Ascaridoid nematodes in mesopelagic fish (Cape Verde - Bay of Biscay areas): ecological and food safety perspectives

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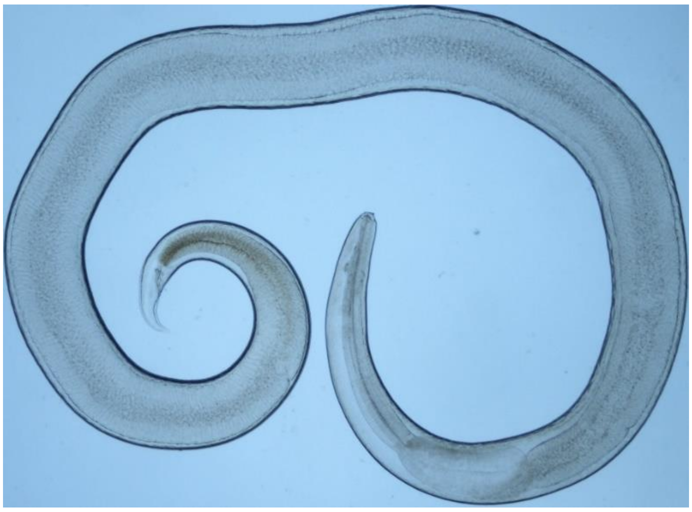

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Front cover motive: A third larval stage of *Pseudoterranova* cf. *ceticola* (For more information see section 3.3.)

Abstract

The total amount of mesopelagic fish biomass is estimated to be between 1 and 20 billion tons globally. However, there is currently no industrial fishery for them. As the sustainability of harvesting pelagic fishes and the health constraint of replacing marine lipids/proteins with land-based lips/proteins in the feed for farming fish seem to have reach their limits, there is an increasing interest of exploitation of mesopelagic fishes from fishing companies and fish feed producers. Although, some anisakid ascaridoid nematodes (some *Anisakis* and *Pseudoterranova* spp.) infect many marine teleosts worldwide and are causative agents of a fish-borne zoonotic disease to humans known as anisakidosis. This gastrointestinal disease may result from ingestion of live larvae with lightly cooked or raw fishery products. The *Anisakis* species *A. simplex* (*s.s.*) and *A. pegreffii* may also cause allergic reactions in sensitized patients. Considering mesopelagic fish as new fish recourse, it is then important to know their ecological role as well as the possible health risks that may be associated with ascaridoid parasites. In May 2019, a research cruise was carried out from Cape Verde to the Bay of Biscay, trawling mesopelagic fish. A total of 1271 fish specimens of 32 fish species were sampled for the presence of ascaridoids. From these, 13 species were found infected with a total of 177 ascaridoid individuals, all larvae. Molecular identification (*Cox2*, ITS) suggests the larvae to belong to 8 different species: *Anisakis pegreffii*, *A. typica*, *A. ziphidarum*, *A. brevispiculata*, *A. paggiae*, *A. cf. paggiae*, *Pseudoterranova* cf. *ceticola* and *Raphidascarididae* sp. *Diretmus argenteus* and *Diaphus rafinesquii* were the most infected fish species with an ascaridoid prevalence of 78% and 36%, respectively. The total density was estimated at 71 ascaridoids/Kg fish.

It appears that there is a high diversity of ascaridoid nematodes in mesopelagic fish and that these are likely important transport hosts in the life cycle of these parasites. Results suggest the existence of spatial variation in the abundance and diversity of ascaridoids in mesopelagic fish i.e. *D. argenteus*. In the perspective of food safety, the potential presence of i.e. *A. simplex* thermostable cross-allergens in mesopelagic fishes should be considered if derived products are meant to be destined as feed for farmed fish.

Acknowledgements

The present study was funded and organized by The Institute of Marine Research (IMR) in connection with an already arranged cruise with the research vessel Kronprins Haakon in May 2019 focused on the study of mesopelagic organisms. The work was conducted at the Parasitology and Molecular labs, at the Department Contaminants and Biohazards of the IMR. I would like to thank Eva Garcia-Seoane (IMR) and Rupert Wienerroither (IMR), the two taxonomists on the cruise who did the taxonomic work of the mesopelagic fishes for me. Especially thanks to Eva, who helped me organizing my samples and took the role as a co-supervisor for me during the cruise. Also, huge thanks to Natalia Drivenes (IMR), for the invaluable amount of help, kindness, molecular training, and time supervising the practical molecular work that I have performed in this study.
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Kaja Meek Olsen.

Glossary

Accidental host: a host that is not part of the natural chain of infection and do not normally lead to infection of a definitive host, but is accidentally infected and is end in the life cycle of the parasite (EFSA 2010)

Allergic anaphylaxis: A rapid onset and dangerous syndrome characterized by urticaria, angioedema, severe respiratory and gastrointestinal symptoms, collapse and shock (EFSA 2010).

Allergic urticaria: a skin reaction with hives (raised, itchy areas of skin), which are changing and do normally not persist at the same location more than 24 hours. Acute, short-lived urticaria (less than 24 or 48 hours) is allergic and mediated by specific IgE against foods, drugs, insects, gastro-allergic anisakiasis etc. (EFSA 2010).

Appendix?

Caecum (= intestinal caecum): a blind diverticulum or pouch from the intestine (Arai and Smith 2016).

Density: the number of individuals of a parasite in a measured sampling unit (e.g. host weight, volume, skin area) (Bush et al. 1997).

Final host: The host in which the parasite reaches adulthood and reproduce (EFSA 2010).

Food allergen: An antigen than can cause allergic reaction in humans when consumed (EFSA 2010).

Intermediate host: A host in which a parasite develops infectivity for the next host, in nematodes often passing through one or more of its juvenile stages

Abundance: The number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts) (Bush et al. 1997).

Intensity: Average intensity of a particular species of parasite among the infected members of a particular host species (Bush et al. 1997).

Mucron: terminal tail spine (Arai and Smith 2016).

Paratenic host: same as transport host (see above).

Prevalence: The number of hosts infected with one or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species (commonly expressed as percentage) (Bush et al. 1997).

The Spearman's Rank Correlation Coefficient (R_s): a statistical measure of the strength of a link or relationship between two sets of data based on their ranks. **R_s** will always be between 1.0 (a perfect positive correlation) and -1.0 (a perfect negative correlation). An R_s of 0 indicates no association between ranks (Geographyfieldwork 2020).

The Spearman's Rank Correlation Coefficient *p* value: a statistical measure of how probable it is that any observed correlation is due to chance. A p-value >0.05 suggest no correlation other than due to chance and that your null hypothesis assumption is correct. If your p-value <0.05 the observed correlation is unlikely to be due to chance and there is a high probability (>95%) that your null hypothesis is wrong (Geographyfieldwork 2020).

Site: The topological or spatial location on or in a host where a parasite (or a sample of parasites) is collected.

Transport host: A host not needed for the development of the parasite but that sustain the parasite and hence facilitates the parasite's life cycle (see above).

Ventricle (=ventriculus): glandular modification of the distal portion of the oesophagus of some nematodes; it may have a solid appendage of varying length extending posteriorly dorsal to the intestine (ventricular appendix) (Arai and Smith 2016).

Oesophagus: in nematodes, the muscular anterior part of the digestive tract, pumping in fluid food (it is now recognized as a pharynx, but the term sticks).

Abbreviations

bp: Base pairs in the DNA.

DNA: Deoxyribonucleic acid.

cf.: Taxonomic abbreviation for confer (L.), meaning “compare” or narrow down to the affinity to a species.

g: Gram.

IMR: Institute of Marine Research.

ITS: Internal transcribed spacers.

L1-L4: The four larval stages of nematodes.

min: Minutes

ml: Milliliter

mm: Millimeter

n: Number of specimens

NCBI: National Centre for Biotechnology Information

PCR: Polymerase chain reaction

rDNA: Ribosomal DNA, DNA sequence coding for ribosomal RNA.

TS: Trawl Station

UiB: University of Bergen

V: Volt

µl: Microliter

µM: Micromolar

µm: Micrometer

Table of contents

1. Introduction

1.1 Ascaridoid nematodes in mesopelagic fishes and potential food safety issues.

Mesopelagic fishes are a various group of marine teleosts living in the mesopelagic zone (also known as twilight zone) at depths between 200 to 1000 m below the ocean surface (Gjøsaeter and Kawaguchi 1980; Robinson et al. 2010; John et al. 2016). They usually perform dial vertical migration into the epipelagic zone (0 – 200 m) during nighttime for predation on mesozooplanktons, smaller fishes or crustaceans such as euphausiids, and hide down in the dark deep during daytime (Gjøsæter and Kawaguchi 1980; Bernal et al. 2009; Irigoien et al. 2014)**.**

Mesopelagic fishes are believed to dominate the biomass of marine teleost fishes in the world, as their estimated total biomass seem to be between 1 to 20 billion metric tons globally, compared to 1 billion tons estimated fish biomass in surface waters (Irigoien et al. 2014; St. John et al. 2016; Martin et al. 2020). Mesopelagic fishes have been very little exploited so far (Lamhauge et al. 2008; Hidalgo and Browman 2019), even though they have been considered as an enormous harvestable resource since the 1970s (Lamhauge et al. 2008; Hidalgo and Browman 2019; Grimaldo et al. 2020). The sternoptychids (e.g. *Maurolicus spp.*) and myctophids (e.g. *Benthosema* spp.) are considered to be the most abundant mesopelagic fish families (Valinassab et al. 2007; Lamhauge et al. 2008; Standal and Grimaldo 2020). Globally, a few commercial attempts have been made in the Gulf of Oman and south of Iceland, targeting mesopelagic species such as pearlsides (e.g. *Maurolicus muelleri*) (Gjøsæter 1984; Standal and Grimaldo 2020). During the last three years, it has also been carried out trial fisheries in international waters in the North East Atlantic and within the Norwegian EEZ (Grimaldo et al. 2020).

Both *B. glaciale* and *M. muelleri* are considered suitable for human consumption, but mostly they have been valued as raw material for the global fish meal and oil industry for use as fish feed for the aquaculture industry (John et al. 2016; Hidalgo and Browman 2019; Alvheim et al. 2020; Olsen et al. 2020; Ytrestøyl et al. 2015).

Currently, the marine fish protein/oil raw materials are produced from pelagic fish, a resource that seems to have reach its limits of exploitation. Thus, pelagic fishes would not be able to provide enough fish protein/oil to satisfy the increasing demand of these products by the farming industry (Ytrestøyl et al. 2015). Marine lipids and proteins may be partially replaced by land-based lipids and protein sources such as soya in feed for farmed fishes such as salmonids (Shepherd et al. 2017; Egerton et al. 2020). However, the replacement of marine lipids/proteins with land-based lipids/proteins in the feed seems to have also reached its limits. There have been suggested some fish health and welfare issues connected to the high content of soya replacement in the feed, such as intestinal damage and reduced growth performance in different fish species (Refstie et al. 2001; Dersjant-li 2002; Lamhauge et al. 2008; Naylor et al. 2009; FAO 2018). As consequence, there is an increasing interest for the exploitation of mesopelagic fishes as a potential new food/nutrient resource from fishing companies and fish feed producers (Klimpel et al. 2006; John et al. 2016; Standal and Grimaldo 2020). Thus, it is important to identify all possible health risks that may entail when introducing new species as a source of nutrition, either indirectly via feed for farmed fish, or directly as human food.

Living third-stage larva of anisakids is known to be causative agent of a fish-borne zoonotic disease to humans named anisakidosis (Chai et al. 2005; Buchmann and Mehrdana 2016; Bao et al. 2019; Adroher-Auroux and Benítez-Rodríguez 2020). The viable anisakid larvae may reach the humans by consumption of raw or lightly cooked fish meals. Under these circumstances, the larva may infect the human gastrointestinal tract and cause disease that cause abdominal pain due to the larvae trying to penetrate stomach (or gut) wall and be accompanied with allergic symptoms (Audicana and Kennedy 2008; Adroher-Auroux and Benítez-Rodríguez 2020).

In addition, allergy to *Anisakis* spp., in which a sensitized consumer may have allergic symptoms (ranging from urticaria to life-threatening anaphylaxis) after consumption of fish contaminated with dead larvae or with their allergens has been also reported (reviewed by Bao et al., 2019). Fourteen allergens have been described from *A. simplex* (WHO/IUIS 2020), and several of those have been shown to be resistant to pepsin and heath treatments (Caballero and Moneo 2004; Moneo et al. 2005). In relation to this, there are some studies suggesting that some of those allergens can possibly be transmitted to humans by feeding farmed fish with infected fish (Fæste et al. 2015b, 2015a).

To date, a few studies have examined the occurrence of anisakids in mesopelagic fish (Hamre and Karlsbakk 2002; Klimpel et al. 2010; Mateu et al. 2015; Cabrera-Gil et al. 2018; Gaglio et al. 2018). Since mesopelagic fish constitute such a large biomass in the world oceans, it is important to understand/determine their parasite diversity and infection levels. Indeed, Martin et al (2020) suggested as research priority to identify how many organisms live in the twilight zone and how diverse they are (from bacteria to cetaceans). The dominant fish species are small sized, and therefore likely important in ascaridoid parasite transmission through the food webs (Klimpel et al. 2006; Cabrera-Gil et al. 2018; Gaglio et al. 2018), i.e. connecting the various ascaridoid nematodes from zooplankton to larger predatory fishes/squids and cetacean final hosts (Bloodworth and Odell 2008; Fernández et al. 2009; Naito et al. 2013).

Thus, considering a future scenario in which mesopelagic fish can be exploited, either for human consumption or for the production of feed for the fish farming industry, it is important to have qualitative and quantitative data on the ascaridoid nematode occurrence in these fishes, in order to facilitate risk assessments and to gather new epidemiological and ecological data.

1.2 Diversity of ascaridoid nematode larvae

The most important fish ascaridoid parasites belong to the anisakid genera *Anisakis*, *Pseudoterranova* and *Contracaecum*, and the raphidascaridid genus *Hysterothylacium* (Berland 2006), because they are very common and are associated with medical and socioeconomic issues (Bao et al. 2021). In the present study, a special focus is placed on species of the genus *Anisakis*, and some other ascaridoids that were found (further details at section 3).

1.2.1 The genus *Anisakis*

To date, nine *Anisakis* species have been described worldwide based on morphology and the genetics of adult specimens (Mattiucci et al. 2014, 2017b). The biodiversity within the genus *Anisakis* has been inferred based on a multi-locus molecular approach (Mattiucci et al. 2016). Valid nuclear and mitochondrial molecular markers have been developed , allowing to differentiate a number of sibling species (Zhu et al. 2000; Timi et al. 2014; Mattiucci et al. 2017a). Two of the most important diagnostic molecular/genetic markers available are the mitochondrial cytochrome oxidase 2 gene (mtDNA *cox2*) and the ITS region of rDNA (Zhu et al. 2002; Nadler et al. 2005; Timi et al. 2014; Mattiucci et al. 2017a, 2018).
Two morphological types of third stage larvae (L3) belonging to genus Anisakis were found in Norwegian marine fishes by Berland (1961). These, termed type I and type II, were discerned on the basis of ventriculus length, shape of the junction between ventricle and intestine, and the presence/absence of a mucron at the tail tip (Berland (1961). Later, similar larvae have been found in fishes worldwide.

Molecular studies have shown that *Anisakis* type I larvae represent several species, *A. simplex* (*sensu stricto (s.s.)*), *A. pegreffii*, *A. berlandi*, *A. typica*, *A. ziphidarum* and *A. nascettii*.

Anisakis type II larvae have been identified with *A. physeteris*, *A brevispiculata* and *A. paggiae*. However, based on morphology, Shiraki (1974) discerned two more larval types, called Type III and Type IV and Murata et al. (2011) showed that type II, III and IV larvae from Japan could be identified as *A. physeteris*, *A. brevispiculata* and *A. paggiae*, respectively.

In addition, two larval genotypes that may represent two undescribed species of *Anisakis* have been recognized, referred to as *Anisakis* sp.1 and *Anisakis* sp. 2, and these were found to be phylogenetically related to *A. typica* and *A. physeteris*, respectively (Mattiucci et al. 2018). Details about which morphologically features for differentiate within the different ascaridoid genera and *Anisakis* larval types will be explained at the material and methods section.

1.2.2 The genus *Pseudoterranova*

Species member of the genus *Pseudoterranova* are often named seal worms, as common final hosts are pinnipeds (Abollo and Pascual 2002; Berland 2006). To date, six biological species have been recognized in the *Pseudoterranova decipiens* complex species: *P. decipiens (s.s.)*, *P. krabbei*, *P. bulbosa*, *P. azarasi*, *P. cattani* and *P. decipiens* E (Mattiucci and Nascetti 2008; Timi et al. 2014; Mattiucci et al. 2017a), all infecting seals and sea-lions. Two species mature in cetaceans, i.e. *P. ceticola (*previously *Terranova ceticola)* and *P. kogiae* (Abollo and Pascual 2002; Longshaw 2012; Timi et al. 2014; Mattiucci et al. 2017a).

1.2.3 The genus *Hysterothylacium*

To date, there are more than a hundred accepted species of the genus *Hysterothylacium* (WORMS-World Register of Marine Species). However, its taxonomy is unresolved (Klimpel et al. 2007b). One of the most important species is *H. aduncum*. As it has the potential to cause substantial economic losses to the fishing industry as a consequence of cosmetic degradation for the consumers of the fish product, even if it is considered a non-zoonotic parasite (Bao et al. 2021). *Hysterothylacium aduncum* is a very common parasite of fishes from the NE Atlantic waters (Klimpel and Rückert 2005). In addition, according to other authors, some *Hysterothylacium* L3 from marine fishes cannot be differentiated morphologically from nematode larvae of other genera, such as *Lappetascaris* (Hossen and Shamsi (2019) cited in Guardone et al. (2020)).

1.3 Life cycle of ascaridoid nematodes.

The genera *Anisakis*, *Pseudoterranova* and *Hysterothylacium* share a similar life cycle. The anisakids *Anisakis spp.* and *Pseudoterranova spp.*, have in common that their final host are homeotherms, fish-eating, marine mammals like cetaceans or seals (Anderson 2000; Klimpel et al. 2004; Mattiucci et al. 2017a). In the digestive tract (particularly stomach) of the final host, the adult roundworms sexually reproduce, and the fertilized eggs are released to the water with the hosts feces (Højgaard 1999). Free in the water, it is not completely elucidated if the larva undergoes one or two molts within the egg before it hatches (Køie 1993; Measures and Hong 1995). The prevailing view is that it is the L3 which emerges from the egg and that the L3, loosely ensheathed in the cuticle of second-stage larva, may be ingested by the first intermediate host which normally are small crustaceans (i.e. euphausiids, copepods, etc.) (Køie 2001; Mattiucci et al. 2018).

In the fish that ingest infected crustaceans, the L3 become digested free in the stomach or intestine. The larva may then, with the aid of the boring tooth and histolytic enzymes, bore through the wall of the stomach/intestine to the visceral organs or into the flesh. At final sites that vary with species, they may coil up and become encapsulated (Smith 1984; Anderson 2000; Berland 2006).

As the big fish prey on smaller, the L3 may also be transferred. Hence, the role of the fish host may vary. They may be important second intermediate hosts if the larvae grow to infectivity to the final host in them, but they may also carry reestablished larvae that had reached infective size already, and act as paratenic hosts (Klimpel and Palm 2011). Cephalopods may also be involved in the life cycles, with a similar role as fishes (Costa et al. 2014; Mattiucci et al. 2018). The larvae of many ascaridoids live for a long time in their fish hosts, and therefore may accumulate, sometimes in high numbers (e.g. hundreds of L3 in one fish) (Levsen and Berland 2011).

When the fish host is ingested by a potential final host, the L3 are digested free, usually in the stomach. The L3 then molt to the preadult stage, and finally to the mature adult stage that mates and thereby closing their lifecycle (Sprent 1954; Berland 2006; Colón-Llavina et al. 2009).

The raphidascaridid genus *Hysterothylacium* has a similar life cycle, except that marine teleosts are final host animals (i.e. heterotherms), meaning that the L3 ingested by a suitable final fish host may undergo molting to the preadult and to adults (Sprent 1954; Navone et al. 1998; Berland 2006; Shamsi et al. 2013). Thus, when inspecting marine teleosts for ascaridoid parasites, anisakids occur as L3, and raphidascaridids such as *Hysterothylacium* spp. may occur as encapsulated L3, or luminal L3, preadult and adult nematodes.

1.4 The Project Aims

To date, few studies have examined the occurrence of ascaridoids in small mesopelagic fish, which is likely important in transmitting those paradise through the food webs. Mesopelagic fishes may also be relevant in future exploitation for fish meal that could be used e.g., in feed for farmed fish. When considering using a new fish resource, it is important to know the ecological role of the resource in the ecosystem and as well as the possible health risks that may be associated with its consumption. Qualitative and quantitative data on the ascaridoid nematodes in these fishes can aid future assessments into allergen occurrence, and hence the risks from using fish meal of this origin. This study aims to increase our knowledge on ascaridoid nematode epidemiology in mesopelagic fishes, specifically to:

(1) determine the parasite infection levels in various mesopelagic fish species in the Cape Verde to The Bay of Biscay region.

(2) identify the ascaridoid parasite larvae using morphological and molecular methods.

(3) identify factors affecting the parasite abundance in the fish (i.e. host species, size, geographical location).

(4) Provide relevant data for the evaluation of food safety aspects, such as anisakid density (risk posed by using mesopelagic fish as protein source for humans and/or animals).

2. Material and Methods

2.1 Fish sampling

Fish samples were collected in the Eastern Atlantic Ocean from Mindelo (Cape Verde) to The Bay of Biscay (Northeast Atlantic) in May 2019. Eighteen (TS4601-4618) trawl hauls were conducted during a research cruise with the vessel "RV Kronprins Haakon (Fig. 1). A 6x6m macroplankton trawl or a multipelt 380 trawl (fish trawl) were used at depths between 84 and 1650 m. The multipelt 380 trawl was used at three stations (4604, 4606, 4614) as this trawl had the potential to catch larger specimens than the macroplankton trawl.

This could provide an idea of which fish-sizes may not be able to catch the macroplankton trawl. It must be noted that the multipelt trawl is classified as a non-quantitative trawl. It cannot be used for quantitative catch of relatively small organisms and to study the biodiversity of the catch. Hence, it will not be possible to know anything about the species biomass in the ocean when using the multipelt catch (see Table 1 and Fig.1 for more trawling data) (Anonymous 2019).The other thirteen hauls were done with the macroplankton trawl (Anonymous 2019).

2.1.1 Trawling data

Table 1: Overview of the trawl stations from which fish samples were obtained during the research cruise with RV ‘Kronprins Haakon’ 3rd-22nd May 2019. Most samples were obtained using macroplankton Trawl, except those indicated with subscript ‘MT’ which represent Multipelt Trawl samples.

Station Trawl	Station CTD	Date (Day)	Time	Latitude	Longitude	Max Depth (m)
4601	0118	03	Day	17.9692	-23.9560	1650
4603	0120	06	Day	24.9748	-20.3110	1200
4604$_{MT}$	0121	07	Day	26.8987	-19.2319	1200
4605	0122	08	Day	29.1403	-17.9654	1200
4606$_{MT}$	0123	09	Day	29.7668	-16.0871	1200
4607	0124	10	Day	30.6122	-13.5899	1200
4608	0125	11	Day	31.6338	-10.5101	1200
4609	0126	12	Day	32.6997	-11.9357	1200
4610	0127	13	Day	33.6949	-13.2319	1200
4611	0128	14	Night	34.0972	-13.7590	84
4612	0129	15	Day	35.1488	-15.1697	1200
4613	0130	16	Day	36.1140	-16.4944	1200
4614$_{MT}$	0132	18	Day	40.2823	-13.4325	1200
4615	0133	19	Day	42.9822	-12.3180	1200
4616	0134	20	Night	43.6340	-12.2278	290
4617	0135	21	Day	45.9535	-9.5882	1200
4618	0136	22	Day	47.2549	-8.0342	1200

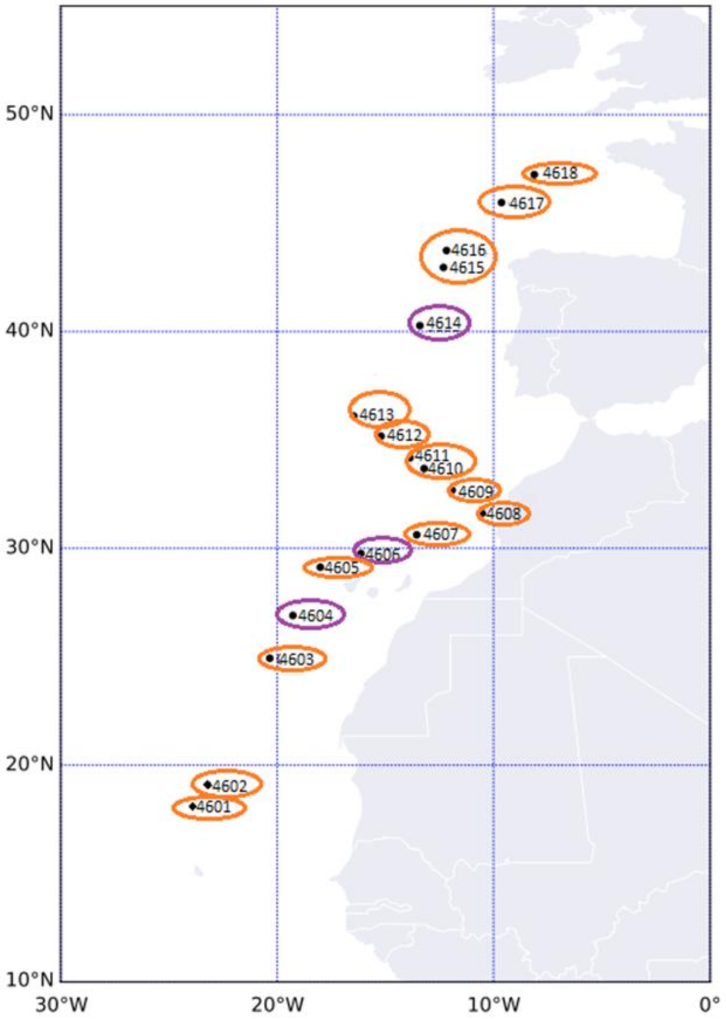

FIGURE 1: MAP OF THE TRAWLING STATIONS FOR THE WHOLE CRUISE AND MARKINGS OF WHERE THE MACROPLANKTON TRAWLS (ORANGE RINGS) AND THE MULTIPELT (FISH) TRAWLS (PURPLE RINGS) WERE USED. MAP HAS BEEN MODIFIED BY AUTHOR, SEE ORIGINAL MAP IN ANONYMOUS 2019.

2.1.2 The fish sampling

A total of 1271 specimens from 32 fish species were collected from TS4601, 4603-4618 and examined for ascaridoid nematodes. Fishes were firstly sorted from the catch and identified to species level by two taxonomists (Eva Garcia-Seoane and Rupert Wienerroither) before they were distributed on the various projects that took part in the cruise. A selection was made on the fish-size (i.e. fishes larger than 1 cm total length) in order to be able to distinguish between infection by consumption or actual patriotization in the fish. As fish smaller than 1 cm total length is virtually impossible to carefully dissect out the whole organ package from. No fish was sampled for this study from TS4602. For overview of the fish sample used in this study see Table 3 and Table 4 in the result section. Fishes were sheated in plastic bags sorted per fish species and trawl stations and stored as soon as possible in a deep freezer (−20°C) onboard.

2.2 Parasite examination

2.2.1 Autopsy of the fishes and nematode inspection

At the lab (IMR, Bergen), the frozen fish were thawed at room temperature and measured to the nearest millimetre (mm) for the total (TL) and standard length (SL) and then weighed (TW) in grams (g) (see table 8). In addition, a few fishes were analysed fresh for parasitic nematodes during the cruise in the RV lab. The body cavity was opened with a scalpel and/or surgical scissors, and all the internal organs were dissected out with tweezers and placed in Petri dishes with some drops of physiological saline. The emptied body cavity and the surfaces of the internal organs were then carefully examined under stereomicroscope for ascaridoids. Stomachs and intestines were opened longitudinally, and the contents scraped out. Identifiable preys were recorded, and any parasite present collected.

Thereafter, the internal organs and carcass were placed into plastic bags (e.g., one bag for the muscle and one bag for the viscera) and inspected by UV-press method (see section 2.2.2). The ascaridoid nematode larvae site was registered and then dissected out.

The body lengths of the nematodes collected were measured to the nearest millimeter on a millimeter paper under a stereomicroscope. They were then examined in a temporal mount on a microscope slide in saline, in a light microscope equipped with a camera. Photos were taken at 40x, 100x and 200x magnification. The nematodes were morphologically assigned to larval types (see section 2.2.3).

After photographing, each larva was placed individually in eppendorf tubes filled with a few drops of water and deep frozen (-20 °C) for later molecular identification (see section 2.2.4).

2.2.2 UV-press method

After parasite examination of the viscera (see above), the gutted fish and viscera were refrozen for later examination by the UV-press method. Briefly, the thawed samples were flattened to 1-2 mm thick layers in a hydraulic press and subsequently inspected under a 366 nm UV-light source in a dark room in order to reveal ascaridoids inside the flesh or viscera, as nematodes that have died by freezing glow when irradiated by UV-light (Pippy 1970; Karl and Leineman 1993; Levsen et al. 2005). Some fish individuals that were too small or in too bad condition to be examined manually, were inspected by UV-press method only.

2.2.3 Morphological identification

The ascaridoids found were assigned to genus or larval morphotype under the light microscope as follows. The morphological characters considered, included presence/absence of lips or boring tooth (e.g., if the ascaridoids were preadult/adult or larval). Then, the presence/absence and appearance of the ventricle, intestinal caecum, ventricular appendix, cuticle ornamentation, mucron, shape of tail, the total length and color of the larva, as well as the position of the excretory pore (Berland, 196; Shiraki, 1974; Cannon, 1977; Murata et al., 2011).

2.2.4 Molecular identification

The larvae varied in size. Hence, for the larvae identified as *Anisakis* spp. and *Terranova* -like (see results section) only the midpart of the larvae were used; the anterior and posterior parts were kept deep frozen in physiological water. The larvae tentatively classified as *Hysterothylacium*-like (see results section) were utilized whole for DNA extraction as they were very small.

2.2.4.1 DNA extraction

DNA extraction were done with totally 109 ascaridoid larvae of all different larval types. The rest of larvae were stored for future studies. The DNeasy® Blood & Tissue kit was used for extraction of DNA, using the protocol: *Extraction of Total DNA from Animal Tissue (Spin-column Protocol)* (Qiagen 2006). A few modifications were made from the protocol. At first step, the process of preparing for lysis of the tissue, the 3 h digestion step was enhanced by addition of three ceramic balls (Precellys ceramic kit 2.8 MM, VWR) in each tube before centrifuging in the *Precellys* 24 lysis & homogenizer (bertin Technologies).

They were added in order to help the enzymatical degradation by homogenization of the tissue. Also, at step six the samples were supposed to centrifuge for 3 min at 14,000 rpm, but the centrifuge machine “Eppendorf® Microcentrifuge Model4514D” do have a maximum rpm limitation on 13,200 rpm. So, at this step the maximum rpm for the machine were used for 3 min as said in the protocol. DNA was eluted with 30 µl AE buffer. DNA amount and quality was measured using a Thermo Scientific NanoDrop Spectrophotometer (Geuther 1977).

2.2.4.2 PCR

A total of 104 larvae were used for molecular identification, as not all the DNA extractions were successful.

The ascaridoid mitochondrial cytochrome c oxidase subunit II (*cox2*) gene of 104 ascaridoids was amplified following procedures of Mattiucci et al. (2014) with some modifications (see below). Polymerase chain reaction (PCR) was carried out using the primers from Nadler & Hudspeth (2000):

- 211F (5′-TTTTCTAGTTATATAGATTGRTTTYAT-3′)
- 210R (5′-CACCAACTCTTAAAATTATC-3′)

The entire internal transcribed spacers of the nuclear ribosomal DNA (ITS rDNA (ITS1, 5.8S rDNA gene and ITS2)) of 88 ascaridoids was amplified following Zhu et al. (2000) using the NC5 F and NC2 R primers:

- NC5F (5’-GTAGGTGAACCTGCGGAAGGATCATT-3’)
- NC2R (5’-TTAGTTTCTTTTCCTCCGCT-3’)

Firstly, PCRs were done using Platinum HiFi Taq polymerase, but amplification did not work well even though PCR conditions were optimized several times. Finally, we used Go Taq Hot Start Polymerase (Promega) which showed good results. The optimized master mix (MM) can be found at Table (2).

TABLE 2: PCR MASTER MIX (MM)

Regents	**Volume(µL) for 1x rxn.**
H20 (Mq)	16,8
25 mM MgCl2	2,5
5x Buffer	1,5
10mM dNTP's	0,5
10µM Forward Primer	0,5
10µM Reverse Primer	0,5
10% DMSO	0,5
5U Go Taq Hot Start Polymerase (Promega)	0,2
DNA	2
Total:	**25µL** (=23µl MM + 2µL DNA)

PCR program used for ITS: initial denaturation at 94°C for 5 min, followed by 30 cycles of: denaturation at 94°C for 30 s, annealing at 54 °C for 30 s., extension at 72°C for 30 s. Followed by final step of final extension at 72°C for 5 min, and hold at 4°C.

PCR program used for *Cox2:* initial denaturation at 94°C for 5 min, followed by 35 cycles of: denaturation at 94°C for 30 s, annealing at 46 °C for 1 min, extension at 72°C for 1.30 min. Followed by final step of final extension at 72°C for 10 min, and hold at 4°C.

2.2.4.3 Electrophoresis

PCR-products were visualized by Gel electrophoresis. PCR products were run in the agarose gel of the mixing ratio of 0.5g agarose to 50mL 1X TAE buffer, before adding 5 µl of GelRed™ giving the finish product of 50mL agarose gel at concentration of 1% (Somma and Querci 2006). This concentration of the gel is recommended for resolving linear DNA molecules to 500-20000bp. The expected size of the present PCR products was c. 629bp for *cox2* and about 936bp for the amplicon containing the ITS (Nadler and Hudspeth 2000; Zhu et al. 2000).

The gel was bathed in 1X TAE-buffer and the wells were loaded with 6 µl PCR product mixed with 2 µl loading dye, gives the total product of 8µl. The first well used in the gel were loaded with 6 µl GelPilot® 100bp Plus ladder with the function being a visual marker for the molecular weight of the PCR products. The electrophoresis was run at 90 V for 60 min. The bands on the gel were visualized using UV-light with Molecular Image Chemi Doc XRS+ (Universal Hood II (Bio-Rad)) and the program Image Lab Software.

PCR-products with correct size, were sent for purification and sequencing to Eurofins (Cologne, Germany). The obtained sequences were searched for similarity using BLAST (Basic Local Alignment Search Tool) at National Center for Biotechnology Information (USA) (Altschul et al. 1990). Reference sequences from adult nematodes recovered from final hosts and deposited in GenBank were used for the blasting (see further details at section 3.3.1).

2.2.5 Morphometric measurements

Measurements of body dimensions were taken from the images of some ascaridoid larvae using the software Image J (https://imagej.nih.gov/ij/). Images of corresponding object micrometer scales were used for calibration. Illustration of how some of the measures were obtained can be observed in Fig.2.

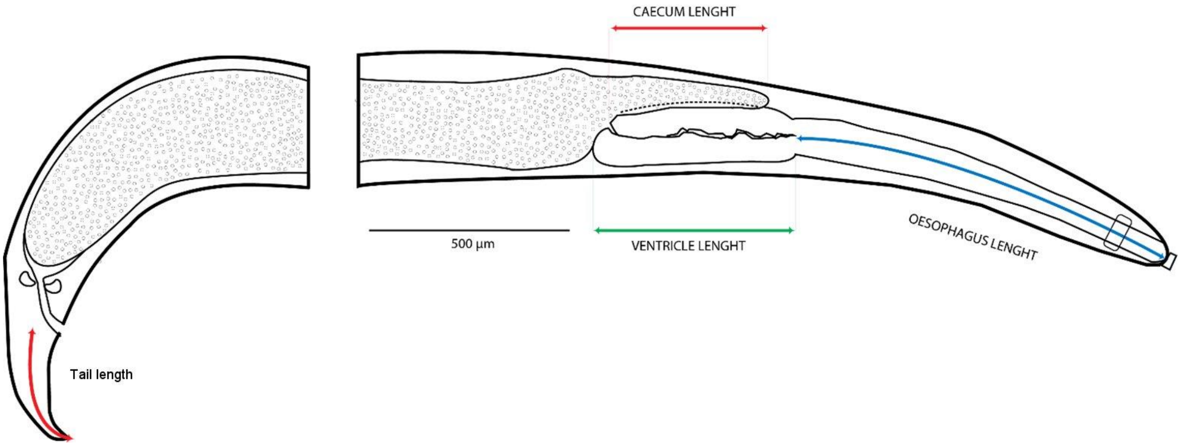

FIGURE 1: MEASUREMENTS TAKEN FROM THE IMAGES OF *P.* CF. *CETICOLA* LARVAE. THE OESOPHAGUS LENGTH IS TAKEN ALONG THE MIDLINE FROM THE START OF THE OESOPHAGUS (E.G. SLIGHTLY SUB TERMINALLY IN THE WORM) TO THE VENTRICLE. THE CAECUM LENGTH WAS MEASURED FROM THE APERTURE INTO THE VENTRICLE AND TO THE CAECUM END. THE TAIL LENGTH REPRESENTS THE DISTANCE ALONG THE MIDLINE, FROM THE LEVEL OF THE ANUS/CLOACA TO THE POSTERIOR END.

2.3 Data analysis

The quantitative descriptors of parasite infection, prevalence, abundance, intensity and density were used as defined in Bush et al. (1997). Correlations between parasite abundance and fish size (i.e. length or weight) were examined for the most parasitized species using Spearman's Rank-Order Correlation test in Spearman’s Rho Calculator (Socscistatistics 2020).

3. Results

3.1 Trawl samples and sampled fishes.

The most dominant biomass in the trawls were shrimp, krill and other crustaceans, as well as jellyfish (See Fig. 3). The majority of the fishes sampled in this study belonged to the family Myctophidae (See Table 3). The stomach contents for the various species consists mostly of different species of crustaceans, except of *Chauliodus* spp. which had rests of smaller fishes. The majority of the fishes were sampled from the trawl catch near the West Sahara (TS4604).

FIGURE 2: BOX FILLED WITH UNSORTED TRAWL CATCH, DOMINATED BY CRUSTACEANS.

TABELL 3: OVERVIEW OF THE TOTAL NUMBER OF INDIVIDUALS OF EACH FISH SPECIES SAMPLED PER TRAWL STATION (TS4601-4618)

Family	**Stations (4601-4618)**																	
Species	1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Tot.
Gonostomatidae																		
Cyclothone microdon									10							29	18	57
Myctophidae																		
Benthosema glaciale	1												21	9		29	85	145
Bolinichthys indicus					9	3			8		8	13						41
Ceratoscopelus warmingii			18		31	1												50
Diaphus brachycephalus			10															10
Diaphus dumerilii	1																	1
Diaphus effulgens			1															1
Diaphus metopoclampus			10															10
Diaphus mollis	1		14	3	40													58
Diaphus rafinesquii	2		48															50
Hygophum hygomii			27			4	2		21	33	7	15						109
Hygophum reinhardtii									1		4	2						7
Hygophum taaningi	21	6		31	13			10										81
Lampanyctus alatus	6																	6
Lampanyctus cuprarius						1			3									4
Lampanyctus lineatus	5						1											6
Lepidophanes guentheri	5																	5
Lobianchia dofleini			7				1	16	14	9	14							61
Lobianchia gemellarii			2	2														4
Nannobrachium atrum	12								5				9					26
Notoscopelus resplendens								7	70	40		8						125
Diretmidae																		
Diretmus argenteus		1	11		8	2		1										23
Sternoptychidae																		
Argyropelecus aculeatus			22		10				10	21		2						65
Argyropelecus hemigymnus												11	10					21
Maurolicus muelleri													102		8		27	136
Sternoptyx sp.			39						2			11						52
Melamphaidae																		
Poromitra crassiceps			10		9	1												20
Platytroctidae																		
Maulisia argipalla			7															7
Eurypharyngidae																		
Eurypharynx pelecanoides			1						7			10	1					19
Stomiidae																		
Chauliodus danae					20	17	3	1	5		3	6						55
Chauliodus sloani	5					2		7										14
Chauliodus schmidti	5																	5
All	64	7	227	36	140	31	7	42	156	103	36	78	142	9	8	58	130	1271

Table 4: Total length (TL) and Total weight (Tw) for every fish species examined in this study. sorted by family**.** SD: standard deviation (SD)

Family Species	TL (mm) ± SD (range)	TW (g) ± SD (range)
Gonostomatidae		
Cyclothone microdon	48.4±8.4 (32-64)	0.5±0.3 (0.2-1.1)
Myctophidae		
Benthosema glaciale	35.1±21.1 (13-78)	0.5±0.2 (0.1-1.5)
Bolinichthys indicus	40.8±4.8 (31-52)	0.7±0.2 (0.3-1.4)
Ceratoscopelus warmingii	47.1±6.4 (36-70)	0.8±0.4 (0.3-3.0)
Diaphus brachycephalus	46.4±3.9 (40-50)	1.8±0.5 (0.9-2.4)
Diaphus dumerilii	48.0±0.0 (48-48)	1.2±0.0 (1.2-1.2)
Diaphus effulgens	55.0±0.0 (55-55)	1.6±0.0 (1.6-1.6)
Diaphus metopoclampus	62.8±3.9 (57-69)	4.0±0.9 (3.0-5.9)
Diaphus mollis	48.7±5.2 (36-66)	1.4±0.4 (0.5-2.4)
Diaphus rafinesquii	62.9±7.6 (51-85)	3.4±1.3 (1.3-6.5)
Hygophum hygomii	38.0±10.2 (23-64)	0.7±0.6 (0.1-3.4)
Hygophum reinhardtii	42.6±8.4 (28-46)	0.7±0.4 (0.1-1.2)
Hygophum taaningi	42.0±7.0 (24-56)	0.8±0.4 (0.1-1.6)
Lampanyctus alatus	50.7±10.8 (34-62)	34.6±54.5 (0.3-125.1)
Lampanyctus cuprarius	75.3±2.1 (73-77)	1.7±0.1 (1.6-1.8)
Lampanyctus lineatus	114.0±49.3 (62-172)	10.3±9.6 (0.8-23.4)
Lepidophanes guentheri	46.4±10.2 (32-57)	0.7±0.4 (0.1-1.0)
Lobianchia dofleini	32,8±6.1 (20-46)	0.4±0.2 (0.1-0.7)
Lobianchia gemellarii	71.5±9.9 (63-82)	4.3±2.2 (2.5-6.4)
Nannobrachium atrum	87.3±26.3 (11-124)	4.5±3.3 (0.2-12.3)
Notoscopelus resplendens	37.3±5.9 (26-56)	0.4±0.2 (0.1-1.2)
Diretmidae		
Diretmus argenteus	74.2±23.5 (25-105)	16.5±8.9 (0.6-30.3)
Sternoptychidae		
Argyropelecus aculeatus	41.2±17.7 (15-73)	2.4±2.5 (0.1-10.7)
Argyropelecus hemigymnus	35.4±4.7 (25-46)	0.5±0.2 (0.2-1.1)
Maurolicus muelleri	44.1±9.5 (14-53)	1.0±0.2 (0.6-1.7)
Sternopty Sp.	91.1±24.5 (63-143)	1.1±1.1 (0.1-8.2)
Melamphaidae		
Poromitra crassiceps	31.8±8.0 (15-65)	12.2±10.7 (3.3-34.9)
Platytroctidae		
Maulisia argipalla	112.9±24.7 (86-157)	10.5±5.2 (4.9-18.0)
Eurypharyngidae		
Eurypharynx pelecanoides	278.4±97.1 (152-555)	5.8±6.5 (0.4-26.6)
Stomiidae		
Chauliodus danae	100.7±14.0 (66-132)	2.4±0.9 (0.6-4.7)
Chauliodus sloani	154.4±62.7 (62-213)	Na*
Chauliodus schmidti	128.4±71.2 (50-274)	18.7±13.6 (0.4-45.1)

* Not weighed

3.2 Nematodes found.

All the nematodes recovered were L3 larvae. A total of 177 ascaridoid larvae were collected from the 1271 fish specimens examined. Infections were registered in 13 out of the 32 fish species. The nematodes were usually easy to see in the viscera, even with the naked eye. The 177 larvae were morphologically assigned as to larval types follows: *Anisakis* type I larvae (n=9) *sensu* Berland (1961) (Figure 5 and 6_A, B, C), *Anisakis* type III (n=51) larvae *sensu* Shiraki (1974) (Figure 7_A, B, C), *Anisakis* type IV (n=38) larvae *sensu* Shiraki (1974) (Figure 8_A, B, C), *Hysterothylacium*-like (n=42) (Figure 9_A, B, C.), and *Terranova*-like (n=37) (Figure 10_A,B,C).

Larvae that had a distinct ventricle without appendix, and a straight intestine without a caecum were considered belonging to genus *Anisakis*. All these had a rounded cephalic end with a ventral boring tooth, subtended by an excretory pore at the base of the lip-anlagen.

Anisakis type I larvae had a long, whitish body (total length range:18-31 mm). The prominent oval ventricle had an (usually visible) oblique transition to the intestine (Fig. 5 and 6 B). The tail was rounded, with a clear mucron at the tail tip (Fig. 5 and 6 C).

Anisakis type III larvae had relatively small body compared to type I (total length range:15-18 mm). Larvae had a light yellow to reddish body, an oval small ventricle relative to body length, and a rounded tail with a small mucron at the tail tip (Fig. 7 C). Their whole body reflected a light ice-blueish color when exposed to UV-light after freezing and thawing. In addition, the larvae showed a less intensity of glowing when UV-lighted, showing a more whiteish/yellowish color rather than clear blueish (Fig. 4), compared to the clear bluish color showed by *Anisakis* type I larvae (e.g. *A. simplex* (*s.s.*) and *A. pegreffii*) (see Fig. 1 at Bao et al. (2015)).

Anisakis type IV larvae had a relatively small body compared to *Anisakis* type I larvae (total length range: 16-20 mm). Larvae had a light yellow to reddish body. Larvae had a violin-shaped ventricle (Fig. 8 B) and a conical and pointed (but curved) tail without any mucron (Fig. 8. C). Their whole body did reflect a light sky-blueish color when exposed to UV-light after freezing and thawing (Fig 4). In addition, the larvae showed a more bluish color than type III when UV-lighted (Fig.4), but less than *Anisakis* type I larvae (e.g. *A. simplex* (*s.s.*) and *A. pegreffii*) (see Figure 1 at Bao et al. (2015)). It was also observed that their anterior "head" part showed less glowing intensity compared to the rest of the body.

Hysterothylacium -like larvae were small (total length range: 4-6 mm long), and with a transparent-whiteish body-color. Larvae had a boring tooth ventrally located (Fig.9 A), a short, round ventricle with both a very long ventricular appendix and long intestinal caecum (Fig 9. B). The excretory pore was seen below the nerve ring. Larvae had a pointed conical tail with a terminal spine (Fig. 9 C).

The larvae categorized as *Terranova*-like resembled *Anisakis*, but they were smaller (total length range: 6-10 mm) and thick-bodied (Fig 11). These larvae had an intestinal caecum extending alongside the ventricle (Fig 10 C), and a pointed tail (Fig. 10 B). For more information see section 3.4.

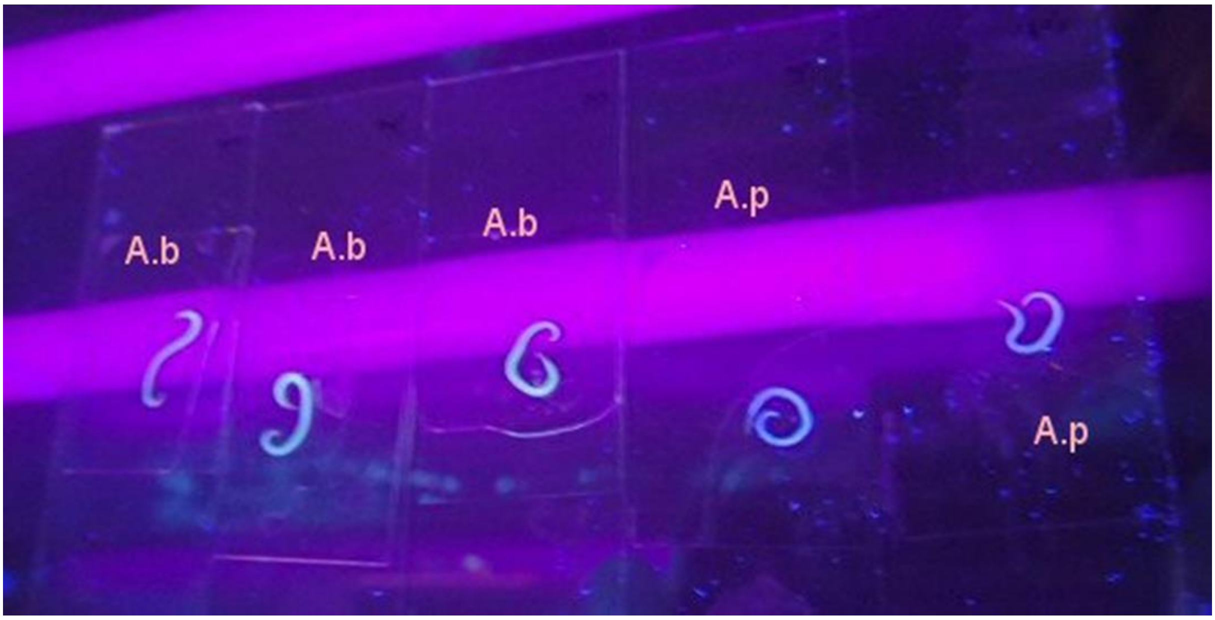

FIGURE 4: UV LIGHTING OF *ANISAKIS BREVISPICULATA* (A.B) AND *ANISAKIS PAGGIAE* (A.P).

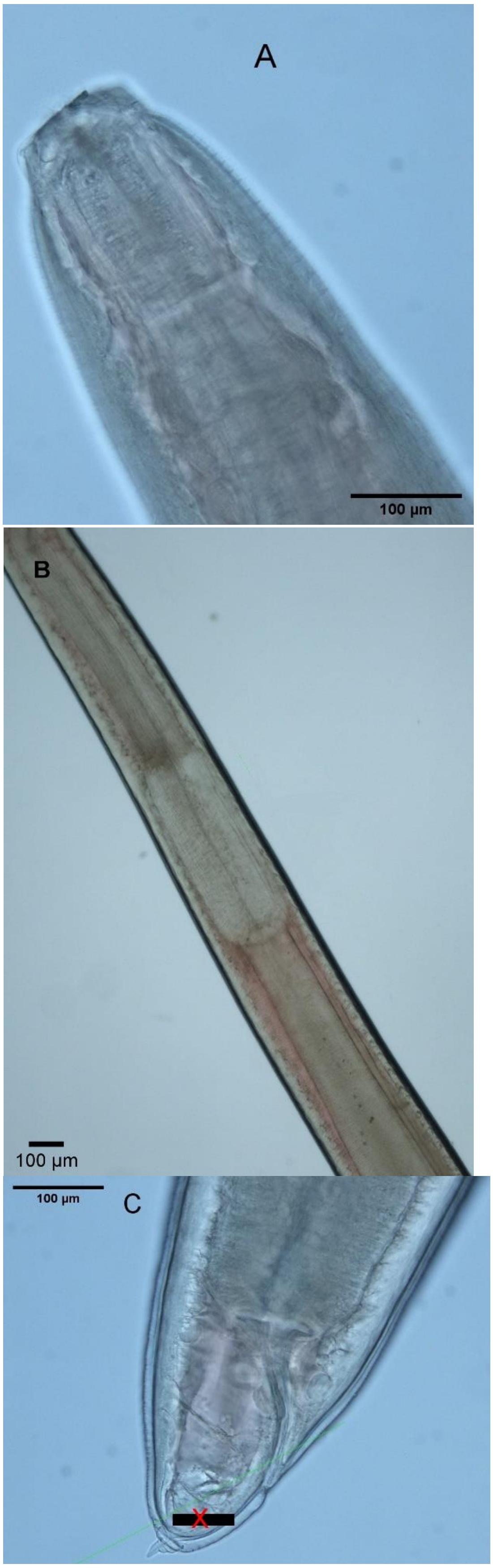

FIGURE 5: *ANISAKIS* TYPE I (*A. ZHIPIDARIUM*): ANTERIOR PART (A), VENTRICULUS PART (B) AND POSTERIOR PART (C). INCORRECT SCALE BAR THAT COULD NOT BE DELETED FROM THE IMAGE (RED X).

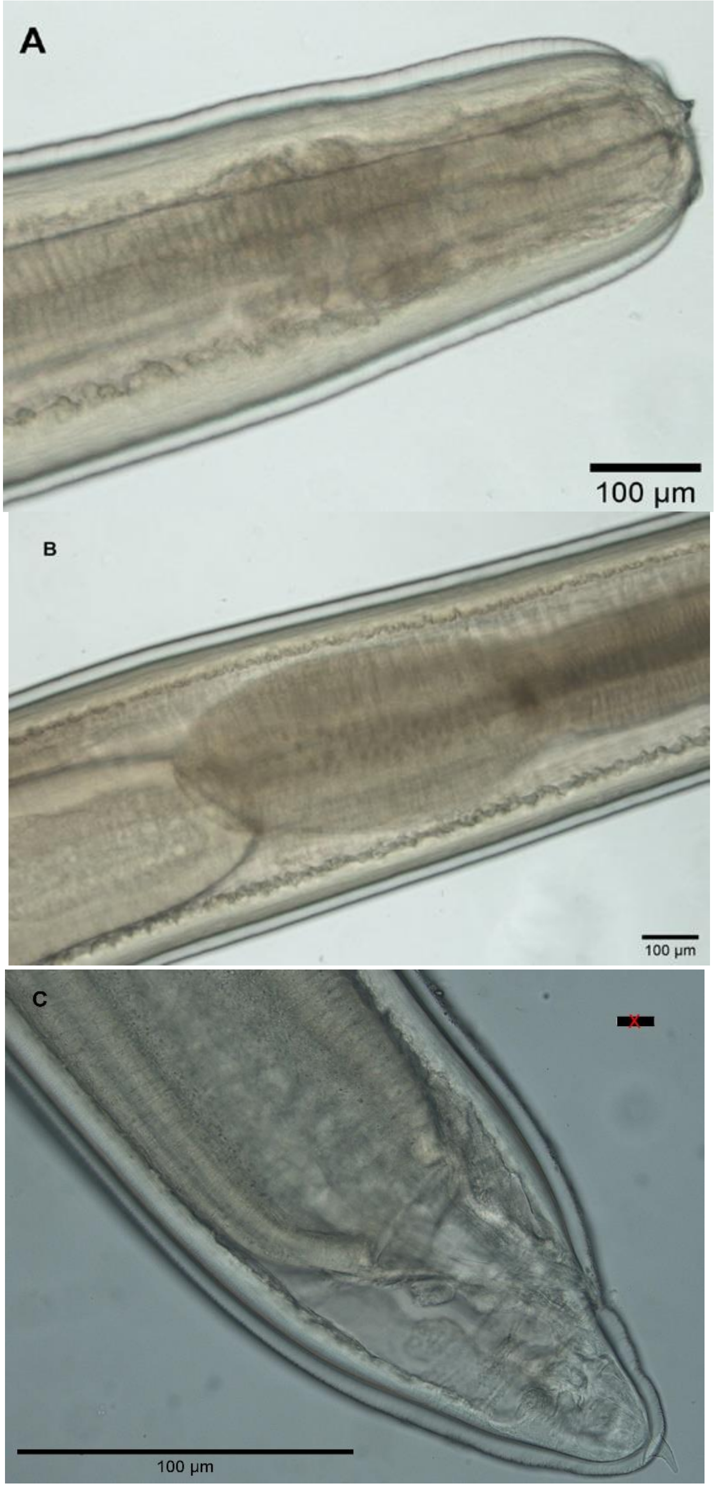

FIGURE 6: *ANISAKIS* TYPE I (*A. TYPICA*): ANTERIOR PART (A), VENTRICULUS PART (B) AND POSTERIOS PART (C). INCORRECT SCALE BAR THAT COULD NOT BE DELETED FROM THE IMAGE (RED X).

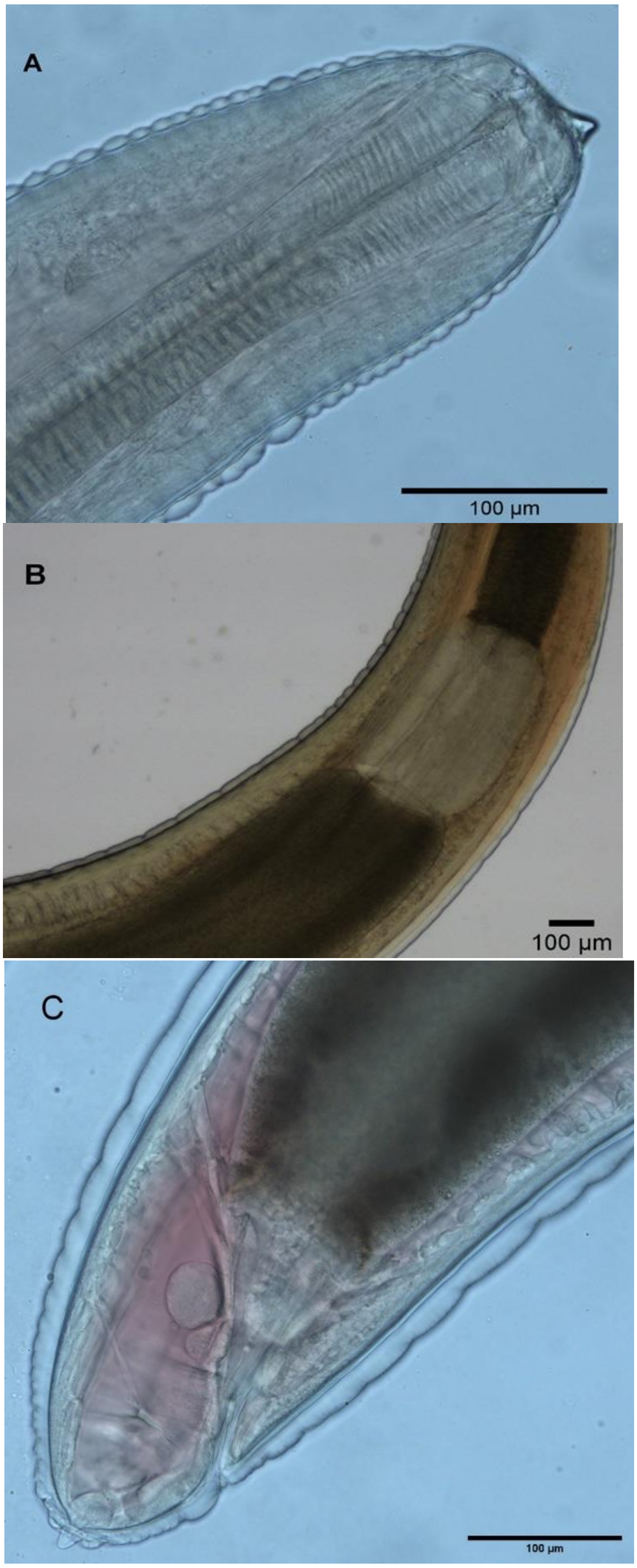

FIGURE 7: *ANISAKIS TYPE III (A. BREVISPICULATA)*: ANTERIOR PART (A), VENTRICULUS PART (B) AND POSTERIOR PART (B).

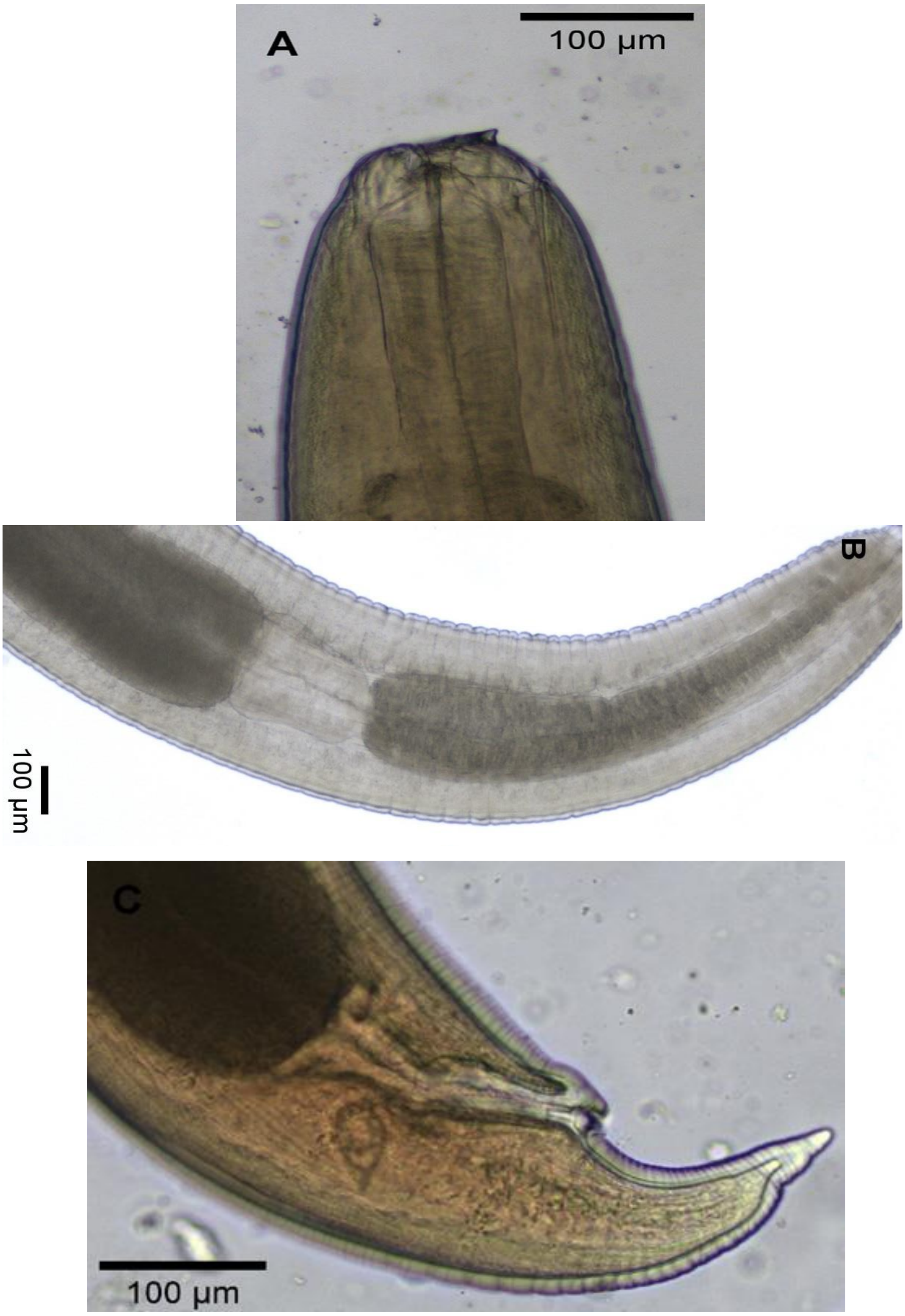

Figure 8: *Anisakis* type IV (*A.cf.Paggiae*): Anterio part (a), ventriculus part (B), and posterio part (c).

Figure 9: *Hysterothylacium* like (Raphidascarididae gen sp.): Anterior part (A), Ventriculus part (B) and Posterior part (C).

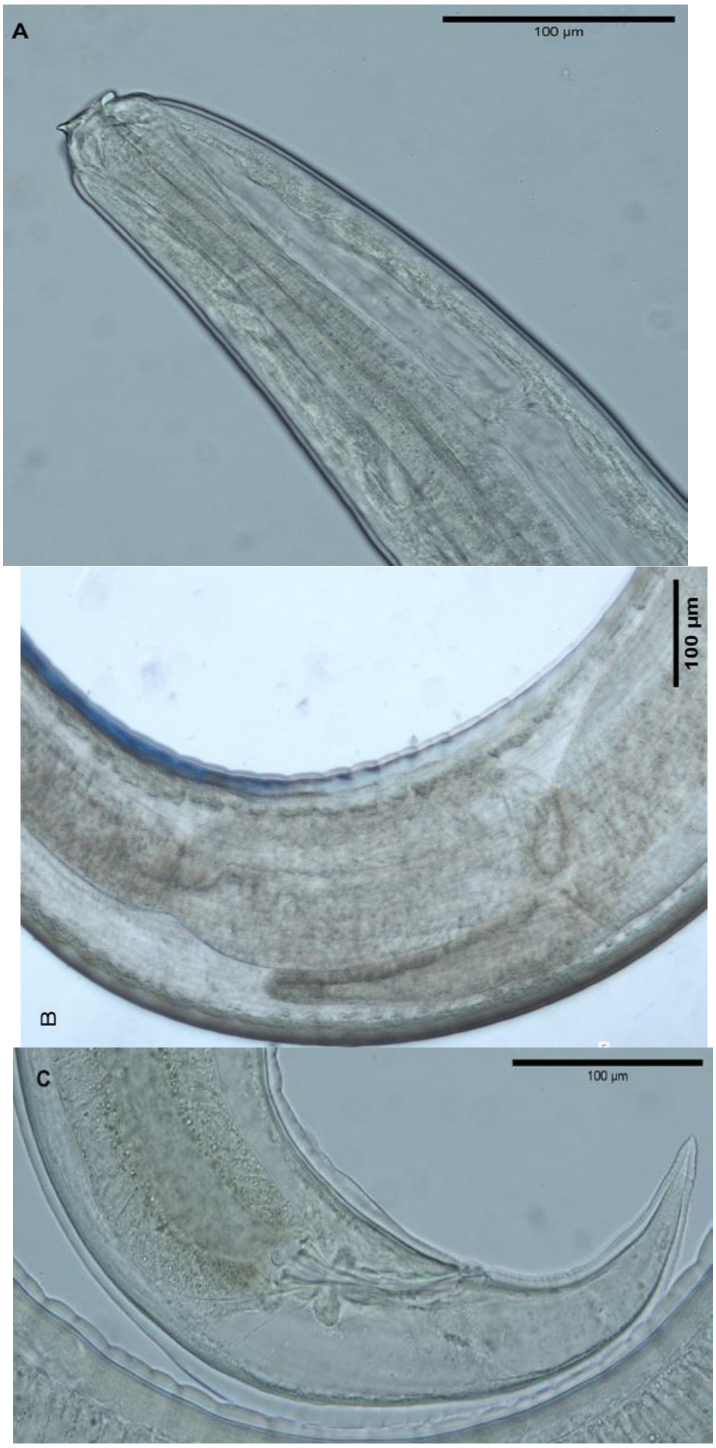

Figure 10: *Terranova* like (*Pseudoterranova* cf. *ceticola*): Anterior part (A), part with ventricle and caecum (B) and posterior part(C)

3.3 Molecular identification

3.3.1 BLAST Results

Blasting the ITS /*Cox2* gene sequences to reference sequences deposited in the GenBank suggested that the 5 nematode types recognized by morphology may belong to 8 different nematode species (Table 5).

Table 5: BLAST RESULT FOR NEMATODE ITS (ITS1-5.8S-ITS2) AND COX2 GENE SEQUENCES. NUMBER OF LARVAE BLASTED (n), MATCH PER. INDENT RANGE IN % (*), MATCHED SPECIES (**), NUMBER OF BASE PAIR BLASTED (BP), ACCESSION SEQUENCE IN GENBANK (R.S. ID), NOT OBTAINED (NO) AND CONCLUSION (CON.)

Nematode species	**ITS**			***Cox2***			**Con.**
	n	BLAST (bp)	R.S ID	n	BLAST (bp)	R.S ID	
Anisakis type I							
i)	4	100* *A.typica*** (884)	JQ912690	4	97.9-99.1* *A. typica*** (557)	DQ116427	*Anisakis typica*
ii)	4	100* *A. ziphidarum*** (856)	JQ912691	4	99.3-99.6* *A. ziphidarum*** (546)	DQ116430	*Anisakis ziphidarum*
iii)	0	NO	NO	1	99.3* *A. pegreffii* ** (567)	MG076946	*Anisakis pegreffii*
Anisakis type III							
i)	30	99.8-100* *A. brevispiculata*** (826)	MH481715	29	97.5-99.8* *A. brevispiculata*** (560)	MH669508	*Anisakis brevispiculata*
Anisakis type IV							
i)	23	99.1-99.5* *A. paggiae*** (851)	JQ912695	23	95.0-98.9* *A. cf. paggiae*** (556)	KF693770	*Anisakis* cf. *paggiae*
ii)	11	98.6-99.8* *A. paggiae* ** (826)	JQ912695	11	95.0-100* *A. paggiae* ** (568)	KF693769	*Anisakis paggiae*
Hysterothylacium like							
i)	8	99.1-99.3* *Hysterothylacium* sp. ** (886)	MT365537	6	86.4-87.8* *Hysterothylacium deardorffoverstreetorum*** (571)	KU886687	Raphidascarididae gen. sp.
Pseudoterranova like							
i)	16	99.9-100* *Anisakis sp.*** (801)	KC342894	15	96.8-97.6* *P. ceticola* ** (570)	DQ116435	*Pseudoterranova* cf. *ceticola*

3.4. Description of *Pseudoterranova* cf. *ceticola* third larval stage (L3)

Small pale-white larvae, with a thick-set appearance (see Fig. 10 and 11). The body was widest near the middle; the body total length: max width ratio was 17-31 (mean 24.7 ± 4.2, N=17). *In-situ*, the larvae were coiled like a watch spring when found in the viscera. Two larvae found in the muscle had a light neon-bluish color when exposed to UV-light (Fig. 12). Lip anlagen (see Figure 10 A) visible through the cuticle, wider than long. A plate like cuticular thickening extend dorsally approximately 21-27 µm (ca.22.6 ±1.9) in U-shape from the tooth, apparently surrounding the mouth (Fig. 10 A).

Boring tooth and excretory pore at ventral side, excretory pore near base of tooth. Oesophagus narrows by the nerve ring which is positioned at around 8.4% of oesophagus length. Oesophagus length constitute 9-14 % (mean 10 %) of body length. The ventricle relatively long and wide; ventricle length about half of oesophagus length (oesophagus length: ventricle length ratio 1.5-2.8 µm (2.0 ± 0.3; N=17)) (Fig. 11B). The length of the intestinal caecum represents 48-85 % (mean 74 ± 13) of the ventricle length. The tail is conical, long and pointed (but curved) without a mucron (Fig. 10C).

Transversal cuticular annulations revealed clearly at the tail. The mean length from the cloak/anus to tail tip (Tail L) were measured to 187 ± 9.8 µm (see table 6) for more information of measurements).

TABLE 6: MEASUREMENTS OF MOLECULARLY IDENTIFIED *PSEUDOTERRANOVA* CF. *CETICOLA* LARVAE. L=LENGTH, W=WIDTH, N=NUMBER OF MEASUREMENTS, SD=STANDARD DEVIATION. MEASUREMENTS IN µM UNLESS SPECIFIED.

	N	Mean	SD	Min.-Max.
Total L (mm)	17	8.5	0.8	7-10
Max. W	17	348.2	50	279-442
Oesophagus L	15	977.7	100	807-1147
Anterior to nerve ring	17	84.8	4.3	78-90
Ventricle L	17	493.5	78	378-647
Ventricle W	17	152.3	21	112-175
Caecum L	17	378.1	86	248-556
Tail L	17	187	9.8	160-204

FIGURE 11: *PSEUDOTERRANOVA CF. CETICOLA (L3*). POSTERIOR PART (A), VENTRICLE (V) AND POSTERIOR PART (P)

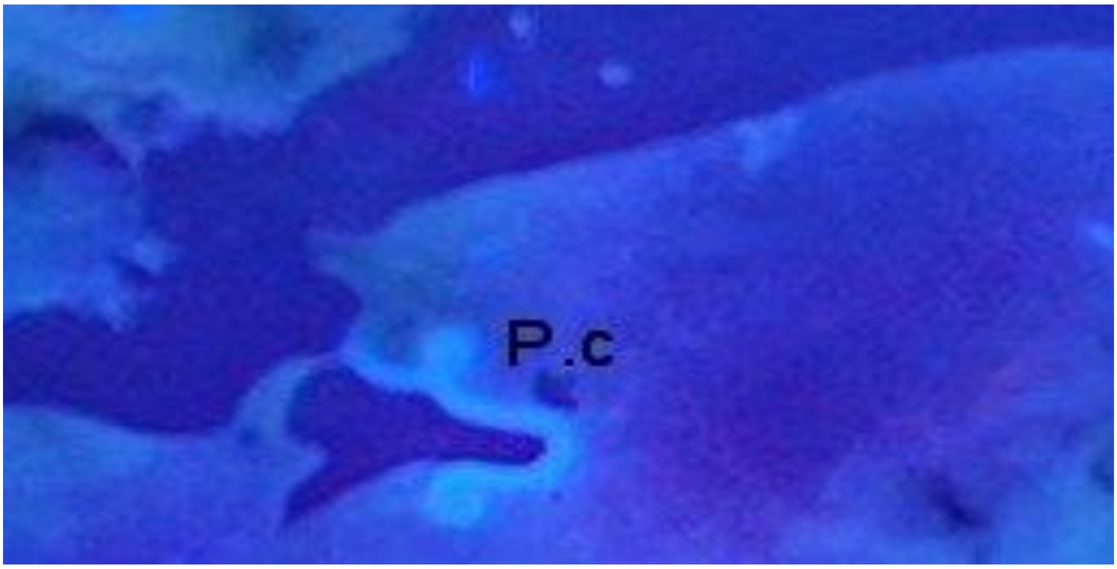

FIGURE 12: UV LIGHTED *PSEUDOTERRANOVA CF. CETICOLA (L3).*

3.5 Epizootic data

3.5.1 Geographical distribution

Fish were sampled from seventeen trawl stations (TS) (TS4601, 4603-4618) (see Table 3 above). Ascaridoid nematode larvae were found in fish from 57 areas. There was some geographical variation in the occurrence of the larval types (Table 7). All five morphotypes of larvae were collected off West Sahara – South Canary Islands waters (TS 4604 and 4606) (see Table 8). *Anisakis typica, A. ziphidarum, A. brevispiculata, A. cf. paggiae* and *P. ceticola* were all found in Cape Verde waters (TS 4601).

Anisakis typica, A. brevispiculata and Raphidascarididae sp. were collected in Canary Island waters (TS 4607). The northernmost location where Raphidascarididae sp. larvae were found were the station 4607 in the Canary Island water.

Anisakis pegreffii was the only species found off Portugal waters (TS 4614), the northernmost parasite finding of the research campaign. *Anisakis ziphidarum* and *P.* cf. *ceticola* were also found in Morocco waters (TS 4610).

3.5.2 Infection data

Ascaridoid L3 (N=177) were collected from a total of 53 fish individuals representing 13 species: *B. indicus; C. warmingii; C. danae; D. dumerilii; D. mollis; D. rafinesquii; D. argenteus; E. pelecanoides; L. guentheri; L. gemellarii; M. muelleri; M. argipalla* and *N. atrum* (see Table 8).
Anisakis pegreffii was only found as a single larva in *M. muelleri*. The larva was found free (not encapsulated) in the viscera of the fish.

Anisakis typica was found in *D. dumerilii, C. warmingii, C. danae* and *L. gemellarii*. The larvae from *D. dumerilii* and *C. warmingii* were emerged from the fishes' gill (likely *post-mortem* migration). Findings from the other fish-species were from the viscera.

Anisakis ziphidarum was found with *E. pelecanoides* (plastic bag harboring the fish, again probably due to *post-mortem* migration). The nematode was also found in *D. rafinesquii*, there laying free over the organs.

Anisakis brevispiculata was found in *D. argenteus, L. guentheri* and *N. atrum*. Larvae were mainly found free, coiled among the pylorus blind sacs in those fishes. In addition, two larvae were found within the stomach of *D. argenteus*, and two larvae were revealed by UV light in the rest of viscera.

Anisakis cf. *paggiae* and *A. paggiae* were found in *D. argenteus* and *A. paggiae* also in *N. atrum*. The larvae were free, coiled in the pylorus blind sacs or in the viscera. In addition, four larvae were found in the stomach of *D. argenteus*. No findings in the musculature.

Raphidascarididae gen. sp. was only found in *D. argenteus*. Larvae were either found encapsulated in yellow/orange capsules inside the stomach wall (Fig. 13), in the pyloric caeca of the fish, or as free larvae on the viscera, or inside the stomach.

Pseudoterranova cf. *ceticola* was found in *B. indicus, C. danae, D. argenteus, D. mollis* and *D. rafinesquii*. Larvae were mainly found free, compact spiral-coiled in the viscera and in a few cases 4/177 worms in the flesh of the fish (revealed by UV-lightening).

Nematode parasites were not found in *A. aculeatus; A. hemigymnus; C. sloani; C. schmidti; C. microdon*; *D. brachycephalus; D. effulgens; D. metopoclampus; H. hygomii; H. reinhardtii; H. taaning; L. cuprarius; L. lineatus; L. dofleini; N. resplendens; P. crassiceps* or *Sternoptyx* sp.

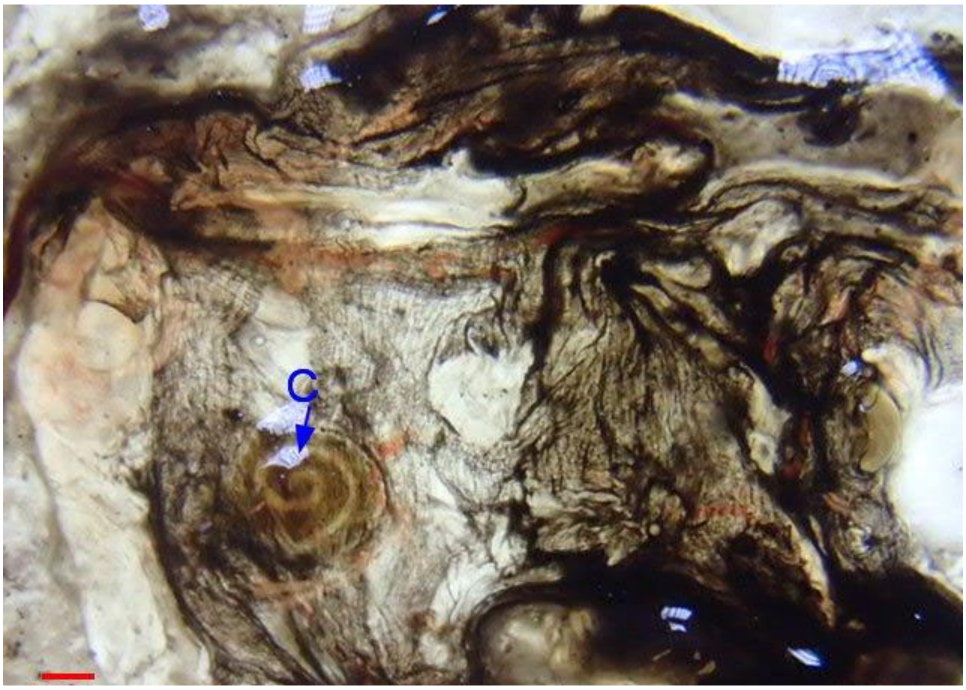

FIGURE 13: RAPHIDASCARIDIDAE GEN. SP. *IN SITU*, WITHIN A CAPSULE (C) IN STOMACH-TISSUE OF *DIRETMUS ARGENTEUS.*

Table 7: Larval morphotypes and species found per fish species and Trawl Station (TS). N= number of fish examined, Ni= number of fish infected, Ps= positive samples, Nl= number of larva found, *A. pegreffii* (A), *A. typica* (B), *A. ziphidarum* (C), *A. brevispiculata* (D), *A. paggiae* (E), *A. cf. paggiae* (F).

Fish species	**N**	**Ni**	***Anisakis* Type I (A; B; C)**		***Anisakis* Type III (D)**		***Anisakis* Type IV (E; F)**		***P.* cf. *ceticola***		**Raphidascarididae gen. sp.**	
			Ps	Nl	Ps	Nl	Ps	Nl	Ps	Nl	Ps	Nl
TS.4601 (Cape verde)												
D. dumerilii	1	1	1	1^{B}	0	0	0	0	0	0	0	0
D. rafinesquii	2	1	0	0	0	0	0	0	1	1	0	0
L. guentheri	5	1	0	0	1	1	0	0	0	0	0	0
N. atrum	12	3	0	0	1	1	2	2^{E}	0	0	0	0
TS.4604 (West Sahara)												
C. warmingii	18	1	1	1^{B}	0	0	0	0	0	0	0	0
D. mollis	14	1	0	0	0	0	0	0	1	1	0	0
D. argenteus	11	8	0	0	8	27	6	8^{E+F}	2	2	4	28
M. argipalla	7	1	0	0	0	0	0	0	1	1	0	0
D. rafinesquii	48	17	2	2^{c}	0	0	0	0	15	25	0	0
TS.4605												
L. gemellarii	2	1	1	1^{B}	0	0	0	0	0	0	0	0
TS.4606 (South Canary Islands)												
C. danae	20	1	0	0	0	0	0	0	1	1	0	0
D. mollis	40	3	0	0	0	0	0	0	3	3	0	0
D. argenteus	8	8	0	0	6	14	5	27^{E+F}	1	1	4	9
TS.4607 (Canary Islands)												
C. danae	17	1	1	1^{B}	0	0	0	0	0	0	0	0
D. argenteus	2	2	0	0	2	8	0	0	0	0	2	6
TS.4610 (Morocco)												
B. indicus	8	1	0	0	0	0	0	0	1	1	0	0
E. pelecanoides	7	1	0	2^{C*}	0	0	0	0	1	1	0	0
TS.4614 (Portugal)												
M. muelleri	102	1	1	1^{A}	0	0	0	0	0	0	0	0

*Two larvae were found in the plastic bag, that harbored the *E. pelecanoides* from the TS. 4610.

TABLE 8: TABLE SHOWING OVERVIEW OF THE INFECTION LEVELS OF ASCARIDOID (ASC.) NEMATODES; ANISAKID (ANI.) OR RAPHIDASCARIDID (RAPH.) PRESENT IN MESOPELAGIC FISHES. PREVALENCE (P) (%), MEAN ABUNDANCE (MA), MEAN WEIGHT (MW), TOTAL WEIGHT OF THE SAMPLE (TW) AND DENSITY (N WORMS/KG). SORTED BY FISH-HOST FAMILY. (FOR INFECTION LEVEL DATA FOR EACH NEMATODE SPECIES SEE TABLE 1 IN APPENDIX).

Fish host: Family Species	**Total N fish**	**Total Asc.**		**mW fish (g)**	**tW fish (g)**	**Total Ani.**	**Total Raph.**	**Density Ani.**	**Density Raph.**	**Density all Asc.**
		P	mA							
Myctophidae										
Bolinichthys indicus	41	2	0.02	0.7	30.2	1	0	33	0	33
Ceratoscopelus warmingii	51	2	0.02	0.8	43.0	1	0	23	0	23
Diaphus dumerilii	1	100	1	1.2	1.2	1	0			
Diaphus mollis	58	7	0.07	1.4	78.3	4	0	7	0	7
Diaphus rafinesquii	50	36	0.50	3.4	167.5	25	0	149	0	149
Lepidophanes guentheri	5	20	0.20	0.7	3.4	1	0	298	0	298
Lobianchia gemellarii	4	25	0.25	4.3	17.4	1	0	58	0	58
Nannobrachium atrum	26	12	0.12	4.5	117.4	3	0	26	0	26
Diretmidae										
Diretmus argenteus	23	78	5.61	16.5	380.5	87	42	229	110	339
Sternoptychidae										
Maurolicus muelleri	137	1	0.01	1.0	135.7	1	0	7	0	7
Platytroctidae										
Maulisia argipalla	7	14	0.14	10.5	73.5	1	0	14	0	14
Eurypharyngidae										
Eurypharynx pelecanoides	19	16	0.16	5.5	105.1	3	0	29	0	29
Stomiidae										
Chauliodus danae	55	4	0.04	2.4	132.9	2	0	15	0	15

3.6 Nematode abundance and host size

At the trawl station with highest parasite abundance (Ts4604), the abundance of ascaridoid larvae in both *D. rafinesquii* and *D. argenteus* was positively related to host weight (see table 9). When analysing the ascaridoid species, the relationship of abundance with weight was particularly strong for *A. brevispiculata* and *A. paggiae* (*sensu lato* (*s. l.*)) in *D. argenteus*. *Pseudoterranova* cf. *ceticola* abundance was positively related to host size (weight) in both these host species. Too few *D. rafinesquii* (n=2) and *D. argenteus* (n=2) were infected with *A. ziphidarum* or *Pseudoterranova* cf. *ceticola* respectively, to reveal any relationship between abundance and size.

Table 9: The relationship of parasite abundance to fish weight in gram for *Diaphus rafinesquii* and *Diretmus argenteus* from TS4604. n= number of larvae, R_s= Spearmann rank correlation coefficient; *p*= p-value; P (%)= prevalence.

Fish species	***Diaphus rafinesquii* (n=48)**				***Diretmus argenteus* (n=11)**			
	n	R_s	*p*	P (%)	n	R_s	*p*	P (%)
Total	25	0.451	0.001*	35	68	0.818	0.002*	73
Anisakis brevispiculata	0			0	28	0.899	0.000*	73
***Anisakis paggiae* (s. l.)**	0			0	7	0.829	0.002*	45
Anisakis ziphidarum	2	0.233	0.110	6	0			0
***Hysterothylacium* sp.**	0			0	27	0.385	0.241	36
Pseudoterranova cf. ceticola	23	0.451	0.001*	31	2	0.670	0.023*	18

3.7 Other parasites

Two specimens of a parasitic pennellid copepod, *Sarcotretes* sp. were found attached to the outer surface of two *L. alatus* at TS4601. Two *Sarcotretes scopeli* were found on two *B. glaciale* at TS4618. Another pennellid, *Cardiodectes medusaeus* was found on a *N. atrium* from TS4610. An unidentified cestode plerocercoid, morphologically a *Scolex pleuronectis* with bilocular bothria, was collected from the intestine of a *C. danae* sampled at TS4606, and a *C. sloani* from TS4601. An acanthocephalan, *Echinorhynchus* sp. was collected from a *C. schmidti* from TS4601.

3.8 Previous records of ascaridoid nematodes in mesopelagic fishes from Mediterranean and north Atlantic waters

TABLE 10: REVIEWED BIBLIOGRAPHY OF PREVIOUS EPIDEMIOLOGICAL STUDIES UPON THE PRESENCE OF ASCARIDOID NEMATODES IN MESOPELAGIC FISHES FROM ATLANTIC WATERS.

Parasite	**Host**	**Geographic area**	**Reference**
Anisakis simplex	*Maurolicus muelleri*	Western Norway	Hamre & Karlsbakk 2002
	Borostomias antarcticus	off Greenland	Klimpel et al. 2006
	Argentina silus	off Greenland	Klimpel et al. 2006
Anisakis simplex (s.s.)	*Maurolicus muelleri*	Norwegian Deep	Klimpel et al. 2007a
	Maurolicus muelleri	Mid-Atlantic Ridge	Klimpel et al. 2007a
	Myctophum punctatum	Mid-Atlantic Ridge	Klimpel et al. 2008, 2010
	Notoscopelus kroyeri	Mid-Atlantic Ridge	Klimpel et al. 2008
	Aphanopus carbo	Madeira	Costa et al. 2003; Pontes et al. 2005
Anisakis pegreffii	*Ceratoscopelus maderensis*	Mediterranean Sea	Mateu et al. 2015
	Notoscopelus elongatus	Mediterranean Sea	Mateu et al. 2015
	Diaphus metopoclampus	Mediterranean Sea	Gaglio et al. 2018
	Myctophum punctatum	Macaronesian	Klimpel et al. 2010
	Aphanopus carbo	Madeira	Costa et al. 2003; Pontes et al. 2005
Anisakis ziphidarum	*Diaphus metopoclampus*	Mediterranean Sea	Gaglio et al. 2018
	Aphanopus carbo	Madeira	Costa et al. 200; Pontes et al. 2005
Anisakis physeteris	*Ceratoscopelus maderensis*	Mediterranean Sea	Mateu et al. 2015
	Notoscopelus elongatus	Mediterranean Sea	Mateu et al. 2015
	Electrona risso	Mediterranean Sea	Gaglio et al. 2018
	Vinciguerria attenuate	Mediterranean Sea	Gaglio et al. 2018
	Aphanopus carbo	Madeira	Costa et al. 2003
Anisakis brevispiculata	*Aphanopus carbo*	Madeira	Costa et al. 2003
Anisakis paggiae	*Anoplogaster cornuta*	Irminger Sea	Klimpel et al. 2011
Hysterothylacium aduncum	*Maurolicus muelleri*	Norwegian Sea	Karlsbakk & Nilsen 1993
	Maurolicus muelleri	Western Norway	Hamre & Karlsbakk 2002
	Maurolicus muelleri	Norwegian Deep	Klimpel et al. 2007a
	Benthosema glaciale	Norwegian Sea	Karlsbakk & Nilsen 1993
Hysterothylacium sp.	*Notoscopelus elongatus*	Mediterranean Sea	Mateu et al. 2015

4 Discussion

4.1 Morphology and genetics

Five nematode larval types were morphologically distinguished from the 1271 fishes examined. Of the 177 larvae collected, 104 specimens were molecularly identified to species level. The few (9) *Anisakis* type I larvae represented 3 species *A. typica (4)*, *A. ziphidarum* (4) and one *A. pegreffii*. All the type III larvae molecularly identified were *A. brevispiculata*, and the type IV larvae were *A. paggiae* or a *A. paggiae* related genotype (i.e. *A.* cf. *paggiae*). The *Terranova*-like larvae were closely related to *Pseudoterranova ceticola*. It was not possible to identify the raphidascaridid larvae to species level, since there is not an identical (or even close) reference sequence from adult individual deposited in GenBank. All larvae were L3, as they lacked lips and presented a boring tooth.

Five different types of larvae were distinguished from each other by morphological characteristics. In addition, comparison of gene sequences of these larvae to corresponding sequences deposited in GenBank showed that the genes belonged to eight different species of nematodes. Means the genetic analyses can show more species diversity than morphology alone can show hence to ascarides species.

4.1.1 *Anisakis* spp.

Anisakis type I, III and IV larvae were morphologically classified mainly attending to differences in shape and length of the ventricle and tail (e.g. present or absence of a mucron at the tail tip) (Murata et al. 2011). *Anisakis* type I larvae englobes 6 *Anisakis* species; i.e. *A. simplex* (*s.s.*), *A. pegreffii*, *A. berlandi*, *A. typica*, *A. ziphidarum* and *A. nascettii* (Mattiucci et al. 2018), which share morphological characteristics (i.e. long ventricle, junction between the ventricle and intestine oblique, rounded tail with a mucron at its tip (Berland (1961)), and are therefore not possible to separate only by morphology.

The use of diagnostic genetic markers (e.g. mtDNA *cox2* and rDNA ITS) is needed for species identification (reviewed in Mattiucci et al. (2018)). For the *Anisakis* type I larvae found in the present study, the genetic results (upon the previous markers) showed the presence of three *Anisakis* spp., consisting in four *A. typica*, four *A. ziphidarum* and one *A. pegreffii*.

The larvae categorized as *Anisakis* type III were recognized by having a stout body, short ventriculus and a horizontal junction between the ventricle and intestine (Shiraki 1974; Murata et al. 2011). Shiraki (1974) reported the *Anisakis* Type III had a tail without mucron. However, Type III larva was previously reported to have a tiny mucron (Oshima (1972) cited in Murata et al. (2011)). Murata et al. (2011) reported that a few (2 out of 10) Type III larvae had a tiny spine-like mucron and concluded that the tail mucron is an unstable characteristic. Sardella & Luque (2016) described 1 larva genetically identified as *A. brevispiculata* and collected from the fish *Pinguipes brasilianus* from Rio de Janeiro (Brazil) , as not having terminal mucron (Dos Reis Sardella and Luque 2016). Recently, Cabrera-Gil et al. (2018) reported that out of 130 *Anisakis* Type III larvae found in myctophids from the Arabian Sea, 11% had a spine-like mucron. In the present study, apart from the characteristics described before, a small mucron was revealed (sometimes clearer than others) at the end of a short, rounded tail for all specimens found.

Murata et al. (2011) found that all *Anisakis* type III larvae from *Beryx splendens* in Japan belonged to *A. brevispiculata*. Here, the sequence analysis of the mtDNA *cox2* gene suggested that all the *Anisakis* type III specimens from Atlantic mesopelagic fishes represent *A. brevispiculata* (See table 5). Therefore, the larvae of this anisakid appears morphologically identifiable to species. However, the rDNA ITS sequences obtained are likely conclusive. They matched 99.8 to 100% with sequences obtained from adult nematodes and deposited in GenBank. A higher rate of evolution and genetic variability is expected for the mitochondrial gene (e.g. *cox2*) than that of the ribosomal gene (e.g. rDNA ITS) (Ceballos-Mendiola et al. 2010), as it was found in the present study. The genetic variability at the rDNA ITS gene should be further studied (e.g. analysis of the different ribosomal haplotypes, phylogenetic studies, morphological and genetic studies from adult nematodes, etc.), but it is out of the scope of this work. Thus, based on their morphology and the mtDNA *cox2* result, our larvae are believed to belong to the species *A. brevispiculata*.

*Anisaki*s type IV larvae here recognized from the description in Murata et al. (2011), were identified as *A. paggiae* (*s. l.*). The specimen’s ITS genes showed relatively high identity (98.6 -99.8%) with *A. paggiae*, a parasite of *K. breviceps* from northwestern Atlantic Ocean (Mattiucci et al. 2014). However, when comparing the *cox2* genes, the majority of the sequences showed highest identity to a genotype called *A.* cf. *paggiae* by Di Azevedo et al. (2017). This genotype could represent a separate species (Di Azevedo et al. 2017) so far only found as adults in *K. sima* from Brazil. Unfortunately, no corresponding ITS sequence of the Brazilian specimen is available for comparison. However, since the present *cox2* sequences diverged by as much as 4.9% from *A. cf. paggiae* and from *A. paggiae*, the diversity among these whale parasites could exceed *A. paggiae* and *A.* cf. *paggiae* based on the *cox2* matches.

4.1.2 *Pseudoterranova* cf. *ceticola*

The larvae molecularly identified as close to *P. ceticola* share some morphological characteristics (e.g. presence of intestinal caecum and excretory pore below tooth) with adults of *Terranova ceticola* (Deardorff and Overstreet 1981) and the *Terranova* larval types (Cannon 1977) so they were categorized as *Terranova*-like. The blasting results for *cox2* gene, revealed 96.8-97.6% identity to a *cox2* sequence from adult *Pseudoterranova ceticola* from Caribbean *K. sima* (Valentini et al. 2006).

Therefore, these anisakid larvae are here tentatively identified as *P.* cf. *ceticola*. However, molecular data does not exist for a related species, *P. kogiae*, originally described from an Australian *K. breviceps* (Johnston and Mawson 1939). These anisakid species were only found as adults in kogiid whales (*K. breviceps* and *K. sima*) (Abollo and Pascual 2002;Colón-Llavina et al. 2009;González-Solís et al. 2006;Mcalpine et al. 1997), but it is at present unknown whether the kogiid whales may carry one widespread, or several host specific or geographically restricted *Pseudoterranova* species.

A molecularly identified larva was found by Costa et al. (2014) in the deep-water shark *Centrophorus squamosus* taken off Madeira, but a sequence was not provided. The larval type of these *Pseudoterranova* spp. has so far not ben described. Therefore, the morphology larvae here molecularly identified as *Pseudoterranova* cf. *ceticola* was described. Further studies on *P. kogiae* and adult nematodes from kogiid whales are needed to completely resolve the species identity problem.

4.1.3 Raphidascarididae gen. sp. larvae

A raphidascaridid larva resembling larvae of some members of the genus *Hysterothylacium* was exclusively found in *D. argenteus*. The nematodes were characterized by a small, rounded ventricle, a long intestinal caecum, and a very long ventricular appendix. The tail was long and had a spine at the tail tip. Such characteristics may be found in several raphidascaridid genera (e.g., *Hysterothylacium*, *Lappetascaris* and *Heterotyphlum*) (Hossen and Shamsi 2019). The ITS genetical results suggests that all belonged to a single species and reveals affinity (99.3%) to a raphidascaridid found in the Mediterranean octopus *Eledone* sp. (Guardone et al. 2020) (see below). The raphidascaridid L3 found here share some features (see above) to the raphidascaridid morphologically assigned as *Lappetascaris* sp. according to (Nagasawa and Moravec 1995, 2002; Culurgioni et al. 2010) or *Hysterothylacium* sp. by (Guardone et al. 2020).

In relation to this, Nagasawa and Moravec (1995; 2002) reported the occurrence of *Lappetascaris* sp. larvae in squids from the Sea of Japan and from Central and Western North

Pacific Ocean. Also, Culurgioni et al (2010) reported the presence of *Lappetascaris* sp. in squids from Mediterranean Sea. The best match of the present *Cox2* gene sequences was only 86.4-87.8%, representing *Hysterothylacium deardorffoverstreetorum* sequences. Hence the present larvae represent a species not sequenced so far, and also does not appear to be congeneric with any of the *Hysterothylacium* spp. from which there are *cox2* sequences in GenBank. Based on both the genetic results and their morphology, the present larva cannot be assigned to a species or genus, and possibly represent a new species. A genetic match with adult specimens is needed to resolve the species identity.

4.2 Biological aspects

4.2.1 Geographical distribution and host records

It appears that *Anisakis* spp. have different host specificity (in the final host) and that the different life stages demand different biological taxa as hosts. For instance, adult *Anisakis* spp. are found as parasites from several species of dolphins, porpoises, and whales, whereas L3 are found as parasites in marine teleosts and squids from pelagic to demersal zone (reviewed by Mattiucci et al. 2018). It also appears to be differences in the geographical distribution in the Atlantic waters among the species. In the present study, the sampling areas with highest diversity of nematode parasites were near Cape Verde (TS 4601), West Sahara (TS 4604) and Canary Islands (TS 4606, 4607), where all five larval types were found. Portugal (TS 4614) was the northernmost sea area having an infected *M. muelleri* with a single *A. pegreffii* larva.

4.2.1.2 *Anisakis pegreffii*

Anisakis pegreffii is found to be the dominating species of *Anisakis* in the Mediterranean Sea (Mattiucci et al. 2018). The Iberian coast seems to be its northern limit of distribution in the Atlantic Ocean (Abollo et al. 2001; Mattiucci et al. 2004, 2018) as *A. pegreffii* is rarely found in northern NE Atlantic waters, e.g. with migratiory fish such as Atlantic mackerel (*Scomber scombrus*) (reviewed by Levsen et al. 2020). *Anisakis pegreffii* appears to be a generalist both for final and transport host. It has been reported as L3 from about sixty pelagic and benthopelagic fish species, and as adults from six dolphins and whales species from the Atlantic and Pacific waters (reviewed by Mattiucci et al. 2018).

4.2.1.2 *Anisakis typica*

The few (4) *Anisakis typica* larvae recovered were found in single infections in four different fish species, i.e. *D. dumerilii*, *C. warmingii*, *C. danae* and *L. gemellarii*. These were also from four different localities of the Macaronesia region, from Cape Verde to the Morocco coast. *Anisakis typica* has previously been reported from *Merluccius merluccius, Platichthys flesus, Scomber scombrus* and *Scomber japonicus* from Morocco-Mauritania Atlantic coast and Portugal waters in the North-East Atlantic Ocean (Mattiucci et al. 2002; Marques et al. 2006; Farjallah et al. 2008). It has been also reported in *Pagellus bogaraveo*, *S. japonicus* and *Trachurus picturatus* off Madeira waters (Mattiucci et al. 2002; Hermida et al. 2012) and several fish species from other waters (see Table 5 in Mattiucci et al. 2018). In terms of final host, *A. typica* is mainly a parasite of oceanic dolphins in warmer temperate and tropical waters (reviewed by Mattiucci et al., 2018). In addition, it has been reported as adult from *K. breviceps* and *K. sima* from Brazilian (Iniguez et al. 2011; Di Azevedo et al., 2017) and Philippine waters (Quiazon et al. 2013; Quiazon 2016).

4.2.1.3 *Anisakis ziphidarum*

Anisakis ziphidarum was found in *D. rafinesquii* off Western Sahara waters (TS4604) and in *E. pelecanoides* off Morocco (TS4610). *Anisakis ziphidarum* has previously been reported from other *Diaphus* species, i.e. *Diaphus metopoclampus* from the Mediterranean Sea (Gaglio et al. 2018). This nematode appears to show specificity to the beaked whales (Ziphiidae) (e.g. *Mesoplodon layardii* and *Ziphius cavirostris*, etc.), as it was found in ziphiids from the South Atlantic Ocean (off the South African coast) and other waters (reviewed by Mattiucci et al. 2018). In addition, it has been reported from *K. sima* from Philippine waters (Quiazon et al., 2013).

4.2.1.4 *Anisakis cf. paggiae, A. paggiae* and *A. brevispiculata*

Anisakis paggiae were collected from *N. atrium* at Cape Verde (TS4601) and the rest eleven specimens were collected from *D. argenteus* off West Sahara (TS4604) and South Canary Islands (TS4606). In addition, the rest twenty-three *Anisakis cf. paggiae* were only sampled from *D. argenteus* from the same sampling areas as *A. paggiae*. Both nematode species occurred in mixed infections in the same fish specimen. Interestingly, *A. paggiae, A. cf. paggiae* and *A. brevispiculata* were mainly found in *D. argenteus*. *Anisakis paggiae* and *A. brevispiculata* did also share *N. atrium* as transport host, but *A. brevispiculata* was found in even more sampling areas and hosts than *A. paggiae/A. cf. paggiae*. *Anisakis brevispiculata* was found in *D. argenteus* at the same areas as *A. paggiae/A. cf. paggiae*, but also at south of Canary Islands (TS4606) and had a higher prevalence in *D. argenteus* of 70% compared to the former (see table 1, at the appendix). *Anisakis brevispiculata* was also found at Cape Verde (TS4601) in *L. guentheri* with 20% prevalence.

Anisakis brevispiculata, *A. paggiae* and *A. cf. paggiae* appear to have specificity to kogiid whales (Kogiidae) (further details at section 4.2.2). *Anisakis brevispiculata* and *A. paggiae* appear to be more generalist at the fish transport host level. They have been reported from swordfish (*Xiphias gladius*) from Atlantic waters, as well as in several other fish species from different waters (reviewed by Mattiucci et al 2018).

4.2.1.5 *Pseudoterranova* cf. *ceticola* and the Raphidascarididae sp.

Pseudoterranova cf. *ceticola* was mainly collected from *D. rafinesquii* reaching a prevalence of 38% for the total fish sample. Specimens of *D. mollis* and *D. argenteus* sampled at TS4604 where infected with *P.* cf. *ceticola* as well. These fish species, including *C. danae*, were found infected as well with *P.* cf. *ceticola* when sampled at the Canary Islands (TS4606). In addition, two single specimens were also collected from *B. indicus* and *E. pelecanoides* at the Madeira Island (Ts4610).

The species *P. ceticola* seems to be strongly connected to the kogiid whales. It has been reported from stranded *K. breviceps* from localities in Canada (Mcalpine et al. 1997)), Mexico (González-Solís et al. 2006) and from NW Spain (Abollo and Pascual 2002), and from *K. sima* stranded in the Caribbean sea (Colón-Llavina et al. 2009). In addition, it has been reported from *K. breviceps* of Florida, representing the only *cox2* sequence of the species available in GenBank (Valentini et al. 2006). The larva of (likely) the latter is for the first time characterized here.

Compared to the other nematode species found in this study, the Raphidascarididae gen. sp. larvae appeared host specific. *Diretmus argenteus* was the only fish species found infected. It is therefore reasonable to assume that *D. argenteus* is an important fish transport host within the life cycle of this species of nematode as its prevalence was 48%.

4.2.2 Ecological determinants of parasite diversity and abundance

There were no fishes parasitized with nematodes at any of the other locations than those previously mentioned. This might reflect an absence or low abundance of suitable final hosts in these geographical studied areas. It could also be the case that the species of nematodes found in this study, use other fish in their transmission than those sampled. However, since some of the fish species were found to be infected at other sampling areas, it appears that there can be large spatial variation in the abundance and diversity of ascaridoids in mesopelagic fish.

Across the present samples, 24 % of the 177 larvae recovered belonged to a raphidascaridid which likely matures in a fish. The rest (76 %) mature in cetaceans. Among those, kogiid whales (i.e. *K. sima* and *K. breviceps*) were found as final hosts to 100% of the anisakids found here, i.e. *A. brevispiculata, A. paggiae* (*s. l.*)*, P. ceticola*, *A. ziphidarum*, *A. typica* and *A. pegreffii* (Cavallero et al. 2011; Iñiguez et al. 2011; Quiazon et al. 2013; Di Azevedo et al. 2015; Quiazon 2016; Santoro et al. 2018; Hossen and Shamsi 2019). Although, *A. typica, A pegreffii* and *A. typica* are more likely to accidental infection as those are found to be parasites from other whales as final hosts (Mattiucci et al. 2018).

In addition, *K. breviceps* has been reported stranded in the studied areas (Fernández et al. 2009; Berrow et al. 2015) and Bloodworth and Odell 2008 (See fig 3 Bloodworth and Odell 2008) and McAlpine (2018) suggested that this area is a suitable habitat for both kogiid whales (Mcalpine 2018) *Kogia sima* is suggested to occur in the tropical Atlantic, Indian and Pacific Oceans (from approximately 45°S to 45°N) (McAlpine 2009; Kiszka and Braulik 2010).

There are some studies which indicated that mesopelagic fish are prey for *K. breviceps* and *K. sima* (Bloodworth and Odell 2008; Mcalpine 2009; West et al. 2009; Naito et al. 2013). West et al. (2009) analyzed the stomach content of stranded *K. breviceps* of the Hawaiian archipelago, and identified among others, *D. argenteus*, *C. warmingii*, *E. pelicanoides*, *Diaphus* sp., *Nannobrachium* sp., *Chauliodus macouni*, etc., which are species that were found here infected with anisakid nematodes, or congenerics to them. In addition, the parasites could be transmitted through the food web from mesopelagic fish to cephalopods and then to the whales, since squids are also known as a very important part of the diet of these kogiids (Cabrera-Gil et al. 2018). Hence, it appears that kogiid whales (e.g. *K. breviceps* and *K. sima*) can be presented as suitable final host.

Since parasites can be used as biological indicators (e.g., abundance and occurrence of the parasites relates to the distribution, migration patterns and ecology of their hosts) Klimpel and Palm 2011 findings suggest that the former kogiid whales are present (at least) in the sampling areas with infected mesopelagic fish. Further research is recommended to investigate *K. breviceps* and *K. sima* relationship to any of the mentioned larvae and fish prey (especially to *D. argenteus*).

4.2.3 Other aspect to consider.

Other aspect that could possibly affect the sampling data, such as the fish biodiversity and biomasses of the catch, is the trawling method. The macroplankton trawl and the multipelt trawl were used to get the samples, and apparently the latter might be more efficient in fishing compared to the macroplankton trawl (Anonymous 2019). However, the size of the fishes was the same regardless of which trawl was used. So, the very largest fish specimens may have been able to avoid the trawls. Hence, the occurrence of the fish hosts in a particular area, and with those also the larvae, appear to be more related to the sea area of sampling than the trawling method, but results should be interpreted with caution as they might be biased by the trawling method.

4.3 Food safety aspects

4.3.1 Risk of anisakidosis

When assessing the risk of anisakidosis (anisakiasis when caused by *Anisakis* spp.) to humans through consumption of fish products, one must consider that the fish product needs to be parasitized by a viable anisakid nematode (EFSA 2010). If so, freezing at -20ºC for not less than 24 hours or heating at >60 °C for at least 1 minute at the core of the fishery product would guarantee the killing of the parasite (EFSA 2010). Other treatments, such as marinating, undercooking, cold-smoking, or salting are considered insufficient to guarantee the killing of all the nematodes that may be present in the product (EFSA 2010). A recent study carried out on dried Northeast Arctic cod (*tørrfisk*) suggest that drying may be an efficient treatment to devitalize the anisakids present in the fish fillets (Bao et al. 2020). In addition, it is important to highlight that one singe larvae of *Anisakis* spp. is sufficient to cause disease to humans (Kołodziejczyk et al. 2020). Thus, consumption of mesopelagic fish as raw or lightly cooked may represent a risk of anisakidosis. To the best of our knowledge, mesopelagic fish are not eaten by humans directly to date, but these circumstances can change, so awareness given above is provided.

4.3.2 Allergy risk

The species *A. simplex* (*s.s.*) and *A. pegreffii* have been found to cause allergic responses to humans (Daschner et al. 2005; Mattiucci et al. 2013). The allergic response induced by *A. simplex* (*s. l.*) allergens can provoke two clinical forms namely gastro-allergic anisakiasis and allergy to *A. simplex* (EFSA 2010). Gastro-allergic anisakiasis (GAA) has been defined as an acute IgE-mediated generalized reaction (urticaria- angioedema-anaphylaxis) after the intake of *A. simplex* (*s. l.*) infected fish, where the live larva induces the symptoms during penetration of the gastric mucosa (Daschner et al. 2000; EFSA 2010; Adroher-Auroux and Benítez-Rodríguez 2020). Allergic urticaria appears to be the most common allergic symptom for GAA, and may or not be accompanied by gastric/abdominal symptoms (i.e. epigastric pain, nausea and vomiting) (EFSA 2010; Fernández-Fígares et al. 2015). Immunoglobulin E-mediated allergy is involved in so-called Type I immune hypersensitivity, hypersensitivities where proteins generally are the allergy trigger (the allergen) (Daschner et al. 2000; EFSA 2010), but other immunoglobulins (e.g. IgG, IgG4) are also involved (Daschner et al. 2014;2002)

Allergy to *Anisakis* spp. varies in symptoms from acute urticaria, angioedema to anaphylaxis (Daschner et al. 2000; EFSA 2010; Fernández-Fígares et al. 2015). Allergic responses have been described from patients which have consumed fish contaminated with dead larva or even just *Anisakis* spp. allergens. This is considered as “true” food allergy and implies that sensitized individuals may potentially suffer allergic symptoms even when the larvae is dead or even not physically present in the food, if *Anisakis* spp. allergens are present (EFSA 2010; Bao et al. 2019). In relation to this, fourteen allergens have been described from *A. simplex* (WHO/IUIS 2020), and several of those have been shown to be resistant to pepsin and heat treatments (Caballero and Moneo 2004; Moneo et al. 2005).

Farmed salmon from Norway and Japan has for now been considered to have very low risk of exposure to anisakid nematodes (Inoue et al. 2000; Levsen and Maage 2016). By feeding farmed salmon with pellets the transmission of larvae to the fish is prevented (Inoue et al. 2000; Levsen and Maage 2016). However, there are some studies suggesting that *Anisakis* spp. thermostable allergens can be transmitted to humans through consumption of farmed fish that was fed with infected fish or infected fishmeal (Fæste et al. 2015b, 2015a; Polimeno et al. 2021).

In relation to this, it has been reported allergic symptoms in 8 patients highly sensitized to *A. simplex* after eating chicken that were fed with fishmeal (Armentia et al 2006).

Recently, Polimeno et al. (2021) showed that sera from *A. simplex* allergic patients recognized *A. simplex* allergens (i.e. Ani s4) in the edible part of Mediterranean farmed sea bream (*Sparus aurata*) supposedly free of anisakid parasites. Sea breams were fed with commercial flour widely used as fish or poultry feed, from which the presence of antigenic proteins of *A. simplex* was also recognized (Polimeno et al. 2021). Thus, the allergens may remain allergenic after processing of fish meal and potentially, be transmitted by feeding farming salmon in Norway with feed containing fishmeal. Mesopelagic fish may host (as shown here) ascaridoid species, and fishmeal from them could therefore contain ascaridoid derived allergens. However, it remains unknown whether the anisakids found in this study such as *A. paggiae*, *A. brevispiculata*, *A. typica* and *A. ziphidarum*, *P.cf. ceticola* and Raphidascarididae sp. can cause allergies in humans.

Raphidascaridids such as *Hysterothylacium spp.* appear to not cause any harm to humans as they do not have the abilities to survive in warm intestines (e.g. they are adapted to fishes as final hosts). But this aspect remains controversial and not fully understood (Shamsi et al. 2013). *Hysterothylacium aduncum* has been reported as causative agent of fish borne zoonosis (González-Amores et al. 2015; Yagi et al., 1996). However, the parasite has not been genetically identified in the reports, so the diagnosis is debatable.

As mentioned above, *A. simplex* (*s.s.*) and *A. pegreffii* have been shown to be able to cause allergic responses in humans (Mattiucci et al., 2013). However, other ascaridoid species could potentially also be involved in allergic reactions presented by sensitized patients (Valero et al. 2003). For instance, a number of studies suggested that the ascaridoids (i.e. *Anisakis simplex* (*s.l.*), *Anisakis physeteris*, *Hysterothylacium aduncum*) share common allergens and may have a high degree of cross-reactivity among them (Iglesias et al. 1996; Leti and Research Laboratories 1998; Valero et al. 2003; Lozano Maldonado et al. 2004)

Recently, Kochanowski et al. (2020) performed a comparative proteomic profile among L3 of *A. simplex*, *P. decipiens* and *C. osculatum*. They detected several allergens and concluded that in addition to *A. simplex*, *P. decipiens* and *C. osculatum* should be considered potential sources of allergens that could lead to IgE-mediated hypersensitivity (Kochanowski et al. 2020). This increases the likelihood that the ascaridoids found in the present study also release thermostable allergens.

4.3.2 Risk assessment associated with density.

In here, a total of 2.5 kg of mesopelagic fishes (consisting of 32 fish species) were found to have 177 ascaridoids corresponding to a total density of 71 ascaridoids/Kg fish. In particular, the fish species with the highest density values were *D. argenteus* and *D. rafinesquii* with 339 and 155 ascaridoids/kg of fish, respectively. Hamre & Karlsbakk (2002) found a density of 4217 *H. aduncum* L3/Kg *M. muelleri* from Herdlefjorden. Estimated calculation of the data in Klimpel et al. (2007a) shown a density of 142 *A. simplex (s. s.)* and 2884 *H. aduncum* L3/ Kg *M. muelleri* fished from the Mid-Atlantic ridge and Norwegian Deep, respectively (Klimpel et al. 2007a). If compared with the low the density value found in this study off Portugal (5 larvae/Kg *M. muelleri*), it suggests that the ascaridoid parasitism may also be site conditioned.

Maurolicus muelleri and *B. glaciale* have been evaluated as potential good sources to provide marine lipids and protein in salmon feed in Norway (Olsen et al. 2020).

In addition, the former density values appear very high when compared to the value (3 anisakids/kg of fillet) found in fresh Northeast Arctic cod (*skrei*) from Lofoten (Bao et al., 2021). It is important to mention here, that most of ascaridoid larvae were commonly present in the viscera of fishes rather than in flesh in the present study. Commonly, most ascaridoids that may be present in fishes are in the viscera (Levsen et al. 2018). In this sense, if mesopelagic fishes are meant to be used as whole product, they may contain a high number of anisakids, and possibly potentially allergenic anisakid proteins, that might be directly consumed by humans, or may potentially be transferred to the fish feed and then to the farmed fish (and eventually to humans).

There were no findings of parasitic nematodes in either of the following fish species: *A. aculeatus; A. hemigymnus; C. sloani; C. schmidti; C. microdon*; *D. brachycephalus; D. effulgens; D. metopoclampus; H. hygomii; H. reinhardtii; H. taaning; L. cuprarius; L. lineatus; L. dofleini; N. resplendens; P. crassiceps* and *Sternoptyx sp*. This suggests a low risk of transmitting ascaridoid parasites (or their allergens). However, it should not be ruled out that ascaridoid infection may occur to those fish species as well at a low prevalence, or in other sea areas or seasons, as ascaridoid nematodes are known to be able to infect many marine teleosts (Mattiucci et al. 2018). In case of future interest of exploitation of mesopelagic fish species, either for human or aquaculture use, in the studied geographic area, then it may be important to consider both which fish species to exploit and in which geographical areas.

4.4 Conclusion

The data produced in the present study are important for the understanding of the parasite-host relationships and ecology in the marine ecosystems (particularly the twilight zone). The mesopelagic fish species *B. indicus; C. warmingii; C. danae; D. dumerilii; D. mollis; D. rafinesquii; D. argenteus; E. pelecanoides; L. guentheri; L. gemellarii; M. muelleri; M. argipalla* and *N. atrum* caught from Eastern Central Atlantic waters were found infected with ascaridoid nematodes.

The sea areas with highest parasitic infection were around Cape Verde, Canary Islands, Madeira, and the waters off West Sahara and Morocco. The following ascaridoid nematodes were identified: *A. pegreffii*, *A. typica*, *A. ziphidarum*, *A. brevispiculata*, *A. paggiae*, *A. cf. paggiae*, Raphidascarididae *sp.* and *P.* cf. *ceticola*, showing high species diversity. The fish species with higher infection levels were *D. argenteus* and *D. rafinesquii* with total density values of 399 and 155 ascaridoids/kg fish, respectively. Some of the studied fishes, e.g., *M. muelleri* appeared to be much less infected in the studied areas than previously reported from other locations.

As a potential food resource, mesopelagic fish would represent a risk for anisakidosis only if it would be meant for being consumed as raw or lightly cooked. However, the anisakid thermostable allergens that may possibly be present in these fishes (even if meant to be used as fish feed for farmed salmon) may pose a risk for sensitized consumers (potentially even through consumption of farmed fish feed with them) that should be considered and further evaluated. To conclude, findings suggest that mesopelagic fishes are important transport hosts in the life cycle of various species of ascaridoid nematodes, especially *D. argenteus,* which was found to carry possibly 4 anisakid species (i.e., *A. brevispiculata*, *A. paggiae*, *A. cf. paggiae* and *P. cf. ceticola*), and probably a new species of Raphidascarididae yet to be described. The larva of *Pseudoterranova* cf. *ceticola* was herein described for the first time.

4.5. Future studies

The following research topics are recommended.

4.5.1 *Anisakis brevispiculata*

In the present study, it has been found *A. brevispiculata* L3 having a terminal mucron, whilst in the bibliography there is controversy about the stability of this characteristic. In addition, it appears to be two genotypes at the ITS gene. Thus, it arises a question of the possible presence of a sibling species within an *A. brevispiculata* complex that should be studied. Further morphological and genetic work upon adult nematode specimens recovered from whale final hosts would be needed to resolve the possible existence of a sibling species.

4.5.2 *Anisakis paggiae* and *A. cf. paggiae*

The *cox2* sequences from *Anisakis* type IV larvae diverged as much as 5.0% from *A. cf. paggiae* and from *A. paggiae*. Thus, the diversity among these whale parasites could exceed *A. paggiae* and *A.* cf. *paggiae,* and this should be further investigated. In addition, it should be also studied if *A. cf. paggiae* should be considered a valid species within an *A. paggiae* complex. Again, further morphological and genetic work upon adult nematode specimens recovered from whale final hosts would be needed to resolve the possible existence of a complex of sibling species.

4.5.3 *Pseudoterranova* cf. *ceticola* and Raphidascarididae sp.

As above, further work needs to be done on adult specimens of *Pseudoterranova* spp. from whale final hosts and the Raphidascarididae sp. must be found and described from the final fish host. It is identified as particularly important to carry out studies on *P. kogiae* and adult nematodes from kogiid whales as it is at present unknown whether the kogiid whales may carry widespread, several host specific or geographically restricted nematode species.

4.5.4 Parasites as biological indicators and anisakid diversity

It appears that kogiid whales may be important final hosts of most of the anisakids found in the present study, i.e. *A. brevispiculata*, *A. paggiae*, *A. cf. paggiae* and *P. cf. ceticola*. These whales probably predate on mesopelagic fishes in the sampling area. In addition, the parasites could also be transmitted from mesopelagic fish to squids and then to kogiids through the food web. The presence of these parasites in mesopelagic fish could be used as an indicator of presence of these kogiid whales in the area. Further studies to confirm or refute the importance of these kogiid whales in the life cycles of these anisakids should be carried out. Also, the genetic variation within these nematode species could reflect the presence of final host specific genotypes or even species.

The high diversity of anisakids found in mesopelagic fish in the present study provides new knowledge about these poorly known anisakids, therefore, further research should be carried out in other sea areas and fish species.

4.5.5 Risk assessment

Finally, further research should be done to confirm if ascaridoid species like *A. paggiae*, *A. cf. paggiae*, *A. brevispiculata*, *A. typica*, *A. ziphidarum*, *Pseudoterranova cf. ceticola* and Raphidascarididae sp. would also have allergens that may potentially cause allergic symptoms to *Anisakis* sensitized patients. In addition, the presence of *A. pegreffii* (*A. simplex (s.s.)* was already reported in previous "mesopelagic studies"), already known to produce thermostable allergens, suggests the potential transfer of allergens from mesopelagic fish to fish feed, to farmed fish and then to humans that should be further investigated.

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Appendix

TABLE 1: EPIDEMIOLOGICAL DATA FOR EACH NEMATODE SPECIES. PREVALENCE (P), MEAN ABUNDANCE (MA), STANDARD DEVIATION (SD), MAXIMUM NUMBER OF LARVAE FOUND IN ONE HOST = MAXIMUMS INETNSITY (IMAX).

Parasite species Fish host	*Anisakis brevispiculata*			*Anisakis paggiae*			*Anisakis cf. Paggiae*			*Anisakis pegreffii*			*Anisakis typica*			*Anisakis ziphidarum*			*Pseudoterranova cf. ceticola*			Raphidascarididae gen.		
Family	P (%)	mA (SD)	Imax	P (%)	mA (SD)	Imax	P (%)	mA (SD)	Imax	P (%)	mA (SD)	Imax	P (%)	mA (SD)	Imax	P (%)	mA (SD)	Imax	P (%)	mA (SD)	Imax	P (%)	mA (SD)	Imax
Species																								
Gonostomatidae																								
Cyclothone microdon	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Myctophidae																								
Benthosema glaciale	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Bolinichthys indicus	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		2 %	<0.1(0.2)	1	0 %	0	
Ceratoscopelus warmingii	0 %	0		0 %	0		0 %	0		0 %	0		2 %	<0.1(0.1)	1	0 %	0		0 %	0		0 %	0	
Diaphus brachycephalus	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Diaphus dumerilii	0 %	0		0 %	0		0 %	0		0 %	0		100 %	1 (0)	1	0 %	0		0 %	0		0 %	0	
Diaphus effulgens	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Diaphus metopoclampus	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Diaphus mollis	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		7 %	0.1(0.3)	1	0 %	0	
Diaphus rafinesquii	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		4 %	<0.1(0.2)	1	32 %	0.5(0.8)	3	0 %	0	
Hygophum hygomii	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Hygophum reinhardtii	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Hygophum taaningi	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Lampanyctus alatus	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Lampanyctus cuprarius	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Lampanyctus lineatus	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Lepidophanes guentheri	20 %	0.2(0.4)	1	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Lobianchia dofleini	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Lobianchia gemellarii	0 %	0		0 %	0		0 %	0		0 %	0		25 %	0.3(0.5)	1	0 %	0		0 %	0		0 %	0	
Nannobrachium atrum	4 %	<0.1(0.2)	1	8 %	0.1(0.3)	1	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Notoscopelus resplendens	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Diretmidae																								
Diretmus argenteus	70 %	5.6(2.4)	8	26 %	0.5(1.0)	4	30 %	0.9(3.0)	17	0 %	0		0 %	0		0 %	0		13 %	0.1(0.3)	1	48 %	1.8(2.7)	8
Sternoptychidae																								
Argyropelecus aculeatus	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Argyropelecus hemigymnus	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Maurolicus muelleri	0 %	0		0 %	0		0 %	0		1 %	<0.1(0.1)	1	0 %	0		0 %	0		0 %	0		0 %	0	
Sternopty Sp.	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Melamphaidae																								
Poromitra crassiceps	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Platytroctidae																								
Maulisia argipalla	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		14 %	0.1(0.4)	1	0 %	0		0 %	0	
Eurypharyngidae																								
Eurypharyn pelecanoides	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		11 %	0.1(0.3)	1	6 %	0.1(0.2)	1	0 %	0	
Stomiidae																								
Chauliodus danae	0 %	0		0 %	0		0 %	0		0 %	0		2 %	<0.1(0.2)	1	0 %	0		2 %	<0.0(0.2)	1	0 %	0	
Chauliodus sloani	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	
Chauliodus schmidti	0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0		0 %	0	