The University of South Bohemia in České Budějovice
Faculty of Science

BIOLOGY AND PHYLOGENY OF ANISAKIS NEMATODES

Bachelor Thesis

Viktoriia Vyshnevska

Supervisor: Roman Kuchta

Co-Supervisor: Jesus Hernández-Orts

České Budějovice 2023

Anotace

This thesis is focused on the anisakid nematodes. The study concluded current knowledge on the biology and systematics of this group of parasites with a focus on zoonotic genera causing anisakidosis and the practical part using molecular techniques for the identification of anisakid larvae obtained from Antarctic fishes.

Declaration

I declare that I am the author of this qualification thesis and that in writing it, I have used the sources and literature displayed in the list of used sources only.

České Budějovice …08.12.2023……….   …………………………………

                                                                          Viktoriia Vyshnevska

Acknowledgments

CONTENTS

1. INTRODUCTION

Nematodes or roundworms are one of the most specious metazoan groups belonging to the Ecdysozoa, with around 40 including both free-living and parasitic species (Dezfuli et al. 2021). These organisms are characterized by unsegmented, elongated, and cylindrical worm-like bodies. They live in various environments, including soil, freshwater, and marine habitats, and even act as parasites in plants, animals, and humans (Hodda et al. 2009).

Nematodes are a highly diverse group of organisms, with many species yet to be discovered and classified. Their ecological role ranges from beneficial soil organisms to harmful parasites, making them an important group to study in various fields of biology and ecology.

The phylogenetic relationships within many nematode lineages remained unresolved for a long time despite numerous morphology and molecular analyses. Still, Ahmed et al. (2022) recently performed analyses based on all available genomic and transcriptomic data. According to these authors, nematodes are divided into three lineages: Chromadoria, Dorylamia, and Enoplia (Fig. 1). The present study focuses on anisakid nematodes (Chromadoria: Spirulina: Ascaridomoprha) (Fig. 1).
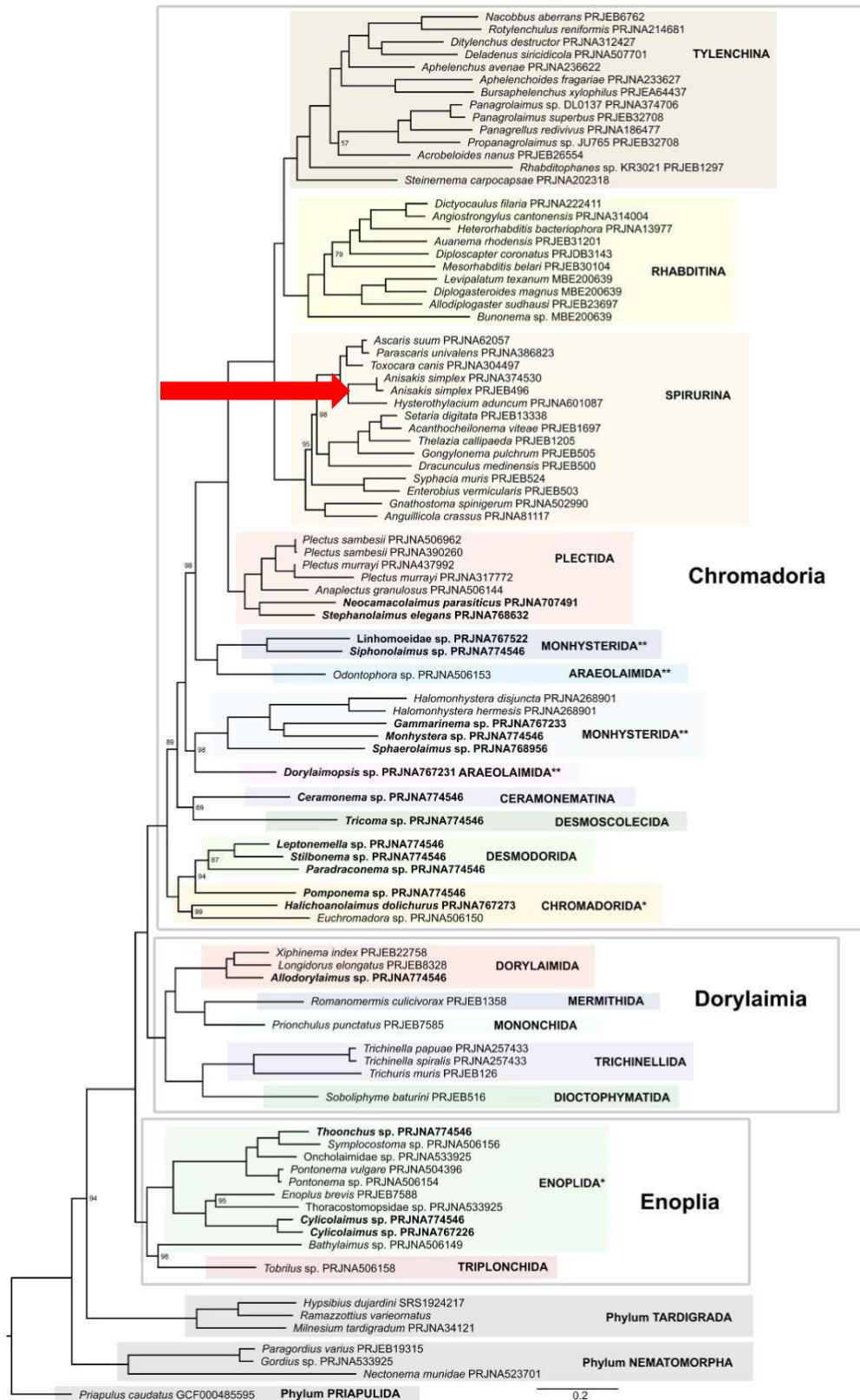
Figure 1. Phylogeny of the Nematoda after Ahmed et al. (2022) based on 18S rRNA and mitogenomes. Paraphyletic orders are marked with *. Polyphyletic orders are marked with **.

2.   OBJECTIVES

1. Compile a literature review on the biology and systematics of anisakid nematodes.

2. Genotyping of anisakid larvae from Antarctic fishes.

3.   LITERATURE REVIEW

3.1   Life cycle of anisakid nematodes

Anisakids are heteroxenous parasites using a wide range of aquatic organisms as their host (McClelland 2005). These nematodes have evolved to exploit a broad spectrum of hosts to continue their life cycle. They employ a network of relationships, where vertebrates (mainly marine mammals and birds, see Tab. 1) act as definitive hosts, and planktonic or pelagic crustaceans serve as first intermediate or transport hosts, with cephalopods or fish serving as transport hosts (Levsen a Berland 2012).

After fertilization in the definitive hosts, females release unembryonated released into the stool to the aquatic environment (Køie 2001). An *Anisakis* female produces around 1.5 million eggs per life (Ángeles-Hernández et al. 2020). While ascaridoid eggs, which are transmitted in terrestrial habitats, have a thick shell, *Anisakis* eggs found in aquatic habitats have a thinner shell. Embryonic development and the first molt of *Anisakis* larvae occur within the floating egg in the water. Available evidence suggests that with the eggs, *Anisakis* larvae undergo two additional molts (L1–L2–L3), resulting in the development of free-swimming L3 larvae. However, other studies reported only one molt (L1–L2) of these larvae in the egg (Klimpel et al. 2004).

The outcome of these freely floating larvae often hinges upon their interactions with marine crustaceans, such as copepods and euphausiids (krill), but also tunicates, which act as the first intermediate host (Fig. 2). These larvae of infect krill such as *Nyctiphanes couchii* (Bell, 1853), or tunicates *Salpa fusiformis* (Cuvier, 1804) (Ángeles-Hernández et al. 2020). Upon successful entry into the intermediate host's body, the larvae undergo a transformative phase characterized by the shedding of their sheathed exterior. This transition occurs within the hemocoel (body cavity) of the invertebrate host but without additional molting of the larvae (Køie et al. 1995).

Third-stage larvae are then transmitted through trophic chains to fish or cephalopod mollusks (second intermediate host), which, as a result, become infectious to the final host (Fig. 1; Gregori et al. 2015). Since anisakid larvae do not develop or molt within fish or cephalopod mollusks, these hosts should be considered paratenic regarding the nematode's life cycle (Pontone et al. 2023). When krill are consumed by various species of fish, such as

herring, mackerel, cod, salmon, or cephalopods like squids, the L3 penetrate the gut wall of these intermediate hosts and migrate to the flesh or body cavity, where they encase themselves in cysts (Anderson 2000). Nevertheless, if any of these paratenic hosts are subsequently consumed by numerous species of marine mammals or birds, the contained larvae undergo maturation into adult worms within the stomach (Muller 2002).

The definitive host, but also men as accidental hosts, are infected alimentary by ingestion of intermediate or paratenic host with L3 larvae (Ángeles-Hernández et al. 2020). Upon ingesting the L3-infected intermediate host, the larvae further develop, transitioning into either larval stage L4 or the adult form. Adult worms reside in the stomachs of their definitive host (Levsen a Berland 2012). This marks the completion of the life cycle, bringing the process full circle. Adult worms reside in the stomachs of their definitive host (Levsen a Berland 2012).
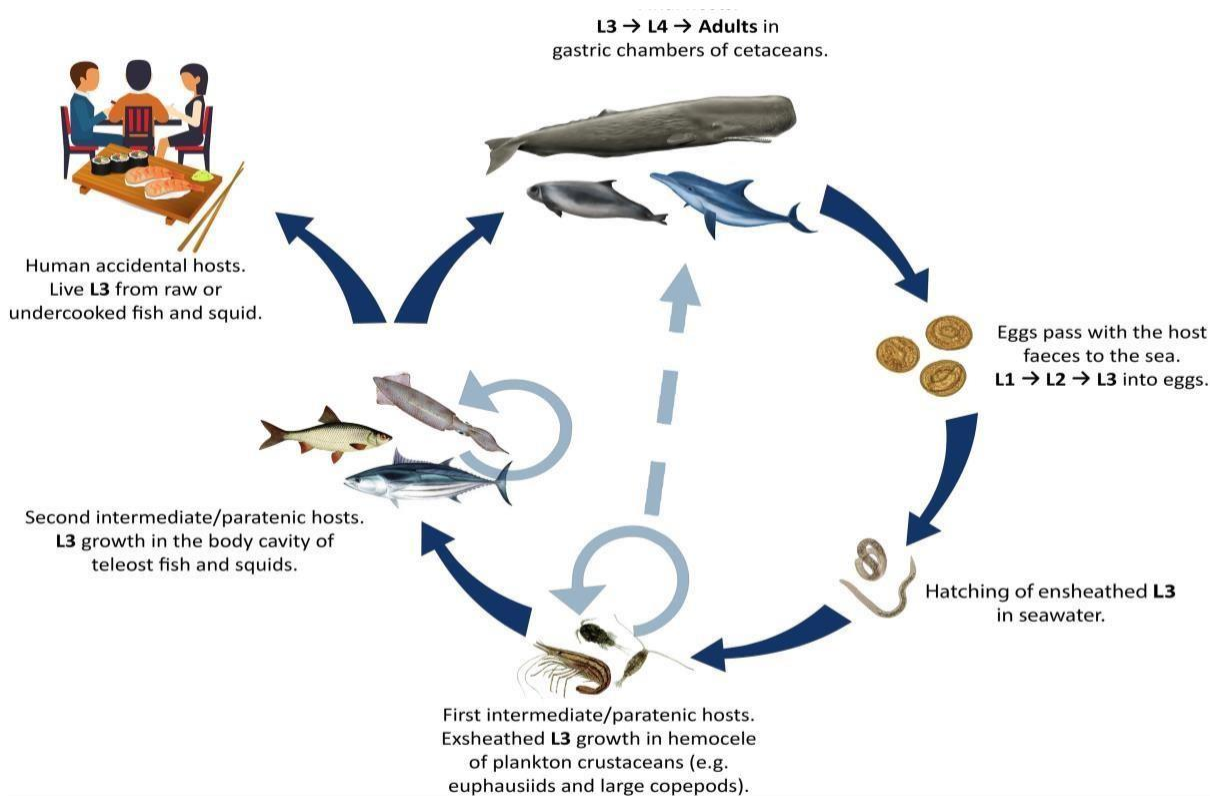
Figure 2. The life cycle of anisakid nematodes from Adroher-Auroux and Benítez-Rodríguez (2020).

3.2 Anisakidosis (anisakiosis) as a human disease

Fish and seafood can serve as potential sources of human infection. Parasitic zoonosis, caused by larvae of anisakids, can infect humans through the consumption of undercooked, partially raw marine animals, including fish, cephalopods, crustaceans (e.g., crabs, shrimp), mollusks (e.g., clams, mussels) (Lymbery a Walters 2014).

So far, only three genera have been reported from humans, namely *Anisakis* (nearly 97% of all cases) and, less commonly, *Phocanema* and *Contracaecum* (Brunet et al. 2017; Tak 2022).

Back in 1988, a group of standardized nomenclature experts put forth three distinct terms: (1) **anisakidosis**, which denotes an infection caused by any members of the family Anisakidae; (2) **anisakiosis**, referring to the infection caused by members of the genus *Anisakis*, (3) **pseudoterranovosis**, to the infection caused by members of the genus *Phocanema* (earlier *Pseudoterranova*) should be known named **phocanemosis**, and (4) **contracoecosis** caused by members of the genus *Contracaecum* (Shamsi a Barton 2023).

The fishes such as herring *Clupea harengus* (from here came the alternative name of the disease as "herring worm disease»), mackerels *Scomber scomber*, *Scomber japonicus*, cod fish *Gadus morhua*, *Theragra chalcogramma*, salmon *Oncorphynchus keta*, skipjack tuna (*Katsuwonus pelamis*), and sardine *Sardina pilchardus* and also cuttlefish *Ascarosepion apama*, or squid *Todarodes pacificus*, are among the primary sources of infections in a different part of the world (Jíra 1998). For example, the most common causative agents of anisakidosis in Japan are mackerel (*Scomber* spp.), skipjack tuna, and "pickled mackerel" (Murata et al. 2021).

The first reported case of anisakidosis was most probably a child experiencing vomiting caused by an "ascarid", identified as as *Ascaris maritima* (Leuckart 1876), documented in 1876 by Leuckart (Myers 1976). Ascaris maritima is an invalid species now considered an anisakid nematode (Shamsi and Barton, 2023). However, the causative agent was not identified until 1960, when Van Thiel in the Netherlands identified the nematode in the eosinophilic phlegmon of the intestine in a patient who had consumed raw herring. This discovery shed light on the connection between the consumption of raw herring and the presence of this nematode larva in the human body (Van Thiel et al. 1960). Later, the first anisakid allergy was documented in 1990 in Japan by Kasuya et al. (1990), who highlighted

the significance of considering anisakids as a potential causative agent of allergic reactions in humans. In 1995, Audicana reported the first documented case of anaphylaxis induced by anisakids. This case provided evidence of the severe allergic reaction that can occur during anisakid infection (Audicana et al. 1995). These studies played a crucial role in raising awareness of anisakids as a potential allergenic agent and contributed to further research and understanding of *Anisakis*-induced allergic reactions.

In most cases, however, the causative agent is simply identified as *Anisakis* sp. L3, and even when the diagnosis indicated *A. simplex*, there was often no conclusive evidence regarding the species' identity. However, identifying the anisakid larvae at the species level is almost impossible by morphology. Molecular methods are necessary to accurately identify these larvae at the species level (Falla-Zuñiga et al. 2021).

Most diagnoses are made based on the presumption that the larvae should be *A. simplex*, but the correct identification is mostly missing (Rahmati et al. 2020). In addition, it is challenging to attempt identification even at the genus level because larvae that infect humans are frequently injured or fractured when removed (Mattiucci et al. 2013). Typically, non-specific serology tests are used to diagnose allergic anisakidosis, as pointed out by Ubeira (2014). So far, there are at least several hundred cases caused by *A. simpex* worldwide and more than 50 cases caused by *A. pegreffii, mainly in the* Mediterranean, Japan, and Korea (Mattiucci et al. 2018). As a result, it is impossible to rule out with certainty the possibility that other anisakid species are capable of infecting humans.

The first cases of pseudoterranovosis (now as phocanemosis) were first identified in North America (Margolis 1977; Lee et al. 1985). So far, around 50 cases of phocanemosis have been reported worldwide, mainly from Japan, Korea, Chile, the USA, France, Italy, Iceland, Denmark, and Canada (Mattiucci et al. 2017). Moreover, three cases caused by *Ph. cattani* in Chile and two cases caused by *Ph. azarasi* in Japan were reported (Mattiucci et al. 2017). Most recently, Nordholm et al. (2020) reported the first infection of the nasal cavity of a 33-year-old patient suffering from rhinitis for 1.5 years, recovered a worm larva from the nose that was also molecularly characterized as *Ph. recipes* (*s.s.*).

Approximately 7 cases of contracoecosis have been reported from the Baltic region, France, Korea, Australia, and Japan, but the causative agent is most probably *C. osculatum* (Nagasawa 2012; Shamsi 2019).

3.2.1 Distribution of anisakidosis

Anisakidosis is reported to have a global incidence of 0.32 cases per 100,000 people (Orphanet 2016). Subsequently, numerous cases of this zoonotic infection have been reported in various countries. However, the highest number of cases have been reported in Japan, where the consumption of raw fish is a common practice (Aibinu et al. 2019) (Fig. 3). The most recent data has been estimated in Japan, with an average annual incidence of 19,737 cases annually from 2018–2019 (Sugiyama et al. 2022). The number of such registrations has increased rapidly in recent years, but only a small number of cases were identified at the species level using molecular methods (Pontone et al. 2023).
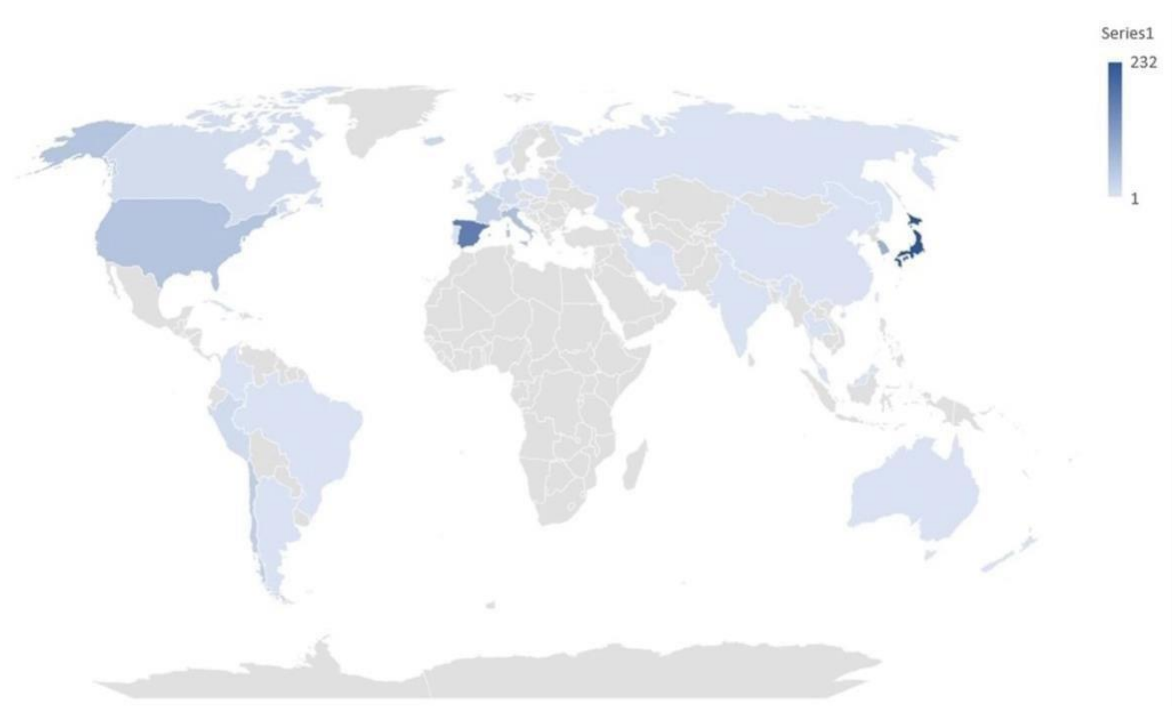
Figure 3. Worldwide distribution of anisakidosis cases (in blue) from Shamsi and Barton (2023).

The first highest incidence of anisakidosis in Europe is reported in Spain, with a total of 2,471 cases reported between 1997 and 2015, with a median incidence rate of 2.93 per 1.000.000 population (Fig. 5A; Herrador et al. 2018). This high incidence of this disease is attributed to the popularity of dishes like "boquerones en vinagre" (marinated anchovies), which often involve the consumption of raw or marinated fish or shellfish (Shamsi a Barton 2023).
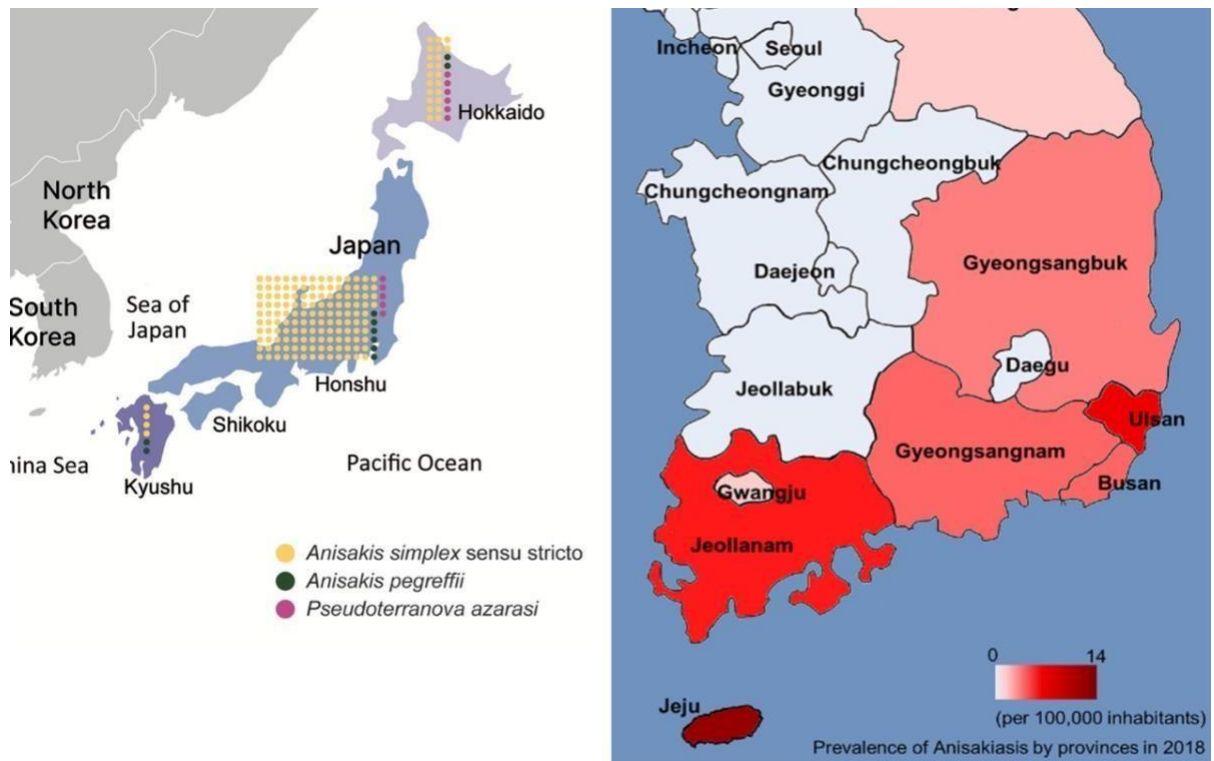
Figure 4. Anisakidosis in Japan and South Korea. Patient number and causative agents of anisakiasis in Japan, 2018–2019 (Sugiyama et al. 2022) on left. Prevalence of anisakidosis by provinces of South Korea, 2011–2018 (Kim et al. 2019) on the right.

Anisakidosis is also very common in South Korea (Fig. 4; Kim et al. 2019). Since the first case was reported in 1971, around 200 cases have been reported annually (Kim et al. 1971). However, the number of cases has increased in recent years to 409 in 2011 and 818 in 2018, making this disease the fastest-growing parasitic disease in the country (Kim et al. 2019). The source of infections in South Korea is most commonly the consumption of raw seafood, like conger, squid, or mackerel (Song et al. 2019). The unexpectedly high proportion of *A. pegreffii* infecting common conger suggests that this species may be responsible for most cases of anisakidosis in South Korea (Lim et al. 2015).

Italy is another European country where anisakidosis has been commonly reported, particularly in regions with a strong tradition of consuming raw or lightly cooked fish and seafood. The number of hospitalizations at the national level has varied from 1.3 to 15.5 out of every 100,000 people annually, with the highest recorded in 2011 (Fig. 5B; Cavallero et al. 2018). Dishes like "sarde a beccafico" (stuffed sardines) and "baccalàmantecato" (creamed cod) are examples of high risk of anisakidosis. The first case in Italy was reported

in 1996 (Guardone et al. 2018). The number of cases of infection in Italy is difficult to estimate because scientific data are scattered, and hospital reports tend to be not published.
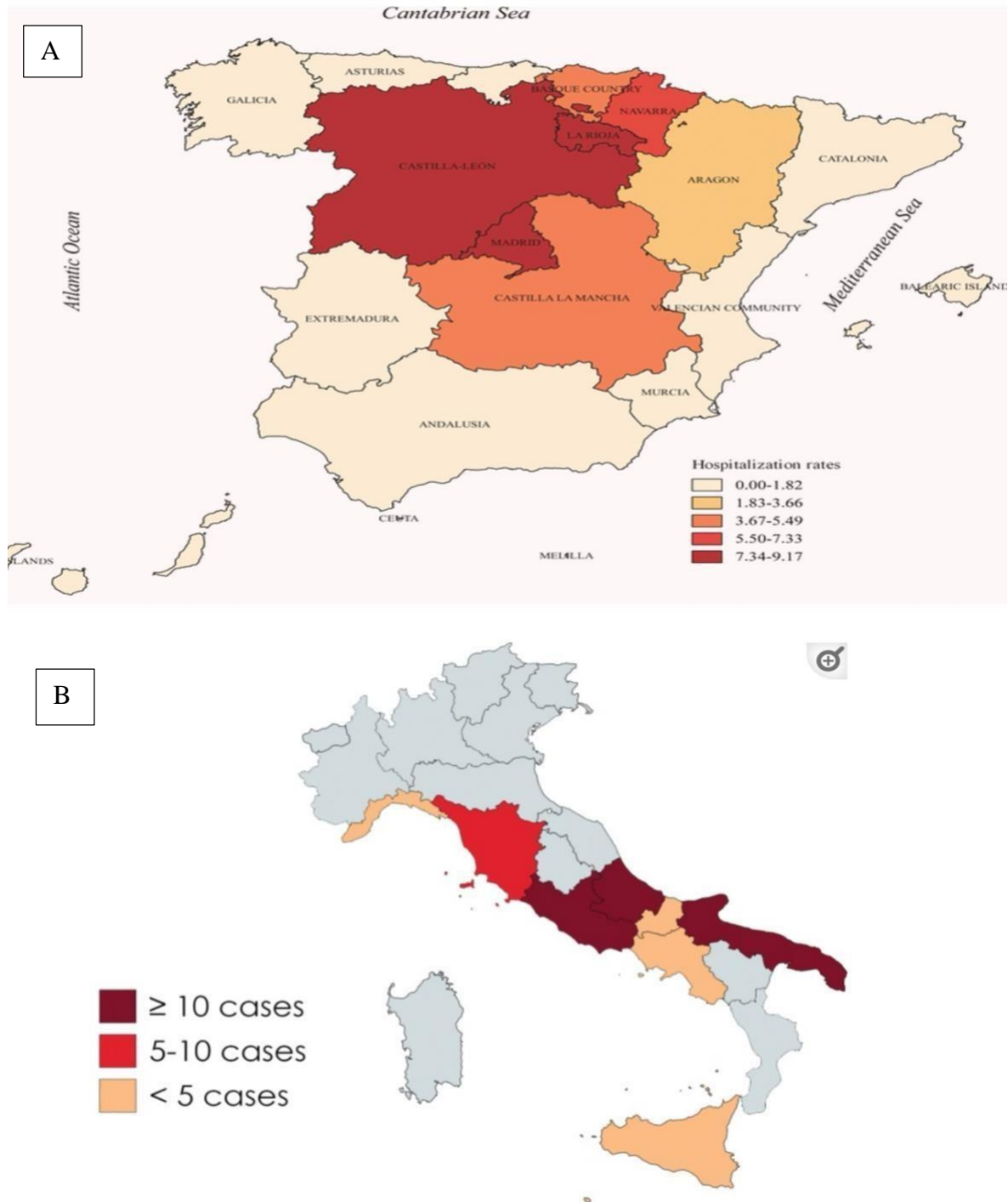
Figure 5. A. Incidence of anisakidosis per 100,000 people in Spain, 1997–2015 (Herrador et al. 2018). B. Map of the distribution of anisakidosis Italy, 1996–2018 (Guardone et al. 2018).

3.2.2 Pathology of anisakidosis

When a human consumes anisakid larva with raw or undercooked fish or seafood, the infested larva enters the tissues of the human digestive tract, specifically the stomach or intestines, and may penetrate the walls of the digestive and reach the surrounding tissues such as the liver, spleen, lungs, vessel walls, or even the brain (Bogitsh et al. 2013). Here, the larva attempts to penetrate the lining of the intestine or stomach and causes inflammation. Depending on where the larvae is located, the first symptoms of the disease may include a feeling of something between the teeth, respiratory symptoms, nasal congestion, «tingling throat syndrome», epigastralgia, nausea, gastric reflux, cough, dysphagia, vomiting, and in some cases, hematemesis due to gastric ulceration (Ángeles-Hernández et al. 2020). Serious illness, sometimes with a severe outcome, can appear when alive larvae penetrate internal organs. In exceptional cases, the parasite may also encyst the esophagus and cause palatine tonsillar infection and eosinophilic esophagitis (Fukui et al. 2020).

The pathogenicity of anisakidosis results from two main mechanisms: direct tissue damage and an allergic response. Four categories of clinical pictures were defined: (i) most common gastric, (ii) intestinal, (iii) ectopic or extra-intestinal by larva migration to the abdomen, and (iv) the generation of allergic responses (Menéndez et al. 2005; Shimamura et al. 2016).

Anisakidosis can manifest as an illness with no symptoms, acute (or subacute) symptoms, or persistent symptoms. Once the larvae enter the internal organs, various degrees of inflammatory reactions, as well as modifications in the permeability of blood vessels, occur. As a response, the body may produce eosinophilic granulomas (granulomas with significant eosinophilic infiltration), granulomas in the submucosa, and edema at the injury site. After being consumed, the larva can survive in the human body for two to three weeks (Arizono et al. 2012).

The first symptoms mainly appeared between 7 and 36 hours after consummation (Brunet et al. 2017; Mizumura et al. 2018).

The larva may penetrate gastric mucosa in around 95% of clinical cases, where it can then be relatively easily spotted attached, causing the host's epigastric pain until it dies or is surgically removed. When an intestinal penetration occurs, the patient may have abdominal distention and severe discomfort that lasts for 5 to 7 days (Ahmed et al. 2016).

The use of computed tomography has been beneficial to view the area with the inflammatory process, which would suggest the existence of the parasite. Gastroscopy in the early stages of the disease allows one to observe the mucosa and the presence of the nematode *in situ* (Mizumura et al. 2018).

Although allergic reactions caused by helminth infection are rare, anisakids frequently do (Baird et al. 2014). The immune system responds to anisakid larvae as an invading pathogen and an allergen, suggesting that these are two aspects of the same response. Clinical signs include urticaria, rhinitis, bronco-constriction, cough, and/or gastrointestinal responses (Audicana a Kennedy 2008). Acute allergic reactions are frequently observed, with no or minimal gastrointestinal issues. However, gastrointestinal symptoms can also happen without an allergic component (Baird et al. 2014). *Aanisakis simplex* (*s.s.*) has been recognized as the parasite with the highest number of known allergens (Pontone et al. 2023). These allergens can be categorized into two groups: (i) somatic allergens originating from dead or live larvae and (ii) excretory-secretory antigens released from larvae. Currently, 14 allergens associated with *A. simplex* have been identified. Notably, certain allergens such as Ani s 1, Ani s 4, Ani s 5, Ani s 8, Ani s 9, and Ani s 10 are thermostable, meaning that allergic reactions can still occur even after consuming dead *A. simplex* larvae. Among these, the following are considered "major allergens": Ani s 1, Ani s 2, Ani s 7, Ani s 12, Ani s 13, and Ani s 14, Ani s 4, and Ani s 6 exhibit resistance to gastric pepsin. Additionally, Ani s 2 and Ani s 3 are recognized as pan-allergens, contributing to cross-reactivity with other sources of food and inhalants (Pontone et al. 2023).

The main immunological response with respect to anisakidosis appears to be Th_2-mediated, leading to the expulsion of the parasite. IgE antibody synthesis, eosinophil, mast cell, and basophil recruitment, goblet cell hyperplasia, smooth muscle contraction, and vascular fluid release are all examples of Th_2-driven immune responses that aid in parasite elimination (Audicana a Kennedy 2008). Patients sensitive to a variety of allergenic proteins produce certain IgE antibodies. These antibodies bind to receptors on the outer surfaces of many different cells, such as mast cells in the skin and digestive system and blood-circulating basophils. When exposed to allergens, the IgE antibody interacts with the allergen, causing a variety of inflammatory mediators to be released by cells. This causes clinical symptoms such as mucus secretion, eosinophil accumulation, and the release of cytokines and histamine. IgE immunoblotting has been used as a diagnostic tool, along with

skin prick tests and direct measurement with the ImmunoCAP system, to examine patients for allergic reactions to recognized *A. simplex* allergens (Baird et al. 2014).

To ensure effective prevention and diagnosis, it is crucial for medical professionals to be knowledgeable about anisakidosis. Specific diagnostic procedures can be employed to identify the causative agent accurately. In cases where medical staff may lack experience with seafood parasites, consulting an expert can be beneficial in achieving accurate diagnoses. By staying informed and seeking appropriate expertise, medical professionals can effectively address cases related to anisakidosis (Ángeles-Hernández et al. 2020).

3.3   Family Anisakidae Railliet et Henry, 1912

The family Anisakidae is a well-known group of parasitic nematodes, mainly of mammals and birds, some of which can cause fish-borne zoonosis (Murata et al. 2011; Shamsi et al. 2020).

The family comprises ten valid genera with approximately 102 valid species (Tab. 1). As adults, they parasitize mainly marine mammals or birds, and members of at least three genera have zoonotic potential (Ferrantelli et al. 2015) (Tab. 1; Fig. 2). The third-sage larvae (L3) of the genus *Anisakis* can cause anisakiosis.

Table 1. List of genera and species included in the family Anisakidae according to the World Register of Marine Species (WoRMS) database.

Genus	Definitive hosts	No. spp.	Zoonotic[1]
Anisakis Dujardin, 1845	cetaceans and pinnipeds	9	×
Contracaecum Railliet et Henry, 1912	pinnipeds and birds	66	×
Euterranova Moravec et Justine, 2020	elasmobranchs	5	
Neoterranova Moravec et Justine, 2020	sharks and reptiles	2	
Phocanema Myers, 1959	pinnipeds	6	×
Phocascaris Höst, 1932	pinnipeds	3	
Pseudoterranova Mozgovoi, 1951	cetaceans	2	
Pulchrascaris Vicente et Santos, 1972	elasmobranchs	4	
Skrjabinisakis Mozgovoi, 1951	cetaceans and pinnipeds	4	
Sulcascaris Hartwich, 1957	sea turtles	1	

[1]Genera with zoonotic potential.

The interrelationships and phylogeny of anisakid nematodes are still not fully understood, but recent molecular studies resolved several problems with polyphyly mainly in genera *Anisakis* and *Pseudoterranova* and resurrected two genera, *Skrjabinisakis* for *Anisakis* clade 3 ("*A.*" *brevispiculata*, "*A.*" *paggiae*, and "*A.*" *physeteris*) by Takano and Sata (2022) and *Phocanema* for most species previously listed in the genus *Pseudoterranova* from pinnipeds by Bao et al. (2023) (Fig. 6).

So far, molecular data and interrelationships are available for 8 of 10 known genera, except genus *Euterranova* and *Phocascaris* (Takano a Sata 2022) (Fig. 6).
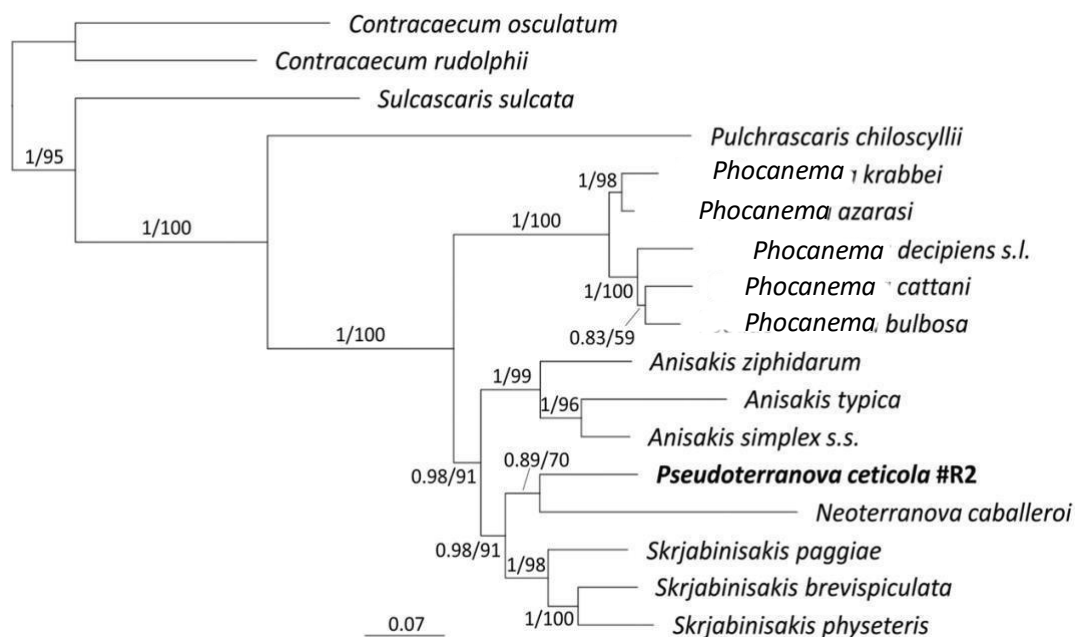
Figure 6. Bayesian phylogeny of Anisakinae based on *cox*1, *cox*2, 12S, and nuclear 28S genes. Modified from Takano and Sata (2022).

Anisakid nematodes are characterized by a dense cross-striated cuticle. The cuticle has rows of spines in certain forms, contributing to their distinctive appearance. They possess three well-developed lips around the mouth. The shape, location, and armament of these lips, along with the presence/absence of interlabia, represent important taxonomic features for classifying these nematodes. The mouth leads into a straight, cylindrical oesophagus, comprising an anterior muscular region and a posterior glandular region known as the ventriculus. The intestine then takes the form of an elongated tube. The posterior

region of this intestinal tract called the rectum, opens into an anus at the posterior end of the nematode body, with three anal glands.

An important morphological difference between anisakid genera is the transition from the ventriculus to the intestine. In some genera (e.g., genus *Contracaecum*), a gastric protrusion, known as the ventricular appendix, and an intestinal process, known as intestinal caecum, is observed (Fig. 7), whereas in others (e.g., genera *Pseudoterranova* a *Phocanema*) only an intestinal caecum is present (Fig. 7). In the genus *Anisakis*, the ventricular appendix and the intestinal caecum are absent. It should also be noted that most of the morphological diagnostic features needed for identification are usually found in adults. For instance, some typical features of adult males, like oral apparatus, spicules, and postanal papillae with a certain number, do not show up in their larval development stages (Grabda 1976). Those features, among others, contribute to the systematic classification and differentiation of anisakids, enhancing our understanding of their biology, ecology, and potential impact on both aquatic ecosystems and human health.

The length of male anisakids ranges between a few millimeters and a few centimeters, depending on the species. They have a slender, cylindrical body with a pointed anterior end and a more rounded posterior end (Fig. 7). The anterior end is characterized by the presence of a mouth surrounded by three small lips. They possess ventrally curved copulatory spicules, which are essential for mating. The spicules are located in the tail region. Females are generally larger than males and have a similar cylindrical body shape. They also possess an anterior mouth, but their posterior end is more rounded and expanded to accommodate the developing larvae. They are characterized by the presence of well-developed vulvae, and the ovaries contain numerous developing eggs, which are later released into the host's digestive system.

Other morphological traits that have been mentioned are those that pertain to the parasites' eggs. The egg surface of *Contracaecum multipapillatum sensu lato* is covered by microvillous and has an uneven look under scanning electron microscopy (SEM) (Molina-Fernández et al. 2017). It has been hypothesized that the eggshell of ascarid parasites contains proteins and mucopolysaccharides, giving it a rough look, as well as grooves or «opercular regions», which act as weak spots that enable the parasites to hatch. The size of the embryonated eggs and the fibrous substance that comprises the eggs might differ from one genus to another, making it challenging to identify certain species based exclusively on

physical traits. The length of the parasite and its association with certain genera may also be related to variations in egg size, structure, and surface. Therefore, in order to develop a more morphological understanding of anisakids, it would be necessary to conduct a more in-depth study of the various eggs of various genera to build a pattern between the shapes, sizes, surfaces, and attributes of embryonated eggs (Ángeles-Hernández et al. 2020).
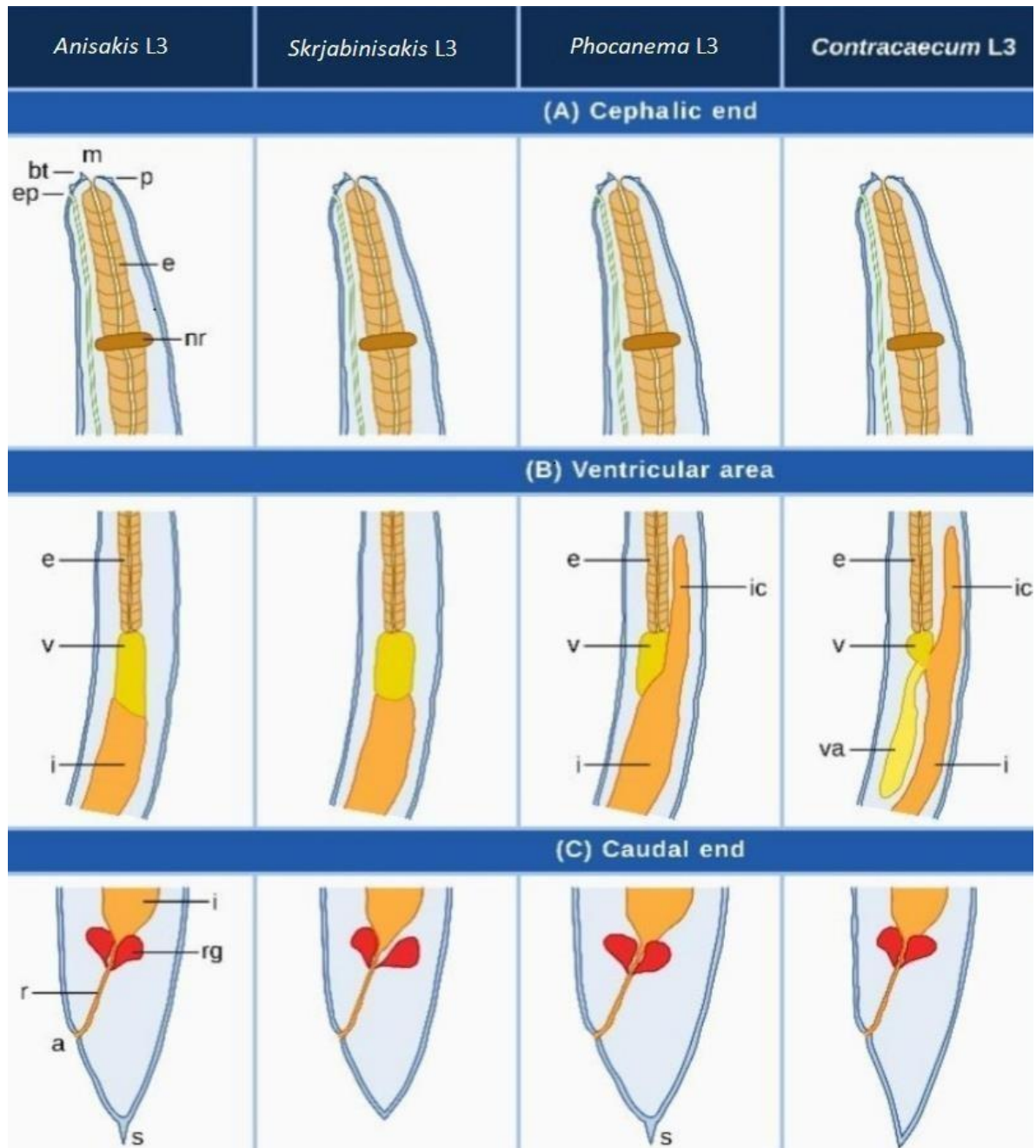
Figure 7. Main morphological features among third-stage larvae (L3) of anisakid nematodes with zoonotic potential. Abbreviations: a, anus; bt, boring tooth; e, esophagus; ep, excretory pore; i, intestine; ic, intestinal caecum; m, mouth; nr, nerve ring; p, papilla; r, rectum; rg; rectal gland; s, spine or mucron; v, ventriculus; va, ventricular appendage. Modified from Javier Adroher-Auroux and Benítez-Rodríguez (2020).

3.3.1 Genus *Anisakis* Dujardin, 1845

Synonyms: *Conocephalus* Diesing, 1860; *Filocapsularia* Deslongchamps, 1824; *Peritrachelius* Diesing, 1851; *Stomachus* Goeze, 1800.

**Type species**: *Anisakis simplex* (Rudolphi, 1809).

The genus *Anisakis* was proposed almost 180 years ago by the French helminthologist Felix Dujardin (1801–1860), who proposed the name *Anisakis* from the Greek ànisos = different and akon = hook.

In total, there are eight valid species described (Tab. 2). Previously, larvae of the genus *Anisakis* were classified into two types, type I and II, based on the length of the ventriculus and the presence or absence of the tailspin (mucron) (Van Hien et al. 2021). However, now the three species ("*A.*" *brevispiculata*, "*A.*" *paggiae*, and "*A.*" *physeteris*) of the type II belong to *Skrjabinisakis* Mozgovoi, 1951 (Takano a Sata 2022).

One of the morphological characteristics of the members of the genus *Anisakis* that vary from other genera is that it lacks interlabia, like was observed above and does not have a ventriculus appendix or intestinal caecum (Davey 1971). The elongated esophagus consists of an anterior muscular part and a posterior glandular one, clearly demarcated into a "ventriculus" (Grabda 1976). In males, the spicules exhibit uneven lengths (Davey 1971). Numerous preanal papillae are present, and postanal papillae include a cluster of three or four pairs situated near the tail tip on the ventral side (Grabda 1976).

Table 2. The list of valid species of the genus *Anisakis* and their definitive hosts is based on the WoRMS database (2023).

Species	Definitive host (family)
Anisakis berlandi Mattiucci, Cipriani, Webb, Paoletti, Marcer, Bellisario, Gibson et Nascetti, 2014	cetacea (Kogiidae)
Anisakis nascettii Mattiucci, Paoletti et Webb, 2009	cetacea (Ziphiidae)
Anisakis oceanica (Johnston et Mawson, 1951)	cetacea and pinnipeds
Anisakis pegreffii Campana-Rouget et Biocca, 1955	cetaceans and pinnipeds
Anisakis similis (Baird, 1853)	pinnipeds
Anisakis simplex (Rudolphi, 1809)	cetaceans and pinnipeds
Anisakis typica (Diesing, 1860)	cetacea (Delphinidae)
Anisakis ziphidarum Paggi, Nascetti, Webb, Mattiucci, Cianchi et Bullini, 1988	cetacea (Ziphiidae)
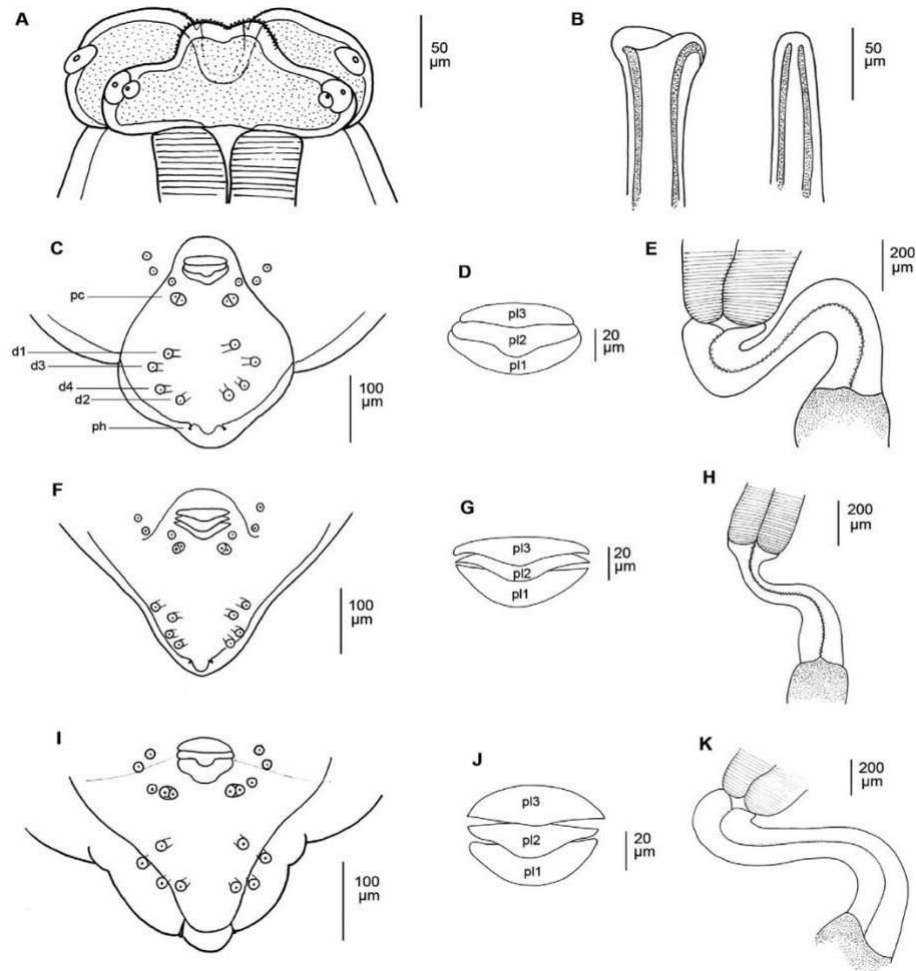
Figure 8. Adults of *Anisakis berlandi*. (A) Anterior end, dorsal view. (B) Proximal and distal ends of the spicules. (C) Posterior end of the male, ventral view. (D) Plectanes - PLs. (E) Ventriculus, anterior end. Adults of *Anisakis pegreffii*. (F) Posterior end of the male, ventral view. (G) PLs; (H) Ventriculus, anterior end. Adults of *Anisakis simplex* (*s.s.*). (I) Posterior end of the male, ventral view. (J) PLs. (K) Ventriculus, anterior end (Mattiucci et al. 2014).

In the field of nematode taxonomy, *Anisakis simplex* is acknowledged as a species complex, denoted as *Anisakis simplex* (*s.l.*) ("*sensu lato*" means "in the broad sense"), indicating a broader classification that incorporates genetic diversity within the species (Mattiuci et al. 2018). This complex included various lineages or genetic variants. Within this broader context, *Anisakis simplex* (*s.s.*) ("*sensu stricto*" means "in the strict sense") designates a more specific and well-defined subset or lineage within the *Anisakis simplex* complex, representing a distinct genetic group.

3.3.2 Genus *Contracaecum* Railliet et Henry, 1912

Synonyms: *Cerascaris* Cobb, 1929; *Kathleena* Leiper et Atkinson, 1914.

Type species: *Contracaecum rudolphii* Hartwich, 1964.

They infect various piscivorous birds and mammals that inhabit freshwater, brackish, and marine environments, including cormorants, pelicans, and seals. Its spread is connected to the distribution of shags or cormorants, the primary definitive host (Baruš et al. 1978).

There are 40 species in this genus, making it the most specious genus in the family (Tab. 3). Furthermore, some species of the genus are represented as complex species based on molecular data. For example, *C. osculatum* is represented by five lineages named A–E and *C. rudolphii* with six lineages named A–F (Mattiucci and Nascetti in 2008; Roca-Geronès et al. 2023). The identification of multiple lineages within a species based on molecular data suggests that what was once perceived as a single species may be a complex of closely related yet genetically distinct species.

Additional notable characteristics of taxonomic significance in members of the genus *Contracaecum* include the development of the ventricular appendix, as shown in Figure 9 in *C. bancrofti*, the length of the cecum of the intestine is two to six times the length of the ventricular appendix (Shamsi et al. 2009). Spicules in males are almost the same length (Shamsi et al. 2009). There are no dentigerous ridges (Anderson et al. 2009). Interlabia and labia are present (Shamsi et al. 2019).

Table 3. The list of valid species of the genus *Contracaecum* Railliet et Henry, 1912 and spectrum of their definitive hosts based on the WoRMS database (2023).

| Species	Definitive host
Contracaecum accipiterum Shen, 1981	fish-eating birds (Accipitridae)
Contracaecum anasi Mawson, 1956	fish-eating birds
Contracaecum andersoni Vevers, 1923	fish-eating birds (Ardeidae)
Contracaecum asturi Xu, 1957	fish-eating birds (Accipitridae)
Contracaecum australe Garbin, Mattiucci, Paoletti, González-Acuña et Nascetti, 2011	fish-eating birds (Phalacrocoracidae)
Contracaecum bancrofti Johnston et Mawson, 1941	fish-eating birds (Pelecanidae)
Contracaecum bioccai Mattiucci, Paoletti, Olivero-Verbel, Baldiris, Arroyo-Salgado et Garbin, 2008	fish-eating birds (Pelecanidae)
Contracaecum bubakii Akram, 1996	fish-eating birds (Phalacrocoracidae)
Contracaecum caballeroi Bravo-Hollis, 1939	fish-eating birds (Anhingidae)
Contracaecum cantonense Xu, 1957	fish-eating birds

Table 3. (Continued).

Contracaecum carlislei Ortlepp, 1938	fish-eating birds (Phalacrocoracidae)
Contracaecum ceylanicum (Linstow, 1904)	fish-eating birds (Australia)
Contracaecum chinense Shen et Wu, 1964	fish-eating birds (Accipitridae)
Contracaecum chubutense Garbin, Diaz, Cremonte et Navone, 2008	fish-eating birds (Phalacrocoracidae)
Contracaecum engonium Baylis et Daubney, 1922	fish-eating birds (Ciconiidae)
Contracaecum erraticum Johnston et Mawson, 1940	marsupials (Australia)
Contracaecum eudyptes Johnston et Mawson, 1953	fish-eating birds (Spheniscidae)
Contracaecum eudyptulae Johnston et Mawson, 1942	fish-eating birds (Spheniscidae)
Contracaecum fagerholmi D'Amelio, Cavallero, Dronen, Barros et Paggi, 2012	fish-eating birds (Pelecanidae)
Contracaecum gibsoni Mattiucci, Paoletti, Solorzano et Nascetti, 2010	fish-eating birds (Pelecanidae)
Contracaecum hagedashiae Sandground, 1933	fish-eating birds (Africa)
Contracaecum haliaeti Baylis et Daubney, 1923	fish-eating birds
Contracaecum heardi Mawson, 1953	fish-eating birds (Spheniscidae)
Contracaecum himeu Yamaguti, 1941	fish-eating birds (Phalacrocoracidae)
Contracaecum jorgei Sardella, Mancini, Salinas, Simões et Luque, 2020	fish-eating birds (Phalacrocoracidae)
Contracaecum magnicollare Johnston et Mawson, 1941	fish-eating birds (Laridae)
Contracaecum magnipapillatum Chapin, 1925	fish-eating birds (Laridae)
Contracaecum margolisi Mattiuci, Cianchi, Paggi, Sardella, Timi, Webb, Bastida, Rodriguez et Bullini, 2003	seals (Otariidae)
Contracaecum matwejewi Layman et Mudrezova, 1926	fish-eating birds (Laridae)
Contracaecum microcephalum (Rudolphi, 1809)	fish-eating birds
Contracaecum micropapillatum (Stossich, 1890)	fish-eating birds (Pelecanidae)
Contracaecum mirounga Nikolskij, 1974	fish-eating birds
Contracaecum mulli (Wedl, 1855)	seals
Contracaecum multipapillatum (Drasche, 1882)	seals
Contracaecum milvi Yamaguti, 1935	fish-eating birds (Accipitridae)
Contracaecum milviense Karokhin, 1937	fish-eating birds (Accipitridae)
Contracaecum mirounga Nikolskij, 1974	seals (Phocidae)
Contracaecum mulli (Wedl, 1855)	fish-eating birds
Contracaecum multipapillatum (Drasche, 1882)	fish-eating birds
Contracaecum nycticoracis Johnston et Mawson, 1941	fish-eating birds (Ardeidae)
Contracaecum ogmorhini Johnston et Mawson, 1941	seals
Contracaecum oschmarini Mozgovoi, 1950	fish-eating birds
Contracaecum osculatum (Rudolphi, 1802)	seals and birds (Spheniscidae)
Contracaecum ovale (Linstow, 1907)	fish-eating birds (Podicipedidae)

20

Table 3. (Continued).

Contracaecum overstreeti Mattiucci, Paoletti, Solorzano et Nascetti, 2010	fish-eating birds
Contracaecum pandionis Sobolev et Sudarikov, 1939	fish-eating birds
Contracaecum papilligerum (Creplin, 1846)	fish-eating birds (Pandionidae)
Contracaecum pelagicum Johnston et Mawson, 1942	fish-eating birds (Spheniscidae)
Contracaecum pelecani Hu, Liu et Qiu, 1997	fish-eating birds (Pelecanidae)
Contracaecum pingi Xu, 1957	fish-eating birds (Accipitridae)
Contracaecum plagiaticium Lent et Freitas, 1948	seals
Contracaecum praestriatum Monnig, 1923	fish-eating birds (Podicipedidae)
Contracaecum prevosti Tchéprakoff, 1966	fish-eating birds (Spheniscidae)
Contracaecum punctatum (Gedoelst, 1916)	fish-eating birds (Pelecanidae)
Contracaecum pyripapillatum Shamsi, Gasser et Beveridge, 2008	fish-eating birds (Pelecanidae)
Contracaecum qudripapillatum Saad, Younis et Rabei, 2018	fish-eating birds (Pelecanidae)
Contracaecum quincuspis Lucker, 1941	fish-eating birds (Anhingidae)
Contracaecum radiatum (Linstow, 1907) Baylis, 1920	seals
Contracaecum rectangulum (von Linstow, 1907)	seals (Phociidae)
Contracaecum rodhaini (Gedoelst, 1916)	fish-eating birds (Anhingidae)
Contracaecum rudolphii Hartwich, 1964	fish-eating birds and seals (Phocidae)
Contracaecum scotti (Leiper et Atkinson, 1914)	fish-eating birds (Diomedeidae)
Contracaecum septentrionale Kreis, 1955	fish-eating birds (Phalacrocoracida)
Contracaecum sinulabiatum Johnston et Mawson, 1941	fish-eating birds (Phalacrocoracida)
Contracaecum tricuspe (Gedoelst, 1916)	fish-eating birds (Anhingidae)
Contracaecum turgidum Chapin, 1925	seals (Phocidae)
Contracaecum umiu Yamaguti, 1941	fish-eating birds
Contracaecum variegatum (Rudolphi, 1809)	fish-eating birds
Contracaecum yamaguti Yamaguti, 1941	fish-eating birds (Anatidae)
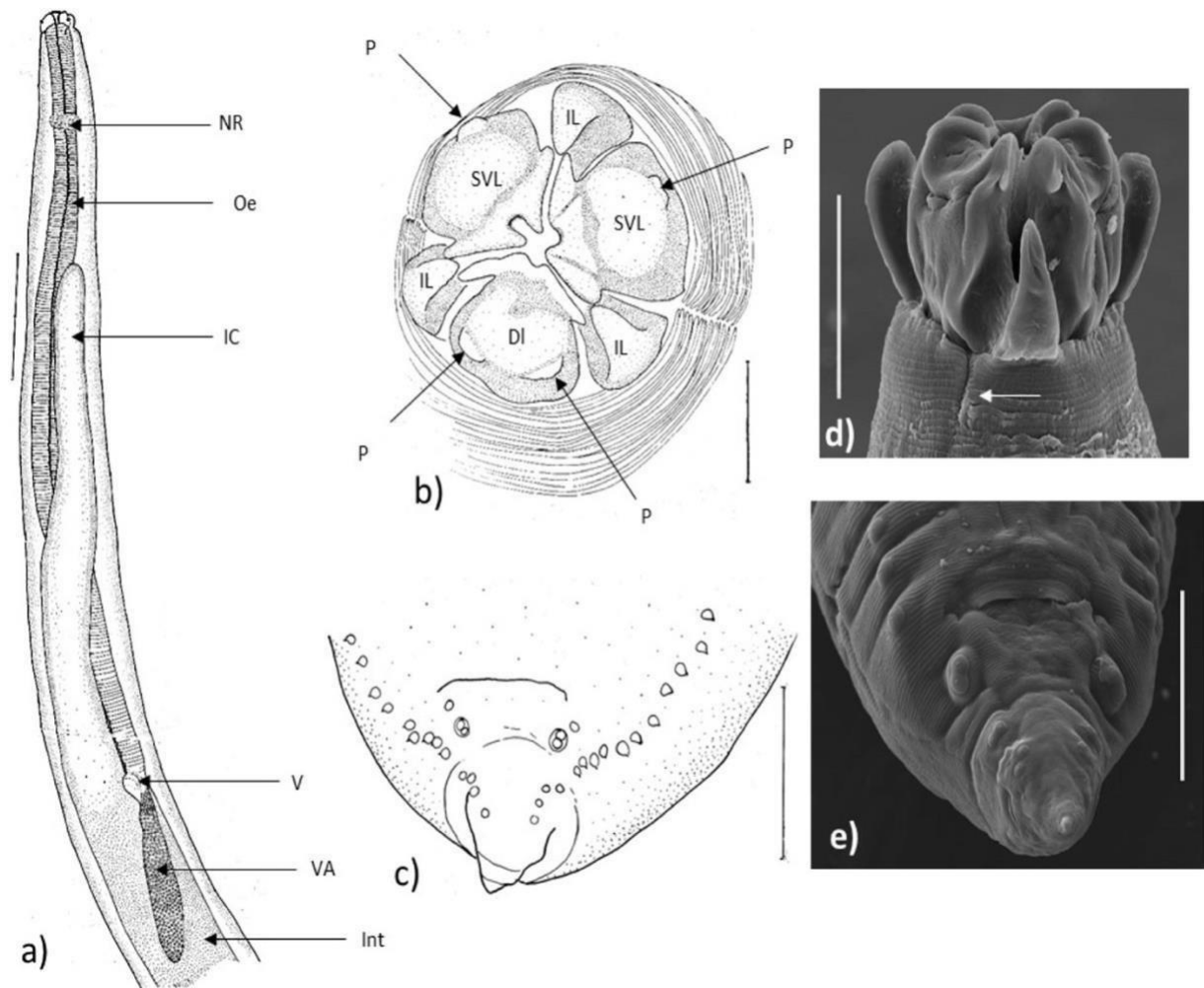
Figure 9. Morphology of *Contracaecum* nematodes: (a) anterior end of *C. bancrofti* showing oesophagus, ventricular appendix, and intestinal caecum; (b) apical view of lips in *C. bancrofti*; (c) posterior end of male (*C. pyripapillatum*), ventral view showing cloacal papillae; (d and e) scanning electron micrographs of *C. bancrofti*, c, showing folded interlabium. Arrow indicating lateral interruption in annulation of collar; (e) ventral view of male tail with three double pairs of post-cloacal papillae. Abbreviations: NR: nerve ring, Oe: oesophagus, IC: intestinal caecum, V: ventriculus, VA: ventricular appendix, Int: intestine, IL: interlabium, SVL: subventral labium, DL: dorsal labium, P: papillum. Modified from Shamsi (2019).

3.3.3 Genus *Phocanema* Myers, 1959

Type species: *Phocanema decipiens* (Krabbe, 1878) Myers, 1959

Five species from pinnipeds have been previously listed in this genus *Pseudoterranova*, but recently, Bao et al. (2023) resurrected the genus *Phocanema* to accommodate species of *Pseudoterranova* from pinnipeds (Tab. 4).

Genus *Pseudoterranova* currently consists of only two species from kogiid whales, namely *Pseudoterranova kogiae* (Johnston et Mawson, 1939) and *Pseudoterranova ceticola* (Deardorff et Overstreet, 1981) (Bao et al. 2023).

According to Bao et al. (2023), nematodes of the genus *Phocanema* are larger than those of *Pseudoterranova*. These worms have a rounded bilobed projection at the end of their dorsal and two ventral lips, with small teeth along the jagged edges. The excretory pore is located between the subventral lips, and one notable anatomical feature is that they lack interlabial lips. Regarding digestive tract characteristics, *Phocanema* nematodes have a projecting cecum of varying lengths among different species, as well as a ventricle but no ventricular process (Bao et al. 2023). Male nematodes have spicules that are approximately equal in length.

Some species may represent a complex of species, such as *P. decipiens*, that is represented by five lineages named A–E (Paggi et al. 1991). These lineages have similar characteristics but are genetically distinct from each other.

Table 4. The list of valid species of the genus *Phocanema* Myers, 1959, and the spectrum of their definitive hosts is based on the WoRMS database (2023) and Bao et al. (2023).

Species	Definitive host
Phocanema azarasi (Yamaguti et Arima, 1942)	pinnipeds
Phocanema bulbosum (Cobb, 1889)	pinnipeds
Phocanema cattani (George-Nascimento et Urrutia, 2000)	pinnipeds
Phocanema decipiens (Krabbe, 1878)	pinnipeds
Phocanema krabbei (Paggi, Mattiucci, Gibson, Berland, Nascetti, Cianchi et Bullini, 2000)	pinnipeds
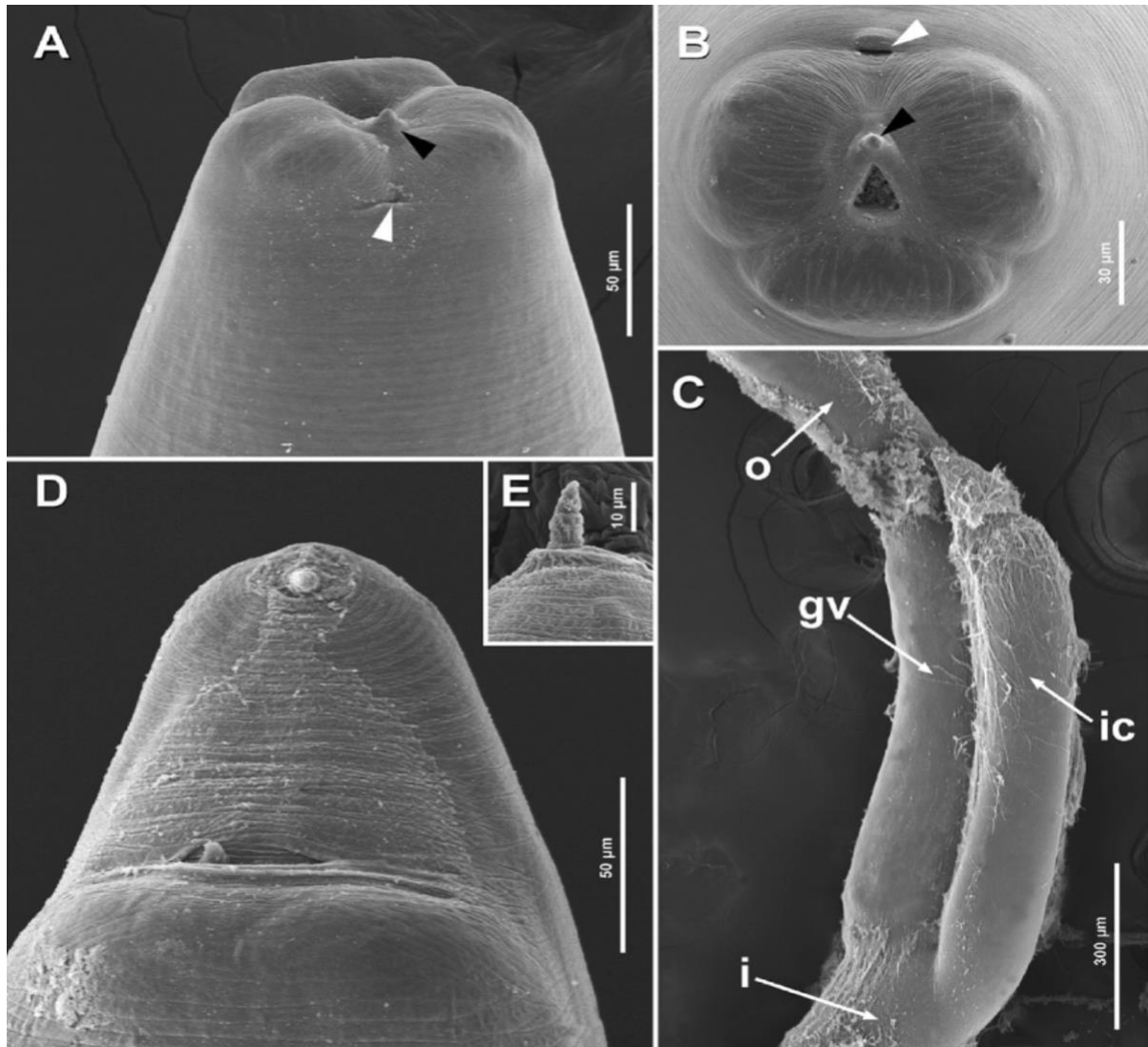
Figure 10. Morphology of *Phocanema cattani* from *Prionotus nudigula*, moreover note that this are L3 larvae collected from fish. A. Anterior end, ventral view. B. Anterior end, apical view. C. Detail of the area of the glandular ventriculus and intestinal caecum of a dissected worm, lateral view. D. Posterior end, ventral view. E. Mucron, lateral view. Black arrowheads point to the boring tooth, and white arrowheads point to the excretory pore. Abbreviations: i, intestine; ic, intestinal caecum; gv, glandular ventriculus; o, oesophagus. Modified from Hernández-Orts et al. (2013).

3.3.4 Other anisakid genera

Most of the genera are known as parasites of marine mammals (genera *Anisakis*, *Phocanema*, *Phocascaris*, *Pseudoterranova*), or less commonly fish-eating birds (*Contracaecum*), but members of the genera *Neoterranova*, *Sulcascaris*, and *Euterranova* genera parasitize sharks and/or reptiles (Tab. 1) (Takano a Sata 2022; Moravec and Justine 2020).

The life cycle of these unique genera is mostly unknown, but larvae of *Sulcascaris* has been identified in the waters of Japan in Ballot's saucer scallop *Ylistrum balloti*, which is a commercial species and may represent some zoonotic potential (Sata a Nakano 2023).

3.4   Identification of larvae

The third-stage larvae (L3) of these nematodes are commonly found in the body cavity, mesenteries, and branchial chambers of various fish species of all oceans. The L3 larvae has a relatively elongated and slender cylindrical body shape with a pointed anterior end and a more rounded posterior end (Tunya et al. 2020). The shape of the mucron found at the posterior end of the nematodes may be different in the members of the genus *Anisakis* and *Contracaecum* mucron are cylindrical-shaped (Tunya et al. 2020). The posterior end of the L3 *Phocanema decipiens* is characterized by a slender mucron that is longer and thinner compared to *Anisakis simplex* (Ishii et al. 1989). This mucron typically exhibits a curved shape in its distal section (Ishii et al. 1989). Morphological traits of anisakid nematodes L3 larvae comprise transverse grooves on the cuticle, contributing to a finely textured surface (Grabda 1976). The ventrolateral (lower) lips in larvae of the genus *Anisakis* feature both single and double papillae, while the dorsal (upper) labium presents two double papillae (Borges et al. 2012). In the L3 larvae of the genus *Contracaecum*, the lips have four papillae – two on the dorsal lip and one on each ventrolateral lip (Younis et al. 2017). A clearly defined, a boring tooth in anisakid larvae is situated between the ventrolateral lips (Borges et al. 2012). The base of the ventrolateral lips in L3 larvae of *Anisakis* and *Phocanema* contains an excretory pore (Fig. 11B, D, E). The excretory pore in members of the genus *Contracaecum* is located anteriorly, specifically below the boring tooth (Younis et al. 2017). At the anterior end of the larvae, a straight and slender esophagus is succeeded by a thicker ventriculus in the posterior direction (Ishii et al. 1989). The ventriculus forms in an oblique junction with the intestine at its posterior end (Ishii et al. 1989). As previously stated, members of the genus *Phocanema* differ from others in the presence of the intestinal cecum, which position extends to the middle section of the ventriculus (Ishii et al. 1989). Their ventricles show an elongated shape (Fig. 11A–C). Members of the genus *Contracaecum* also have a ventricular appendix, which is usually located at the posterior end of the ventricle.
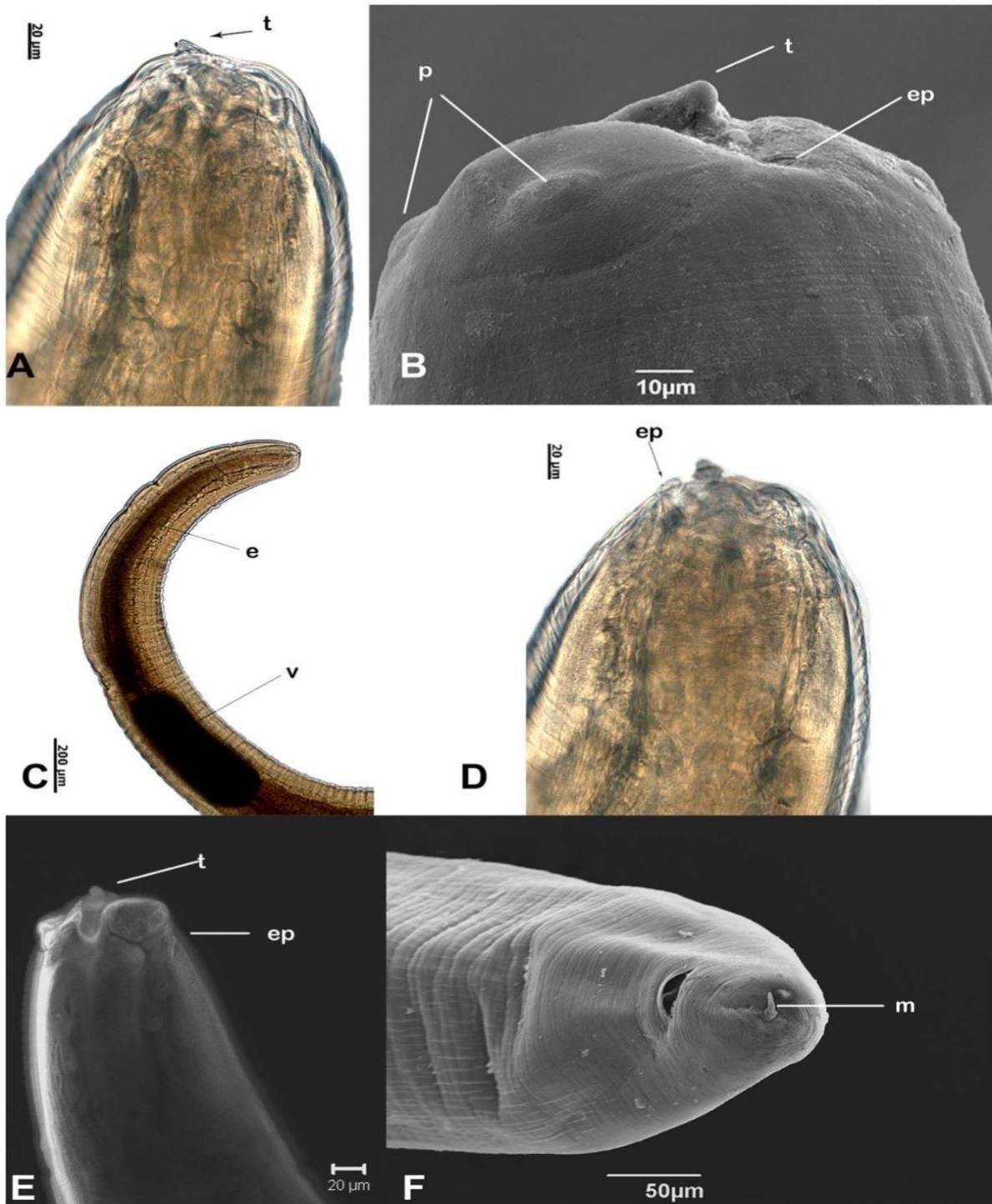
Figure 11. Light, scanning (SEM), and confocal (CLSM) microphotographs of *Anisakis typica* L3 larvae. A. Aanterior end with boring tooth; B. Lips with papilla, boring tooth, and excretory pore; C. Esophagus and ventriculus; D. Position of the excretory pore; E. Detail boring tooth and excretory pore; F. Tail with mucron terminal. Abbreviations: e - esophagus; ep - excretory pore; t - tooth; p - papilla; v - ventriculus; m – mucron. Modofied from Borges et al. (2012).

3.5 Molecular identification

Significant challenges exist in the precise identification of anisakids, primarily stemming from the limitations associated with the morphological characterization of their larval stages (Mattiuci a Nascetti 2008). Consequently, the application of molecular identification techniques is essential for correct species identification of adults as well as larvae of anisakids (Ángeles-Hernández et al. 2020).

Isoenzyme analysis was among the important methods used in the past for species discrimination and/or population studies. Isoenzymes are enzyme variants that differ in amino acid sequence but perform the same chemical reaction. Nonetheless, molecular approaches, primarily based on DNA analysis, prevail today. With the use of specific techniques such as DNA sequencing and PCR, it is now possible to determine more accurate genealogy among anisakids.

Various genetic markers, such as the internal transcribed spacer region (ITS), large and small ribosomal rDNA (28S, 18S), cytochrome *c* oxidase subunits (*cox*1, *cox*2), or mitogenomes have been used to determine the identity of anisakids (Zhang et al. 2018; Seesao et al. 2017; Ahmed et al. 2022).

The *cox*2 gene plays a crucial role in the identification of anisakids. This mitochondrial encodes a component of the cytochrome *c* oxidase enzyme involved in cellular respiration. By analyzing a specific fragment of the *cox*2 gene through PCR methods, we can identify anisakid larvae at the species level (Mattiuci et al. 2011). Furthermore, it has been proven that it provides extra genetic characteristics beneficial for a molecular epidemiological approach to examining anisakiasis in humans (Audicana a Kennedi 2008). This molecular marker is highly polymorphic and particularly helpful for determining the parental species in genera *Anisakis* and *Phocanema* (Aibinu et al. 2019; Bao et al. 2023). In *Contracaecum*, the genes *cox*1 and *cox*2 are often used as molecular markers for identification (Deng et al. 2022). These mitochondrial markers are very useful in identifying closely related species, including potential sibling species of anisakids (Mattiucci et al. 2009).

3.6   World-wide distribution of anisakids

Anisakids have been discovered in all oceans, including the Arctic, Atlantic, Pacific, Indian, and Southern Oceans (Fig. 12, 13; Sakanari a McKerrow 1989). Each ocean has its own unique species composition and distribution patterns of these parasites.
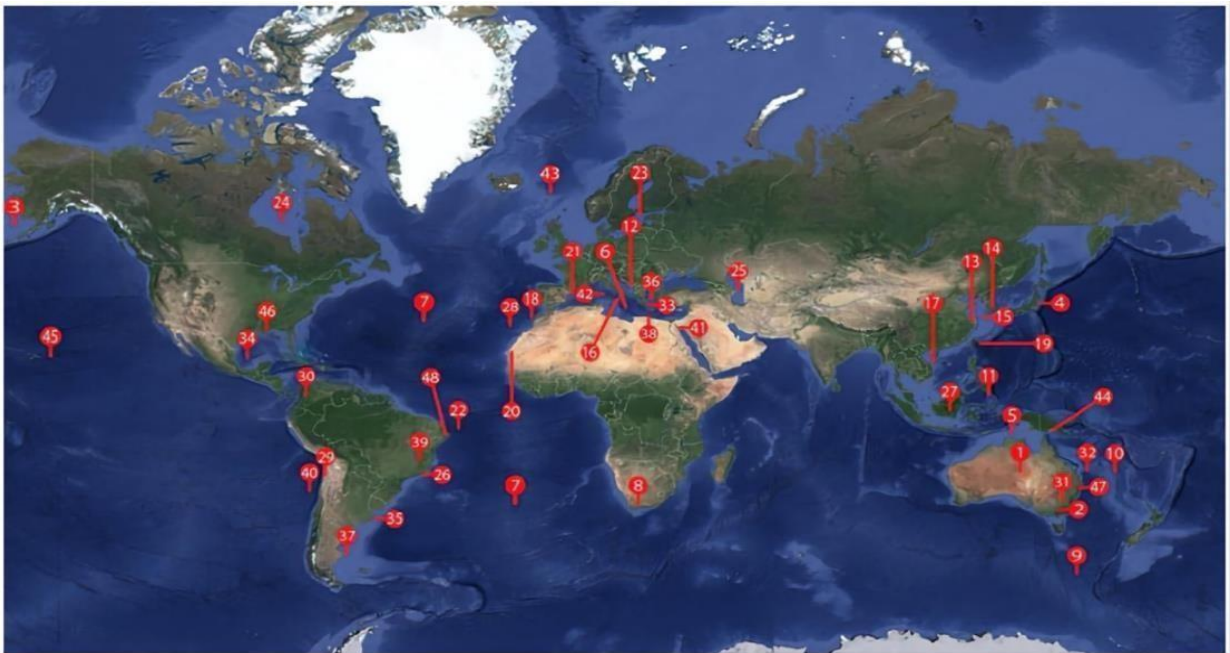
Figure 12. World map with the geographical location of the anisakids after Ángeles-Hernández et al. (2020).

Anisakis species like *A. simplex* predominantly parasitize cetaceans in temperate-cold and polar waters (Davey 1971). The *A. simplex* (*s.s.*) is more dominant in the North of the Northern hemisphere, but *A. pegrefffii* is more common in the tropics, Southern hemisphere, but also in the Mediterranean Sea, and *A. berlandi* is reported predominately from the Southern hemisphere (Fig. 13; Mattiucci et al. 2018).
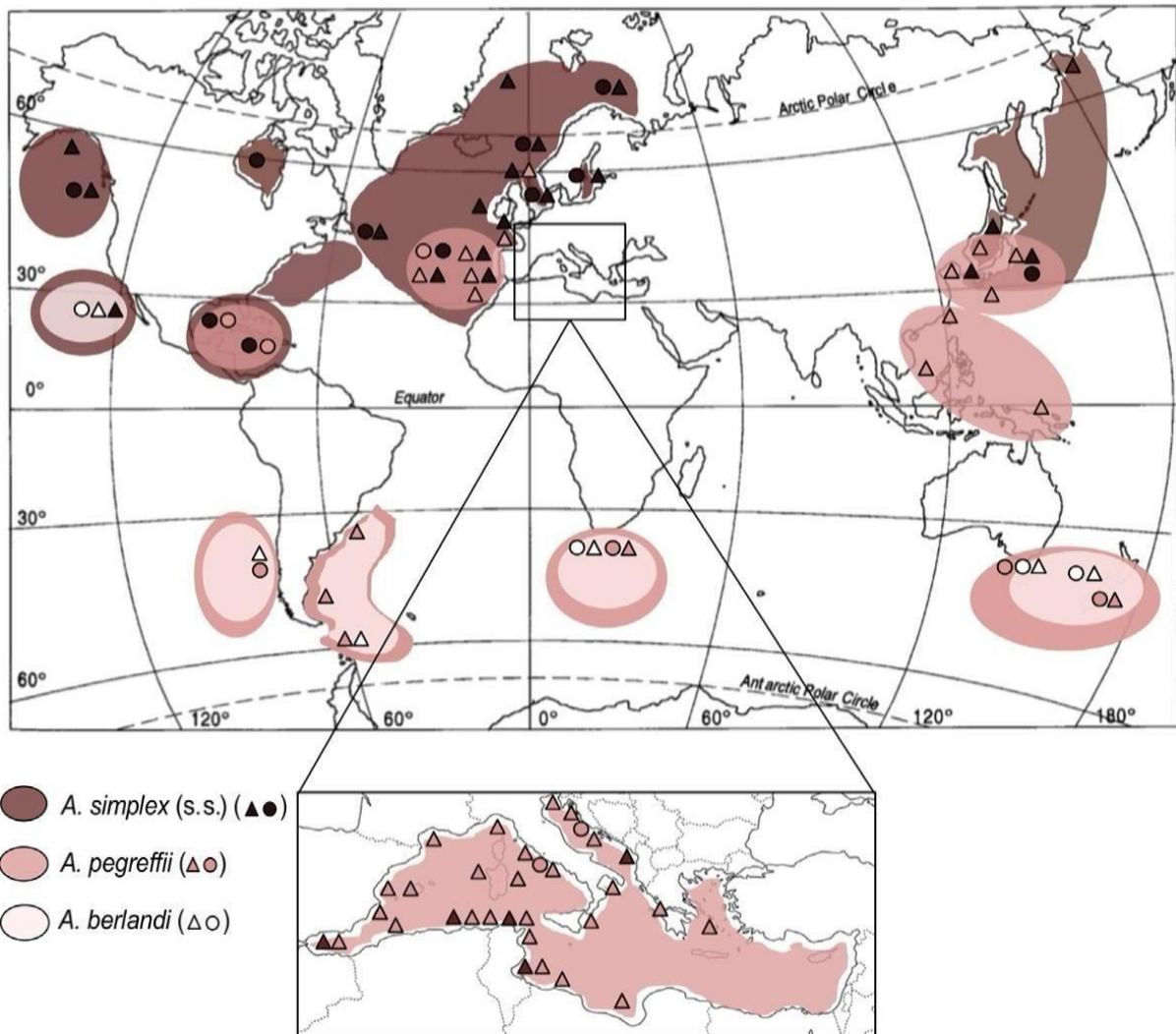
Figure 13. Distribution of *Anisakis simplex* (*s.s.*), *A. pegreffii*, and *A. berlandi*. The symbols are related to the sampling localities of their definitive (circle) and intermediate/paratenic (triangle) hosts, so far identified by molecular markers after Mattiucci et al. (2018).

The species of the genus *Phocanema* also have cosmopolitan distribution with affinity to Arctic waters. Three species, *Phocanema decipiens* (*s.s.*), *Phocanema bulbosa*, and *Phocanema krabbei* are known from the northeastern Atlantic Ocean (Mattiucci et al. 2017). *Phocanema decipiens* (*s.s.*) exhibited the highest suitability in temperate northern regions, particularly in the vicinity of the Baltic and North Seas, as well as in various other areas within the North Atlantic and North Pacific (Alt et al. 2019). Remarkably, high suitability was also discovered along the coasts of Patagonia and the Falkland Islands in the southern hemisphere (Alt et al. 2019). This distribution closely aligns with the habitats of its primary definitive hosts, which are the harbor seal *Phoca vitulina* and the gray seal

Halichoerus grypus (Mattiucci et al. 2017). Furthermore, *P. decipiens* (*s.s.*) has been genetically detected in various fish hosts, such as Atlantic cod (*Gadus morhua*), particularly in the Norwegian and Baltic Seas (Mehrdana et al. 2014). *Phocanema bulbosa* shows similar distribution but has spread towards polar regions, and *Ph. cattani* has a wide distribution in the South hemisphere, particularly in coastal areas of South America like Peru, Chile, and Argentina (Alt et al. 2019). *Phocanema krabbei* is highly distributed in the North Sea, Northeast and Northwest Atlantic, Pacific coast of Alaska, Sea of Japan west of Hokkaido, and northern China Sea and the southern hemisphere off the coasts of Chile, Argentina, and the Bass Strait between Australia and Tasmania (Alt et al. 2019).

Members of the genus *Contracaecum* are most widely distributed because they are distributed in all oceans and freshwater ecosystems of all continents except Antarctica (Shamsi 2019). Detailed data exist from several species, such as *C. osculatum* (*s.s.*) distribution in the Baltic Sea (Mattiucci and Nascetti 2008; Mehrdana et al. 2014; Mattiucci et al. 2017) or *C. chubutensis* and *C. australe* parasitizing cormorants in Latin America (Garbin et al. 2023).

The distribution of anisakids can vary within a specific area due to factors such as water temperature, salinity, and food availability. These factors affect the presence and abundance of both – definitive and intermediate hosts, ultimately influencing the distribution of these parasites. Therefore, to gain insights into their distribution, it is essential to study the feeding behavior of these hosts (Mattiucci a Nascetti 2008). Also, the prevalence of anisakids is influenced by various environmental factors, including hydrographic and climatic conditions. The transmission routes of these parasites are closely connected to the trophic relationships between the final hosts, intermediate hosts, and transport hosts (Mattiucci a Nascetti 2008).

The main factors contributing to the distribution of anisakids include the intentional or accidental discarding of fish and remains during fishing activities (Ángeles-Hernández et al. 2020). Ocean temperature changes resulting from global warming, leading to alterations in latitude, oceanographic conditions, water circulation, and salinity percentages, induce the migration of marine hosts, which, in some cases, can even lead to mortality in species unable to adapt to temperature changes (Gregori et al. 2015). Consequently, this may result in discovering anisakids in previously unobserved geographic areas (Gregori et al. 2015). Distances between continents, ocean surface temperatures, and host depth are other variables

linked to the spread of parasites in parasite-host relationships (Kuhn et al. 2016). Specifically, concerning the distribution of *A. simplex* eggs, one study suggests that *A. simplex* larvae have a limited survival time in freshwater, making their spread into estuaries or rivers improbable due to their intolerance to low salinity (Højgaard 1998). This might be related to the fact that higher water salinity in oceans and seas helps maintain the optimal level of egg hydration, which is vital for their survival and development. Conversely, *Ph. decipiens* can tolerate lower salinity, and salinity has a minimal impact on their hatching success (Højgaard 1998). Additionally, the research confirms that warmer water can increase the likelihood of their life cycle's successful completion.

3.6.1 Anisakids in Antarctic

Antarctica (and the adjacent Southern Ocean) provides a singular natural laboratory to investigate the connections between evolutionary processes and species adaptability to harsh environmental and climatic conditions (Clarke et al. 2007). Low water temperatures, the absence of a coastal zone due to shelf-ice cover, and the frequently stranding drifting icebergs that affect the benthic organisms are just a few of the unusual characteristics that require exceptional adaptations of the flora and fauna. Another distinctive feature is the lack of a clear boundary between the continental shelf and the deep sea. As a result, deep-sea species can also occur in shallower waters, particularly bentho-demersal shallow-water animals can expand their range into the deep sea (Klimpel et al. 2010).

The area of the Southern Hemisphere below 60° S is known for its high diversity of marine mammals (Erbe et al. 2019). There are a total of 18 species of cetaceans and six species of pinnipeds that may serve as definitive hosts of anisakids (Tab. 5; Erbe et al. 2019).

According to the study of Duhamel et al. (2014), the fish of the family Nototheniidae Günther family is the most prevalent and diverse fish family in Antarctic waters. This largely endemic family consists of 107 species primarily associated with demersal habitats, followed by Liparidae Scopoli and Zoarcidae Swainson, with 85 and 40 described species, respectively (Duhamel et al. 2014). These families dominate the benthic fish populations found in the Southern and Antarctic Oceans (Duhamel et al. 2014). As for the meso- and bathypelagic zone, Myctophidae Gill are the most diverse family with 24 known species (Duhamel et al. 2014).

Table 5. List of marine mammals of the Antarctic after Ropert-Coudert et al. (2014).

Species Latin Name	Species Common Name	Sightings <60°S
Mysticetes		
Balaenoptera acutorostrata	Dwarf minke whale	common
Balaenoptera bonaerensis	Antarctic minke whale	regular
Balaenoptera borealis	Sei whale	common
Balaenoptera musculus brevicauda	Pygmy blue whale	rare
Balaenoptera musculus intermedia	Antarctic blue whale	regular
Balaenoptera physalus	Fin whale	regular
Eubalaena australis	Southern right whale	common
Megaptera novaeangliae	Humpback whale	regular
Odontocetes		
Berardius arnuxii	Arnoux's beaked whale	common
Globicephala melas	Long-finned pilot whale	common
Hyperoodon planifrons	Southern bottlenose whale	regular
Lagenorhynchus cruciger	Hourglass dolphin	common
Mesoplodon grayi	Gray's beaked whale	rare
Mesoplodon layardii	Strap-toothed whale	rare
Orcinus orca	Killer whale	regular
Phocoena dioptrica	Spectacled porpoise	rare
Physeter macrocephalus	Sperm whale	regular
Ziphius cavirostris	Cuvier's beaked whale	rare
Pinnipeds		
Arctocephalus gazella	Antarctic fur seal	regular
Hydrurga leptonyx	Leopard seal	regular
Leptonychotes weddellii	Weddell seal	regular
Lobodon carcinophaga	Crabeater seal	regular
Mirounga leonina	Southern elephant seal	regular
Ommatophoca rossii	Ross seal	regular

Several studies have been conducted on helminths of Antarctic fish. Laskowski and Zdzitowiecki (2005) studied helminths from *Notothenia coriiceps* Richardson, 1844 and discovered 16 species of helminths, including two species of anisakids, *Phocanema decipiens*, and *Contracaecum* sp. Recently, Kuzmina et al. (2020) also studied parasite fauna of *N. coriiceps* and morphologically recognized 18 adult helminth species and additional 12 species parasitize it in the larval stage, including at least three unidentified species of nematodes larvae, namely *Phocanema* sp. with prevalence around 95–97%, *Contracaecum* sp. (31–59%), *Anisakis* sp. (2–6%). And later, Kuzmina et al. (2021) reported the diversity of helminths in the fish *P. charcoti*. Among the seven most prevalent species, nematodes of two varieties, namely larvae of *Contracaecum* sp. and *Pseudoterranova* sp., were also identified.

However, in the study notes that none of these species is a specific parasite of this particular host.

Mattiucci et al. (2017) concluded knowledhe about anisakids distributed in Austral hemispheare reported five species of *Anisakis* (*A. berlandi*, *A. pegreffii*, *A. nascettii*, *A. brevispiculata*, and *A. paggiae*), four species *Contracaecum* (*C. osculatum* D, E, *C. miroungae*, and *C. radiatum*), and two species of *Phocanema* (*P. cattani* and *P. decipiens* E).
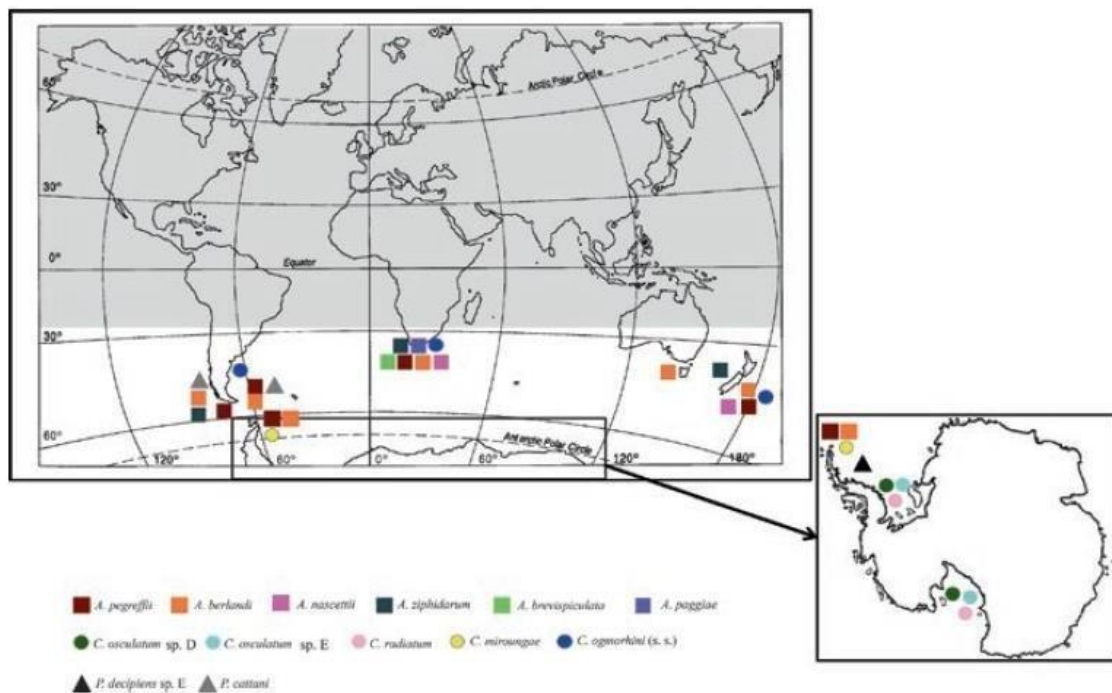
Figure 14. World map highlighting the so far known distribution areas of the anisakids of the genera *Anisakis* (□), *Phocanema* (Δ), and *Contracaecum* (○) in the Austral Region. The geographical areas indicated are those related to the sampling localities for their definitive and intermediate hosts. Modified from Mattiucci et al. (2017).

4. EXPERIMENTAL PART

4.1 Sample collection

Larvae of anisakid nematodes were obtained by T. A. Kuzmina from the mesenteries of 22 fishes of six species of the family Nototheniidae, namely the blackfin icefish *Chaenocephalus aceratus* (Lönnberg) (n = 4), the black rockcod *Notothenia coriiceps* Richardson (n =10), the marbled rockcod *Notothenia rossii* Richardson (n = 2), the Antarctic dragonfish *Parachaenichthys charcoti* (Vaillant) (n = 3), then he emerald rock cod *Trematomus bernacchii* Boulenger (n = 2) and the dusky rock cod *Trematomus newnesi* Boulenger (n = 1) off Galindez Island (Argentine Islands, Wilhelm Archipelago, West Antarctica; 65°15′S, 64°16′W) (Tab. 6). Fishes were captured by angling (depth 10–30 m) in 2019 and 2021. Encapsulated nematode larvae were collected from the body cavity, liver, and stomach wall and were fixed in 70% ethanol. In the laboratory, the nematodes were removed from the capsules, preliminary identified to genus level following Anderson et al. (2009), and then post-fixed in molecular-grade ethanol (96%). This part of the study was done by T. A. Kuzmina from the Schmalhausen Institute of Zoology National Academy of Sciences of Ukraine.

4.2 DNA extraction and sequencing

Total genomic DNA was isolated from small pieces at the midlevel of the body of 41 specimens of *Phocanema* sp. and 44 specimens of *Contracaecum* sp. collected from the six fish species (Tab. 6). The remaining parts of anisakids are kept as hologenophores *sensu* Pleijel et al. (2008) in the Helminthological Collection of the Institute of Parasitology (IPCAS), Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic. Total genomic DNA was extracted using a Monarch® DNA Purification Kit (New England Biolabs, Ipswich, Massachusetts, USA) following the manufacturer's instructions or a standard phenol-chloroform protocol.

Partial fragments of the mitochondrial cytochrome *c* oxidase subunit 2 (*cox*2) gene (600 nt) were amplified using the primers 211 (5′-TTT TCT AGT TAT ATA GAT TGR TTT YAT-3′) and 210 (5′-CAC CAA CTC TTA AAA TTA TC-3′) (Nadler and Hudspeth, 2000). Polymerase chain reaction (PCR) amplifications were performed with 25 µl reactions

34

containing 12.5 µl of AccuStart II PCR SuperMix (Quanta bio, Beverly, Massachusetts, USA), 8.0 µl of RNAse/DNAse free water, 1.5 µl of each primer (10 µM) and 1.5 µl of diluted template DNA. The following thermocycling profile was used for amplification: denaturation of DNA (94°C for 4 min), 25 cycles of amplification (94°C for 30 s, 45°C for 30 s, and 72°C for 50 s), and a final extension at 72°C for 5 min. Results of PCR were verified by electrophoresis of 2 µl of the products in 1 % agarose gel. The rest of the amplicons were purified before sequencing using Exo-SAP-IT Kit (GE Healthcare Life Sciences, UK), following the manufacturer's instructions, and Sanger sequenced with PCR primers at SEQme (Dobříš, Czech Republic). Contiguous sequences were assembled and edited using Geneious® and were compared to those in GenBank using BLASTN (Basic Local Alignment Search Tool) (Altschul et al. 1990)

Table 6. L3 larvae of anisakids from Antarctic fish were used for DNA extraction, and a number of obtained sequences of *cox*2 were in the present study.

Fish host (number of fish)	*Phocanema* sp.		*Contracaecum* sp.	
	isolated	sequenced	isolated	sequenced
Chaenocephalus aceratus (4)	13	1	13	–
Notothenia coriiceps (10)	13	1	11	6
Notothenia rossii (2)	5	–	5	5
Parachaenichthys charcoti (3)	5	–	6	–
Trematomus bernacchii (2)	5	–	5	–
Trematomus newnesi (1)	–	–	4	–
Total (22)	41	2	44	11

4.3   Sequence alignment and phylogenetic analyses

For each anisakid genera, sequences of representative species and lineages were downloaded from GenBank to build a reference dataset (Tab. 7, 8). The outgroup of each dataset was selected following previous analyses on the phylogeny of each anisakid genera (e.g., Mattiucci et al. 2015; Timi et al. 2014). The newly generated sequences were added, and the resulting datasets aligned using the default settings of MAFFT (Katoh a Standley 2013) implemented in Geneious®. The *cox*2 sequences were aligned concerning the amino acid translation using the invertebrate mitochondrial code (transl_table 5). The alignments were trimmed to match the shortest sequence prior to phylogenetic analyses, resulting in 480 nt positions for the dataset of *Contracaecum* spp. and 471 nt positions for the dataset of *Phocanema* spp.

Phylogenetic affinities for the *Contracaecum* spp. and *Phocanema* spp. datasets were evaluated by maximum likelihood (ML) analysis. The best-fit nucleotide substitution models for each gene dataset were determined using ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE v1.6.12 (Chernomor et al. 2016), according to the corrected Akaike information criterion (AICc). Based on the results of the ModelFinder analyses, the model of evolution used for the *cox*2 dataset for *Contracaecum* was TIM3 + F + I + G4, and the model K3Pu + F + I + G4 for the dataset of *Phocanema*. Maximum likelihood analyses were run in IQ-TREE, with 1,000 bootstrap replicates to estimate support values, and the resulting trees were visualized in FigTree v.1.4.4 (Rambaut 2018). Genetic distances (uncorrected p-distance) were calculated with MEGA v11 (Tamura et al. 2021).

Table 7. Species of *Contracaecum* Railliet et Henry, 1912 included in the phylogenetic analyses with data on the host, locality, and GenBank accession number (*cox*2), including the present study in bold. Abbreviations: A, adult; L3, third-stage larva.

Species	St.	Host	Locality	GenBank	Reference
C. osculatum	L3	*Gadus morhua*	Baltic Sea	MT259583	Mohamed et al. (2020)
	A	*Leptonychotes weddellii*	Antarctica	KF718940	Rengifo-Herrera et al. (2014)
	L3	*Theragra chalcogramma*	South Korea	MG012360	Nurhidayat et al. (2018)
C. osculatum A	L3	*Reinhardtius hippoglossoides*	Barents Sea, Norway	JN786334	Rengifo-Herrera et al. (2014)
	A	*Erignathus barbatus*	Canada	EU477203	Mattiucci et al. (2008)
C. osculatum B	L3	*G. morhua*	Greenland	MT448512	Severin et al. (2020)
	L3	*G. morhua*	Greenland	MT448514	Severin et al. (2020)
	L3	*G. morhua*	Greenland	MT448516	Severin et al. (2020)
	L3	*Mallotus villosus*	Barents Sea	KX158866	Levsen et al. (2016)
C. osculatum D	L3	*Chionodraco hamatus*	Antarctica	KC412223	Santoro et al. (2013)
	A	*L. weddellii*	Antarctica	EU477207	Mattiucci et al. (2008)
	L3	*Trematomus bernacchii*	Antarctica	KF771361	unpublished
	L3	*T. hansoni*	Antarctica	KC412227	Santoro et al. (2013)
	L3	*T. hansoni*	Antarctica	KF771354	unpublished
	L3	*T. hansoni*	Antarctica	KT285808	Mattyiucci et al. (2015)
	L3	*T. newnesi*	Antarctica	KF771356	unpublished
	L3	*T. newnesi*	Antarctica	KT285810	Mattyiucci et al. (2015)
C. osculatum E	L3	*C. hamatus*	Antarctica	KF771353	unpublished
	L3	*C. hamatus*	Antarctica	KT285805	Mattyiucci et al. (2015)
	A	*L. weddellii*	Antarctica	EU477205	Mattiucci et al. (2008)
	L3	*Notothenia coriiceps*	Antarctica		**present study**
	L3	*Notothenia rossii*	Antarctica		**present study**
	L3	*T. bernacchii*	Antarctica	KT285807	Mattyiucci et al. (2015)
	L3	*T. hansoni*	Antarctica	KF771355.	unpublished
	L3	*T. newnesi*	Antarctica	KC412229	Santoro et al. (2013)
	L3	*T. newnesi*	Antarctica	KT285811	Mattyiucci et al. (2015)
Outgroup					
Ascaris suum	A	pig	USA	NC001327	Okimoto et al. (1992)
Toxocara canis	A	dog	Sri Lanka	JN593098	Wickramasinghe et al. (2014)

Table 8. Species of *Phocanema* Mozgovoi, 1951, were included in the phylogenetic analyses with host, locality, and GenBank accession number (*cox*2), including the present study in bold. Abbreviations: A, adult; L3, third-stage larva.

| Species	St.	Host	Locality	GenBank	Reference
P. azarasi	A	*Zalophus californianus*	California, USA	MT912411, MT912443	Hrabar et al. (2021)
P. bulbosa	A	*Erignathus barbatus*	Newfoundland, Canada	KU558720	unpublished
P. decipiens	L3	*Gadus morhua* L.	Norway	MT347695, MT347696	Bao et al. (2020)
	L3	*Gadus morhua* L.	Denmark	OK338699, OK338713	Karami et al. (2022)
	L3	man	Denmark	MT624317	Nordholm et al. (2020)
	A	*Cystophora cristata*	Canada	HM147278	Mattiucci et al. (2012)
P. decipiens E	A	*Leptonychotes weddellii*	Antarctica	HM147282	Mattiucci et al. (2012)
	L3	*Chaenocephalus aceratus*	Antarctica		**present study**
	L3	*Notothenia coriiceps*	Antarctica	KC782948	Timi et al. (2014)
	L3	*Notothenia coriiceps*	Antarctica		**present study**
P. cattani	L3	*Acanthistius patachonicus*	Argentina	KC782947	Timi et al. (2014)
	L3	*Atlantoraja castelnaui*	Argentina	MF353898	Irigoitia et al. (2018)
	A	*Arctocephalus australis*	Brazil	KP402087	Jacobus et al. (2016)
	A	*Otaria flavescens*	Argentina	JX500060, JX500061	Timi et al. (2014)
	A	*O. flavescens*	Chile	KC782949	Timi et al. (2014)
	A	*O. flavescens*	Chile	KU558721	unpublished
	L3	*Paralichthys patagonicus*	Argentina	JX500058	Timi et al. (2014)
		Pseudopercis semifasciata	Argentina	JX500059	Timi et al. (2014)
	L3	*Sympterygia bonapartii*	Argentina	MF353899	Irigoitia et al. (2018)
P. krabbei	A	*Halichoerus grypus*	Iceland	HM147279	Mattiucci et al. (2012)
	A	*H. grypus*	Norway	KU558724	unpublished
Phocanema sp.	A	*L. weddellii*	Antarctica	KF718941	Rengifo-Herrera et al. (2014)
Outgroup					
Anisakis nascettii	A	*Mesoplodon mirus*	South Africa	GQ118165	Mattiucci et al. (2009)
A. ziphidarum	A	*Kogia sima*	Philippines	KC821737	Quiazon et al. (2013)

5. RESULTS

A total of 13 *cox*2 (581–624 nt) sequences of anisakids from six species of Antarctic fishes were generated, namely 11 isolates of *Contracaecum* from *Notothenia coriiceps* and *N. rossii*, and two of *Phocanema* from *Chaenocephalus aceratus* and *N. coriiceps* (Tab.6–8).

The phylogenetic analysis for the *cox*2 dataset of *Contracaecum osculatum* species complex resolved at least five lineages (species) (37 sequences; Tab. 7). Our 11 novel sequences clustered in not well-supported clade *C. osculatum* E *sensu* D'Amelio et al. (1990) from several Antarctic fish and one adult isolate from *Leptonychotes weddellii* (Fig. 15). The genetic divergence within this clade was 0–2.5 (0–12 nt).

The phylogenetic analysis of the *cox*2 dataset of *Phocanema* spp. included five clades (species), but mostly with low support (27 sequences; Tab. 8). Our two new sequences clustered in a strongly supported clade *P. decipiens* E *sensu* D'Amelio et al. (1990) with two isolates of adult from Antarctic *L. weddellii* and one isolate from *N. coriiceps* off Chile (Fig. 16). The sequence divergence within this clade was 0–0.6% (0–3 nt). Species of the *P. cattani* from fish and eared seals (Otariidae) off South America form the most basal but non-monophyletic clade with negligible nodal support in the present analysis (Fig. 16).
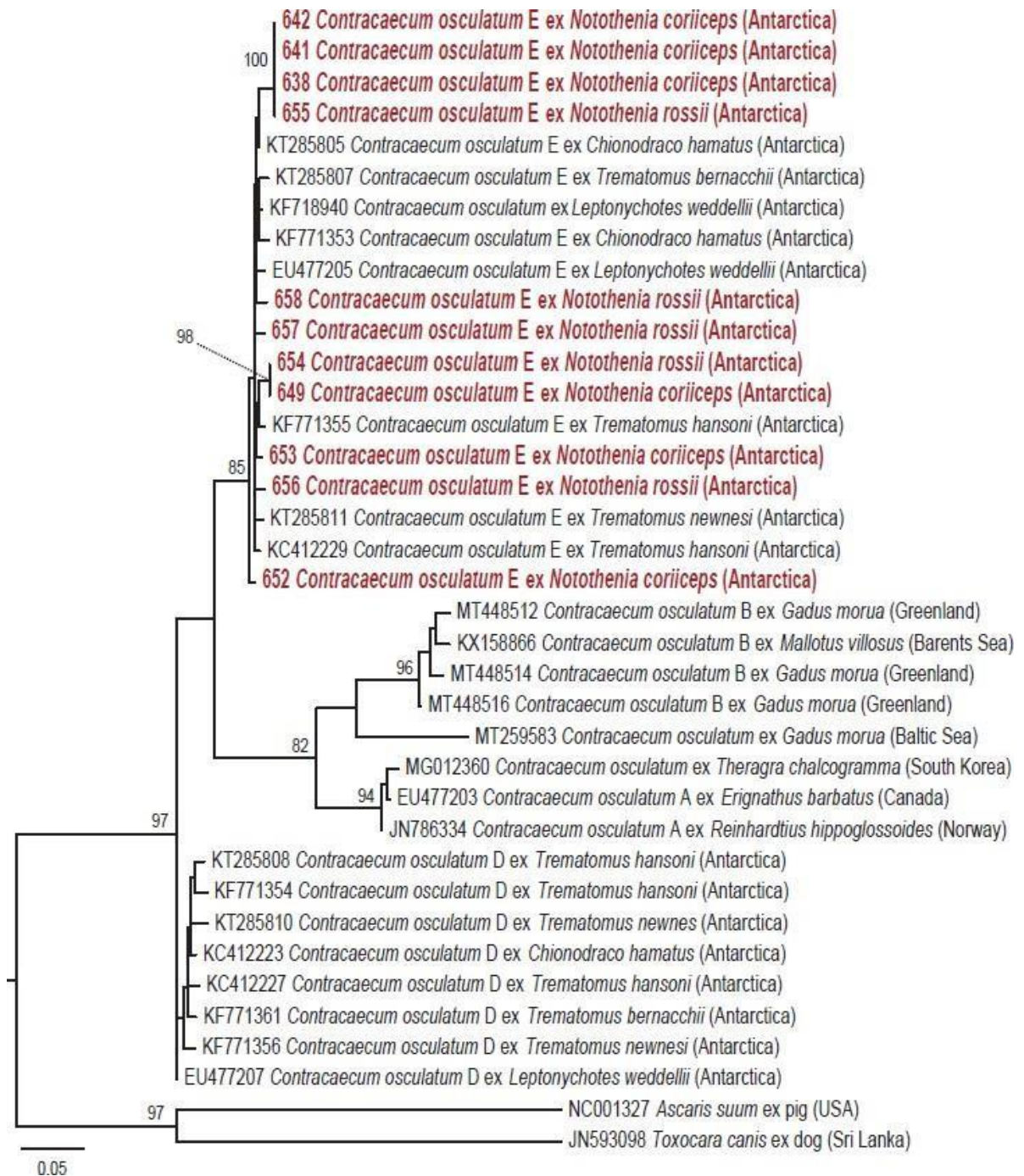
Figure 15. Maximum likelihood (ML) phylogram for the *cox*2 dataset for *Contracaecum* species. Numbers indicate nodal supports from ML analysis (bootstrap values >60% shown only). The newly generated sequences are indicated in red. The scale bar indicates the number of substitutions per site.
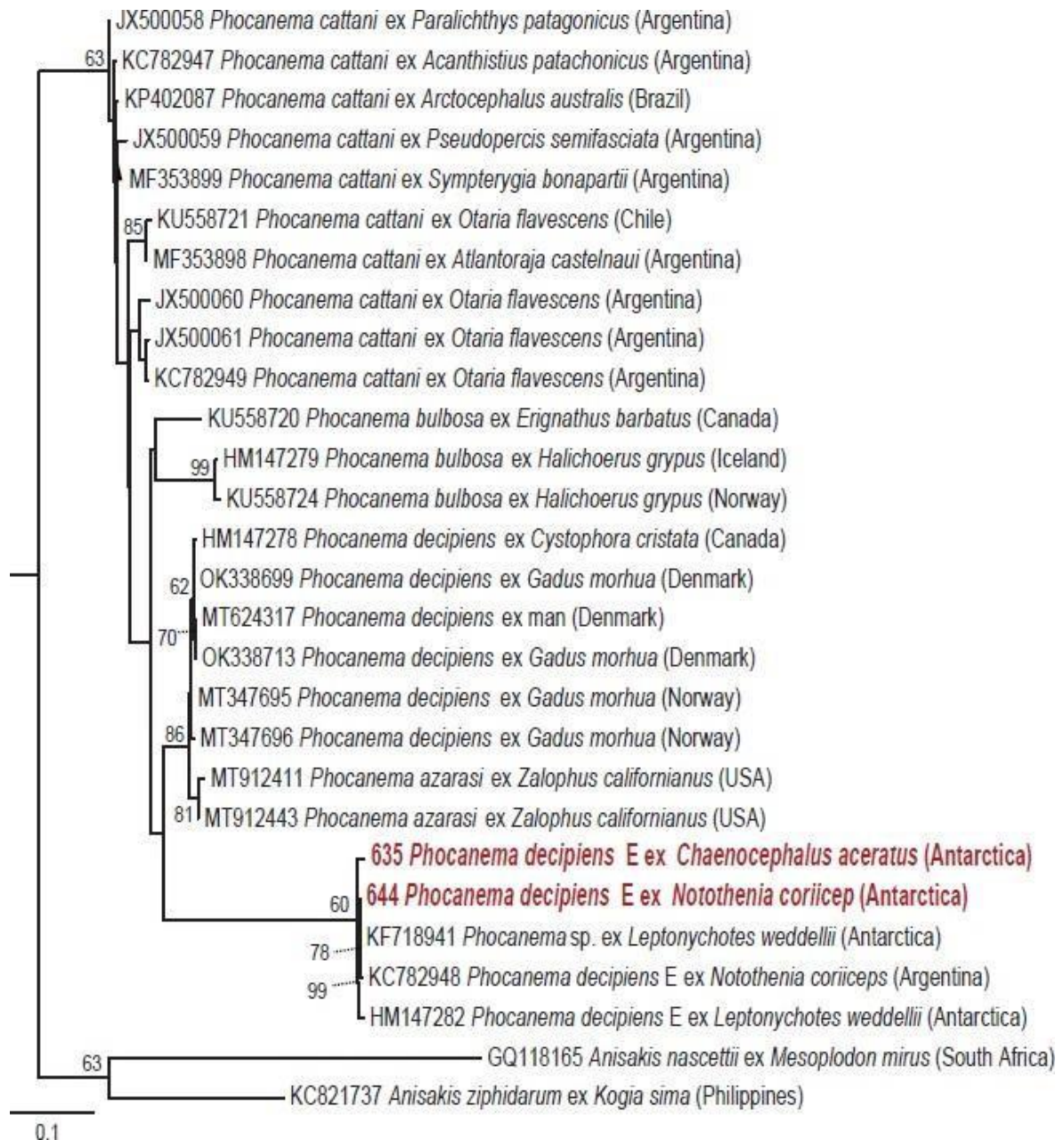
Figure 16. Phylogenetic tree using maximum likelihood for the *cox*2 dataset for *Phocanema* species. Numbers indicate nodal supports from ML analysis (bootstrap values >60% shown only). The newly generated sequences are indicated in red. The scale bar indicates the number of substitutions per site.

6. DISCUSSION

The present study confirmed two species of anisakid larvae from Antarctic nototeniids off Galindez Island, namely from *Chaenocephalus aceratus*, *Notothenia coriiceps*, and *N. rossii* (Tab. 6). 11 isolates from *N. coriiceps* and *N. rossii* belong to *Contracaecum osculatum* E previously confirmed from several Antarctic fishes and Weddell seal (Fig. 15). However, at least one more lineage was confirmed from Antarctic fishes and Weddell seal, namely *Contracaecum osculatum* D, but this taxon was not confirmed in our study. The life cycle of these two taxa was studied in detail by Mattiucci et al. (2015), who distinguished them based on ecology. The life cycle of *C. osculatum* D mainly infects the pelagic intermediate host, however, *C. osculatum* E infects the benthic intermediate host (Fig. 17).
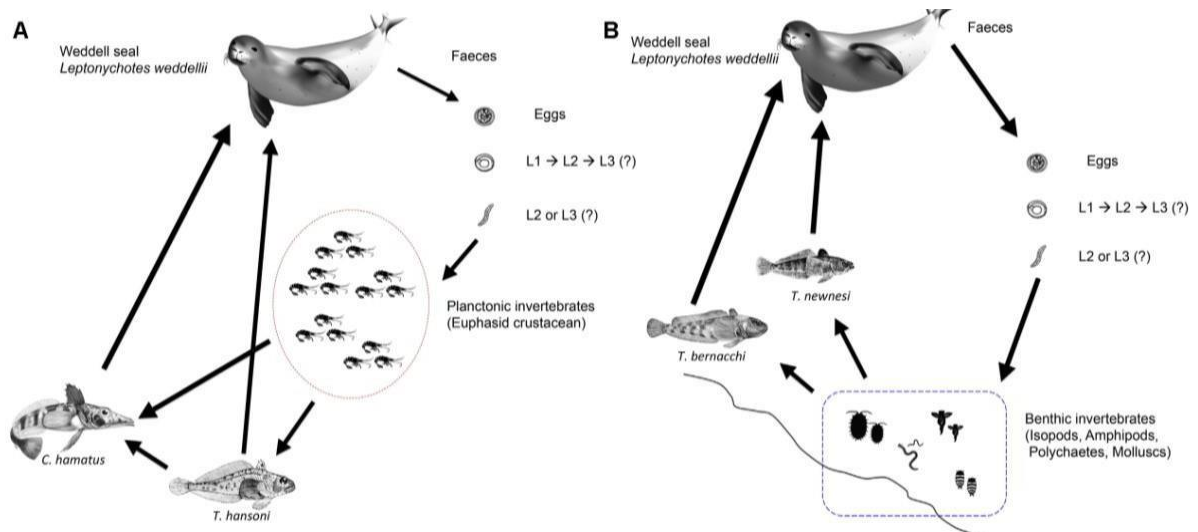
Figure 17. A – Pelagic life cycle of *Contracaecum osculatum* D; B – betic life cycle of *C. osculatum* E (modified from Mattiucci et al. 2015).

In the case of the genus *Phocanema*, only two sequences clustered with *P. decipiens* E from two hosts were obtained (Fig. 16). This lineage is specific for antarctic nototeniids (probably benthic) and Weddell seal in the sub-Antarctic. However, another species, *P. cattani* has been reported from Patagonian waters close to the sub-Arctic from several fish species (but not nototeniids) and several eared seals such as South American fur seal *Arctocephalus australis* (Zimmermann) and South American sea lion *Otaria flavescens* (Péron), but these hosts are not distributed in Antarctic (see Tab. 5; Mattiucci et al. 2017).

Morphometric study between *P. cattani* from Argentina and *P. decipiens* sp. E from Antarctic seas show considerable variations in larval morphology, highlighting the possibility of differentiating them based on exterior traits (Mattiucci et al. 2017).

Previous studies of Kuzmina et al. (2000, 2021) based on morphological evaluation of the helminths from the same material as the present study reported at least three unidentified species of anisakid nematodes in *Notothenia coriiceps* (from 106 hosts) with the highest prevalence of *Phocanema* sp. (95.3%), followed by *Contracaecum* sp. (31.1%), and *Anisakis* sp. (3.9%), and in *Chaenocephalus aceratus* (from 106 hosts) also with the highest prevalence of *Phocanema* sp. (95.5%), followed by *Contracaecum* sp. (72.7%), and *Anisakis* sp. (13.6%). Our results molecularly identified two species of anisakids, namely *C. osculatum* E and *P. decipiens* E (Fig. 15, 16), but future studies may identify much higher diversity. Mattiucci et al. (2017) reported at least seven species of anisakids in the Antarctic, namely *Anisakis berlandi*, *A. pegreffii*, *Contracaecum osculatum* D, E, *C. miroungae*, *C. radiatum*, and *Phocanema decipiens* E (Fig. 14; Mattiucci et al. 2017).

Nevertheless, the results of research on anisacid nematodes illustrate the characteristics of cladogenesis, where morphological differentiation is limited due to the influence of environmental factors (D'Amelio et al. 2000). This process produces a large number of species that are morphologically similar, resulting in a high level of relatedness between them (D'Amelio et al. 2000). However, homoplasy – i.e., the similarity of morphological characters that occur independently of each other due to adaptation to environmental conditions, which can be deceptive in recognising phylogenetic relationships – is also found at a higher taxonomic level (D'Amelio et al. 2000). This underscores the fact that some physical traits are not trustworthy predictors of a species or genus' phylogeny (D'Amelio et al. 2000). The importance of incorporating genetic and molecular techniques to better understand the taxonomy and evolutionary relationships of these nematodes and, consequently, for diagnosing human cases at the species level is evident (Liu et al. 2012).

Molecular markers like ITS-1, ITS-2, and mtDNA *cox*2 genes are useful for phylogenetic analysis and identifying various anisakid nematode species (Timi et al. 2014; Mattiucci et al. 2017). However, the possibility of transcriptomic data, which includes hundreds of genes, gives a more comprehensive and in-depth technique for investigating parasites than using specific molecular markers (Takano a Sata 2022). Transcriptomic data is information about which genes are active in a host organism at any given moment. This

information may include which genes are expressed (generating RNA, which is subsequently translated into proteins) and in what quantities. This makes it possible for researchers to learn more about the genetic mechanisms underlying interactions between parasites and their hosts as well as within parasite populations.

Nototheniid fishes are predators that primarily feed on small pelagic or benthic fish and krill, leading to the active accumulation of anisakid larvae in the fish as paratenic hosts (Klöser et al. 1992). This allows host species to achieve higher population sizes, maintaining a high and stable density of anisakid populations with consistent genetic variability over time. It is also important to note that the presence of these anisakids in Weddell seals does not necessarily imply that clinical symptoms will develop due to the infection (Kerry a Riddle 2009). Dearborn (1965) reported that a high load of nematodes is characteristic of *L. weddellii* in the Antarctic conditions. This shows that regarding reproduction, both anisakid nematodes species can successfully complete their life cycles, as well as sustain high parasitic densities in the Antarctic food web.

Contracaecum osculatum D and E are two species restricted to the waters of Antarctica (Orecchia et al. 1994). The distribution of these lineages has been documented in the Weddell and Ross Seas (Mattiuci a Nascetti 2007). *Contracaecum osculatum* D is presumed to be closely associated with fish species such as *Trematomus hansoni* and *Chionodraco hamatus*, exhibiting a benthopelagic lifestyle and preying on other small fish and Antarctic krill, likely *Euphausia crystallorophias*, the most prevalent euphausiid in the Ross Sea. This observation indicates that a potential first invertebrate host could be an amphipod/polychaete/isopod with a benthic lifestyle (Mattiucci et al. 2015). Larval stages of *C. osculatum* D were identified by allozyme and molecular markers as the main parasite of pelagic and mesopelagic fish hosts belonging to the families Channicthydae and Bathydraconidae (Mattiuci a Nascetti 2007; Timi et al. 2014). Meanwhile, *C. osculatum* E has been identified in *T. bernacchii*, *Notothenia coriacopsis,* and *N. rossii* in the present study, suggesting that benthic organisms are probably the first intermediate hosts for this parasite species (Mattiuci et al. 2017). *Contracaecum osculatum* E has been identified as a major parasite of Antarctic bottom-dwelling fish hosts belonging to the Nothotenidae family in its larval stage. The findings of this research, particularly the detection of this parasite type in fish from Nothotenidae, add to this knowledge (Mattiuci a Nascetti 2007). These findings add to our knowledge of the possible life cycles of *C. osculatum* sp. D and *C. osculatum* sp. E in the Antarctic ecosystem.

The Antarctic region, characterized by its extreme climate and remote geographical location, poses inherent challenges to commercializing its marine resources. Exporting fresh fish from this environment is infrequent, with Antarctic fish resources primarily serving scientific objectives such as ecosystem monitoring and small-scale subsistence activities. Tin et al. (2009) illustrates a significant decrease in *N. rossi* catches from 1970 to 2006. A more recent paper by Hollyman et al. (2021) mentioned the beginning of catch recovery in the Southern Ocean. However, the latest biomass estimate is less than half of the recorded annual catch during 1969–1971. Commercial export of other fish species with anisakids from the Antarctic is rare. However, it is imperative to acknowledge the existence of local subsistence fishing operations and the presence of indigenous communities in proximity to Antarctica. Despite restrictions on exportation, these local communities' consumption of fresh fish introduces a potential avenue for zoonotic transmission. Accordingly, it can be inferred that the absence of documented cases of human infection with these two species may be associated with limited exportation.

7. CONCLUSION

1. The aim of this bachelor thesis was to provide an overview of the biology and systematics of anisakid nematodes with a focus on zoonotic genera. This goal was successfully achieved through a critical review of the existing literature. This study has contributed to a better understanding of the diversity of anisakids, including those found in Antarctica, as well as their life cycle, systematics, key morphological characteristics, and their zoonotic potential.

2. The aim of genotyping anisakid nematodes from Antarctic fish was partially achieved in this study. In particular, two species have been identified, *Contracaecum osculatum* E was successfully identified in 11 samples from *Notothenia coriiceps* and *N. rossii*, while *Phocanema decipiens* E was identified in 2 samples from *Chaenocephalus aceratus* and *Notothenia coriiceps*. However, as only a small proportion of the nematodes were successfully molecularly identified, this study sheds light on the relationships between these parasites in the Antarctic, providing preliminary targets for future, more comprehensive investigations.

8. REFERENCES

Adroher-Auroux, F. J., Benítez-Rodríguez, R. 2020: Anisakiasis and *Anisakis*: An underdiagnosed emerging disease and its main etiological agents. *Research in Veterinary Science*, 132: 535–545.

Ahmed, M., Ayoob, F., Kesavan, M., Gumaste, V., Khalil, A. 2016: Gastrointestinal anisakidosis – watch what you eat. *Cureus*, 8: 1–4.

Ahmed, M., Roberts N. G., Adediran, F., Smythe A, B., Kocot K. M., Holovachov, O. 2022: Phylogenomic analysis of the phylum Nematoda: conflicts and congruences with morphology, 18S rRNA, and mitogenomes. *Frontiers in Ecology and Evolution*, 9: 1–18.

Aibinu, I. E., Smooker, P. M., Lopata, A. L. 2019: *Anisakis* nematodes in fish and shellfish – from infection to allergies. *International Journal for Parasitology: Parasites and Wildlife*, 9: 384–393.

Alt, K. G., Kochmann, J., Klimpel, S., Cunze, S. 2019: Improving species distribution models of zoonotic marine parasites. *Scientific Reports*, 9: 1–7.

Altschul, S. F., Gish, W., Miller, W, Myers, E. W., Lipman, D.J. 1990: Basic local alignment search tool. *Journal of Molecular Biology*, 215: 403–410.

Anderson, R. C., Chabaud, A. G., Willmott, S. 2009: Keys to the nematode parasites of vertebrates. CABI International, Wallingford, pp. 315 + 110.

Ángeles-Hernández, J. C., Gómez-de Anda, F. R., Reyes-Rodríguez, N. E., Vega-Sánchez, V., García-Reyna, P. B., Campos-Montiel, R. G., Calderón-Apodaca, N. L., Salgado-Miranda, C., Zepeda-Velázquez, A. P. 2020: Genera and species of the Anisakidae family

and their geographical distribution. *Animals*, 12: 1–15.

Arizono, N., Yamada, M., Tegoshi, T., Yoshikawa, M. 2012: *Anisakis simplex* sensu stricto and *Anisakis pegreffii*: biological characteristics and pathogenetic potential in human anisakiasis. *Foodborne Pathogens and Disease*, 9: 517–521.

Audicana, M. T., de Corres, L. F., Muñoz, D., Fernández, E., Navarro, J. A., Pozo, M. D. 1995: Recurrent anaphylaxis caused by *Anisakis simplex* parasitizing fish. *Journal of Allergy and Clinical Immunology*, 96: 558–560.

Audicana, M. T., Kennedy, M. W. 2008: *Anisakis simplex*: From obscure infectious worm to inducer of immune hypersensitivity. *Clinical Microbiology Reviews*, 21: 360–379.

Baird, F. J., Gasser, R. B., Jabbar, A., Lopata, A. L. 2014: Foodborne anisakiasis and allergy. *Molecular and Cellular Probes*, 28: 167–174.

Bao, M., Cipriani, P., Giulietti, L., Roiha, I. S., Paoletti, M., Palomba, M., Levsen, A. 2020: Air-dried stockfish of Northeast Arctic cod do not carry viable anisakid nematodes. *Food Control*, 116: 1–6.

Bao, M., Giulietti, L., Levsen, A., Karlsbakk, E. 2023: Resurrection of genus *Phocanema* Myers, 1959, as a genus independent from *Pseudoterranova* Mozgovoĭ, 1953, for nematode species (Anisakidae) parasitic in pinnipeds and cetaceans, respectively. *Parasitology International*, 97: 1–6.

Baruš, V., Sergeeva, P. T., Sonin, D. M., 1978: Helminths of fish-eating birds of the Palaearctic Region I. Nematoda, Springer Netherlands, p. 69.

Bogitsh, B. J., Carter. C.E., Oeltmann, T.N. 2012: Human Parasitology 4. ed., Academic Press, pp. 320–321.

Borges, J. N., Cunha, L. F. G., Santos, H. L. C., Monteiro-Neto, C., Santos, C. P. 2012: Morphological and molecular diagnosis of anisakid nematode larvae from cutlassfish (*Trichiurus lepturus*) off the coast of Rio de Janeiro, Brazil. *PloS ONE*, 7: 1–13.

Brunet, J., Pesson, B., Royant, M., Lemoine, J. P., Pfaff, A. W., Abou-Bacar, A., Candolfi, E. 2017: Molecular diagnosis of *Pseudoterranova decipiens* in human, France. *BMC Infectious Diseases*, 17: 1–5.

Bruschi, F. 2014: Anisakiasis. *Helminth Infections and Their Impact on Global Public Health*, pp. 325–365.

Cavallero, S., Martini, A., Migliara, G., De Vito, C., Iavicoli, S., D'Amelio, S. 2018: Anisakiasis in Italy: analysis of hospital discharge records in the years 2005-2015. *PloS ONE*, 13: 1–11.

Tak, V. 2022: Anisakiasis. *Textbook of Parasitic Zoonoses*. Springer Nature Singapore, pp. 521–528

Chernomor, O., Von Haeseler, A., Minh, B. Q. 2016: Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, 65: 997–1008.

Clarke, A., Murphy, E. J., Meredith, M. P., King, J. C., Peck, L. S., Barnes, D. K., Smith, R. C. 2007: Climate change and the marine ecosystem of the western Antarctic Peninsula. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362: 149–166.

D'Amelio, S., Mathiopoulos, K. D., Santos, C. P., Pugachev, O. N., Webb, S. C., Picanço, M., 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. *International Journal for Parasitology*, 30: 223–226

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., Mathiopoulos, K. D., Santos, C. P., Pugachev, O. N., Webb, S. C., Picanço, M., 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. *International Journal for Parasitology*, 30: 223-226.

Davey, J. T. 1971: A revision of the genus *Anisakis* Dujardin, 1845 (Nematoda: Ascaridata). *Journal of Helminthology*, 45: 51–72.

Dearborn, J. H. 1965. Food of Weddell seals at McMurdo sound, Antarctica. *Journal of Mammalogy*, 46: 37–43.

Deng, Y. P., Li, R., Wang, H. M., Liu, G. H., Tu, Y. 2022. Complete mitochondrial genome of *Contracaecum* sp. (Nematoda: Ascarididae) from Night Herons in China. *Journal of Nematology*, 54: 1–9.

Dezfuli, B. S., Giari, L., Bosi, G. 2021: Survival of metazoan parasites in fish: putting into context the protective immune responses of teleost fish. *Advances in Parasitology*, 112: 77–132.

Duhamel, G., Hulley, P. A., Causse, R., Koubbi, P., Vacchi, M., Pruvost, P., Vigetta S., Irisson J.O., Mormède S., Belchier M., Dettai A., Detrich H.W., Gutt J., Jones C.D., Kock

K.H., Lopez Abellan L.J., Van de Putte, A. 2014: Biogeographic patterns of fish. *Biogeographic Atlas of the Southern Ocean*, 7: 328–329 + 358–359.

Erbe, C., Dähne, M., Gordon, J., Herata, H., Houser, D. S., Koschinski, S., Leaper, R., McCauley, R., Miller, B., Müller, M., Murray, A., Oswald, N.J., Scholik-Schlomer, R.M., Schuster, M., Van Opzeeland, C.I., Janik, V. M. 2019: Managing the effects of noise from ship traffic, seismic surveying and construction on marine mammals in Antarctica. *Frontiers in Marine Science*, 6: 1–4.

Falla-Zuñiga, L, F., Castellanos-Garzón, J, A., Salazar, L., Pustovrh, M, C. 2019: Anisakis and anisakidosis: Case reports and hosts in South America. Systematic review. *Revista de La Facultad de Medicina*, 69: 16–17.

Ferrantelli, V., Costa, A., Graci, S., Buscemi, M. D., Giangrosso, G., Porcarello, C., Palumbo, S., Cammilleri, G. 2015: Anisakid Nematodes as possible markers to trace fish Products. *Italian Journal of Food Safety*, 4: p. 49.

Fukui, S., Matsuo, T., Mori, N. 2020: Palatine tonsillar infection by *Pseudoterranova azarasi*. *The American Journal of Tropical Medicine and Hygiene*, 103: p. 8.

Garbin, L. E., Diaz, J. I., Servian, A., Fusaro, B., Navone, G. T. 2023: The genus *Contracaecum* Raillet et Henry, 1912 (Nematoda: Anisakidae): host and geographical distribution on Neotropical and Antarctic species. *Zootaxa*, 1: 43–62.

Grabda, J. 1976: Studies on the life cycle and morphogenesis of *Anisakis simplex* (Rudolphi, 1809) (Nematoda: Anisakidae) cultured in vitro. *Acta Ichthyologica et Piscatoria*, 6: 119–141.

Gregori, M., Roura, Á., Abollo, E., González, Á. F., Pascual, S. 2015: *Anisakis simplex*

complex (Nematoda: Anisakidae) in zooplankton communities from temperate NE Atlantic waters. *Journal of Natural History*, 49: 755–773.

Guardone, L., Armani, A., Nucera, D., Costanzo, F., Mattiucci, Bruschi, F. 2018: Human anisakiasis in Italy: a retrospective epidemiological study over two decades. *Parasite*, 25: 1–5.

Hernández-Orts, J. S., Aznar, F. J., Blasco-Costa, I., García, N. A., Víllora-Montero, M., Crespo, E. A., Montero, Crespo, A. E., Raga, A. J., Montero, F. E. 2013: Description, microhabitat selection and infection patterns of sealworm larvae (*Pseudoterranova decipiens* species complex, Nematoda: Ascaridoidea) in fishes from Patagonia, Argentina. *Parasites and Vectors*, 6: 1–15.

Herrador, Z., Daschner, Á., Perteguer, J. M., Benito, A. 2018: Epidemiological scenario of Anisakidosis in Spain based on associated hospitalizations: the tip of the iceberg. *Clinical Infectious Diseases*, 1: 69–76

Hodda, M., Peters, L., Traunspurger, W. 2009: Nematode diversity in terrestrial, freshwater aquatic, and marine systems. Nematodes as environmental indicators, CABI Publishing, Oxfordshire, UK: pp. 45–93.

Hollyman, P. R., Hill, S. L., Laptikhovsky, V. V., Belchier, M., Gregory, S., Clement, A., Collins, M. A. 2021. A long road to recovery: dynamics and ecology of the marbled rockcod (*Notothenia rossii*, family: Nototheniidae) at South Georgia, 50 years after overexploitation. *ICES Journal of Marine Science*, 78: 2745–2756.

Højgaard, D. P. 1998: Impact of temperature, salinity, and light on hatching of eggs of *Anisakis simplex* (Nematoda, Anisakidae), isolated by a new method, and some remarks on

survival of larvae. Sarsia, 1: 21–28.

Hrabar, J., Smodlaka, H., Rasouli-Dogaheh, S., Petrić, M., Trumbić, Ž., Palmer, L., Sakamaki, K., Pavelin, T., Mladineo, I. 2021. Phylogeny and pathology of anisakids parasitizing stranded California sea lions (*Zalophus californianus*) in Southern California. *Frontiers in Marine Science*, 8:1–16.

Irigoitia, M. M., Braicovich, P. E., Lanfranchi, A. L., Farber, M. D., Timi, J. T. 2018: Distribution of anisakid nematodes parasitizing rajiform skates under commercial exploitation in the Southwestern Atlantic. *International Journal of Food Microbiology*, 267: 20–28.

Ishii, Y., Fujino, T., Weerasooriya, M. V. 1989: Morphology of Anisakine larvae. *Gastric Anisakiasis in Japan: Epidemiology, Diagnosis, Treatment*, pp. 19–29.

Jacobus, K., Marigo, J., Gastal, S. B., Taniwaki, S. A., Ruoppolo, V., Catão-Dias, J. L., Tseng, F. 2016: Identification of respiratory and gastrointestinal parasites of three species of pinnipeds (*Arctocephalus australis*, *Arctocephalus gazella*, and *Otaria flavescens*) in southern Brazil. *Journal of Zoo and Wildlife Medicine*, 47: 132–140.

Jíra, J. 1998: Lékařská Helmintologie, Helmintoparazitární Nemoci, Galén: pp. 358–359.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K., Von Haeseler, A., Jermiin, L. S. 2017: ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods*, 14: 587–589.

Karami, A. M., Marnis, H., Korbut, R., Zuo, S., Jaafar, R., Duan, Y., Mathiessen, H., Aj-Jubury, A., Kania W.P., Buchmann, K. 2022: Absence of zoonotic parasites in salmonid aquaculture in Denmark: Causes and consequences. *Aquaculture*, 549: 1–5.

Kasuya, S., Hamano, H., Izumi, S. 1990: Mackerel-induced urticaria and Anisakis. Lancet (British edition), 335: p. 665.

Katoh, K., Standley, D. M. 2013: MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30: 772–780.

Kerry, K. R., Riddle, M. J. 2009. Health of Antarctic wildlife: an introduction. *Health of Antarctic Wildlife: A Challenge for Science and Policy*, pp. 1–10.

Kim, C. H., Chung, B. S., Moon, Y. I., Chun, S. H. 1971: A case report on human infection with Anisakis sp. in Korea. *The Korean Journal of Parasitology*, 9: 39–43.

Kim, J. Y., Yi, M-h., Yong, T-S. 2019: Parasitic infections and medical expenses according to health insurance. Review assessment claims data in South Korea, 2011–2018. *PloS ONE*, 14: 1–13.

Klimpel, S., Busch, M. W., Kuhn, T., Rohde, A., Palm, H. W. 2010: The *Anisakis simplex* complex off the South Shetland Islands (Antarctica): endemic populations versus introduction through migratory hosts. *Marine Ecology Progress Series*, 403: 1–11.

Klimpel, S., Palm, H., Rockert, S., Piatkowski, U. 2004: The life cycle of *Anisakis simplex* in the Norwegian Deep (northern North Sea). *Parasitology Research*, 94: 1–9.

Klöser, H., Plötz, J., Palm, H., Bartsch, A., Hubold, G. 1992. Adjustment of anisakid nematode life cycles to the high Antarctic food web as shown by *Contracaecum radiutum* and *C. osculatum* in the Weddell Sea. *Antarctic Science*, 4: 171–178.

Køie, M. 2001: Experimental infections of copepods and sticklebacks *Gasterosteus aculeatus* with small ensheathed and large third-stage larvae of *Anisakis simplex*

(Nematoda, Ascaridoidea, Anisakidae). *Parasitology Research*, 87: 32–36.

Køie, M., Berland, B. and Burt, M.D.B. 1995: Development to third-stage larvae occurs in theeggs of *Anisakis simplex* and *Pseudoterranova decipiens* (Nematoda, Ascaridoidea, Anisakidae). *Canadian Journal of Fisheries and Aquatic Sciences*, 52: 134–139.

Kuhn, T., Cunze, S., Kochmann, J., Klimpel, S. 2016: Environmental variables and definitive host distribution: a habitat suitability modelling for endohelminth parasites in the marine realm. *Scientific Reports*, 1: 1–14.

Kuzmina, T. A., Salganskij, O. O., Lisitsyna, O. I., Korol, E. M. 2020: Helminths of Antarctic rockcod *Notothenia coriiceps* (Perciformes, Nototheniidae) from the "Akademik Vernadsky" Station area (Argentine Islands, West Antarctica): new data on the parasite community. *Zoodiversity*, 54: 99–110.

Kuzmina, T. A., Dykyy, I. V., Salganskij, O. O., Lisitsyna, O. I., Korol, E. M., Kuzmin, Y. I. 2021: Helminth Diversity in Teleost Fishes from the Area of the Ukrainian Antarctic Station "Akademik Vernadsky", Argentine Islands, West Antarctica. *Zoodiversity*, 55: 251–264.

Kuzmina, T. A., Salganskij, O. O., Dykyy, I. V., Lisitsyna, O. I., Korol, E. M., Faltýnková, A., Kuzmin, Y. I. 2021. Helminths of the Antarctic dragonfish, *Parachaenichthys charcoti* (Perciformes, Notothenioidei, Bathydraconidae) studied near Galindez Island (Argentine Islands, West Antarctica). *Acta Parasitologica*, 66: 1424–1430.

Laskowski, Z., Zdzitowiecki, K. 2005: The helminth fauna of some notothenioid fishes collected from the shelf of Argentine Islands, West Antarctica. *Polish Polar Research*, 26: 315–324.

Leuckart, R. 1876: Die menschlichen Parasiten und die von ihren herrührenden Krankheiten, 2,3 lief, Leipzig, pp. 513–882. (In German).

Levsen, A., Berland B. 2012: *Anisakis* species. Fish parasites: pathobiology and protection, CABI Books, pp. 298–309.

Levsen, A., Paoletti, M., Cipriani, P., Nascetti, G., Mattiucci, S. 2016: Species composition and infection dynamics of ascaridoid nematodes in Barents Sea capelin (*Mallotus villosus*) reflecting trophic position of fish host. *Parasitology Research*, 115: 4281–4291.

Lim, H., Jung, B. K., Cho, J., Yooyen, T., Shin, E. H., Chai, J. Y. 2015: Molecular diagnosis of cause of anisakiasis in humans, South Korea. *Emerging Infectious Diseases*, 21: 342–344.

Liu, D. (Ed.). 2011. Molecular detection of human bacterial pathogens. CRC press, pp. 491–492.

Lymbery, A. J., Walters, J. A. 2014: Helminth–Nematode: Anisakid nematodes. Encyclopedia of Food Safety, Elsevier, pp. 78–82.

Mattiucci, S., Cipriani, P., Webb, S. C., Paoletti, M., Marcer, F., Bellisario, B., Gibson, D.I., Nascetti, G. 2014: Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. (Nematoda: Anisakidae). *Journal Parasitology*, 100: 199–214.

Mattiucci, S., Paoletti, M., Webb, S. C., Nascetti, G. 2013: *Pseudoterranova* and *Contracaecum*. Molecular detection of human parasitic pathogens, pp. 645–656.

Mattiucci, S., Nascetti, G. 2008: Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Advances in Parasitology*, 66: 47–148.

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., Nascetti, G. 2018: Molecular epidemiology of *Anisakis* and Anisakiasis: an ecological and evolutionary road map. *Advances in Parasitology*, 99: 3–263.

Mattiucci, S., Cipriani, P., Paoletti, M., Levsen, A., Nascetti, G. 2017: Reviewing biodiversity and epidemiological aspects of anisakid nematodes from the North-east Atlantic Ocean. *Journal of Helminthology*, 4: 422–439.

Mattiucci, S., Cipriani, P., Paoletti, M., Nardi, V., Santoro, M., Bellisario, B., Nascetti, G. 2015: Temporal stability of parasite distribution and genetic variability values of *Contracaecum osculatum* sp. D and *C. osculatum* sp. E (Nematoda: Anisakidae) from fish of the Ross Sea (Antarctica). *International Journal for Parasitology: Parasites and Wildlife*, 4: 356–367.

Mattiucci, S., Fazii, P., De Rosa, A., Paoletti, M., Megna, A. S., Glielmo, A., De Angelis, M., Costa, A., Meucci, C., Calvaruso, V., Sorrentini, I., Palma, G., Bruschi, F., Nascetti, G. 2013: Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection, Italy. *Emerging infectious diseases*, 19: 496–499.

Mattiucci, S., Paoletti, M., Webb, S. C. 2009: *Anisakis nascettii* n. sp. (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. *Systematic Parasitology*, 74: 199–217.

Mattiucci, S., Paoletti, M., Borrini, F., Palumbo, M., Palmieri, R. M., Gomes, V., Casati, A., Nascetti, G. 2011: First molecular identification of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in a paraffin-embedded granuloma taken from a case of human intestinal anisakiasis in Italy. *BMC Infectious Diseases*, 11: 1–6.

Mattiucci, S., Paoletti, M., Cipriani, P., Webb S. C., Timi, J. T., Nascetti, G. 2017. Inventorying biodiversity of anisakid nematodes from the Austral Region: a hotspot of genetic diversity? Biodiversity and evolution of parasitic life in the Southern Ocean. *Parasitology Research Monographs*, 9: 109–140.

Mattiucci, S., Paoletti, M., Webb, S. C., Sardella, N., Timi, J. T., Berland, B., Nascetti, G. 2008: Genetic relationships among species of *Contracaecum* Railliet et Henry, 1912 and *Phocascaris* Höst, 1932 (Nematoda: Anisakidae) from pinnipeds inferred from mitochondrial *cox*2 sequences, and congruence with allozyme data. *Parasite*, 15: 408–419.

McClelland, G., Rohde, K., 2005: Nematoda (roundworms). Marine parasitology. CABI Publishing, Wallingford, pp. 104–115.

Mehrdana, F., Bahlool, Q. Z., Skov, J., Marana, M. H., Sindberg, D., Mundeling, M., Overgaard, C. B., Korbut, R., Strøm, B.S., Kania, W.P., Buchmann, K. 2014: Occurrence of zoonotic nematodes *Pseudoterranova decipiens*, *Contracaecum osculatum* and *Anisakis simplex* in cod (Gadus morhua) from the Baltic Sea. *Veterinary Parasitology*, 205: 581–587.

Menéndez, P., Pardo, R., Delgado, M., León, C. 2005: Mesenteric tumor due to chronic anisakiasis. *Revista Española de Enfermedades Digestivas*, 107: 570–572.

Mizumura, N., Okumura, S., Tsuchihashi, H., Ogawa, M., Kawasaki, M. 2018: A second

attack of *Anisakis*: intestinal anisakiasis following gastric anisakiasis. *ACG Case Reports Journal*, 5: 1–3.

Mohamed, A., Zuo, S., Karami, A. M., Marnis, H., Setyawan, A., Mehrdana, F., Kirkeby, C., Kania, P., Buchmann, K. 2020: *Contracaecum osculatum* (sensu lato) infection of *Gadus morhua* in the Baltic Sea: inter-and intraspecific interactions. *International Journal for Parasitology*, 50: 891–898.

Molina-Fernández, D., Valles-Vega, I., Hernández-Trujillo, S., Adroher, F. J., Benítez, R. 2017: A scanning electron microscopy study of early development in vitro of *Contracaecum multipapillatum* s.l. (Nematoda: Anisakidae) from a brown pelican (*Pelecanus occidentalis*) from the Gulf of California, Mexico. *Parasitology Research*, 116: 2733–2740.

Moravec, F., Justine, J. L. 2020: Erection of *Euterranova* n. gen. and *Neoterranova* n. gen. (Nematoda, Anisakidae), with the description of *E. dentiduplicata* n. sp. and new records of two other anisakid nematodes from sharks off New Caledonia. *Parasite*, 27: 1–13.

Murata, R., Suzuki, J., Kodo, Y., Kobayashi, K., Sadamasu, K., Takano, T., Takashi, I., Tsukasa, W., Ogawa, K. 2021: Probable association between *Anisakis* infection in the muscle of skipjack tuna (*Katsuwonus pelamis*) and human anisakiasis in Tokyo, Japan. *International Journal of Food Microbiology*, 337: 1–6.

Murata, R., Suzuki, J., Sadamasu, K., Kai, A. 2011: Morphological and molecular characterization of *Anisakis* larvae (Nematoda: Anisakidae) in *Beryx splendens* from Japanese waters. *Parasitology International*, 60: 193–198.

Murata, Y., Ando, K., Usui, M., Sugiyama, H., Hayashi, A., Tanemura, A., Hiroyuki, K.,

Naohisa, K., Masashi, K., Shugo, M., Hiroyuki, S., Isaji, S. 2018: A case of hepatic anisakiasis caused by *Pseudoterranova decipiens* mimicking metastatic liver cancer. *BMC Infectious Diseases*, 18: 1–7.

Myers, B. J. 1976: Research then and now on the Anisakidae nematodes. *Transactions of the American Microscopical Society*, 95: 137–142.

Nagasawa, K. 2012: The biology of *Contracaecum osculatum* sensu lato and *C. osculatum* A (Nematoda: Anisakidae) in Japanese waters: a review. *Biosphere Sci*, 51: 61–69.

Nordholm, A., Kurtzhals, J. A. L., Karami, A. M., Kania, P. W., Buchmann, K. 2020: Nasal localization of a *Pseudoterranova decipiens* larva in a Danish patient with suspected allergic rhinitis. *Journal of Helminthology*, 94: 1–3.

Nurhidayat, S. W., Nam, U. H., Kim, J. H. 2018: Occurrence of anisakid nematodes in walleye pollock (*Gadus chalcogrammus*) caught off the East Sea of Korea: their molecular identification and biological implication. *Ocean Science Journal*, 53: 679–689.

Okimoto, R., Macfarlane, J. L., Wolstenholme, D. R. 1992: Evidence for the frequent use of TTG as the translation initiation codon of mitochondrial protein genes in the nematodes, *Ascaris suum* and *Caenorhabditis elegans*. *Nucleic Acids Research*, 18: 6113–6118.

Orecchia, P., Mattiucci, S., D'Amelio, S., Paggi, L., Plötz, J., Cianchi, R., Nascetti, G., Arduino, Bullini, L. 1994: Two new members in the *Contracaecum osculatum* complex (Nematoda, Ascaridoidea) from the Antarctic. *International Journal for Parasitology*, 24: 367–377.

Orphanet. 2016: Prevalence and incidence of rare diseases: Bibliographic data. Report Series: p. 8.

Paggi, L., Nascetti, G., Cianchi, R., Orecchia, P., Mattiucci, S., D'amelio, S., Bersland, B., Brattey, J., Smith, W.J. Bullini, L. 1991. Genetic evidence for three species within *Pseudoterranova decipiens* (Nematoda, Ascaridida, Ascaridoidea) in the North Atlantic and Norwegian and Barents Seas. *International Journal for Parasitology*, 21: 195–212.

Pontone, M., Giovannini, M., Barni, S., Mori, F., Venturini, E., Galli, L., Valleriani, C., Vecillas, L. L., Sackesen, C., Lopata, A. L., Buyuktiryaki, B. 2023: IgE-mediated *Anisakis* allergy in children. *Allergologia et Immunopathologia*, 51: 98–109.

Quiazon, K. M. A., Santos, M. D., Yoshinaga, T. 2013: *Anisakis* species (Nematoda: Anisakidae) of Dwarf Sperm Whale Kogia sima (Owen, 1866) stranded off the Pacific coast of southern Philippine archipelago. *Veterinary Parasitology*, 197: 221–230.

Rahmati, A. R., Kiani, B., Afshari, A., Moghaddas, E., Williams, M., Shamsi, S. 2020: World-wide prevalence of *Anisakis* larvae in fish and its relationship to human allergic anisakiasis: a systematic review. *Parasitology Research*, 119: 3585–3594.

Rambaut, A. 2018: FigTree version 1.4. 4. Computer program and documentation distributed by the author.

Ropert-Coudert Y., Hindell M.A., Phillips R., Charrassin J.B., Trudelle L., Raymond B., 2014. Chapter 8. Biogeographic patterns of birds and mammals. Biogeographic atlas of the Southern Ocean. Scientific Committee on Antarctic Research, Cambridge, pp. 364–387.

Rengifo-Herrera, C., Ferre, I., Ortega-Mora, L. M., Rojo-Montejo, S., García-Moreno, F. T., García-Párraga, D., Garcia-Pena, J. F., Pereira-Bueno, J., Pedraza-Díaz, S. 2014: Helminth parasites found in faecal samples of phocids from the Antarctic Peninsula. *Polar Biology*, 37: 685–695.

Roca-Geronès, X., Fisa, R., Montoliu, I., Casadevall, M., Tobella, C., Bas, J. M., Palomba, M., Mattiucci, S. 2023: Genetic diversity of *Contracaecum rudolphii* sp. A (Nematoda: Anisakidae) parasitizing the European Shag *Phalacrocorax aristotelis desmarestii* from the Spanish Mediterranean coast. *Frontiers in Veterinary Science*, 10: 1–8.

Sakanari, J.A., McKerrow, J.H. 1989: Anisakiasis. *Clinical Microbiology*, 3: 278–84.

Santoro, M., Mattiucci, S., Work, T., Cimmaruta, R., Nardi, V., Cipriani, P., Bellisario, B., Nascetti, G. 2013: Parasitic infection by larval helminths in Antarctic fishes: pathological changes and impact on the host body condition index. *Diseases of Aquatic Organisms*, 105: 139–148.

Sata, N., Nakano, T. 2023: Molecular analysis of larvae suggests the existence of a second species of *Sulcascaris* (Nematoda: Anisakidae: Anisakinae) in the Japanese moon scallop (*Ylistrum japonicum*) from Japanese waters. *Parasitology International*, 92: 1–5.

Seesao, Y., Gay, M., Merlin, S., Viscogliosi, E., Aliouat-Denis, C. M., Audebert, C. 2017: A review of methods for nematode identification. *Journal of Microbiological Methods*, 138: 37–49.

Severin, N. L., Yurchenko, M., Sørensen, J. S., Zuo, S., Karami, A. M., Kania, P. W., Buchmann, K. 2020: Anisakid nematode larvae in the liver of Atlantic cod *Gadus morhua* L. from West Greenland. *Parasitology Research*, 119: 3233–3241.

Shamsi, S., 2019: Parasite loss or parasite gain? Story of *Contracaecum* nematodes in antipodean waters. *Parasite Epidemiology, and Control*, 4: 1–6.

Shamsi, S., Barton, D. P. 2023: A critical review of anisakidosis cases occurring globally. *Parasitology Research*, 122: 1733–1745

Shamsi, S., Norman, R., Gasser, R., Beveridge, I. 2009: Genetic and morphological evidence for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) (Nematoda: Anisakidae) in Australia. *Parasitology Research*, 105: 529–538.

Shimamura, Y., Muwanwella, N., Chandran, S., Kandel, G., Marcon, N. 2016: Common symptoms from an uncommon infection: gastrointestinal anisakiasis. *Canadian Journal of Gastroenterology and Hepatology*: pp. 1–5.

Song, H., Jung, B. K., Cho, J., Chang, T., Huh, S., Chai, J. Y. 2019: Molecular identification of *Anisakis* larvae extracted by gastrointestinal endoscopy from health check-up patients in Korea. *The Korean Journal of Parasitology* 57, 207: 207–211.

Sugiyama, H., Shiroyama, M., Yamamato, I., Ishikawa, T., Morishima, Y. 2022: Anisakiasis Annual Incidence and Causative Species, Japan, 2018–2019. *Emerging Infectious Diseases Journal – CDC*, 10: 2105–2108.

Takano, T., and Sata, N. 2022: Multigene phylogenetic analysis reveals non-monophyly of *Anisakis* s. l. and *Pseudoterranova* (Nematoda: Anisakidae). *Parasitology International*, 91: 1–5.

Tamura, K., Stecher, G., Kumar, S. 2021: MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38: 3022–3027.

Timi, J. T., Paoletti, M., Cimmaruta, R., Lanfranchi, A. L., Alarcos, A. J., Garbin, L., George-Nascimento, M., Rodríguez, H. D., Giardino, V.G., Mattiucci, S. 2014: Molecular identification, morphological characterization, and new insights into the ecology of larval *Pseudoterranova cattani* in fishes from the Argentine coast with its differentiation from the Antarctic species, *P. decipiens* sp. E (Nematoda: Anisakidae). *Veterinary*

Parasitology, 199: 59–72.

Tin, T., Fleming, Z. L., Hughes, K. A., Ainley, D. G., Convey, P., Moreno, C. A., Pfeiffer, S., Scott, J., Snape, I. 2009. Impacts of local human activities on the Antarctic environment. *Antarctic Science*, 21: 3–33.

Tunya, R., Wongsawad, C., Wongsawad, P., Chai, J. Y. 2020: Morphological and molecular characteristics of *Anisakis typica* larvae in two species of threadfin bream, *Nemipterus hexodon* and *N. japonicus*, from the Gulf of Thailand. *The Korean Journal of Parasitology*, 58: 15–25.

Ubeira, F. M. 2014: Travelling with *Anisakis* allergens. *International Archives of Allergy and Immunology*, 163: 243–244.

Van Hien, H., Dung, B. T., Ngo, H. D., Doanh, P. N. 2021: First morphological and molecular identification of third-stage larvae of (Nematoda: Anisakidae) from marine fishes in Vietnamese water. *Journal of Nematology*, 53: 1–9.

Van Thiel, P. H., Kuipers, F. C., Roskam, R. 1960: A nematode parasitic to herring, causing acute abdominal syndromes in man. *Tropical and Geographical Medicine*, 12: 97–113.

Wickramasinghe, S., Yatawara, L., Agatsuma, T. 2014: *Toxocara canis* (Ascaridida: Nematoda): Mitochondrial gene content, arrangement and composition compared with other *Toxocara* species. *Sri Lankan Journal of Infectious Diseases*, 4: 90–98.

Younis, A. E., Saad, A. I., Rabei, J. M. 2017. The occurrence of *Contracaecum* sp. larvae (Nematoda: Anisakidae) in four teleostean species from Lake Nasser, Egypt: morphological and molecular studies. *The Journal of Basic and Applied Zoology*, 78: 1–13.

Zhang, K., Xu, Z., Chen, H. X., Guo, N., Li, L. 2018: Anisakid and raphidascaridid nematodes (Ascaridoidea) infection in the important marine food-fish *Lophius litulon* (Jordan) (Lophiiformes: Lophiidae). *International Journal of Food Microbiology*, 284: 105–111.