *Anisakis physeteris, A. brevispiculata* and *A. paggiae*. The position of *A. typica* as forming a distinct phylogenetic lineage with respect to the other species has also been demonstrated; its position in the phylogenetic tree, as representing a sister taxon to the other *Anisakis* species, has been discussed in recent phylogenetic analyses based on different genetic data sets (Cavallero et al. 2011; Mattiucci et al. 2014a).

Moreover, in recent years, both Palm et al. (2008) and Mattiucci and Nascetti (2008) have genetically detected the existence of one additional taxon, closely related to *A. typica*, which has been recovered at larval stage from nonmigratory fish species in Balinese, Javanese and Malaysian waters of the Pacific Ocean. The preliminary results appear to indicate that the third taxon (*Anisakis* sp. 1) may be a sibling species of *A. typica* occurring in central Pacific waters (Mattiucci and Nascetti 2008).

A further gene pool, referred to as *Anisakis* sp. 2, has been genetically detected by means of allozyme markers and mtDNA *cox2* sequence analysis based on larvae of Type II from swordfish in the Atlantic equatorial area (Mattiucci et al. 2007; Garcia et al. 2011).

Interestingly, while those *Anisakis* spp. which have been included in Clade I exhibit larvae of morphoType I, those species comprising Clade II have larvae of morphoType II. This means that, at present, five species of *Anisakis* have Type I and three species have Type II larval morphology. In other words, the larval stages of *Anisakis* spp. cannot be identified by means of morphological features but only by genetic/molecular markers.

However, despite the limited morphological characters available in adults of *Anisakis* spp., in the recent years, a 'reconciliation' between genetic and morphological traits has been possible with the use of more detailed morphological and morphometric analyses of sibling species which has resulted in the finding of diagnostic features which can be used for species recognition at the adult stage. This is the case for *A. paggiae* with respect to the closely related taxa *A. brevispiculata* and *A. physeteris* (see Mattiucci et al. 2001), for *A. nascettii* vs *A. ziphidarum* (see Mattiucci et al. 2009) and for *A. pegreffii, A. simplex (s. s.)* and *A. berlandi* (see Quiazon et al. 2008; Mattiucci et al. 2014a).

11.3 Current Methods Used for the Identification of *Anisakis* spp.

The limited value of morphological analyses makes the use of genetic and molecular methods absolutely necessary for the identification of species of *Anisakis*. The most used molecular/genetic methods are briefly reported below.

11.3.1 Multilocus Allozyme Electrophoresis

Multilocus allozyme electrophoresis (MAE) (19–24 enzyme loci) has been used extensively to identify large number of *Anisakis* spp. populations sampled from many geographical regions in the Boreal and Austral hemispheres, detect 'sibling species', discover new species and address questions concerning population genetics, evolutionary biology and the relationship between genetic variability and habitat disturbance (Mattiucci and Nascetti 2008). These genetic markers have proved to be a cheap, effective tool for the identification of large numbers of *Anisakis* spp. larvae; for example, they have been used to identify thousands of *Anisakis* spp. larvae used as biological tags in fish stock assessment (Mattiucci et al. 2004, 2007, 2008).

11.3.2 PCR-RFLPs Analysis

Notwithstanding the huge amount of data which have been obtained from the application of MAE, the development of molecular markers for the accurate identification of related species using PCR-based approaches is in some cases preferable, especially as this approach requires only small amounts of fresh or ethanol-fixed parasite material for analysis. For example, PCR-based restriction fragment length polymorphism (PCR-RFLP) (D'Amelio et al. 2000; Pontes et al. 2005) and sequence analyses of the ribosomal DNA (rDNA) internal transcribed spacers (ITS-1 and ITS-2) (Nadler et al. 2005; Cavallero et al. 2011; Mattiucci et al. 2014a) provide useful approaches for the specific identification of species of *Anisakis* from different definitive and intermediate/paratenic hosts.

11.3.3 DNA Sequencing

Direct DNA sequencing of some genes has proved to be a fruitful tool for the identification of the different sibling and morphospecies of *Anisakis*. Sequence data are now available for almost all of the nine species recognised within the genus, the

only exception being *A. schupakovi*. In particular, the sequences of all nine species available in GenBank represent both nuclear and mitochondrial genes.

As for the nuclear genes, the region of the nuclear ribosomal DNA, spanning the final part of the 18S subunit, the first internal transcribed spacer (ITS-1), the 5.8S subunit, the second internal transcribed spacer (ITS-2) and the very beginning of the 28S subunit, has been sequenced for all of the *Anisakis* spp. (Cavallero et al. 2011; Mattiucci et al. 2014a). The ITS region of the rDNA exhibits a significant degree of variation between closely related species and between the different morphospecies of *Anisakis*, and it is therefore useful for species discrimination. On the other hand, concerted evolution tends to minimise the intraspecific variation in this genomic region, thus allowing an unambiguous attribution of one specified sequence to one corresponding species.

In the case of the mitochondrial DNA, two regions have been sequenced for all of the *Anisakis* taxa; these are the mitochondrial gene *cox2* (cytochrome oxidase 2) (Valentini et al. 2006; Mattiucci et al. 2009, 2014a) and the *rrnS* (the small subunit of the ribosomal DNA in the mitochondrial genome) (Nadler et al. 2005; Mattiucci et al. 2014a). These mitochondrial markers have been able to distinguish all of the taxa which have been characterised genetically as belonging to *Anisakis*. The mtDNA *cox2* region shows a high degree of polymorphism at the intraspecific level; this finding supports the possible use of this gene in further studies of the population genetics and phylogeography of *Anisakis* spp., as recently suggested for some species by Baldwin et al. (2011).

11.3.4 Multiplex and Species-Specific PCR

Umehara et al. (2008) have developed a method based on multiplex PCR which was able to recognise six different species of anisakids, including *A. simplex* (*s. s.*) and *A. pegreffii*. The specificity of these primers for discriminating between the two sibling species is increased due to the introduction of artificial mismatched bases. Recently, PCR-sequence-specific primers have been developed by Abe (2008) in order to establish a quick method for the discrimination of *A. simplex* (*s. s.*) from *A. pegreffii*.

Once genetically detected and characterised, species of *Anisakis* have proved to be ecologically different in terms of host, life cycle and geographical distribution. These data are presented in the next section.

11.4 Life Cycle, Hosts and Geographical Distribution of *Anisakis* spp.

Anisakis species have complex, indirect life cycles which involve various marine organisms at different levels of the trophic web in the marine ecosystem (Fig. 11.1). The adults live in the stomach of marine mammals, mainly cetaceans. The life cycle begins when female worms release eggs which are passed in the faeces of their definitive host into the sea. According to some experimental studies, the eggs are embryonated, and the larvae moult within the eggs, resulting in the third-stage larva (Køie et al. 1995; Højgaard 1998). The eggs are ingested by crustaceans, such as copepods and euphausiids (krill), in which they grow into their haemocoel. Fish or squid (Cephalopoda, Decapodiformes) become infected after eating an infected crustacean; the third-stage larva bores through the digestive tract wall of the fish or squid and passes into the visceral body cavity and undergoes host-induced encapsulation (Levsen and Berland 2012). The life cycle is completed after the intermediate/paratenic host (fish, squid or, directly, a crustacean) is predated by the definitive host. Inside the stomach or the intestine of its final mammalian host, *Anisakis* spp. undergo two final moults and develop into a sexually mature adult nematode.

Since *Anisakis* larvae do not undergo any development or moult inside the fish or squid, these hosts should be regarded as paratenic in terms of the nematode life cycle. Small fish and squid are frequently predated by larger fish species, which form an additional paratenic host in the cycle. This is important from an epidemiological point of view, because the repeated transmission of *Anisakis* larvae between hosts in the prey–predatory system enables an extensive bioaccumulation of infection in fish of a greater size. Several pelagic and demersal fish species show an increase in the prevalence and abundance of *Anisakis* larvae with age and size (Mattiucci et al. 2004; Levsen and Lunestand 2010; Levsen and Berland 2012). Such fish hosts can accumulate hundreds of *Anisakis* spp. larvae during their lifespan. In contrast, an opposite trend has been observed in the fish species *Scomber scombrus* in the North Sea, where it has been suggested that infection levels could be influenced by host- and/or age-specific fish immunological characteristics (Levsen and Berland 2012).

In infected fish, the majority of the larvae are found in the visceral body cavity, typically encapsulated outside the organs; however, a certain number of larvae may migrate from the visceral cavity to the flesh of the fish, mostly to the belly flap of the fish, but also to the dorsal musculature. It has been demonstrated that in some fish hosts migration occurs during the life of the fish (Karl et al. 2011) and not post-mortem as commonly believed. The occurrence of larvae in fish fillets does, however, represent a biological hazard to man, following the consumption of raw or inadequately cooked fish. However, it has been suggested that different species of *Anisakis* larvae may have a different capacity to migrate and infect the fish fillets (Chou et al. 2010; Quiazon et al. 2011a; Mattiucci et al. personal observation).
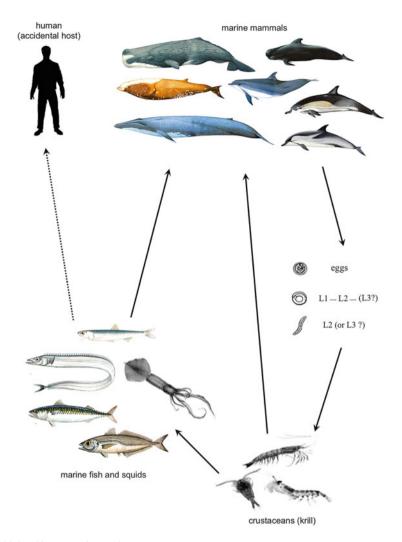
Fig. 11.1 Life cycle of *Anisakis* spp

While an extensive literature has been produced on the systematics and epidemiology of *Anisakis* spp., very little is known of the pathological significance of the occurrence of the parasite in its fish host in relation to condition factor and general fitness. Some pathobiological changes have been reported in commercially important fish hosts in relation to *A. simplex* (*s. s.*), such as 'stomach crater syndrome' in larger fish, e.g. cod (Berland 1980). This consists of a host-induced encapsulation in the gastric mucosa of the fish, due to the fact that the thickness of the stomach wall impedes larval migration. This finding has been observed in other fish species, such as large swordfish (Mattiucci et al. 2014b) and bluefin tuna (Mattiucci personal observation). 'Red vent syndrome' (RVS, i.e. bleeding, swollen and haemorrhagic

vents) of wild Atlantic salmon has been correlated with large numbers of encapsulated *A. simplex* (*s. s.*) larvae in fish tissue at the level of vent and urogenital papilla (Beck et al. 2008; Noguera et al. 2009). In histopathological terms, the red vent exhibits gross lesions characterised by haemorrhages and moderate to severe inflammation dominated by eosinophilic granular cells and melanomacrophages around encapsulated *Anisakis* larvae in the host tissue (Noguera et al. 2009). It has been noted, however, that despite the severity of the infection of *Anisakis* spp. larvae in parasitised hosts, infected fish were generally in good overall condition.

11.4.1 Host Preference

Presented below is a synopsis of ecological factors, in terms of their definitive and intermediate/paratenic hosts and known geographical distribution, for the nine species of *Anisakis* which have been genetically determined.

Generally, *Anisakis* spp. larvae have been detected worldwide in Gadiformes, Perciformes, Clupeiformes, Pleuronectiformes, Scorpaeniformes, Zeiformes, Bericiformes, Lophiiformes, Anguilliformes and Atheriniformes (Table 11.1). *Anisakis* spp. larvae have been also detected in a variety of cephalopods and, rarely, in elasmobranchs. Among squids, they have been found mainly in the Ommastrephidae (Table 11.1). The known data on the occurrence of the species *A. pegreffii* and *A. simplex* (*s. s.*), i.e. those known as etiological agents of human anisakiasis, in fish and squid species from different fishing grounds are summarised in Table 11.1.

In the stomach of cetaceans, adults of *Anisakis* spp. (Table 11.2) are often found free in the lumen and sometimes in clusters embedded in the mucosa and submucosa.

The presence of the two main clades (Clade I and Clade II), as presented above in the section on phylogenetic relationships between *Anisakis* spp., is also supported by ecological data relating to specific definitive host–*Anisakis* spp. relationships.

Sperm whales, *Physeter catodon, Kogia breviceps* and *K. sima* (Table 11.2), are the main definitive hosts for *A. physeteris, A. brevispiculata* and *A. paggiae,* respectively, which cluster in Clade II of the phylogenetic tree of *Anisakis* spp. (Mattiucci and Nascetti 2008; Mattiucci et al. 2009, 2014a; Cavallero et al. 2011). Several oceanic dolphins of the Delphinidae, Arctic dolphins of the Monodontidae and porpoises of the Phocoenidae (Table 11.2) are hosts of the species *A. pegreffii*, *A. simplex* (*s. s.*) and *A. berlandi* (Mattiucci et al. 1997, 2014a; Mattiucci and Nascetti 2008; Cavallero et al. 2011), which are the most derived group of species included in the main Clade I obtained in the *Anisakis* phylogenetic tree analysis.

Beaked whales, *Ziphius cavirostris, Mesoplodon layardii, M. mirus, M. grayi,* *M. densirostris* and *M. europaeus,* are hosts of *A. ziphidarum* (Table 11.2) (Paggi et al. 1998a; Mattiucci et al 2009; Cavallero et al. 2011) and *A. nascettii* (Mattiucci et al. 2009, 2014a; Pontes et al. 2005) which are separated as a subclade and included in the main Clade I of the *Anisakis* phylogenetic tree. Thus, the

Table 11.1 Intermediate/paratenic hosts so far detected, using molecular genetic markers, for *Anisakis* spp.

	A. simplex (s. s.)	A. pegreffii	A. berlandi	A. typica	A. ziphidarum	A. nascettii	A. physeteris	A. brevispiculata	A. paggiae
Cephalopods									
Sepiidae									
Sepia officinalis	IC	–	–	–	–	–	–	–	–
Ommastrephidae									
Illex coindetii	IC	–	–	–	–	–	–	–	–
Illex illecebrosus		WM							
Moroteuthis ingens									
Ommastrephes angolensis	–	SA	–	–	–	–	–	–	–
Ommastrephes sagittatus	IC	NAM	–	–	–	–	CM	–	–
Todarodes pacificus	KYS	KYS	–	KYS	–	–	–	–	–
Todaropsis eblanae	IC, SA	IC, SA	–	–	–	–	–	–	–
Fishes									
Anguillidae									
Synaphobranchus kaupi	–	–	–	–	–	–	CSA	–	–
Anoplogastridae									
Anoplogaster cornuta	–	–	–	–	–	–	–	IRS	–
Anoplopomatidae									
Anoplopoma fimbria	NEP	–	NEP	–	–	–	–	–	–

Belonidae								
Belone belone	IC	IC	–	–	–	–	–	–
Berycidae								
Beryx splendens	–	–	–	–	JA	JA	JA	JA
Bothidae								
Arnoglossus laterna	PC	–	–	–	–	–	–	–
Arnoglossus imperialis	–	PC	–	–	–	–	–	–
Bramidae								
Brama brama	–	SA	–	–	–	–	–	–
Carangidae								
Decapterus macarellus	–	–	–	PNG	–	–	–	–
Trachurus capensis	–	SA	–	–	–	–	–	–
Trachurus mediterraneus	MOR	CM, MOR	–	–	–	–	–	–
Trachurus picturatus	AZ, MD	AZ, MD	–	AZ, MD	–	–	–	–
Trachurus trachurus	NEA, IC, MA, WM	CM, EM, WM, IC, MA, NZ, MOR	–	EM	AZ	CM		
Selar crumenophthalmus	–	–	–	CHS, HWA, CSA	–	–	–	–
Caesionidae								
Caesio cuning	–	–	–	IND	–	–	–	–

Table 11.1 (continued)

	A. simplex (s. s.)	A. pegreffii	A. berlandi	A. typica	A. ziphidarum	A. nascettii	A. physeteris	A. brevispiculata	A. paggiae
Centrophoridae									
Deania profundorum	AZ	–	–	–	–	–	–	–	–
Citharidae									
Citharus linguatula	PC	PC	–	–	–	–	–	–	–
Clupeidae									
Clupea harengus	NEA, BS, NEP, BAL, NS	–	–	–	–	–	–	–	–
Errumeus whiteheadi	–	SA	–	–	–	–	–	–	–
Sardinops sagax	NEP	NEP	NEP	–	–	–	–	–	–
Congridae									
Conger conger	IC	CM	–	–	–	–	–	–	–
Astroconger myriaster	–	CHS	–	–	–	–	–	–	–
Coryphaenidae									
Coryphaena hippurus	–	–	–	SC	–	–	–	–	–
Emmelichthyidae									
Emmelichthys nitidus nitidus	–	SA	–	–	–	–	–	–	–
Engraulidae									

Engraulis encrasicolus	–	CM, WM	–	–	–	–	–	–	–
Etmopteridae									
Etmopterus spinax	AZ	–	–	–	–	–	AZ	–	–
Etmopterus pusillus	AZ	–	–	–	–	–	–	–	–
Etmopterus princeps	AZ	–	–	–	–	–	–	–	–
Gadidae									
Boreogadus saida	IC	–	–	–	–	–	–	–	–
Gadus macrocephalus	–	CHS	–	–	–	–	–	–	–
Gadus morhua	BS, IC	–	–	–	–	–	–	–	–
Melanogrammus aeglefinus	BS	–	–	–	–	–	–	–	–
Micromesistius poutassou	IC	CM, IC, NAM	–	–	–	–	–	–	–
Theragra chalcogramma	NEP, JA, BE	JA	–	–	–	–	–	JA	JA
Trisopterus luscus	IC	–	–	–	–	–	–	–	–
Gempylidae									
Thyrsites atun	–	–	SA	–	–	–	–	–	–
Gerreidae									
Gerres oblongus	–	–	–	PNG	–	–	–	–	–
Hexagrammidae									
Pleurogrammus azonus	NEA, JA	–	–	–	–	–	–	–	–

(continued)

Table 11.1 (continued)

	A. simplex (s. s.)	A. pegreffii	A. berlandi	A. typica	A. ziphidarum	A. nascettii	A. physeteris	A. brevispiculata	A. paggiae
Lampridae									
Lampris guttatus	–	WM	–	–	–	–	CM, WM	–	–
Lophiidae									
Lophius piscatorius	IC	NAM, MOR	–	–	–	–	–	–	–
Lophius vomerinus	–	SA	–	–	–	–	–	–	–
Lotidae									
Molva dypterygia	IC	–	–	–	–	–	–	–	–
Brosme brosme	NEA	–	–	–	–	–	–	–	–
Lutjanidae									
Pinjalo lewisi	–	–	–	PNG	–	–	–	–	–
Pinjalo pinjalo	–	–	–	PNG	–	–	–	–	–
Macrouridae									
Macruronus novaezelandiae	–	–	NZ	–	–	–	–	–	–
Trachyrincus scabrus	MOR	MOR	–	–	–	–	–	–	–
Merlucciidae									
Merluccius capensis	–	SA	–	–	–	–	–	–	–
Merluccius hubbsi	–	FA	–	–	MA, MOR	IC	–	MA	IC

Merluccius merluccius	NEA, IC, MA, NAM, MOR	CM, EM, WM, IC, NEA, MA, NAM, MOR	MA, EM, NAM, MOR	CM, MA, WM, IC, EM, NAM, MOR	–
Myctophidae					
Myctophum punctatum	MAR	–	–	–	–
Gymnoscopelus nicholsi	–	SHI	SHI	–	–
Electrona carlsbergi	–	–	SHI	–	–
Muraenidae					
Muraena helena	–	NAM	–	–	–
Moridae					
Pseudophycis bachus	–	NZ	NZ	–	–
Dicentrarchus labrax	NEA, BB	CM	–	–	–
Nemipteridae					
Nemipterus virgatus	–	–	CHS	–	–
Nemipterus bathybius	–	–	CHS	–	–
Nototheniidae					
Notothenia coriiceps	–	–	SHI	–	–
Notothenia rossii	–	–	SHI	–	–
Ophidiidae	SA	–	–	–	–

Table 11.1 (continued)

	A. simplex (s. s.)	A. pegreffii	A. berlandi	A. typica	A. ziphidarum	A. nascettii	A. physeteris	A. brevispiculata	A. paggiae
Genypterus capensis									
Osmeridae									
Hypomesus pretiosus japonicus	JA	–	–	–	–	–	–	–	–
Phycidae									
Phycis phycis	–	NAM	–	NAM	–	–	NAM	–	–
Phycis blennoides	–	NAM	–	–	–	–	NAM	–	–
Pinguipedidae									
Parapercis colias	–	NZ	NZ	–	–	–	–	–	–
Pleuronectidae									
Hippoglossus hippoglossus	BE	–	–	–	–	–	–	–	–
Platichthys flesus	–	–	–	PC	–	–	–	–	–
Rachycentridae									
Rachycentron canadum	TW	–	–	–	–	–	–	–	–
Petromyzonthidae									
Petromyzon marinus	IC	–	–	–	–	–	–	–	–
Salmonidae									
Oncorhynchus gorbuscha	SI, ALA	–	–	–	–	–	–	–	–
Oncorhynchus keta	SI, KYS, ALA	–	–	–	–	–	–	–	–

Species											
Oncorhynchus nerka	ALA	–	–	–	–	–	–	–	–	–	–
Salmo salar	NWA	–	–	–	–	–	–	–	–	–	–
Scophtalmidae											
Lepidorhombus boscii	IC	IC, NAM	–	–	–	–	–	–	–	–	–
Scomberesocidae											
Scomberesox saurus	NWA	–	–	–	–	–	–	–	–	–	–
Scombridae											
Auxis rochei	–	–	–	IND	–	–	–	–	–	–	–
Auxis thazard	–	–	–	BR, IND	–	–	–	–	–	–	–
Euthynnus affinis	–	–	–	SC	–	–	–	–	–	–	–
Euthynnus alletteratus	MOR	MOR	–	–	–	–	–	–	–	–	–
Katsuwonus pelamis	–	–	–	HWA, CSA	–	–	–	–	–	–	–
Rastrelliger kanagurta	–	–	–	THA	–	–	–	–	–	–	–
Sarda orientalis	–	–	–	SC	–	–	–	–	–	–	–
Scomber australasicus	–	TW	–	TW	–	–	–	–	–	–	–
Scomber japonicus	AZ, MD, JA, MOR	AZ, MD, JA, KYS, NAM, MOR	–	AZ, MD, MOR	–	AZ, MD, MOR	–	MD, JA	–	–	–
Scomber scombrus	NEA, IC, NAM, MOR	CM, IC, NAM, MOR	–	NAM, MOR	–	MOR	–	NAM, JA	–	–	–
Scomberomorus commerson	–	–	–	SC	–	–	–	–	–	–	–
Scomberomorus maculatus	–	–	–	PNG	–	–	–	–	–	–	–
Scomberomorus niphonius	–	CHS	–	–	–	–	–	–	–	–	–

(continued)

Table 11.1 (continued)

	A. simplex (s. s.)	A. pegreffii	A. berlandi	A. typica	A. ziphidarum	A. nascettii	A. physeteris	A. brevispiculata	A. paggiae
Thunnus albacares	–	–	–	PNG	–	–	–	–	–
Thunnus thynnus	JA	CM, BR, NAM	–	BR, CM	–	–	–	–	–
Scorpaenidae									
Scorpaena scrofa	IC	IC	–	–	–	–	–	–	–
Sebastidae									
Helicolenus dactylopterus	–	CM, SA	–	–	–	–	–	–	–
Sebastolobus alascanus	–	–	NEP	–	–	–	–	–	–
Soleidae									
Dicologoglossa cuneata	–	PC	–	–	–	–	–	–	–
Solea senegalensis	PC	–	–	–	–	–	–	–	–
Sparidae									
Spondyliosoma cantharus	IC	–	–	–	–	–	–	–	–
Sternoptychidae									
Maurolicus muelleri	NEA	–	–	–	–	–	–	–	–
Trachichthyidae									
Hoplostethus atlanticus	–	–	TA	–	–	–	–	–	–
Hoplostethus mediterraneus	–	CM	–	–	–	–	–	–	–
Trachinidae									
Echiichthys vipera	–	NAM	–	–	–	–	–	–	–
Trachinus draco	–	NAM	–	–	–	–	–	–	–

Family / Species						
Trichiuridae						
Lepidopus caudatus	–	CM, SA	–	–	–	–
Aphanopus carbo	MD	MD	MD	AZ, MD	AZ	AZ
Trichiurus lepturus	JA	NAM, JA, KYS	BR, TW, JA, KYS, IND	–	–	–
Lepturacanthus savala	–	–	IND	–	–	–
Triglidae						
Eutrigla gurnardus	IC	–	–	–	–	–
Xiphiidae						
Xiphias gladius	NEA, NWA, CNA	CM, NAM	–	CM, IC, NEA, NWA, CNA, TEQ, CSA	NEA, NWA, TEQ, CSA	NEA, TEQ, CSA

Sampling locality codes: *ALA* Alaska, *AZ* Azores Islands, *BB* Biscay Bay, *BE* Bering Sea, *BR* Brazilian Atlantic coast, *BS* Barents Sea, *CHS* China Sea, *CM* Central Mediterranean Sea, *CNA* Central North Atlantic Ocean, *CS* Caribbean Sea, *CSA* Central South Atlantic Ocean, *EM* East Mediterranean Sea, *FA* Falkland Islands, *FL* Florida coast, *HAW* Hawaii, *IC* Iberian Atlantic Coast, *IND* Indian Sea, *IRS* Irminger Sea (Greenland), *JA* Japan Sea, *KYS* Korea Yellow Sea, *MA* Mauritanian coast, *MAR* Mid–Atlantic Ridge, *MI* Macquarie Island, *MD* Madeira, *MOR* Morocco, *NAM* North African Mediterranean coast, *NEA* Northeast Atlantic, *NEP* Northeast Pacific, *NS* North Sea, *NWA* Northwest Atlantic, *NZ* New Zealand, *PC* Portuguese coast, *PNG* Papua New Guinea, *SA* South Africa, *SC* Somali coast, *SHI* Shetland Islands, *SI* Sakhalin Islands, *TA* Tasman Sea, *TEQ* Tropical Equatorial Atlantic, *THA* Thailand, *TW* Taiwan, *WM* West Mediterranean [Data from: Bao et al. (2013), Bernardi et al. (2011), Cavallero et al. (2012), Chou et al. (2011), Dzido et al. (2009), Farjallah et al. (2008a, b), Garcia et al. (2011), Klimpel et al. (2007), Kuhn et al. (2011), Levsen and Karl (2013), Mattiucci et al. (1986, 1997, 2001, 2002, 2004, 2005), Mladineo et al. (2012), Nascetti et al. (1986), Orecchia et al. (1986), Paggi et al. (1998a, b), Abollo et al. (2001), Pontes et al. (2005), Marques et al. (2006), Mattiucci and Nascetti (2006, 2008), Serracca et al. (2013), Setyobudi et al. (2011), Shih et al. (2010)]

Table 11.2 Definitive hosts so far detected, using molecular genetic markers, for *Anisakis* spp.

	A. simplex (s. s.)	A. pegreffii	A. berlandi	A. typica	A. ziphidarum	A. nascettii	A. physeteris	A. brevispiculata	A. paggiae
Cetaceans									
Balaenopteridae									
Balaenoptera acutorostrata	NEA	–	–	–	–	–	–	–	–
Delphinidae									
Delphinus capensis	–	NZ	–	–	–	–	–	–	–
Delphinus delphis	IC	IC	–	–	–	–	–	–	–
Globicephala melas	IC, SA, NEA	NZ	SA, NZ	–	–	–	–	–	–
Globicephala macrorhynchus	FL	–	–	FL	–	–	–	–	–
Lagenodelphis hosei	–	FL	–	FL	–	–	–	–	–
Lagenorhynchus albirostris	NEA	–	–	–	–	–	–	–	–
Lissodelphis borealis	–	–	NEP	–	–	–	–	–	–
Orcinus orca	NEP	–	–	–	–	–	–	–	–
Peponocephala electra	–	–	–	BR	–	–	–	–	–
Pseudorca crassidens	NEP	–	NEP	–	–	–	–	–	–
Sotalia fluviatilis	–	–	–	BR	–	–	–	–	–
Sotalia guianensis	–	–	–	BR	–	–	–	–	–
Stenella clymene	–	–	–	BR	–	–	–	–	–
Stenella coeruleoalba	IC	SA, MS	–	EM, FL	–	–	–	–	–
Stenella attenuata	–	–	–	FL, CS	–	–	–	–	–
Stenella longirostris	–	–	–	BR, FL	–	–	–	–	–

Steno bredanensis	FL	–	–	CS, BR, FL	–	–	–	–
Tursiops truncatus	FL	CM, SA, WM	–	FL, CS	–	–	–	–
Kogiidae								
Kogia breviceps	FL, NWA	–	–	BR	–	FL, NWA	SA, IC, FL, NWA	SA, FL, NWA
Kogia sima	–	–	–	NWA, FL	–	–	CS, NWA, FL	FL, CS, NWA
Monodontidae								
Delphinapterus leucas	NWA	–	–	–	–	–	–	–
Neobalaenidae								
Caperea marginata	–	SA	–	–	–	–	–	–
Phocoenidae								
Phocoena phocoena	NEP, NS	–	–	–	–	–	–	–
Physeteridae								
Physeter macrocephalus	–	–	–	–	–	CM, FL	–	–
Ziphiidae								
Mesoplodon bowdoini	–	–	–	NZ	NZ	–	–	–
Mesoplodon densirostris	–	–	–	SA	NZ	–	–	–
Mesoplodon europaeus	–	–	–	FL, CS	–	–	–	–
Mesoplodon grayi	–	–	–	–	SA	–	–	–
Mesoplodon layardii	–	–	–	SA	NZ	–	–	–
Mesoplodon mirus	–	–	–	–	NZ	–	–	–

(continued)

Table 11.2 (continued)

	A. simplex (s. s.)	A. pegreffii	A. berlandi	A. typica	A. ziphidarum	A. nascettii	A. physeteris	A. brevispiculata	A. paggiae
Ziphius cavirostris	–	–	–	–	CM, SA, CS	NZ	–	–	–
Pinnipeds									
Phocidae									
Mirounga angustirostris	–	–	NEP	–	–	–	–	–	–
Mirounga leonina	–	–	SHI	–	–	–	–	–	–

Sampling locality codes: *AN* Antarctica, *AZ* Azores, *BE* Bering Sea, *BR* Brazilian 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* Sea of Japan, *MA* Mauritanian coast, *MD* Madeira, *MS* Mediterranean Sea. *NAM* North African Mediterranean coast, *NEA* Northeast Atlantic, *NEP* Northeast Pacific, *NS* North Sea, *NWA* Northwest Atlantic, *NZ* New Zealand, *PC* Portuguese coast, *SA* South Africa, *SC* Somali coast, *SI* Sakhalin Islands, *TA* Tasman Sea, *WM* West Mediterranean [Data from: Garvalho et al. (2010), Cavallero et al. (2011, 2012), Colom-Llavina et al. (2009), Klimpel et al. (2011), Quiazon et al. (2009), Mattiucci and Nascetti (2006), Mattiucci and Nascetti (2007), Mattiucci et al. (1986, 1997, 2001, 2002, 2004, 2005), Nascetti et al. (1986), Orecchia et al. (1986), Paggi et al. (1998a, 1998b, 1998c)]

phylogenetic relationships proposed elsewhere (Mattiucci and Nascetti 2008; Mattiucci et al. 2014a) for species of *Anisakis* mirror, in relation to several host–parasite associations, that proposed for their cetacean definitive hosts (Milinkovitch 1995; Cassens et al. 2000; Nikaido et al. 2001; Arnason et al. 2004). Elaboration of these empirical results in order to assess the global congruence of the co-phylogenetic relationship between the host and parasite trees determined by ParaFit (Legendre et al. 2002) was statistically significant ($P < 0.05$) (Mattiucci and Nascetti 2008). Individual host–parasite associations which contributed more to the co-phylogenetic cetacean–*Anisakis* spp. mapping were represented by those between *A. physeteris* and *Physeter catodon*, *A. brevispiculata* and *Kogia breviceps* and *A. ziphidarum* and *Mesoplodon* spp., suggesting host–parasite co-speciation events, whereas a less significant contribution to the total test was that formed by the host–parasite association *A. simplex* (*s. s.*) and *Balaenoptera acutorostrata*, suggesting a possible host-switching event (Mattiucci and Nascetti 2008; Mattiucci et al. 2014a).

11.4.2 Geographical Distribution of Nine Species of Anisakis

Despite the fact that only *A. simplex* (*s. s.*) and *A. pegreffii* are known causative agents of human anisakiasis (see Sect. 11.5), a pathogenic role for other species belonging to the genus cannot be excluded. This is also due to the presence of the above species in a wide range of intermediate/paratenic hosts of commercial importance and to the fact that the molecular identification to the species level of the etiological agent causing human anisakiasis has been possible only in recent years. Therefore, we consider useful to summarise below the geographical distribution of all *Anisakis* species which have been detected genetically.

Anisakis simplex (sensu stricto) is widespread between 35°N and the Arctic Circle; it is present in both the western and eastern Atlantic and Pacific Oceans (Mattiucci et al. 1997, 1998; Abollo et al. 2001; Nadler et al. 2005; Umehara et al. 2006, 2008; Abe et al. 2005, 2006; Quiazon et al. 2009, 2011b). The southern limit of this species in the Northeast Atlantic Ocean is the waters around the Gibraltar region. *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 et al. 2004, 2007; Mattiucci and Nascetti 2008). It has so far been genetically recognised as occurring in several species of cetacean hosts (Table 11.1). Several squid and fish species have been found harbouring larvae of this species throughout its geographical range. A sympatric area between *A. simplex* (*s. s.*) and *A. pegreffii* has been identified along the Spanish and Portuguese Atlantic coasts (Mattiucci et al. 1997, 2004, 2007, 2008; Abollo et al. 2001; Pontes et al. 2005; Marques et al. 2006; Hermida et al. 2012), in the Alboran Sea (Mattiucci et al. 2004, 2007) and in the Sea of Japan (Umehara et al. 2006; Quiazon et al. 2009, 2011b). In areas of sympatry, few F1 hybrid individuals between *A. pegreffii* and *A. simplex* (*s. s.*) have been recognised using allozymes (Mattiucci et al. 2004);

however, several individuals exhibiting recombinant genotypes at the ITS of the ribosomal DNA between *A. pegreffii* and *A. simplex* (*s. s.*) have been recorded using PCR-RFLP markers, but their status as hybrid forms between the two species has been not so far been confirmed (Abollo et al. 2003). *A. simplex* (*s. s.*) also occurs in sympatry with *A. berlandi* in the Eastern Pacific Ocean, where it has been identified in definitive and intermediate/paratenic hosts (Mattiucci et al. 1997, 1998, 2014a; Paggi et al. 1998a).

Anisakis pegreffii is the dominant species of *Anisakis* in the Mediterranean Sea, being widespread in many fish species. Indeed, it is presently the most important anisakid nematode in several pelagic and demersal fish from Mediterranean waters (Paggi et al. 1998b; Mattiucci and Nascetti 2008; Farjallah et al. 2008a; Chaliggiannis et al. 2012; Cavallero et al. 2012; Mladineo et al. 2012; Serracca et al. 2013). It is also widely distributed at both adult and larval stages in the Austral Region between 35°N and 55°S (Mattiucci et al. 1997, 2014a). In Atlantic waters, the northerly limit of its geographical range is represented by the Iberian and Portuguese coasts (Mattiucci et al. 1997, 2004, 2007; Abollo et al. 2001; Pontes et al. 2005; Marques et al. 2006; Hermida et al. 2012). It has been detected, at the larval stage, in some fish hosts from Japanese marine waters (Abe et al. 2006; Umehara et al. 2006, 2008; Quiazon et al. 2009, 2011b) and in Chinese marine waters (Zhu et al. 2007).

Anisakis berlandi Mattiucci et al. 2014a (= *A. simplex* C of Mattiucci et al. 1997) 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 observation; Nadler et al. 2005). This species has been identified at the adult stage in cetaceans as occurring syntopically with *A. pegreffii* and as a larva in some fish species (Table 11.1). It has also occasionally been identified in the seals *Mirounga leonina* from the sub-Antarctic area (Mattiucci and Nascetti 2008) and in *M. angustirostris* from Northeast Pacific Ocean (Nadler et al. 2005).

Anisakis typica has a range extending from 30°S to 35°N in warmer temperate and tropical waters (Mattiucci et al. 2002; Cavallero et al. 2012). In these areas it has been found as an adult in species of dolphin and as a larva in several fish species (Tables 11.1 and 11.2). *A. typica* has also been identified in cetaceans and fish 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 has also been recognised in flatfishes captured in central Portuguese waters of the Northeast Atlantic (Marques et al. 2006) and only rarely in some fish species caught along the North African coast (Tunisia and Lybia) of the Mediterranean Sea (Farjallah et al. 2008a). *A. typica* larvae have also been identified in fish from Chinese marine waters (Zhu et al. 2007).

Anisakis ziphidarum Paggi et al. 1998a was first described, both genetically and morphologically, as an adult in beaked whales from the South Atlantic Ocean (off the South African coast) and in the Mediterranean Sea. This species has also been found in Central Atlantic, including the Caribbean Sea (Colom-Llavina, et al. 2009; Cavallero et al. 2011), and in the South Pacific waters (off the New Zealand coast)

(Mattiucci et al. 2014a). Thus, its geographical range appears to be wide and related to that of its definitive hosts. Only very limited 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 in Central Atlantic waters (Mattiucci et al. 2004; Pontes et al 2005; Hermida et al. 2012). 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 (Mattiucci and Nascetti 2008).

Anisakis nascettii Mattiucci et al. (2009) has been detected at the adult stage in beaked whales from New Zealand waters and from off the South African coast. It has also been identified at L4 stage in ziphiid cetaceans from the Central Atlantic Ocean (Iglesias et al. 2008). This species has been identified genetically, at the larval stage, as heavily infecting the squid *Moroteuthis ingens* in the Tasman Sea this appears to support the hypothesis that this species involves squids rather than fish in its life cycle (Mattiucci et al. 2009).

Anisakis physeteris was first genetically characterised in its main definitive host, the sperm whale *Physeter macrocephalus,* from Mediterranean waters (Mattiucci et al. 1986). Genetically identified adults have also been recorded in the Central Atlantic Ocean (Mattiucci and Nascetti 2008; Cavallero et al. 2011). Type II larvae of *A. physeteris* have been genetically identified in the swordfish *Xiphias gladius* from Mediterranean and Atlantic waters (Garcia et al. 2011).

Anisakis brevispiculata has been characterised genetically using allozymes (Mattiucci et al. 2001), the mtDNA *cox2* gene (Valentini et al. 2006) and ITS rDNA sequence analysis (D'Amelio et al. 2000; Nadler et al. 2005) based on material from a pygmy sperm whale, *Kogia breviceps,* in South African and Northeast Atlantic waters (Iberian coast). Type II *Anisakis* larvae corresponding to *A. brevispiculata* have been recognised using allozyme markers as rare parasites of the fish *Merluccius merluccius* (see Mattiucci et al. 2004) and heavily infecting the swordfish *Xiphias gladius* in tropical–equatorial Atlantic waters (Garcia et al. 2011).

Anisakis paggiae Mattiucci et al. (2005) was first genetically characterised and described morphologically as an adult parasite of the pygmy sperm whale and the dwarf sperm whale off both Florida and the South African Atlantic coast (Mattiucci et al. 2005). It has also been identified in kogiids from the Caribbean Sea (Cavallero et al. 2011). In recent years, this parasite species was also identified in *Kogia sima* from the Sea of Japan (Quiazon et al. 2013a).

11.5   The Human Response to the Parasite

Third-stage larvae of *Anisakis* spp. infecting the flesh of marine fish or squid, if ingested alive by humans, can cause the zoonotic disease 'anisakiasis'. The transmission of this fish-borne pathogen is particularly associated with the tradition of consumption of raw or undercooked fish. A number of fish dishes are considered to be of high risk for the contraction of human anisakiasis. They include, among

others, the Scandinavian gravlax, Dutch salted and marinated herring, Japanese sushi and sashimi, Spanish boquerones and anchovies and Italian marinated anchovies. First reported in the Netherlands, anisakiasis has acquired an increasing health and economic relevance especially in countries such as Japan, where the consumption of raw fish and squid is frequent, although human cases are increasingly reported from many European countries (Spain, Italy, United Kingdom and France). In recent years, due to the popularity and increased consumption of Japanese dishes, as well as the consumption of small pickled fish, more and more cases of anisakiasis have been reported worldwide (Audicana and Kennedy 2008). However, notification of human anisakiasis is not actually mandatory, and consequently, despite several reported cases over time, it remains an underestimated zoonosis.

As indicated above, of the nine species of *Anisakis* which have been characterised genetically, only two, i.e. *A. simplex* (*s. s.*) and *A. pegreffii,* have so far been reported as causative agents of human anisakiasis (D'Amelio et al. 1999; Moschella et al. 2004; Umehara et al. 2008; Fumarola et al. 2009; Mattiucci et al. 2011, 2013a).

11.5.1 Immunopathological Processes

The pathological changes occurring within the gastrointestinal tract during an infection by *Anisakis* app. larvae are the combined result of the direct invasive capacity of the larva and the interaction between the host's immune response and the antigens released by the infective larvae during the invasion. *Anisakis* larvae release proteolytic enzymes in order to invade the gastrointestinal mucosa. These antigenic proteins have been isolated and characterised as excretory/secretory antigens (E/S) (Moneo et al. 2000; Shimakura et al. 2004; Rodriguez-Perez et al. 2008; Cavallero et al. 2011; Kobayashi et al. 2011). Humoral and cellular responses are also involved in the infections with *Anisakis* larvae. Th2 cytokines production and the resulting mastocytosis, IgE response and eosinophilia characterise local inflammatory lesions produced by *Anisakis* spp. larvae. Eosinophilic infiltration in the tissues surrounding the parasite has been reported in both acute and chronic infections. Eosinophilic cell concentration in damaged areas is related not only to the production of chemotactic factors released by T lymphocytes, mast cells and basophils, but also to some chemotactic substances produced directly by *Anisakis* larvae which attracts these cells. Eosinophilic infiltration is the most effective process in the destruction of larvae at the local level (gastrointestinal tract). The presence of eosinophilic cells characterises a late stage of Type I immune hypersensitivity in response to *Anisakis* infection.

11.5.2 Histopathology of Anisakiasis

From the histopathological point of view, anisakiasis may be classified into the following four sequential stages. The first stage is 'phlegmon formation', and the second is the 'abscess formation', which is rather frequent in gastric anisakiasis and is characterised by abundant necrotic tissue around the larvae and by a rich population of eosinophils. The third stage is 'abscess–granuloma formation', which corresponds to the flogistic evolution of the disease at least 6 months after the ingestion of the larva; at this stage, the larva is in the form of few remnants which are invaded by eosinophils, surrounded by giant cells and abundant inflammatory parvicellular infiltrate. Finally, the most advanced stage is 'granuloma formation', characterised by a further decrease in the presence of eosinophils, but with abundance of lymphocytes, giant cells and significant collagenisation (Kikuchi et al. 1990).

11.6 Clinical Manifestations of Anisakiasis

On the basis of the site reached by the live ingested *Anisaki*s larva, the disease could be subdivided into in gastric anisakiasis (GA) and intestinal anisakiasis (IA). The acute form appears to be gastric and is characterised by nausea, vomiting and epigastric pain. These symptoms appear 1–6 h after the ingestion of the infected fish. In IA, acute signs start to appear about 7 days after infection in the form of abdominal pain, nausea, vomiting, fever, diarrhoea and faecal occult blood. Several, rarely occurring, extragastrointestinal sites have been also documented (i.e. oropharyngeal, abdominal cavity, mesenteries and omentum). Anisakiasis of the digestive tract is classified, from the clinical point of view, as 'acute' or 'moderate' and 'invasive' or 'not invasive', depending on the location reached by the larva, if it has remained in the gastric and/or intestinal lumen, and by its capacity to invade or not the submucosa layer of the gastric or intestinal wall.

11.6.1 Gastric Anisakiasis

Epigastric pain is the most frequent sign of acute gastric anisakiasis (GA). Other symptoms are nausea, vomiting, abdominal fullness or distension, anorexia and chest pain High fever is not a classical sign of GA, although it is quite frequently slightly higher than normal (37.5 °C). As for the localisation in the gastric mucosa, Shibata et al. (1989) divided the stomach into four main parts, namely, anterior wall, lesser curvature, posterior wall and greater curvature. The majority of larvae were found in the greater curvature, followed by the posterior wall. These authors also described several endoscopic findings as tissue events, such as oedematous

hypertrophic gastric folds, increase in gastric secretion and peristalsis and mucosal lesions, including oedema, redness, coagulation, haemorrhage and ulceration. Cases of GA described recently from Italy (Mattiucci et al. 2013a) were characterised clinically by epigastric pain after 2 h following the ingestion of raw seafood, vomiting and other digestive symptoms. Endoscopic findings have showed that, in most of the cases, the *Anisakis* larvae were mainly located in the lumen of the stomach and had not invaded the submucosal layer of the gastric wall.

11.6.2 Intestinal Anisakiasis

Most of the cases of intestinal infection with *Anisakis* are characterised by symptoms such as nausea, vomiting and abdominal bulging, although numerous cases of asymptomatic IA have also been observed. The 'mild form' of IA is characterised by eosinophilic granulomas forming 'tumour-like' formations in the intestinal wall, whereas the 'fulminant form' has the symptoms of acute ileus, acute appendicitis, acute abdomen or regional ileitis. Cases of IA reported from Spain (Rosales et al. 1999) and from Italy (Moschella et al. 2004; Mattiucci et al. 2011) were characterised by a clinical picture of acute abdominal pain, acute appendicitis or acute abdomen. Although difficult to evaluate, some biochemical tests based on leucocyte counts or percentage of eosinophils, as well as of the enzymes GOT and GPT, can be of help in suspected cases of IA (Ishikura and Kikuchi 1990). In radiography or ultrasonographic images, showing the thickness of the intestinal wall, a marked dilatation of the intestine, the so-called key board sign, and ascites pooling between the dilated intestines have been reported, characterising both 'mild' and 'fulminant' forms of IA (Ishikura and Kikuchi 1990).

11.6.3 Gastro-allergic Anisakiasis

This is an acute allergic reaction in the context of an acute gastric presence of an *Anisakis* larva, when the live parasite attempts to invade the submucosal layer of the gastric wall (Daschner et al. 2011). GAA is characterised by urticaria, angioedema and anaphylaxis; it consists of an acute IgE-mediated, generalised reaction. In this type of anisakiasis, the allergic reactions take place starting from 2 or 3 h up to 2 or 3 days after the ingestion of an infected fish (Daschner et al. 2011; Mattiucci et al. 2013b). Recently, two GAA cases, characterised by urticaria and oedema of the oral mucosa, due to *A. pegreffii,* were recognised by molecular methods in Italian patients after they had consumed 'marinated anchovies' (Mattiucci et al. 2013b). In those cases, the endoscopical findings showed *Anisakis* larva invading the submucosa layer of the gastric wall. In addition, the serum samples from the patients showed IgE reactivity in WB analysis against *Ani s 1* antigen of *A. pegreffii* (see Table 11.3 and Sect. 11.6.4).

Table 11.3 Allergens of *Anisakis* (*Ani s*) so far characterized, with the percentage of IgE reactivity in human sera

Allergen	MW (kDa)	Location of the products	Major allergen	Panallergen	IgE reactivity (%)
Ani s 1	24	E/S	Yes		85
Ani s 1 isoform	21	E/S			?
Ani s 2	97	S	Yes	Yes	88
Ani s 3	41	S	Yes	Yes	4
Ani s 4	9	E/S			27
Ani s 5	15	E/S			25–49
Ani s 6	7	E/S			18
Ani s 7	139	E/S	Yes		83–100
Ani s 8	15	E/S			25
Ani s 9	14	E/S			13
Ani s 10	22	S (?)			39
Ani s 11	55	S (?)			47
Ani s 11-li	(?)	S (?)			?
Ani s 12	(?)	(?)	Yes		57

E/S excretory/secretory products, *S* somatic, *(?)* indicates unknown location in the larva or molecular weight

11.6.4 Anisakis *Allergy*

Live or dead anisakid larvae ingested with fish can lead to the onset of allergic reactions which are reported as frequently associated with high levels of the immunoglobulin IgE (Audicana and Kennedy 2008). 'Anisakis allergy' was described for the first time in Japan (Ishikura and Kikuchi 1990), but was followed by a plethora of contradictory publications and research (for reviews, see Daschner et al. 2011; Nieuwenhuizen and Lopata 2013). In Spain, since 1995, more than 150 cases of allergy due to *Anisakis* have been reported (Del Pozo et al. 1997; Audicana and Kennedy 2008); more than 50 % of the *Anisakis* allergic patients required emergency treatment, with five of 64 being hospitalised due to respiratory failure (Fernández de Corres et al. 2001). Although the usual signs of anisakiasis are characterised by urticaria, anaphylactic shock and respiratory failure due to oedema, Kikuchi et al. (1990) also referred to a possible association between *Anisakis* and rheumatic pathology. Additionally, cases of occupational *Anisakis* allergy were described in fishmongers or were related to exposure (either by contact or inhalation) to fish meal in chicken feed (Anibarro and Seoane 1998; Armentia et al. 1998). Clinical symptoms of allergic anisakiasis range from urticaria to anaphylactic shock. The diagnosis of anisakiasis can be complicated, as infections with helminths, in general, are associated with high levels of IgE and other immunoglobulins. Additionally, cross-reactivity occurs with other parasite antigens.

To date, 12 *Anisakis (Ani s)* allergens have been characterised, numbered from *Ani s 1* to *Ani s 12* (Table 11.3), according to the allergen nomenclature designated

by the WHO and IUIS. They include both somatic (S) and excretory/secretory (E/S) antigens, whereas some remain not well defined (Table 11.3). Purified allergens have been proved to be useful in the diagnosis of *Anisakis* allergy, especially in combination (Moneo et al. 2007).

The *Ani s 2* and *Ani s 3* allergens have a somatic (muscular) location in the *Anisakis* larva and have been shown to be paramyosin and tropomyosin. These are considered as 'panallergens'. A phylogenetic comparison of tropomyosin amino acid sequences, within and between different invertebrates, demonstrates that nematode tropomyosins of *Anisakis* and *Ascaris* are closely related to those of insect, crustaceans and mites (Nieuwenhuizen and Lopata 2013). This indicates a possible immunological cross-reactivity. Indeed, *Anisakis* muscle proteins paramyosin and tropomyosin are thought to be responsible for the cross-reactivity between *Anisakis* and other invertebrates and for the IgE hypersensitivity detected in blood sera often reported in allergic patients (Asturias et al. 2000; Guarneri et al. 2007; Mattiucci and Bruschi Personal Observation).

The major allergens of *Anisakis*, which are recognised at a high percentage level by IgE and IgG in serum samples of patients, are *Ani s1* (24 kDa) and *Ani s7* (139 kDa); they are located in excretory/secretory glands (ES) (Audicana and Kennedy 2008). *Ani s1* was recognised by IgE in sera of GAA patients (Moneo et al. 2000; Mattiucci et al. 2013b). *Ani s 1* has also been detected at a high percentage in patients from Morocco sensitised to *Anisakis* (Abattouy et al. 2012). *Ani s 1* has been recognised by both IgE and IgG in patients with *Anisakis* allergy (Mattiucci and Bruschi personal observation). *Ani s 1* also seems to be a heat-stable allergen (Moneo et al. 2000); therefore, allergic reactions could occur not only after the consumption of undercooked fresh fish but also from infected fish which have been cooked or frozen. An isoform of *Anis 1* also exists at 21 kDa (Shimakura et al. 2004). It has been suggested that the 'mild' form of GA and the allergic condition in Spain are related to the isoform of *Ani s 1* (Moneo et al. 2000; Shimakura et al. 2004).

Ani s 7, a glycoprotein, is also considered as major allergen, having being recognised in up to 100 % of sera samples in patients with *Anisakis* allergy (Rodríguez et al. 2008). The amino acid sequence similarity of *Ani s 1, Ani s 7* and *Ani s 12* in *A. pegreffii* and *A. simplex* (*s. s.*) has recently been described and compared by Quiazon et al. (2013b).

Other minor allergens are represented by *Ani s 4*, a heat-stable protein, which has been recognised in 27–30 % of patients (Moneo et al. 2005), and *Ani s 5*, *Ani s 8* and *Ani s 9*, all heat-stable E/S proteins, but less frequently recognised in patient sera (Caballero et al. 2008; Kobayashi et al. 2007; Rodriguez-Perez et al. 2008). However, their role could be relevant as in terms of an allergic reaction after the ingestion of cooked, frozen or canned fish products (Rodriguez-Perez et al. 2008). These allergens can be considered as food allergens.

Additional allergens have been identified and named *Ani s 10–12*; however, little knowledge is so far available concerning their function and location in *Anisakis* larvae. Furthermore, a haemoglobin from *A. pegreffii* was recently characterised, which seems to be responsible for a high immunoactivity in hypersensitive patients,

and it has been shown to have a phylogenetic similarity with other invertebrate haemoglobins (Nieuwenhuizen and Lopata 2013).

Finally, healthy individuals can have high levels of anti-*Anisakis* IgE in their serum without the development of allergic symptoms. On the other hand, individuals with low levels of specific IgE antibodies may show clinical manifestations of anisakiasis. For example, minimal symptoms of allergy have been observed in a patient shown by endoscopy to have a heavy parasite burden (200 *Anisakis* larvae) in the stomach (Jurado-Palomo et al. 2010). Here the specific humoral response to *Anisakis* was weak, a finding congruent with a previous experimental model (Amano et al. 1995) in which it was observed that a high parasite load could lead to a poor IgE response, suggesting a possible immunomodulation role for *Anisakis* spp. larvae. Finally, other immunoglobulins, such as IgA, IgG1, IgG2b and IgG2c, can also be detected in allergic reactions due to *Anisakis* (Anadón et al. 2009).

11.7 Diagnosis of Human Anisakiasis

11.7.1 Histological Diagnosis

Histological sections of haematoxylin–eosin-stained granulomas, removed after the surgical treatment at both gastric and intestinal levels, often revealed the presence of worms with the morphological features characteristic of *Anisakis* larvae. This happened when the nematode in the removed nodule is in a very good state of preservation. The following characters in particular, when visible at the microscopical level, enable identification at the generic level:

> In transverse section: a thin cuticle lacking lateral alae; polymyarian muscle cells, separated into four quadrants by chords with two wing-like distal lobes; intestine circular with a triangular lumen and 50–70 tall columnar epithelial cells; and excretory cell (renette cell) banana-shaped and situated ventrally to the intestine
>
> In sagittal section: the muscular part of the oesophagus followed by the glandular part (ventriculus) and absence of a ventricular appendix and/or intestinal caecum

These microscopical findings permitted the identification of *Anisakis* larva in granulomas removed from the gastric wall in the first documented case of GA in Italy (Stallone et al. 1996), in several cases of IA and GA reported later (Pampiglione et al. 2002), in a granuloma surgically removed from near the ileocaecal valve (Moschella et al. 2004), in an extragastrointestinal case of anisakiasis (Cancrini et al. 1997), and, finally, in a granuloma lesion provoked by *A. pegreffii* in a case of IA in Italy (Mattiucci et al. 2011). Unfortunately, identification to the specific levels of the etiological agent in these cases was not possible, except for the last one reported by Mattiucci et al. (2011) (see also Sect. 11.7.2).

11.7.2   Molecular Diagnosis

The very limited specific diagnostic features of individual *Anisakis* spp. larvae available on the basis of morphological examination mean that it is impossible to identify them as aetiological agents of anisakiasis using microscopy. Furthermore, when larvae infect humans, they can become spoiled or fragmented. This often happens, for instance, when they are removed by endoscopy, making it impossible to identify them morphologically, even at the generic level. Likewise, in histological sections of granuloma examined after intestinal surgery, it is sometimes very hard even to recognise the aetiological agent as a nematode.

In contrast, our knowledge of the causative agents of human anisakiasis was greatly advanced by the application of molecular methodologies. The first molecular identification of a larva recovered following a gastric endoscopy was reported by D'Amelio et al. (1999) in Italy. The larva was identified as *A. pegreffii* by PCR amplification of the entire ITS and subsequent RFLP analysis, as expected since this is the most frequent species in fishes from Italian marine waters. The same approach was applied by Farjallah et al. (2007), who recognised a larva recovered from the oesophagus of a patient as belonging to *A. pegreffii*. On the basis of the same molecular method, further two cases of GA in Italy caused by *A. pegreffii* were reported by Fumarola et al. (2009). Six additional cases of gastric anisakiasis (GA) and two cases of gastro-allergic anisakiasis (GAA), removed by endoscopy from eight Italian patients, have been diagnosed as belonging to the same species, i.e. *A. pegreffii*, by PCR amplification and the sequencing of the ITS region of the rDNA and the mitochondrial mtDNA*cox2* gene by Mattiucci et al. (2013b).

The widest survey was conducted by Umehara et al. (2007) in Japan, where 99 larvae from human patients were identified as *A. simplex* (*s. s.*), and one case had *A. pegreffii* as the aetiological agent, using the same method described by D'Amelio et al. (2000).

Finally, the first molecular identification of *A. pegreffii* in a paraffin-embedded granuloma as the aetiological agent of an IA case was performed by Mattiucci et al. (2011). In this technical advance, the PCR development allowed, for the first time, the molecular identification of an *Anisakis* larva in formalin-fixed and paraffin-embedded tissue.

Thus, according to the molecular identification of cases of human anisakiasis undertaken so far, both of the sibling species *A. pegreffii* and *A. simplex* (*s. s.*) have been shown to be causative agents of human anisakiasis. No data are so far available for the third species of the *A. simplex c*omplex, i.e. *A. berlandi* (= *A. simplex* C). We can also assume that *A. pegreffii* is able to provoke in humans gastric, intestinal and gastro-allergic anisakiasis (Mattiucci et al. 2013b).

However, although human infection is highest in countries where eating raw fish is widespread, the molecular identification of human cases remains very limited, especially in those European countries where allergic symptoms and hypersensitivity associated with the parasite are frequently reported. Yet, surprisingly, in Europe the obligatory notification of the human anisakiasis does not apply.

11.7.3 Serodiagnosis

Currently, most serodiagnostic tests for *Anisakis* reactivity include the use of ImmunoCAP systems, immunoblotting (WB), ELISA and skin prick test (SPT) (Audicana and Kennedy 2008). All of these methods use partially purified antigens and crude extract of *Anisakis* larvae. This gives these methodologies a poor specificity value, due to cross-reactivity with antigens from many other parasites and allergens. It should especially be noted that specific IgE detection using ImmunoCAP assay can overestimate the number of human cases sensitised to *Anisakis* allergens. In other words, the sensitivity of these tests can be exaggerated and result in false-positive results. Some authors have preferred to use IgE and IgG detection via immunoblotting (WB) to differentiate, for instance, between anisakiasis or *Anisakis* allergy and asymptomatic *Anisakis* IgE-sensitised patients (Del Pozo et al. 1997; García et al. 1997; Moneo et al. 1997). In recent years, purified *Anisakis* allergens have proven to be useful in diagnosis using WB, especially when combined antigens are used (Moneo et al. 2007). Further analysis using more-refined methods and serodiagnostic tools are needed.

11.8 Treatment

The endoscopic removal of larvae in gastric anisakiasis and the surgical treatment of intestinal granulomas appear to remedy the disease. On the other hand, the effective use of anthelmintic drugs is not supported by large surveys, although recent studies have highlighted a significant success of albendazole against cultured *Anisakis* larvae in vitro (Arias-Diaz et al. 2006). This suggests the possible use of this chemical in treating clinical manifestations of human anisakiasis, at least when the *Anisakis* larvae are still in the stomach, a short time after ingestion of the infected fish.

11.9 Prevention and Control

The consumption of fish infected by *Anisakis* larvae is a biological hazard that can be prevented by control measures under the supervision of health authorities and by the use of proper storage and processing methods that enable the inactivation of the larvae. EU (European Community) regulation nos 853/2004 and 1276/2011, regarding treatment to kill viable parasites in fishery products intended for human consumption, state that 'all wild caught seawater or freshwater fish must be considered at risk of containing viable parasites of human health hazard if these products are to eaten raw or almost raw…'. Cooking at 70 °C is used to kill the larvae within a short time. However, it needs to be considered that some allergens

(e.g. *Ani s 1*, *Ani s 4* and *Ani s 9*) have been demonstrated to be thermostable, and allergens released from the larvae into the surrounding tissue have retained their allergenicity even after the larvae have been killed by heat treatment (Vidaček et al. 2011). With regard to deep-freezing, Adams et al. (2005) reported a low survival of live *Anisakis* larvae per fillet (0–3 %) after 6 h at −40 °C, but up to 30 % of them survived after 48 h at 0 °C. Similarly, Wharton and Aalders (2002) demonstrated that larvae can survive at temperatures down to −10 °C. Whereas deep-freezing and cooking for sufficiently long periods are retained as the most effective methods, cold smoking and marinating procedures are unable to safety kill the larvae, unless high food-grade acetic acid concentrations are used (Sánchez-Monsalvez et al. 2005). Dry-salting can devitalise the parasite, provided that the salt is widely distributed in all parts of the muscle and is used at correct concentrations (>20° Baumé; see ICMSF 1996). Recently, Brutti et al. (2010) demonstrated a complete inactivation of *Anisakis simplex* larvae in raw fish using high hydrostatic pressure treatments, and the effects of microwave treatments has been reported by Adams et al. (1999), Tejada et al. (2006) and Vidaček et al. (2011).

Finally, the same EU regulation (1276/2011) states that '...in the case that epidemiological data show that the fishing grounds do not represent a health hazard with regard to the presence of parasites, the competent authority may adopt national measures which authorise an exemption from the required freezing treatment on fishery products derived from the wild catches'. It should be emphasised that these control measures should be established in the future, when they can be based on data on the following: (1) there are infection levels of genetically identified *Anisakis* spp. larvae in food fish, (2) the fish are from a defined fishing ground, (3) the location and percentage of infection by larvae in fish fillets (the edible part) are known, (4) the pathogenicity to humans of different species of *Anisakis* is fully established and (5) the risk of *Anisakis* allergy due to antigenic proteins released by these parasites in fish products is fully clarified.

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