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Title: Larval ascaridoid nematodes in horned and musky octopus (Eledone. cirrhosa and E. moschata) and longfin inshore squid (Doryteuthis pealeii): safety and quality implications for cephalopod products sold as fresh on the Italian market

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Abstract: The aim of this study was to evaluate the occurrence, infection level and distribution of ascaridoid larvae in cephalopod products sold in Italy. Data on the species most commonly commercialized as whole and fresh on the Italian market were collected. After comparing commercial and literature data, Eledone spp., comprising E. cirrhosa and E. moschata (horned octopus and musky octopus, respectively) and Doryteuthis pealeii (Longfin inshore squid) were selected, also considering that they had been rarely investigated. Overall, 75 Eledone spp. caught in the Mediterranean Sea (FAO area 37) and 70 D. pealeii from the Northwest Atlantic Ocean (FAO area 21) were examined by visual inspection and artificial digestion (viscera and mantle separately). Parasites were submitted to morphological and molecular analysis. Prevalence (P), mean intensity (MI) and mean abundance (MA) were calculated. In Eledone spp. 9 nematodes morphologically resembling Lappetascaris spp. were found in the mantle of 5 specimens (P: 6.7%; MI: 1.8; MA: 0.12). However, the molecular identification did not allow to confirm this identification due to lack of reference sequences. In D. pealeii, 2 nematodes molecularly identified as Anisakis simplex s.s. were found in the viscera and in the mantle (P: 2.8%; MI: 1; MA: 0.028) of two specimens. All the larvae were detected after artificial digestion. This is the first report of A. simplex s.s. in D. pealeii. Despite the low prevalence, the potential public health risk should not be disregarded, considering the zoonotic potential of A. simplex s. s. larvae and their localization in the edible part (mantle). The presence of larvae resembling Lappetascaris spp., although not considered to represent a public health hazard, paves the way for further studies to investigate the phylogenetic relationship within the family Raphidascarididae. Considering also the increasing economic value of cephalopods, both Food Business Operators and control authorities must put in place procedures to prevent the commercialization of products at risk due to the presence of larval ascaridoid nematodes.

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Pisa, 27th November 2019

Dear Editor,

please find enclosed the manuscript entitled "**Larval ascaridoid nematodes in horned and musky octopus (*Eledone. cirrhosa* and *E. moschata*) and longfin inshore squid (*Doryteuthis pealeii*): safety and quality implications for cephalopod products sold as fresh on the Italian market"** to be considered for publication in the International Journal of Food Microbiology.

The commercial importance of cephalopods has risen significantly in the last decades, following an increased request from the market due to an excellent palatability, a high nutritional value and the continuously growing popularity of Japanese sushi and sashimi that may also include raw octopus, squid or cuttlefish. The global production largely depends on Asian, North African, North and South American countries. In addition, they are highly traded and appreciated in southern Europe, such as in Spain and Italy.

Among the most important biohazards related to the consumption of cephalopods is the presence of viable zoonotic nematode larvae belonging to the *Anisakis* genus. Although the parasitological risk associated to the presence of such larvae can be prevented applying a freezing treatment, the presence of dead visible parasites represents a defect that alters the overall quality, causes consumers' rejection and damage of the reputation of the brand. In addition, the allergenic potential of dead larvae is still debated.

Considering the rising market request and value of cephalopods and the scarcity of data on the presence of parasitic nematodes in products commercialized in Italy, the aim of this study was to evaluate the occurrence, infection intensity and distribution of parasitic nematodes in edible and non-edible parts of cephalopod species selected among those most commercialized as fresh and whole on the Italian market.

Firstly, the cephalopods species most commonly sold as fresh whole were identified by a market survey. At the same time, a revision of the existing literature on nematodes in cephalopods was performed, to select the less investigated host species. After the comparison of commercial and literature data, the Mediterranean horned and musky octopus (*Eledone cirrhosa* and *E. moschata*, respectively) and the longfin inshore squid (*Doryteuthis pealeii*), imported from the North-west Atlantic, were selected as target species for the present study, as they had been rarely investigated. Overall, 75 *Eledone* spp. caught in the Mediterranean Sea (FAO area 37) and 70 *D. pealeii* from the Northwest Atlantic Ocean (FAO area 21) were examined by visual inspection and artificial digestion (viscera and mantle separately). Parasites were submitted to morphological and molecular analysis. Prevalence (P), mean intensity (MI) and mean abundance (MA) were calculated. In two specimens of *D. pealeii,* 2 nematodes molecularly identified as *Anisakis simplex* s.s. were found in the viscera and in the mantle (P: 2.8%; MI: 1; MA: 0.028), respectively. In the mantle of 5 specimens of *Eledone* spp. 9 nematodes morphologically resembling *Lappetascaris* spp. were found (P: 6.7%; MI: 1.8; MA: 0.12). Unfortunately, the molecular identification did not confirm this identification, due to the lack of reference sequences. Interestingly, all the larvae were detected after artificial digestion.

The present study represents, to the best of our knowledge, the first report of *A. simplex* s.s. in *D. pealeii* and also the first study investigating parasitic nematodes in this Atlantic species of relevant commercial interest in Italy. Despite the low prevalence of *A. simplex s.s.,* the aroused public health risk should not be disregarded, considering its zoonotic potential and the fact that one larva was found in the edible part (mantle). As regards the larvae resembling *Lappetascaris* spp. found in *Eledone* spp., which had already been described in cephalopods from the Mediterranean Sea, they are not considered to represent a public health hazard, but their presence calls for further studies to better investigate the phylogenetic relationship within the family Raphidascarididae.

Overall, the present study provides data on the distribution of ascaridoid larvae in cephalopod products sold on the market that may be of interest for both the seafood industry and the health authorities. In fact, considering also the increasing economic value of cephalopods, the results of the present study suggest that both Food Business Operators and control authorities must put in place procedures to prevent the commercialization of products at risk due to the presence of larval ascaridoid nematodes.

The manuscript has not been published elsewhere nor is it being considered for publication elsewhere. All authors have approved this manuscript, agree to the order in which their names are listed, declare that no conflict of interests exists and disclose any commercial affiliation.

Yours sincerely,
Andrea Armani and co-authors

***Highlights (for review)**

- *Doryteuthis pealeii* and *Eledone* spp. were selected as they are sold whole fresh in Italy
- All the specimens were analysed by visual inspection and by digestion
- Two *Anisakis simplex* larvae were found in the 70 *Doryteuthis pealeii* examined
- Nine larvae resembling *Lappetascaris* spp. were isolated from the 75 *Eledone* spp.
- The finding of *A. simplex* in *Doryteuthis pealeii* represents a new host record

Larval ascaridoid nematodes in horned and musky octopus (*Eledone cirrhosa* and *E. moschata*) and longfin inshore squid (*Doryteuthis pealeii*): safety and quality implications for cephalopod products sold as fresh on the Italian market

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Abstract

The aim of this study was to evaluate the occurrence, infection level and distribution of ascaridoid larvae in cephalopod products sold in Italy. Data on the species most commonly commercialized as whole and fresh on the Italian market were collected. After comparing commercial and literature data, *Eledone* spp., comprising *E. cirrhosa* and *E. moschata* (horned octopus and musky octopus, respectively) and *Doryteuthis pealeii* (Longfin inshore squid) were selected, also considering that they had been rarely investigated. Overall, 75 *Eledone* spp. caught in the Mediterranean Sea (FAO area 37) and 70 *D. pealeii* from the Northwest Atlantic Ocean (FAO area 21) were examined by visual inspection and artificial digestion (viscera and mantle separately). Parasites were submitted to morphological and molecular analysis. Prevalence (P), mean intensity (MI) and mean abundance (MA) were calculated. In *Eledone* spp. 9 nematodes morphologically resembling *Lappetascaris* spp. were found in the mantle of 5 specimens (P: 6.7%; MI: 1.8; MA: 0.12). However, the molecular identification did not allow to confirm this identification due to lack of reference sequences. In *D. pealeii,* 2 nematodes molecularly identified as *Anisakis simplex* s.s. were found in the viscera and in the mantle (P: 2.8%; MI: 1; MA: 0.028) of two specimens. All the larvae were detected after artificial digestion. This is the first report of *A. simplex* s.s. in *D. pealeii*. Despite the low prevalence, the potential public health risk should not be disregarded, considering the zoonotic potential of *A. simplex* s. s. larvae and their localization in the edible part (mantle). The presence of larvae resembling *Lappetascaris* spp., although not considered to represent a public health hazard, paves the way for further studies to investigate the phylogenetic relationship within the family Raphidascarididae. Considering also the increasing economic value of cephalopods, both Food Business Operators and control authorities must put in place procedures to prevent the commercialization of products at risk due to the presence of larval ascaridoid nematodes.

Keywords: *seafood, biological risk, public health, Anisakis simplex, Lappetascaris* spp.

1.Introduction

Cephalopods are characterized by a rapid growth, short lifespan and strong life-history plasticity; thus, they are quickly adaptable to changing environmental conditions. A global increase of their populations has been observed over the last sixty years: the factors influencing such phenomenon are likely to be complex, but ocean warming and the decrease of fish competitors and predators due to intensive fishery practices are believed to have played a role (Doubleday et al., 2016).

The commercial importance of cephalopods has risen significantly in the last decades (Hunsicker et al. 2010), thanks to an increased request from the market due to an excellent palatability and an high nutritional value. In fact, their high-protein content, the abundance of essential amino acids, and the low-fat content make cephalopods an ideal component of healthy and balanced diets (Vieites et al., 2019).

According to recent data from the Food and Agriculture Organization of the United Nations (FAO), cephalopods constituted 6.4% (live weight) of the total world trade of fish and fish products in 2016 (FAO, 2018). The world cephalopod production has shown a relatively stable growth since 1950, reaching a peak in 2014, then dropping in 2016 (Peng & Mu, 2019). Also in 2018 octopus and squid catches have been poor (FAO, 2019a). However, the global demand is high and this production decrease is causing a strong rise in trading prices (FAO, 2018; FAO, 2019a).

The species of main economic interest belong to two distinct superorders (Decapodiformes and Octopodiformes) and, for commercial and catch statistics purposes, they are grouped in three macro categories: squids (shortfin; longfin and bobtail squids), cuttlefish and octopus (Arkhipkin et al., 2015; FAO, 2018). Squids' category was the most represented on the global market (total production of 3385003 tons) in 2015, followed by octopus (400404 tons) and cuttlefish (331824 tons) (http://www.fao.org/fishery/topic/16140/en). Similar proportions were reported in 2017 (FAO, 2019a).

The global production of cephalopods largely depends on Asian (China, Vietnam, Thailand, Indonesia, India), North African (Morocco, Mauritania), North American (California) and South American (Argentina, Mexico and Peru) countries (FAO, 2016). Although traditionally they have represented a minor catch in European waters, they are particularly important in southern countries (Lougovois et al., 2008), being an important element of the Mediterranean diet (Picó-Durán et al., 2016). In particular, Spain and Italy are the most important consumer markets (FAO, 2018): squid flows from the first to the latter country were among the top 15 of the total intra-EU exchanges in terms of value and showed an upward trend since 2014 (EUMOFA, 2017). In addition, the recent growing worldwide popularity of Japanese sushi and sashimi, as well as Spanish tapas, has helped to further boost demand for cephalopods (FAO, 2018). In a recent survey conducted in Italy it was found that common octopus (*Octopus vulgaris*), squid (*Loligo vulgaris*) and cuttlefish (*Sepia officinalis*) are currently used for the preparation of such Japanese recipes (Armani et al., 2017).

Interest in the parasite communities associated with cephalopods is growing (Guillén-Hernández et al., 2018), and several studies investigating biological/ecological aspects and public health issues are available (Table 1). In fact, it is well documented that cephalopods act as intermediate, paratenic, or definitive hosts with a variable role that depends on the type of parasite and the specific life cycle (Guillén-Hernández et al., 2018). Nematodes from the superfamily Ascaridoidea belonging to the families Anisakidae and Raphidascarididae (according to the classification of Fagerholm, 1991) are common parasites in the marine environment (Picó-Durán et al., 2016). Larval ascaridoid nematodes may be found in cephalopods' viscera and mantle (Roumbedakis et al., 2018) and they are commonly generically referred to as anisakids (Picó-Durán et al., 2016). The most relevant anisakids from a public health point of view belong to the genera *Anisakis* and *Pseudoterranova* (family Anisakidae) (EFSA, 2010). In particular, *Anisakis* sp. is the most studied in cephalopods (Roumbedakis et al., 2018). The life cycle of this genus is indirect, with crustaceans acting as first intermediate hosts, fishes and squids as intermediate and/or paratenic hosts and marine mammals as definitive hosts. Fish and cephalopods generally host the third larval stage (L3) of *Anisakis* spp. encysted on the visceral organs, although L3 migration into the muscle or mantle is possible. If live larvae are accidentally ingested when eating raw, marinated or undercooked seafood infected by L3, human infection can occur (Mattiucci et al., 2018). Other ascaridoid larvae have been described in cephalopods, such as *Contracaecum* spp. (family Anisakidae), as well as the genera *Hysterothylacium* and *Lappetascaris* (family Raphidascarididae) (Table 1). While zoonotic infections by *Contracaecum* spp. are uncommonly described (Nagasawa, 2012), *Hysterothylacium* spp. is generally believed to be not zoonotic (Cipriani et al., 2019). To the best of our knowledge, no human cases due to *Lappetascaris* spp. have been reported.

To manage the parasitological risk, Food Business Operators (FBOs) must submit fishery products to a visual examination for the detection of visible parasites before commercialization (Commission Regulation (EC) No. 2074/2005; Regulation (EC) No. 853/2004). However, the checks performed by FBOs does not ensure that the product is completely free from risks (D'Amico et al., 2014); thus, products intended for raw consumption must undergo a preventive freezing treatment (Commission Regulation (EC) No. 2074/2005; Regulation (EC) No. 853/2004). Moreover, the allergenic potential of dead larvae is still debated (Audicana & Kennedy, 2008; Dashner et al., 2012; EFSA, 2010).

In addition, beside public health issues, the presence of parasitic nematodes may also negatively impact the seafood quality, causing economic losses (Bao et al., 2018). In fact, fishery products containing live or dead visible larvae are unattractive for consumers and unsuitable as food (Regulation (EC) No178/2002).

Considering that data on the distribution of ascaridoid larvae in cephalopod products sold on the market are of interest for both the seafood industry and the health authorities, the aim of the present study was to evaluate the occurrence, infection intensity and distribution of parasitic nematodes in edible and non-edible parts of cephalopod species selected among those most commercialized as fresh and whole in Italy.

2.Materials and methods

2.1 Species selection: market and literature analysis

The cephalopods species most commonly sold as fresh whole on the Italian market were identified by contacting national large-scale retailers and import companies, the Border Inspection Point (BIP) of Malpensa (Milan) and the Wholesale fish market of Milan. At the same time, a revision of the existing literature on nematodes in cephalopods was performed, to assess available epidemiological data and select the less investigated species (Table 1). After the comparison of commercial and literature data, the horned and musky octopus (*Eledone cirrhosa* and *E. moschata*, respectively) and the longfin inshore squid (*Doryteuthis pealeii*) were selected as target species for the present study.

2.2 Sampling

The cephalopod specimens were collected as whole fresh at the Wholesale fish market of Milan and at the distribution platforms of two national leading brands in the organized distribution. Totally, 145 specimens were collected in June 2019, including 70 *D. pealeii* from the Northwest Atlantic (FAO area 21) and 75 *Eledone* spp. (36 *E. cirrhosa* and 39 *E. moschata*) caught in the Mediterranean Sea (FAO area 37.1 and 37.2). The samples collected at the Wholesale fish market of Milan were immediately submitted to visual inspection (see section 2.3.1), then frozen and transferred to the FishLab for further analysis (see section 2.3.2). Specimens collected at the platforms were instead directly transferred to the FishLab, where they were visually examined as fresh and then frozen (see section 2.3). Each specimen was measured registering the total length (TL) and the dorsal mantle length (DML), which was measured from the tip of the mantle to the midpoint between the eyes, and weighted (total weight, viscera weight, mantle weight).

2.3 Parasites detection

2.3.1 Visual inspection. A visual inspection under natural light was conducted on both viscera and mantle of fresh specimens to detect visible parasites (non-encapsulated nematodes longer than 1 cm or parasites with a capsular diameter of at least 3 mm according to the definition given by the Codex Alimentarius Commission, 1971) according to the Commission Regulation (CE) n. 2074/2005. In detail, once separated, the viscera and the body (mantle and arms) were both placed in a Petri dish and left at room temperature for 15 minutes to allow the mobilization of the larvae. In a second moment, after freezing and defrosting, viscera were also checked under a 365 nm UV-light source (Gómez-Morales et al., 2018; Karl and Leinemann, 1993).

2.3.2 Artificial digestion. Viscera and mantle of each specimen were separately submitted to artificial digestion using Trichineasy (CTSV srl, Brescia) slightly modifying the protocol described in Guardone et al., (2017), which was based on the Commission Regulation (EC) No 2075/2005. Residues from the sieve were transferred to Petri dishes and examined under natural and UV (365 nm) light.

2.4 Parasites identification

2.4.1 Morphological identification. The parasitic nematodes were counted, washed and maintained in 0.9% NaCl solution for morphological identification. Parasites were observed under a light microscope at 200×magnifications for identification at the genus and/or species level on the basis of the following morphological characteristics: the size, the position of the excretory pore, the shape of the tail, the shape of the cephalic end, the length and shape of the ventricle, the presence and position of the mucron (Berland, 1961; Fagerholm, 1991; Nagasawa and Moravec, 1995; 2002). After microscopic observations, larvae were preserved in 70% ethanol and stored at −20 °C until further analysis.

2.4.2 Molecular identification. All the collected larvae were submitted to molecular identification. Total DNA extraction was performed according to the protocol used in Guardone et al. (2016). DNA concentration and purity were determined by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). A 629-bp fragment of the mitochondrial cytochrome *c* oxidase subunit II (*cox2*) gene was selected as molecular target and amplified using the primers 211F and 210R (Cipriani et al., 2019). In addition, a fragment of about 900-bp of the ITS-1 region, the 5.8S gene and the ITS-2 region plus approximately 70 nucleotides of the 28S gene (further as ITS), was amplified according to Zhu et al., (1998). PCR products were analysed by electrophoresis in 2% agarose gel and those presenting the expected length were sent for standard forward and reverse Sanger sequencing to an external company. The obtained sequences were analyzed, edited and assembled with the Geneious R7 software (Kearse et al., 2012) and compared with sequences deposited in GenBank.

2.5 Statistical analysis

The prevalence (P), mean abundance (MA) and mean intensity (MI) for the investigated species were calculated (separately for viscera and mantle). To assess the relationship between the weight and the length of the cephalopod specimens and the parasite number, the Pearson's correlation coefficient was calculated.

3. Results and discussion

3.1 Species selected after market and literature analysis

According to our analysis, the species most commonly commercialized as whole and fresh on the Italian market are: *Octopus vulgaris, Sepia officinalis, Illex coindetii, Todarodes sagittatus, Loligo vulgaris, E. cirrhosa* and *E. moschata*, commonly sold as *Eledone* spp. and often appearing mixed on the market (https://www.faoadriamed.org/html/Species/EledoneCirrhosa.html), and *Doryteuthis pealeii*. While for *S. officinalis*, *I. coindetii*, *T. sagittatus* and *L. vulgaris* data on anisakids occurrence are available, very few data exist for *O. vulgaris*, *Eledone* spp. and *D. pealeii*. In particular, to the best of our knowledge, only four studies on parasitic nematodes targeting *Eledone* spp. are available, while no studies targeting *D. pealeii* have been conducted (Table 1). Therefore, these species were selected, in order to investigate a product locally fished (*Eledone* spp.) and one imported (*D. pealeii*) belonging to two different superorders (Octopodiformes and Decapodiformes, respectively).

3.1.1. Eledone spp. *E. cirrhosa* (superorder Octopodiformes, order Octopoda, family Eledonidae), also known as ‘curled’, ‘horned’ or ‘lesser’ octopus, is distributed partly in the North East Atlantic and mainly in the Western part of the Mediterranean Sea (Sealifebase, 2016a), where it is one the most commonly fished benthic cephalopod species. It has a commercial relevance primarily in the central and southern Adriatic (https://www.faoadriamed.org/html/Species/EledoneCirrhosa.html), as well as in France and in Tunisia (Souidenne et al., 2016). In Italy, sales of *E. cirrhosa* represent 17% of the total sale volume of Octopodidae (EUMOFA, 2018) and in the Ligurian Sea and the central Tyrrhenian Sea it contributes to 50% of total seafood landings (Libralato et al., 2018).

E. moschata has a smaller geographical range. Although, differently from *E. cirrhosa*, it extends to the whole Mediterranean Sea (Sealifebase, 2016b), its distribution in the Atlantic is limited to the southern coasts of Portugal, the west coast of Gibraltar and the Gulf of Cadiz (https://www.faoadriamed.org/html/Species/EledoneMoschata.html). It is a typical commercial species for the Mediterranean demersal fishery (Belcari and Sbrana, 1999), being particularly important in the Northern and Central Adriatic (Libralato et al., 2018). In Italy, sales of *E. moschata* represent 15% of the total volume of sale of Octopodidae products (EUMOFA, 2018).

3.1.2 Doryteuthis pealeii. D. pealeii (superorder Decapodiformes, order Teuthida, family Loliginidae, former name *Loligo pealeii*), commonly known as longfin inshore squid, is mainly distributed in the North West and Central West Atlantic Ocean, as well as, to a less extent, in the Western Central Pacific (Sealifebase, 2016c). In fact, it inhabits the continental shelf and upper slope waters between southern Newfoundland and the Gulf of Venezuela, including the Gulf of Mexico and the Caribbean Sea. *D. pealeii* is a high valued species that, together with *Illex illecebrosus* (Northern shortfin squid), has been commercially exploited since the late 1800s. Fishing activities increased rapidly until the 1970s when trawlers from Japan, former USSR and

Western Europe, including Italy, targeted both species. This widespread presence of an international fishing fleets urged the USA to manage the fishery from 1977, imposing catch and bycatch allocations, gear limitations, and time-area restrictions (Arkhipink et al., 2015). Currently, most of the landings are sold domestically for food and the rest is exported. Interestingly, even though this species is available as fresh on the Italian market only for a short period of time, usually between May and June (authors' personal communication), foreign trade statistics for the New England and the Mid-Atlantic Customs Districts combined indicate that in the period 1991-2012 Italy was the first importer of *D. pealeii* products, accounting for 29% of the exports, followed by China (19%), Spain (16%). Greece (6%), and Japan (4%) (Arkhipink et al., 2015).

3.2 Parasites detection and identification

A variety of methods, including classical ones such as visual inspection, slicing, candling, UV-press and chloro-peptic digestion, as well as more innovative imaging technologies (X-rays, electromagnetism, spectroscopy and Magnetic Resonance Imaging) and, recently, biomolecular analysis, have been used to recover larvae from fishery products (Bao et al., 2017). However, nowadays only visual inspection under natural light is included in EU food hygiene and safety regulations (Commission Regulation (EC) No 2074/2005) while different analytical protocols are used by the international scientific community (Table 1).

Even though chloro-peptic digestion and UV press methods are considered the most sensitive ones in fish species (Gómez-Morales et al., 2018; Llarena-Reino et al., 2013; parasite-project.eu), such techniques have not been commonly applied in cephalopods examination. In fact, only 3 (11%) and 1 (4%) out of the 27 revised studies explicitly mentioned to have used the digestion or the UV method, respectively (Table 1). On the contrary, the visual inspection under natural light (9 studies, 33%), frequently coupled with dissection under a stereomicroscope (12 studies, 44%), was more commonly used. No detailed description of the used methods was instead provided in 2 studies (7%) that generally referred to "standard procedures".

In the present study the viscera and the mantle of all the 145 collected specimens (70 *D. pealeii* and 75 *Eledone* spp.) were visually examined (under natural and UV light) and digested. Overall 11 nematode larvae were found: 9 larvae in *Eledone* spp. and 2 larvae in *D. pealeii* (Table 2). Interestingly, all the larvae were detected after artificial digestion. In addition, 18 cestode plerocercoid larvae were found in *D. pealeii* during visual inspection (see section 3.2.2).

3.2.1. Eledone spp. During the visual inspection of the 75 specimens of *Eledone* spp. (mean weight 198.8 g, mean total length 35.8 cm, mean DML 8.3 cm) no nematode larvae were found (Table 2). The artificial digestion of the viscera was also negative for all the specimens. On the contrary, 9 nematodes (P: 6.7%, MA: 0.12, MI: 1.8) were found in the mantle of 5 specimens (3 *E. cirrhosa* and 2 *E. moschata*) after digestion. Details of the measured parameters and parasitological results are reported in Table 3.

The body of the collected larvae was elongated, white and measured between 15 and 28 mm. Microscopic observations, especially of the shape of the cephalic and caudal end, allowed to observe features different from *Anisakis* spp. In particular, the 9 larvae presented an asymmetric, obliquely truncated cephalic end and a recurved conical tail with a slightly inflated extremity and a small spike (Fig. 1). In addition, a very elongated ventricular appendix was visible along the most part (70-80%) of the body. The morphological features of the larvae found in this study corresponded to those described by Nagasawa and Moravec (1995) as third-stage larvae of *Lappetascaris* spp. in *Todarodes pacificus* from the Sea of Japan. The same kind of larvae were then described as *Lappetascaris* sp. Type A by Nagasawa and Moravec (2002) in the mantle of four species of squids (*Thysanoteuthis rhombus*, *Ommastrephes bartramii*, *Onychoteuthis borealijaponica*, and *Gonatopsis borealis*) in Central and Western Pacific Ocean. To the best of our knowledge, the same type of larvae had been only described once in the Mediterranean Sea, by Culurgioni et al., (2010) in *Histioteuthis bonnelli* and *H. reversa* caught in the Sardinian Channel (Western Mediterranean Sea), with prevalence values (4.26% and 1.22%, in *H. bonnelli* and *H. reversa*, respectively) comparable to the prevalence found in the present study in *Eledone* spp. The molecular analysis of the larvae found in *Eledone* spp. in the present study could only be based on ITS sequences, as no amplifications were obtained using the primer pair commonly applied for targeting the mtDNA *cox2* of *Anisakis* spp. (see section 2.4.1). However, the comparison of the obtained ITS sequences with the sequences in GenBank database did not allow a specific identification. In fact, the highest identity values (92.92-93.36%) were obtained with sequences belonging to *Hysterothylacium deardorffoverstreetorum*. Similarly, also in the study of Setyobudi et al., (2013), the *cox2* sequence of an unknown nematode found in common squids from the East Sea, showed 83.0-84.0% similarity with *H. deardorffoverstreetorum*. Interestingly, the RFLP patterns obtained by Setyobudi et al., (2013) for this nematode did not correspond to any known anisakid. The authors hypothesized that the unknown species could be *Lappetascaris* sp., also considering that parasites of that genus had been morphologically identified by Takahara & Sakurai, (2010) from common squids in geographically adjacent Japanese waters (Table 1). Unfortunately, a morphological examination of the nematode found in the study of Setyobudi et al., (2013) was not conducted, as the samples had been directly transferred to molecular analysis. In addition, several confirmed or potential reports from the Pacific Ocean are cited in Nagasawa and Moravec (1995; 2002). In particular, according to Nagasawa and Moravec (1995), many previous records of anisakid larvae from the mantle of squids in the western North Pacific Ocean reported as *Contracaecum*, *Thynnascaris* or *Hysterothylacium* were, in fact, identical to *Lappetascaris* sp. (Nagasawa and Moravec, 2002). In the light of such not fully clear picture, a focused molecular investigation would be needed to better clarify the phylogenetic relationship of *Lappetascaris* type larvae with other genus of the family Raphidascarididae, in particular with the genus *Hysterothylacium*, which is known to be a very abundant and diverse group of marine ascaridoids (Ghadam et al., 2018). In addition, *Hysterothylacium* larvae are generally believed to not migrate into the fish flesh (Cipriani et al., 2019), but Picó-Durán et al. (2016) reported the presence of *Hysterothylacium* sp. larvae in the mantle of *I. coindettii*. Further investigations could thus also help in defining preferred localization of Raphidascarididae.

In the few other available studies on nematodes in *Eledone* spp., no parasites were found by Goffredo et al. (2019) in 5 specimens of *E. moschata* from the South Adriatic and Ionian Sea examined by visual inspection of the celomic cavity and candling technique under stereomicroscope. Also, a single *E. cirrhosa* from Sardinia examined by visual inspection and digestion (of the body cavity and viscera) was negative (Angelucci et al., 2011). In two older studies a low prevalence (1.5% out of 67 specimens) of *Anisakis simplex* s.l. was found along the Spanish coasts of Galicia (Atlantic Ocean) with visual and stereomicroscopic examination (Abollo et al., 1998), and a prevalence of 28% of *Hysterothylacium* spp. was found in 25 specimens from the northern Tyrrhenian Sea (Gestal et al., 1999) (Table1). However, considering that mantle digestion was never conducted in these previous studies, their results are not fully comparable with ours, and the absence of *Lappetascaris* spp. and of other anisakids in some studies might be related to the different techniques applied.

Finally, as regards the sizes, the total weight and the mantle weight as well as the total length and the DML of the positive specimens of *E. cirrhosa* were higher than the average of that species; on the contrary, in the case of *E. moschata*, the total and the mantle weight and the total length were lower than the species average, while the DML was lower or equal. The calculation of Pearson's coefficient showed a moderate positive relationship between total weight, total length, DML of *Eledone* spp. (the two species were analysed together due to the number of samples) and number of found larvae (0.5; 0.5; 0.7), and a weak positive relationship between the mantle weight and the number of larvae (0.2). No comparison with the previous works on *Eledone* spp. can be conducted as sizes of the examined specimens were not reported.

3.2.2 Doryteuthis pealeii. During the visual inspection of the 70 specimens of *D. pealeii* (mean weight 106.5 g, mean total length 41.2 cm, mean dorsal mantle length 16.8) no nematodes were found, while 18 visible cestode plerocercoid larvae were found in the visceral cavity in 10 specimens (P: 14.28% MA: 0.26; MI: 1.8). Since the aim of this paper was to investigate the occurrence of nematode larvae and also considering the lack of previous reports of larval cestodes in *D. pealeii*, data regarding these parasites will be discussed in a dedicated article.

The artificial digestion instead allowed to detect 2 nematodes (1 in the viscera and 1 in the mantle) in 2 specimens of *D. pealeii* (P: 2.8%; MA: 0.01; MI: 1). Details of the measured parameters and parasitological results are reported in Table 3. The body of the recovered third-stage larvae was white, cylindrical in shape, attenuated at both ends, and measured 15 - 30 mm in length and approximately 0.40–0.60 mm in width. The larvae were covered with a rigid cuticle with an annular transverse striation beginning from the cephalic region to the anus and ending with a short mucron. Based on the microscopic observations both larvae were identified as *Anisakis* sp. Amplicons and sequences were obtained for both isolated larvae for both genes (ITS and COX2). The comparison of the obtained sequences with GenBank database allowed to identify both larvae at species level as *A. simplex* s.s. (100% identity for both markers with sequences of *A. simplex* deposited in GenBank).

A wide variety in the prevalence values of *Anisakis* spp. can be observed in cephalopods (Table 1). In the revised studies, the squid category was the most represented with 14 analysed species belonging to three different families (Ommastrephidae, Loliginidae, Histioteuthidae), followed by cuttlefish and octopus, with 4 species and one family each (Table 1SM). Noteworthy is the fact that the squids' category is also the most represented on the global market, followed by octopus and cuttlefish. However, since no data are available for *D. pealeii,* a comparison can only be made with other squid species. In particular, by uniquely observing the 10 studies analysing species belonging to the Loliginidae family (namely *Alloteuthis* spp., *Doryteuthis* spp. and *Loligo* spp.), it may be noted that nematode larvae were only detected in the co-generic *D. gahi* (1 study conducted in the South Atlantic Ocean reporting a prevalence of *Anisakis* spp. of 2.46% for 1096 analysed specimens) and in *Loligo vulgaris* (2 studies conducted in Galicia, Atlantic coast of Spain, with prevalence values of 16% out of 50 specimens and

62.5% out of 8 for *Anisakis* spp., respectively) (Table 1). On the contrary, *L. vulgaris* was found to be negative in studies conducted in the Mediterranean Sea (Angelucci et al., 2011; Gestal et al., 1999; Goffredo et al., 2019; Graci et al., 2019; Picó-Durán et al., 2016), while all samples of the genus *Allotheuthis* resulted negative, both in the Mediterranean area (Gestal et al., 1999; Goffredo et al., 2019) and in the North East Atlantic (Abollo et al., 1998; Abollo et al., 2001). Besides the family Loliginidae, discussed above, species of the family Ommastrephidae have been investigated in a large number of studies, which frequently include the three major market squid species: *D. gigas*, *I. argentinus* and *T. pacificus*. The most relevant data is that prevalence for *Anisakis* spp. higher than 20%, reaching 50-100% in some geographical areas, were observed only for species of Ommastrephidae family, namely *T. sagittatus*, *T. pacificus*, *I. coindettii* and *T. eblanae*. However, in a study on 615 specimens of *T. pacificus*, very different prevalence values have been found in the different geographical areas and were attributed to different migration patterns and different prey items, as already indicated by several authors (Setyobudi et al., 2013).

Beside the origin and the analytical method, another important factor to considered is the host body size. In fish species, large piscivorous fish hosts such as European hake (*Merluccius merluccius*), European anchovy (*Engraulis encrasicolus*), Atlantic herring (*Clupea harengus*), haddock (*Melanogrammus aeglefinus*), whiting (*Merlangius merlangus*) and Atlantic cod (*Gadus morhua*) typically present much higher *Anisakis* spp. infection levels compared to strict plankton feeders fish (Cipriani et al., 2019). In the case of many Ommastrephids an ontogenetic shift in preferred food source occurs at around 200 mm DML: squid below that size feed primarily on crustaceans (amphipods and euphausiids) whereas fish and other squids become the most prevalent prey items in larger host size (Cipriani et al., 2019). In the work of Cipriani et al., (2019), in fact, highest *A. pegreffii* abundance was observed in squids >200 mm DML. Similarly, also Setoybudi et al. (2013) states that the prevalence in the Yellow Sea and the East Sea generally increased with the host body size, due to the fact that common squid juveniles usually prey on crustaceans and switch to fish and cephalopods as they grow. However, in the present study the DML of the two positive species was shorter than the average of all the analysed specimens as well as of that reported by other sources (https://www.sealifebase.ca/summary/Doryteuthis-pealeii.html), while the other parameters did not show a clear tendency. However, considering the low number of positive samples in the present study and the absence of literature data for *D. pealeii*, additional observations would be required to investigate this aspect. Finally, the very broad feeding pattern of *D. pealeii* should also be taken into account. Larger specimens may be at higher risk of harbouring L3, as it has been shown that adults between 12 and 16 cm long feed on fish and squid larvae or juveniles, while adults larger than 16 cm feed on fish and squid (Jacobson, 2005).

3.3 Parasite in cephalopods: implication for food quality and safety

The present survey provides data on the occurrence and distribution of molecularly identified larvae of *A. simplex* in *D. pealeii* from the Northwest Atlantic Ocean and of larvae morphologically resembling *Lappetascaris* sp. in *Eledone* sp. from the Mediterranean Sea.

To the best of our knowledge, this is the first report of *A. simplex* s.s. in *D. pealeii*, both in the viscera and in the mantle. Considering that this species is sold as fresh to the final consumers and that *A. simplex* s.s., together with *A. pegreffii*, is a well renowned zoonotic species (Guardone et al., 2018; Mattiucci et al., 2013; 2018), findings of our work are of interest both for the seafood industry and for the control authorities. Despite the low prevalence value observed in the present study, the potential public health risk should not be disregarded, as this study showed that *D. pealeii* is able to harbour *Anisakis simplex* s.s. also in the mantle that represents the main edible part of cephalopod body sold to the final consumers. As a newly described proven host for *A. simplex* s.s., this squid should thus be considered at risk of anisakiasis transmission if consumed raw or not well cooked. In fact, considering that in Korea and Japan anisakid nematodes were isolated from patients who had eaten raw common squid, cuttlefish and octopus (Hibi et al. 2009; Im et al.1995; Setyobudi et al., 2013), and that cephalopods are increasingly consumed as raw in typical Asian dishes also in Europe, the public health risk posed by cephalopod consumption should not be underestimated. A recent study showed that octopus and squid are used in sushi preparation in Italy (Armani et al., 2017). Even though the species found did not include *Eledone* spp. or *Doryteuthis pealeii*, the use of other kind of cephalopods cannot be excluded. Such practices have already been observed in Japan, where due to poor catches and consequent market shortage, *Todarodes pacificus* is being replaced by jumbo flying squid (*Dosidicus gigas*) in some sushi products (FAO, 2019b).

While the viscera contamination could be controlled by a rapid evisceration of the specimens, the reduction of the parasite burden in the edible part is quite challenging. In fact, while the removing of belly flaps in fish is a routine practice that does not reduce the fish value, the setup of trimming procedures, aimed at removing the “most contaminated part” of the cephalopods body, could result in a drastic reduction of their commercial value. In addition, the elective site of parasites embedding in the mantle is still unclear, as only few studies described this aspect. Cipriani et al., (2019) found each of the three mantle-infecting *A. simplex* larvae in the posterior half of the mantle (the hind part, roughly mirroring the most common larval infection site in the viscera) in the Argentinian shortfin squid. Interestingly, the larvae were embedded in the muscular tissue and were not readily recognizable neither by plain visual inspection (whitish larvae in whitish muscular tissue), nor candling, but only by UV fluorescence. Thus, even though the dorsal part of the cephalopods (adjacent to the viscera) is a potential “election site of localization”, further studies would be required to confirm this localization.

Finally, while the presence of *A. simplex* s.s. in *D. pealeii*, although with very low prevalence values, suggests that preventive measures should be put in place to avoid anisakiasis risk in the case of raw or undercooked longfin inshore squid consumption, the presence of *Lappetascaris* spp. larvae should not represent a public health risk. However, as in the case of other Raphidascarididae larvae, the possibility of causing allergic syndromes still needs to be clarified.

4. Conclusions

The present study contributes to describe the contamination level of cephalopod products of commercial interest for the Italian market sold as whole fresh in terms of anisakids infection, as requested by the European Food Safety Authority, recommending research on the prevalence, intensity, and anatomical location of parasites of public health importance in wild caught fishery products (EFSA, 2010). Unfortunately, the methodology used in the different studies in the literature varies, hampering direct comparison of the epidemiological data. In addition, most of the studies used visual inspection. Although frequently coupled with stereomicroscopic dissection, this technique may not always be adequate to detect nematode larvae, especially in the mantle, which is however the edible part. Standardization is thus highly need to provide new data for risk analysis, in order to better implement Food Business Operators inspection procedures aimed at guaranteeing products' quality and consumers' safety.

Declarations of interest

None.

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Figure captions

Fig. 1

Characteristics of the nematode larvae found in *Eledone* spp.: asymmetric, obliquely truncated cephalic end (A) and a recurved conical tail with a slightly inflated extremity (B) and a small spike (C)

References

Abollo, E., Gestal, C., López, A., González, A. F., Guerra, A., Pascual, S., 1998. Squid as trophic bridges for parasite flow within marine ecosystems: the case of *Anisakis simplex* (Nematoda: Anisakidae), or when the wrong way can be right. Afr. J. Mar. Sci. 20, 223-232.

Abollo, E., Gestal, C., Pascual, S., 2001. *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. Parasitol. Res. 87(6), 492-499.

Angelucci, G., Meloni, M., Merella, P., Sardu, F., Madeddu, S., Marrosu, R., Petza, F., Salati, F., 2011. Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods sampled from waters off Sardinia. J. Food Prot. 74(10), 1769-1775.

Arkhipkin, A. I., Rodhouse, P. G. K., Pierce, G. J., Sauer, W., Sakai, M., Allcock, L., et al. (2015). World squid fisheries. Rev. Fish. Sci. Aquac. 23(2), 92-252.

Armani, A., Tinacci, L., Lorenzetti, R., Benvenuti, A., Susini, F., Gasperetti, L., Ricci, E., Guarducci, M., Guidi, A., 2017. Is raw better? A multiple DNA barcoding approach (full and mini) based on mitochondrial and nuclear markers reveals low rates of misdescription in sushi products sold on the Italian market. Food Control 79, 126-133.

Audicana, M. T., Kennedy, M. W., 2008. *Anisakis simplex*: from obscure infectious worm to inducer of immune hypersensitivity. Clin. Microbiol. Rev. 21(2), 360-379.

Bao, M., Pierce, G. J., Strachan, N. J., Martínez, C., Fernández, R., Theodossiou, I., 2018. Consumers' attitudes and willingness to pay for *Anisakis*-free fish in Spain. Fish. Res. 202, 149-160.

Bao, M., Strachan, N. J., Hastie, L. C., MacKenzie, K., Seton, H. C., Pierce, G. J., 2017. Employing visual inspection and Magnetic Resonance Imaging to investigate *Anisakis simplex* sl infection in herring viscera. Food Control 75, 40-47.

Belcari, P., Sbrana, M., 1999. *Eledone moschata*. Synthesis of the knowledge on bottom fishery resources in Central Mediterranean (Italy and Corsica). Biol. Mar. Medit. 6(suppl 1), 747-752.

Berland, B., 1961. Nematodes from some Norwegian marine fishes. Sarsia 2, 150.

Brickle, P., Olson, P. D., Littlewood, D. T. J., Bishop, A., Arkhipkin, A. I., 2001. Parasites of *Loligo gahi* from waters off the Falkland Islands, with a phylogenetically based identification of their cestode larvae. Can. J. Zool. 79(12), 2289-2296.

Cipriani, P., Giulietti, L., Palomba, M., Mattiucci, S., Bao, M., Levsen, A., 2019. Occurrence of larval ascaridoid nematodes in the Argentinean short-finned squid *Illex argentinus* from the Southwest Atlantic Ocean (off Falkland Islands). Int. J. Food Microbiol. 297, 27-31.

Choi, S. H., Kim, J., Jo, J. O., Cho, M. K., Yu, H. S., Cha, H. J., Ock, M. S., 2011. *Anisakis simplex* larvae: infection status in marine fish and cephalopods purchased from the Cooperative Fish Market in Busan, Korea. Korean J. Parasitol. 49(1), 39.

Codex Alimentarius Commission, 1971. Report of the eighth session of the joint FAO/WHO Codex Alimentarius commission: Recommended international standard for quick frozen filet of cod and haddock. CAC/RS-50-1971 http://www.fao.org/docrep/meeting/005/c0531e/C0531E09.htm Last accessed: 18/11/2019.

Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004 (Text with EEA relevance). OJ L 338, 27–59.

Commission Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for Trichinella in meat. OJ L 338, 60–82.

Costa, A., Cammilleri, G., Graci, S., Buscemi, M. D., Vazzana, M., Principato, D., Giangrosso, G., Ferrantelli, V., 2016. Survey on the presence of *A. simplex s.s.* and *A. pegreffii* hybrid forms in Central-Western Mediterranean Sea. Parasitol. Int. 65(6), 696-701.

Culurgioni, J., Cuccu, D., Mereu, M., Figus, V., 2010. Larval anisakid nematodes of *Histioteuthis reversa* (Verril, 1880) and *H. bonnellii* (Férussac, 1835)(Cephalopoda: Teuthoidea) from Sardinian Channel (western Mediterranean). Bull. Eur. Ass. Fish Pathol. 30(6), 217.

D'Amico, P., Malandra, R., Costanzo, F., Castigliego, L., Guidi, A., Gianfaldoni, D., Armani, A., 2014. Evolution of the *Anisakis* risk management in the European and Italian context. Food Res. Int. 64, 348-362.

Daschner, A., Cuéllar, C., Rodero, M., 2012. The Anisakis allergy debate: does an evolutionary approach help?. Trends Parasitol. 28(1), 9-15.

Di Donfrancesco, B., Righetti, M., Serracca, L., Gustinelli, A., Fioravanti, M. L., Cencetti, E., Giorgi, I., Dellepiane, M., Ercolini, C., Prearo, M., Pavoletti, E., 2011. Presence of Anisakidae larvae in short-finned squids (*Illex coindetii*) caught in the Ligurian Sea [Conference poster]. In: XIII National Congress

SI Di. LV, Trani, Italy, 12-14 October 2011 (pp. 205-206). Italian Society for Veterinary Laboratory Diagnostic (SIDiLV).

Doubleday, Z. A., Prowse, T. A., Arkhipkin, A., Pierce, G. J., Semmens, J., Steer, M., Leporati, S.C., Lourenço, S., Quetglas, A., Sauer, W., Gillanders, B. M., 2016. Global proliferation of cephalopods. Curr. Biol. 26(10), 406-407.

EFSA, 2010. Scientific opinion on risk assessment of parasites in fishery products. EFSA Journal 8:1543 Available at: http://www.efsa.europa.eu/it/search/doc/1543.pdf Last accessed: 18/11/2019.

EUMOFA, 2017. The EU fish market. 2017 Edition. https://www.eumofa.eu/documents/20178/108446/The+EU+fish+market+2017.pdf Last accessed: 27/11/2019.

EUMOFA, 2018. The EU fish market. 2018 Edition. https://www.eumofa.eu/documents/20178/132648/EN_The+EU+fish+market+2018.pdf Last accessed: 27/11/2019.

Fagerholm, H. P., 1991. Systematic implications of male caudal morphology in ascaridoid nematode parasites. Syst. Parasitol. 19(3), 215-229.

FAO, 2016. Globefish highlights. Issue 1, January 2015, with Jan. – Sept. 2015 Statistics. Available at: http://www.fao.org/3/a-bc119e.pdf Last accessed: 18/11/2019.

FAO, 2018. The State of World Fisheries and Aquaculture 2018. http://www.fao.org/documents/card/en/c/I9540EN/ Last accessed: 18/11/2019.

FAO, 2019a. Globefish highlights. Issue 2, April 2019, with Jan. – Dec. 2018 Statistics. http://www.fao.org/3/ca5307en/ca5307en.pdf Last accessed: 18/11/2019.

FAO, 2019b. Globefish highlights. Issue 1, January 2019, with Jan. – Dec. 2018 Statistics. http://www.fao.org/3/ca4185en/ca4185en.pdf Last accessed: 18/11/2019.

Ferrantelli, V., Costa, A., Graci, S., Buscemi, M. D., Giangrosso, G., Porcarello, C., Palumbo, S., Cammilleri, G., 2015. Anisakid nematodes as possible markers to trace fish products. It. J. Food Safety 4(1), 49-53.

Gestal, C., Belcari, P., Abollo, E., Pascual, S., 1999. Parasites of cephalopods in the northern Tyrrhenian Sea (western Mediterranean): new host records and host specificity. Scientia Marina 63(1), 39-43.

Ghadam, M., Banaii, M., Mohammed, E. T., Suthar, J., Shamsi, S., 2018. Morphological and molecular characterization of selected species of *Hysterothylacium* (Nematoda: Raphidascarididae) from marine fish in Iraqi waters. J. Helminthol. 92(1), 116-124.

Goffredo, E., Azzarito, L., Di Taranto, P., Mancini, M. E., Normanno, G., Didonna, A., Faleo, S., Occhiochiuso, G., D'Attoli, L., Pedarra, C., Pinto, P., Cammilleri, G., Graci, S., Sciortino, S., & Costa, A., 2019. Prevalence of anisakid parasites in fish collected from Apulia region (Italy) and quantification of nematode larvae in flesh. Int. J. Food Microbiol., 292, 159-170.

Gómez-Morales, M. A., Castro, C. M., Lalle, M., Fernández, R., Pezzotti, P., Abollo, E., Pozio, E., The Ring Trial and ß-testing Participants, 2018. UV-press method versus artificial digestion method to detect Anisakidae L3 in fish fillets: Comparative study and suitability for the industry. Fish. Res. 202, 22-28.

González, R. A., Kroeck, M. A., 2000. Enteric helminths of the shortfin squid *Illex argentinus* in San Matias Gulf (Argentina) as stock discriminants. Acta Parasitol. 45(2), 89-93.

Graci, S., Collura, R., Cammilleri, G., Buscemi, M. D., Giangrosso, G., Principato, D., Gervasi, T., Cicero, N., Ferrantelli, V., 2017. Mercury accumulation in Mediterranean Fish and Cephalopods Species of Sicilian coasts: correlation between pollution and the presence of *Anisakis* parasites. Nat. Prod. Res. 31(10), 1156-1162.

Guardone, L., Malandra, R., Costanzo, F., Castigliego, L., Tinacci, L., Gianfaldoni, D., Guidi, A., Armani, A., 2016. Assessment of a sampling plan based on visual inspection for the detection of anisakid larvae in fresh anchovies (*Engraulis encrasicolus*). A first step towards official validation? Food Anal. Method. 9, 1418–1427.

Guardone, L., Nucera, D., Pergola, V., Costanzo, F., Costa, E., Tinacci, L., Guidi, A., Armani, A., 2017. Visceral larvae as a predictive index of the overall level of fish batch infection in European anchovies (*Engraulis encrasicolus*): a rapid procedure for Food Business Operators to assess marketability. Int. J. Food Microbiol. 250, 12-18.

Guardone, L., Armani, A., Nucera, D., Costanzo, F., Mattiucci, S., Bruschi, F., 2018. Human anisakiasis in Italy: a retrospective epidemiological study over two decades. Parasite, 25, 41.

Guillén-Hernández, S., López-Struck, A., González-Salas, C., Aguirre-Macedo, M. L., 2018. *Octopus maya* parasites off the Yucatán Peninsula, Mexico. I. Faunal assemblages. Dis. Aquat. Organ. 130(1), 37-43.

Hibi, Y., Ogata, J., Mimuro, A., Ito, K., Hakamada, Y., 2009. A case of small intestinal intussusceptions caused by anisakidosis. Jpn J. Gastroenterol. Surg. 42, 669-673. (In Japanese)

Hunsicker, M. E., Essington, T. E., Watson, R., Sumaila, U. R., 2010. The contribution of cephalopods to global marine fisheries: can we have our squid and eat them too?. Fish Fish. 11(4), 421-438.

Im, K. I., Yong, T. S., Shin, H.J., Kim, B. H., Moon, S. I. 1990. Gastric anisakiasis in Korea, with review of 47 cases. Yonsei Rep. Trop. Med. 21, 1-7.

Im, K. I., Shin, H. J., Kim, B. H., Moon, S. I. 1995. Gastric anisakiasis cases in Cheju-do, Korea. Korean J. Parasitol. 33, 179-186. (In Korean)

Karl, H., Leinemann, M., 1993. A fast and quantitative detection method for nematodes in fish fillets and fishery products. Arch Lebensmittelhyg. 44(5), 124-125.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647-1649.

Jacobson, L. D., 2005. Longfin inshore squid, *Loligo pealeii*, life history and habitat characteristics. Essential fish habitat source document. NOAA Technical Memorandum NMFS-NE-193. https://repository.library.noaa.gov/view/noaa/4035 Last accessed: 27/11/2019.

Jang, G. L., Chung, J. Y., Kim, W. K., Kim, K. S., Kim, J. G., Kim, Y. J., Park, B. K., Hwang, S. S., Kang, M. K. 1989. Clinical observation of 12 cases of gastric anisakiasis. Korean J. Int. Med. 37, 403-410. (In Korean)

Llarena-Reino, M., Piñeiro, C., Antonio, J., Outeriño, L., Vello, C., González, Á. F., Pascual, S., 2013. Optimization of the pepsin digestion method for anisakids inspection in the fishing industry. Vet. Parasitol. 191(3-4), 276-283.

Libralato, S., Colloca, F., Gücü, A. C., Maravelias, C. D., Solidoro, C., Villasante, S., Cardinale, M., 2018. Challenges and Opportunities for the EU Common Fisheries Policy Application in the Mediterranean and Black Sea. Front. Mar. Sci. 5, 299.

Lougovois, V. P., Kolovou, M. K., Savvaidis, I. N., Kontominas, M. G., 2008. Spoilage potential of ice- stored whole musky octopus (*Eledone moschata*). Int. J Food Sci. Technol. 43(7), 1286-1294.

Mattiucci, S., Fazii, P., De Rosa, A., Paoletti, M., Megna, A. S., Glielmo, A., De Angelis, M., Costa, A., Meucci, C., Calvaruso, V., Sorrentini, I., Palma, G., Bruschi, F., Nascetti, G., 2013. Anisakiasis and gastroallergic reactions associated with *Anisakis pegreffii* infection, Italy. Emerg. Infect. Dis. 19(3), 496.

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., Nascetti, G., 2018. Molecular epidemiology of *Anisakis* and Anisakiasis: an ecological and evolutionary road map. Adv. Parasitol. 99, 93–263.

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

Nagasawa, K., Moravec, F., 1995. Larval anisakid nematodes of Japanese common squid (*Todarodes pacificus*) from the Sea of Japan. J. Parasitol. 69-75.

Nagasawa, K., Moravec, F., 2002. Larval anisakid nematodes from four species of squid (Cephalopoda: Teuthoidea) from the central and western North Pacific Ocean. J. Nat. Hist. 36(8), 883-891.

Peng, D. M., Mu, Y. T., 2019. Predicting the Production of the World's Cephalopod Fisheries by Means of Differences in Level of Development and Production Trends. Trans. Am. Fish. Soc. 148(2), 260-270.

Picó-Durán, G., Pulleiro-Potel, L., Abollo, E., Pascual, S., Muñoz, P., 2016. Molecular identification of *Anisakis* and *Hysterothylacium* larvae in commercial cephalopods from the Spanish Mediterranean coast. Vet. Parasitol. 220, 47-53.

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1–24. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004

laying down specific hygiene rules on the hygiene of foodstuffs. OJ L 139, 55–205.

Roumbedakis, K., Krausova, M., Tyml, T., Di Cristo, C., 2018. A perspective around cephalopods and their parasites, and suggestions on how to increase knowledge in the field. Front. Physiol. 9, 1573.

Sealifebase, 2016a. Computer generated distribution maps for *Eledone cirrhosa* (horned octopus), with modelled year 2100 native range map based on IPCC A2 emissions scenario. www.aquamaps.org, version of Aug. 2016. https://sealifebase.org/summary/Eledone-cirrhosa.html# Last accessed 24/11/2019

Sealifebase, 2016b. Computer generated distribution maps for Eledone moschata (musky octopus), with modelled year 2100 native range map based on IPCC A2 emissions scenario. www.aquamaps.org, version of Aug. 2016. https://sealifebase.org/summary/Eledone-moschata.html# Last accessed 24/11/2019

Sealifebase, 2016c. Computer generated distribution maps for Doryteuthis pealeii (longfin inshore squid), with modelled year 2100 native range map based on IPCC A2 emissions scenario. www.aquamaps.org, version of Aug. 2016. https://sealifebase.org/summary/Doryteuthis-pealeii.html Last accessed 24/11/2019

Setyobudi, E., Jeon, C. H., Choi, K., Lee, S. I., Lee, C. I., Kim, J. H., 2013. Molecular identification of anisakid nematodes third stage larvae isolated from common squid (*Todarodes pacificus*) in Korea. Ocean Sci. J. 48(2), 197-205.

Shukhgalter, O. A., Nigmatullin, C. M., 2001. Parasitic helminths of jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae) in open waters of the central east Pacific. Fish. Res. 54(1), 95-110.

Souidenne, D., Florent, I., Dellinger, M., Romdhane, M. S., Grellier, P., Furuya, H., 2016. Redescription of *Dicyemennea eledones* (Wagener, 1857)(Phylum Dicyemida) from *Eledone cirrhosa* (Lamarck, 1798)(Mollusca: Cephalopoda: Octopoda). System. Parasitol. 93(9), 905-915.

Takahara, H., Sakurai, Y., 2010. Infection of the Japanese common squid, *Todarodes pacificus* (Cephalopoda: Ommastrephidae) by larval anisakid nematodes. Fish. Res. 106(2), 156-159.

Vieites, J. M., Ruiz, C. S., Fernández, F., Alonso, R. C., 2019. Importance of Cephalopod Health and Welfare for the Commercial Sector. In: Handbook of Pathogens and Diseases in Cephalopods (pp. 5-7). Springer, Cham.

Zhu, X., Gasser, R. B., Podolska, M., Chilton, N. B., 1998. Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. Int. J. Parasitol. 28(12), 1911-1921.

Table

Table 1. Available studies investigating the presence of nematods larvae in cephalopod species with relative parasite prevalence and analytical method used. O: overall; V: viscera; M: mantle; NR: not reported; * Variable prevalence values, depending on the site of infection and the geographical location.

Reference	**Species**	**n**	**Origin**	**Period**	***Anisakis* O (P%)**	***Anisakis* V (P%)**	***Anisakis* M (P%)**	***Hysterothylacium* O (P%)**	***Hysterothylacium* V (P%)**	***Hysterothylacium* V (P%)**	**other nematods O (P%)**	**Analytical method**	**Identification method**	**identified parasite**
Cipriani et al. (2019)	*I. argentinus*	70	FAO 41	Feb. 2018	15.7	12.9	4.3	2.1	2.1	0	0	UV press methods (V+M)	microscopy (genus level); Anisakis spp.: mtDNA cox2 and partial EF1 α-1 region; Hysterothylacium spp.: ITS rDNA region	*A. pegreffii, H. aduncum*
Goffredo et al. (2019)	*L. vulgaris*	54	FAO 37.2.2	Sept 2012 - Aug 2013	0	0	0	0	0	0	0	visual examination of the celomic cavity, followed by candling technique under microscope	-	-
	A. media	55												
	S. officinalis	88												
	I. coindetii	137												
	E. moschata	5												
	O. vulgaris	1												
Graci et al. (2019)	*L. vulgaris*	7	FAO 37.2.2; FAO 37.1.3	May - Dec 2015	0	0	0	0	0	0	0	visual examination	microscopy (genus level)	*Anisakis* sp.
	T. sagittatus	8			50	50	0	0	0	0	0			
Guillén-Hernández et al. (2018)	*O. maya*	1202	FAO 31	Aug 2009 - Jun 2010	0.4-5 (Anisakidae)						3 (Spiruridae); 0.5-3 (Philometridae)	stereomicroscope examination	microscopy (parasites identified to lowest taxonomic level possible)	Anisakidae
Costa et al. (2016)	*T. sagittatus*	30	FAO 37.1.1; FAO 37.1.3	Jan 2014 - Apr 2015	25	25	0	0	0	0	0	visual inspection + stereomicroscope (V), digestion (Trichineasy) when negative	optical microscopy; molecular ID: ITS PCR-RFLP and sequencing	*A. pegreffii, A. pegreffii x A. simplex s.s., A. simplex s.s., A. physeteris*
Pico-Duran et al. (2016)	*I. coindetii*	123	FAO 37.1.1	May - Jun 2013	12.2	12.2	0	NR	1, 0.8	3.3	0	enzimatic digestion	molecular ID: ITS sequencing	*A. pegreffii, A. pegreffii x A. simplex s.s., A. physeteris, Hysterothylacium sp.*

	L. vulgaris	34			0	0	0		0	0	0			-
	O. vulgaris	45			0	0	0		0	0	0			-
Ferrantelli et al. (2015)	*T. sagittatus*	21	FAO 37.1.3; FAO 37.2.2	Jan 2013 - Mar 2014				5 (Anisakidae)			NR	visual inspection (V)	microscopy; PCR-RFLP ITS	not specified
Serracca et al. (2013)	*I. coindetii*	60	FAO 37.1.3	Oct 2010 - Sept 2011	0	0	0		1.67	0	0	visual examination + transillumination (M)	microscopy; molecular ID: ITS PCR-RFLP and sequencing of a part of the larvae	Hysterothylacium sp.
Setyobudi et al. (2013)	*T. pacificus*	615	FAO 61	2009-2011	8.37-86.0 (anisakid)	0-84.3*	0-36*	0	0	0	unknown nemtode (83% similarity with *H. deardorffoverstreetorum*)	careful observation with a stereomicroscope after dissection of body cavity, V and M	microscopy; molecular ID: ITS PCR-RFLP and mtcox2 sequencing	*A. pegreffii, A. simplex*
Angelucci et al. (2011)	*Octopus spp.*	26	FAO 37.1.3	Oct 2008 - Jan 2010	0	0	0	3.8	NR	NR	0	visual inspection + digestion (body cavity and V)	microscopy (genus level)	*Anisakis* type 1, 2
	L. vulgaris	6			0	0	0	0	0	0	0		microscopy (genus level)	*Anisakis* type 1, 2
	T. sagittatus	5			20	20	0	60	NR	NR	0		microscopy (genus level)	*Anisakis* type 1, 2
	I. coindetii	4			50	50	0	0	0	0	0		microscopy (genus level)	*Anisakis* type 1, 2
	E. cirrhosa	1			0	0	0	0	0	0	0		microscopy (genus level)	*Anisakis* type 1, 2
Choi et al. (2011)	*T. pacificus*	82	Fish Market in Korea	Aug 2006 - Jul 2007	22	NR	NR	0	0	0	0	visual examin + dissection	microscopy (genus level)	*A. simplex* s.l.
	S. esculenta	36			8.3	NR	NR	0	0	0	0		microscopy (genus level)	*A. simplex* s.l.
	L. bleekeri	69			0	NR	NR	0	0	0	0		microscopy (genus level)	*A. simplex* s.l.
Di Donfrancesco et al. (2011)	*I. coindetii*	581	FAO 37.1.3	Jan - Aug 2011	0.34	0.34	NR	1.2	1.2	NR	0	visual inspection + dissection	microscopy	*Anisakis* sp.
Petric et al. (2011)	*I. coindetii*	439	FAO 37.2.1	Oct 2007 - Oct 2008	30.5	NR	NR	0	0	0	0	visual examination	Molecular id: mtDNA cox2	*A. pegreffii*

Culurgioni et al. (2010)	*H. reversa*	141	FAO 37.1.3	Apr 2005 - Apr 2009	0	0	0	0	0	0	4.26 (Lappetascaris sp. - free in the mantle cavity or encysted)	standard diagnostic techniques	microscopy (genus level)	
	H. bonnelli	164			1.83	1.83	0	0	0	0	1.22 (Lappetascaris sp. - free in the mantle cavity or encysted)		microscopy (genus level)	Anisakis sp. Type 2
Takahara & Sakurai (2010)	*T. pacificus*	2153	FAO 61	Jun - Dec 2008	3.2	3.2	0	0	0	0	5.3 (Lappetascaris sp.)	examination of the inner mantle, stomach, caecum, digestive gland and genital organ (not detailed)	microscopy	*Anisakis simplex* SL
Lee et al. (2009)	*T. pacificus*	15	Korea (wholesale and retail markets)	Mar - Jul 2006	5	NR	NR	0	0	0	0	visual inspection	microscopy	*A. pegreffii*, *A. typica*, *A. simplex* SS
Pardo-Gandarillas et al. (2009)	*D. gigas*	124	FAO 87.2	Jul 2003 - Feb 2004	17.7 (Anisakis type II); 6.5 (Anisakis type I)	17.7 (Anisakis type II); 6.5 (Anisakis type I)	0	0	0	0	0	visual inspection + dissection under stereomicroscope	microscopy (genus level)	Anisakis type I-II
Abollo et al. (2001)	*S. officinalis*	175	FAO 27.9	1997-1998	3.42	3.42	0	NR	NR	NR	NR	visual inspection + dissection under stereomicroscope	microscopy (genus level)	*A. simplex* SS
	S. elegans	15			0	0	0	NR	NR	NR	NR		microscopy (genus level)	
	S. orbignyana	50			0	0	0	NR	NR	NR	NR		microscopy (genus level)	
	A. subulata	75			0	0	0	NR	NR	NR	NR		microscopy (genus level)	
	T. eblanae	650			23.5	23.5		NR	NR	NR	NR		microscopy (genus level)	*A. simplex* SS
	I. coindetii	650			11.07	11.07	0	NR	NR	NR	NR		microscopy (genus level)	*A. simplex* SS
	T. sagittatus	70			34.28	34.28	0	NR	NR	NR	NR		microscopy (genus level)	*A. simplex* SS
Brickle et al. (2001)	*L. gahi*	1096	FAO 41.3.2	Feb 1999 - Jun 2000	2.46	2.46	NR	0	0	0	0	visual examination	microscopy (genus level)	Anisakis sp.
Shukhgalter & Nigmatullin (2001)	*D. gigas*	849	FAO 77; FAO 87	1981 - 1989	9.2 (*A. simplex*); 24.2 (*A. physeteri*)	9.2 (*A. simplex*); 24.2 (*A. physeteris*)	NR	0	0	0	29.4, (*Porrocaecum* sp.); 0.5 (*Contracaecum* sp.); 0.4 (*Spinitectus* sp.)	standard diagnostic techniques	microscopy	*A. simplex*, *A. physeteris*

Gonzàlez & Kroeck (2000)	*I. argentinus*	91	FAO 41.3.1	Jul-Nov 1993	67.7-100	67.7-100	NR	0-33.3	0-33.3	NR	Two other types of not identified nematodes (*Ascarophis* and *Pseudoterranova*)	visual inspection + dissection	microscopy	Anisakis type I
Gestal et al. (1999)	*S. orbignyama*	25	FAO 37.1.3	Oct - Nov 1996	0	0	NR	0	0	NR	0	visual examination + dissection under stereomicroscope	microscopy (genus level)	
	S. elegans	35			0	0	NR	0	0	NR	0		microscopy (genus level)	
	E. cirrhosa	25			0	0	NR	28	28	NR	0		microscopy (genus level)	*Hysterothylacium* sp.
	L. vulgaris	65			0	0	NR	0	0	NR	0		microscopy (genus level)	
	A. subulata	23			0	0	NR	0	0	NR	0		microscopy (genus level)	
	A. media	37			0	0	NR	0	0	NR	0		microscopy (genus level)	
	Alloteuthis sp.	41			0	0	NR	0	0	NR	0		microscopy (genus level)	
	I. coindetii	42			4.8	4.8	NR	0	0	NR	0		microscopy (genus level)	*A. simplex* s.l.
Pascual et al. (1999)	*I. coindetii*	600	FAO 27.9	Nov 1992-Nov 1993	3.8-89*	3.8-89*	NR	0	0	0	0	visual inspection + dissection under stereomicroscope	microscopy (genus level)	*A. simplex* s.l.
	T. eblanae	600			3.9-93.3*	3.9-93.3*	NR	0	0	0	0		microscopy (genus level)	
Abollo et al. (1998)	*O. vulgaris*	150	FAO 27.9	1991-1997	2	NR	NR	NR	NR	NR	NR	visual examination + stereomicroscope	microscopy (genus level)	*A. simplex* s.l.
	E. cirrhosa	67			1.5	NR	NR	NR	NR	NR	NR			*A. simplex* s.l.
	S. officinalis	150			3.5	NR	NR	NR	NR	NR	NR			*A. simplex* s.l.
	S. elegans	13			0	NR	NR	NR	NR	NR	NR			-
	S. orbignyana	35			0	NR	NR	NR	NR	NR	NR			-
	L. vulgaris	50			16	NR	NR	NR	NR	NR	NR			*A. simplex* s.l

	A. subulata	60			0	NR	NR	NR	NR	NR	NR			-
	T. eblanae	600			18.6	NR	NR	NR	NR	NR	NR			*A. simplex* s.l.
	I. coindetii	600			11	NR	NR	NR	NR	NR	NR			*A. simplex* s.l.
	T. sagittatus	65			33.8	NR	NR	NR	NR	NR	NR			*A. simplex* s.l.
Pascual et al. (1996)	*O. vulgaris*	70	FAO 27.9	1992-1995	0	NR	NR	0	0	0	11.4 (*Cystidicola* sp.)	visual examination	microscopy (genus level)	-
	E. cirrhosa	67			1.5	NR	NR	0	0	0	0			*A. simplex* B
	S. officinalis	38			0	NR	NR	0	0	0	0			-
	S. orbignyana	22			0	NR	NR	0	0	0	0			-
	Loglio vulgaris	8			62.5	NR	NR	0	0	0	0			*A. simplex* B
	Illex coindetii	600			2.6-19.3	NR	NR	0	0	0	0			*A. simplex* B
	Todaropsis eblanae	600			12.3-25	NR	NR	0	0	0	0			*A. simplex* B
	Todarodes sagittatus	65			33.3-34.3	NR	NR	0	0	0	0			*A. simplex* B
Pascual et al. (1994)	*Illex coindetii*	70	FAO 27.8	Oct 1991-Apr 1992	10	10	NR	0	0	NR	0	visual examination	microscopy (genus level)	*A. simplex* s.l.
Bower and Margolis (1991)	*Ommastrephes bartrami*	68	FAO 77	NR	13.2	NR	NR	100	NR	NR	0	visual examination	microscopy	*A. simplex* s.l.

Table

Table 2. Summary of the results for the two analysed species at visceral and mantle level

Cephalopod species (n tested)	Total weight range (mean) [g]	Mantle weight range (mean) [g]	Total lenght range (mean) [cm]	Dorsal mantle length range (mean) [cm]	No of specimens positive for Anisakidae in viscera (P%, 95% CI)	N of larvae in the viscera (MA, MI)	No of specimens positive for Anisakidae in mantle (P%, 95% CI)	N of larvae in the mantle (MA, MI)
Eledone spp. (75)	69-464 (198.8)	13-96 (45.8)	12-61 (35.8)	5-13 (8.3)	0 (0%, 0-5.1 95% CI)	0	5 (6.7%, 2.2-14.9 95% CI)	9 (0.12, 1.8)
Doryteuthis pealeii (70)	54-175 g (106.5)	19-115 (62.3)	28-66 (41.2)	10-24 (16.8)	1 (1.4%, 0.00-7.7 95% CI)	1 (0.014, 1)	1 (1.4%, 0.00-7.7 95% CI)	1 (0.014, 1)

Table

Table 3. Detail of the measured parameters and parasitological results of the positive specimens

Sample code	Total length (cm)	DML (cm)	Total weight (g)	Mantle weight (g)	N nematodes in viscera	N nematodes in mantle	Morphological identification	Molecular identification
ECIR15	39	8.5	251	62	-	2	*Lappetascaris* sp. according to Nagasawa and Moravec 1995, 2002; Culurgioni et al., 2010	92-93% identity with *Hysterothylacium deardorffoverstreetorum*
ECIR18	39	9	244	63	-	1		
ECIR25	47	10	271	56	-	3		
EMOS3	14	6.5	143	30	-	1		
EMOS10	16	8	158	32	-	2		
DPEA19	42	13	96	46	1	-	*Anisakis* sp. (larval Type I)	*Anisakis simplex*
DPEA20	35	13	80	42	-	1		

Figure

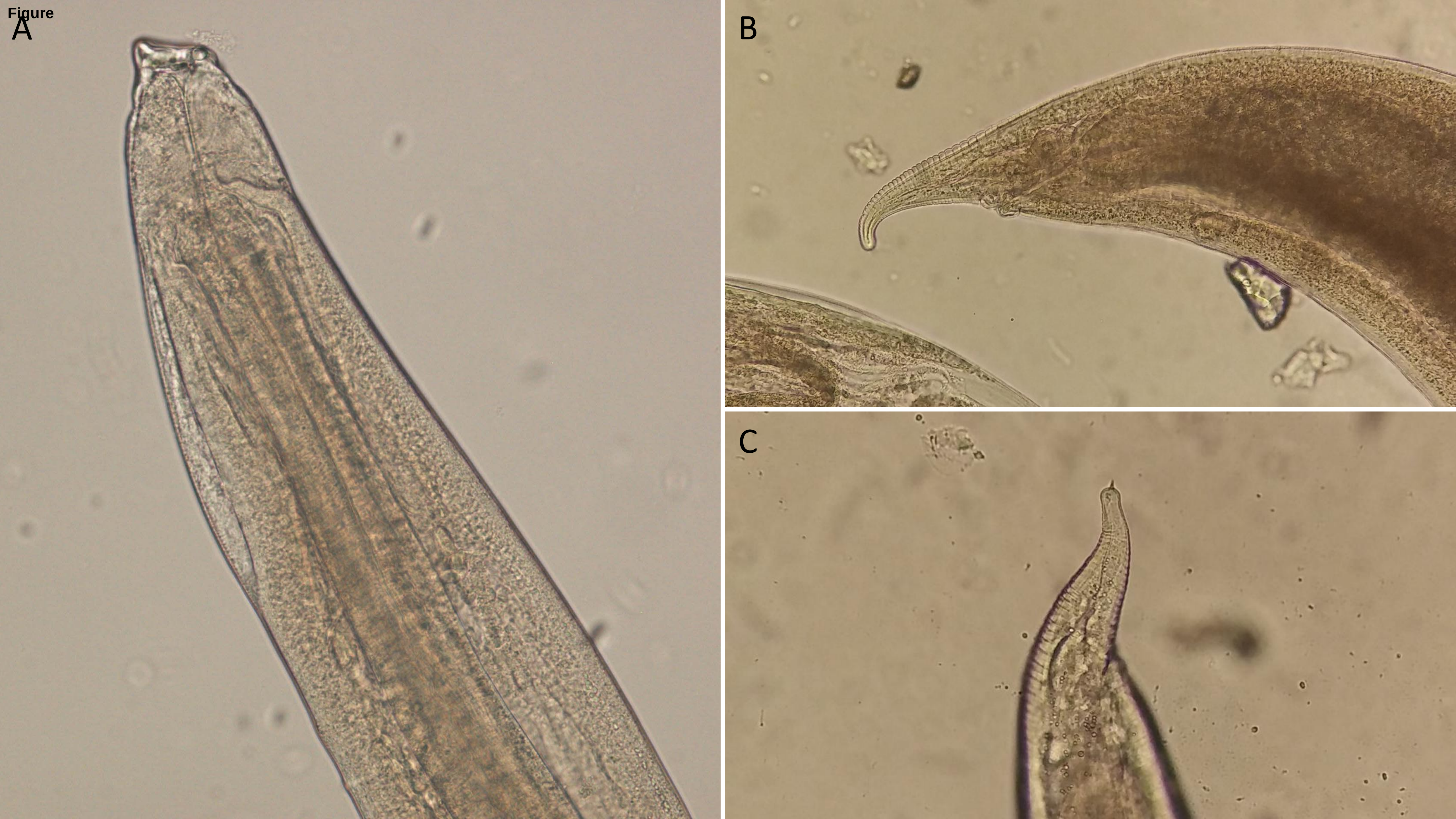

Table 1SM. List of species investigated for the presence of nematod larvae (divided by categories octopus, cuttlefish and squid). CD: commercial designation

Category	Order	Family	Genus	Species	CD
octopus	Octopoda	Octopodidae	Eledone	*E. cirrhosa*	horned octopus
				E. moschata	musky octopus
			Octopus	*Octopus sp.*	-
				O. maya	Mexican four-eyed octopus
				O. vulgaris	common octopus
cuttlefish	Sepiida	Sepidae	Sepia	*S. elegans*	elegant cuttlefish
				S. esculenta	golden cuttlefish
				S. officinalis	common cuttlefish
				S. orbignyana	pink cuttlefish
squid	Teuthida	Histioteuthidae	Histioteuthis	*H. bonnellii*	umbrella squid
				H. reversa	elongate jewell squid
		Loliginidae	Alloteuthis	*Alloteuthis sp.*	-
				A. media	-
				A. subulata	European common squid
			Doryteuthis	*D. gahi*	Patagonian squid
			Loligo	*L. bleekeri*	spear squid
				L. vulgaris	European squid
		Ommastrephidae	Dosidicus	*D. gigas*	jumbo flying squid
			Illex	*I. aregentinus*	Argentine shortfin squid
				I. coindetii	shortfin squid
			Ommastrephes	*O. bartramii*	neon flying squid
			Todarodes	*T. pacificus*	Japanese flying squid
				T. sagittatus	European flying squid
			Todaropsis	*T. eblanae*	lesser flying squid