Food Control 122 (2021) 107807

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

First molecular detection of the parasites *Molicola uncinatus* and *Hepatoxylon trichiuri* (Cestoda: Trypanorhyncha) infecting the silver scabbardfish *Lepidopus caudatus* from the Central Mediterranean Sea: Implications for the seafood quality and safety

Marialetizia Palomba [a,b], Mario Santoro [b], Renato Aco Alburqueque [a], Paolo Cipriani [c], Simonetta Mattiucci [a,*]

[a] Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, 00185, Rome, Italy
[b] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy
[c] Section of Contaminants and Biohazards, Institute of Marine Research (IMR), P.O. Box 1870 Nordnes, 5817, Bergen, Norway

ARTICLE INFO

Keywords:
Lepidopus caudatus
Trypanorhyncha
Molicola uncinatus
Hepatoxylon trichiuri
Molecular markers
Infection levels

ABSTRACT

Trypanorhynch tapeworms are marine heteroxenous parasites, involving sharks and rays as definitive hosts, invertebrates (i.e. crustaceans) as first intermediate hosts, and various teleost fishes as intermediate/paratenic ones. The massive presence of Trypanorhyncha larval forms in the flesh or body cavity of the fish hosts reduces their market value, making them unappealing to consumers, or causing discards of fish products.

The identification of merocercoids (N = 100) of *Molicola uncinatus* and plerocercoids (N = 46) of *Hepatoxylon trichiuri* parasitizing the silver scabbardfish, *Lepidopus caudatus,* from the Central Mediterranean Sea (off Malta coast), was obtained, for the first time, by using sequences analysis of the complete small subunit (ssrDNA) and the partial large subunit (lsrDNA) ribosomal RNA gene loci. The Bayesian Inference from the concatenated sequences obtained at the two gene loci showed that the individuals of *M. uncinatus* and *H. trichiuri* clustered, respectively, in two well distinct and supported phylogenetic lineages, corresponding to the two cestode species. High prevalence (P) and mean abundance (MA) values were observed in the infection with merocercoids of *M. uncinatus* (P = 100%, A = 28.81), with the ventral musculature of the silver scabbardfish as the most parasitized site. Plerocercoids of *H. trichiuri* were found to infect only the mesenteries, attached to various fish tissues, with a mean abundance rate of 1.28.

Even if harmless to humans, a heavy parasitic load with larval stages of Trypanorhyncha, particularly in the edible part of the silver scabbardfish (as here reported for merocercoids of *M. uncinatus*), can compromise the appearance of the fish-food, making it repugnant and unappealing for the final consumer. It has been also discussed that when managing the risk related to parasites affecting the fish quality and safety of fisheries products, the fishing ground where the life cycle of those parasites takes place should be also taken into account, as it would include the ecological drivers of the infection with those parasites. This wider ecological approach would be of help to the Food Business Operators (FBOs), gathering knowledge useful to manage fisheries in a wide "One-Health" perspective, aware of sustainability, quality and consumer safety aspects of sea-food production.

1. Introduction

The order Trypanorhyncha Diesing, 1863 comprises one of the most ubiquitous group of marine metazoan parasites, with more than 270 recognized species inhabiting all the world's oceans (Beveridge & Campbell, 2013; Olson et al., 2010; Palm, 1997; Palm, Waeschenbach, Olson, & Littlewood, 2009). Their life cycle includes several hosts, linked by a prey-predator relationship and thus included in a trophic-web of the marine ecosystem. As adults, they infect the digestive tract of elasmobranchs (sharks and rays), while marine invertebrates (i.

* Corresponding author. Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza - University of Rome, P.le Aldo Moro, 5 00189, Rome, Italy.
E-mail address: simonetta.mattiucci@uniroma1.it (S. Mattiucci).

https://doi.org/10.1016/j.foodcont.2020.107807
Received 8 September 2020; Received in revised form 30 November 2020; Accepted 2 December 2020
Available online 4 December 2020

e. crustaceans) and teleost fishes act as first intermediate and second intermediate and/or paratenic hosts, respectively (Palm, 1997; Palm & Caira, 2008).

The larval forms of Trypanorhyncha are generally found encysted in visceral organs and/or musculature of marine teleosts. Their occurrence in the muscles alters the fish flesh quality, reducing the marketability of commercially important fish species (Dias, Sao Clemente, Pinto, & Knoff, 2011; da Fonseca, de Sao Clemente, Felizardo, Gomes, & Knoff, 2012; Deardorff, Raybourne, & Mattis, 1984; Giarratana et al., 2014; Samn, Metwally, Zeina, & Khalaf Allah, 2014; Abdelsalam et al., 2016; Santoro et al., 2018; Oliveira, Kuraiem, Fonseca, Gomes, & Knoff, 2019; Guardone et al., 2020). Heavily infected fish fillets result unappealing to consumers; thus, the presence of these parasites causes economic loss to the fishing industry (Abdelsalam et al., 2016; Deardorff et al., 1984; Giarratana et al., 2014; Samn et al., 2014; Santoro et al., 2018). Few cases of accidental human ingestion of Trypanorhyncha larvae were reported, showing no clear evidence of pathogenic effects in humans (Bates, 1990; Lima dos Santos & Howgate, 2011). However, species of Trypanorhyncha have been suggested among the possible cause of allergic reactions in humans (Gòmez-Morales et al., 2008; Pelayo, García-Hernández, Puente, Rodero, & Cuébllar, 2009; Vázquez-López, de Armas-Serra, Bernardina, & Rodríguez-Caabeiro, 2001).

The taxonomy of trypanorhynch tapeworms mostly relies on the morphology of the tentacle armature (Campbell & Beveridge, 1994), a feature present both in the adult and larval stages of the parasites (Palm & Caira, 2008). According to Palm (2004), the genus *Molicola* Dollfus, 1935 actually includes three valid species: *Molicola uncinatus* (Linton, 1924) Palm, 2004 (syn. *M. thyrsitae* Robinson, 1959), *Molicola horridus* (Goodsir, 1841) Dollfus, 1935, and *Molicola walteri* Palm, 2004. The genus *Hepatoxylon* Bosc, 1811 includes two species: *Hepatoxylon trichiuri* (Holten, 1802) and *H. megacephalum* (Rudolphi, 1819).

However, the identification of the larval stages of trypanorhynch tapeworms may be difficult when based on morphological characters, especially due to the retraction of the tentacular apparatus inside the tentacular sheaths (Palm, 1997; Santoro et al., 2018). In this concern, the genetic/molecular methodologies are a valuable tool for the characterization and identification of the species of Trypanorhyncha, largely contributing to disentangle their systematics (Olson et al., 2010; Palm et al., 2009), as well as to establish phylogenetic relationships among taxa. The molecular/genetic approach has also offered the chance to deepen the knowledge on the evolutionary and ecological aspects of those tapeworms, as their definitive host preference and geographical distribution (Olson et al., 2010; Palm et al., 2009).

The silver scabbardfish (*Lepidopus caudatus* Euphrasen, 1788) is a carnivorous mesopelagic trichiurid, widely distributed throughout temperate seas of the world, including the Mediterranean Sea (Demestre, Moli, Recases, & Sanches, 1993). Its diet comprises various preys at different trophic levels, mostly represented by mesopelagic crustaceans, fish and cephalopods (Demestre et al., 1993; Torre, Sicuro, & Kallianiotis, 2019). The silver scabbardfish is an important commercial species in the North-Eastern Atlantic, Southern Atlantic off Namibia coast, and Southern Pacific off New Zealand coast (Nakamura & Parin, 1993). In the Mediterranean Sea, the commercial exploitation of this species is limited to Italy, Spain, Albania and Tunisia, where larger specimens (length 1000–2000 mm) reach a considerable commercial value (Fisher, Bauvhot, & Schneider, 1987; Demestre et al., 1993; D'Onghia, Mastrotaro, & Maiorano, 2000; Rosa, Menezes, Melo, & Pinho, 2006; Iwamoto, 2015). According to FAO statistics, its fishery in the Mediterranean Sea and the Black Sea reached a peak of almost 5000 tons in 2011. During the 2015, the total fishery production accounted for 2964 tons with Italy as first country, catching the 52% of the total capture (http://appsso.eurostat.ec.europa.eu/nui/submitViewTableAction.do). In the Central Mediterranean Sea, the silver scabbardfish is the third species in term of contribution (%) to long-line fishery landings (Alvito et al., 2018). Indeed, this fish species is largely consumed at the local scale level and commonly commercialized as fresh fish in the Italian markets (Bottari et al., 2019).

Considering that data on the parasites affecting the fresh fish sold on the market are of interest to both the Health Authorities and the Food Business Operators (FBOs), the aims of the present study were to: *i)* identify, for the first time by using genetic/molecular markers, the Trypanorhyncha metacestodes species infecting the edible and non-edible parts of the silver scabbardfish commercialized in Italy; *ii)* evaluate the infection levels of those metacestodes and their site of infection in the fish.

2. Materials and methods

2.1. Samples collection

During July–November 2019, a total of 36 specimens of silver scabbardfish were obtained from the Central Mediterranean Sea, off Malta coast (FAO 37.2 fishing area, 36°17′17″ N – 13°35′03″ E). Fish were frozen and then delivered to the Laboratory of Parasitology at the Department of Public Health and Infectious Diseases "Sapienza University of Rome", where parasitological examination was performed. Following measurement (total length), each specimen of silver scabbardfish was filleted and eviscerated to be macroscopically examined for the presence of trypanorhynch tapeworms in the fish muscles and body cavity. The tapeworm larvae located in the muscle were collected removing the conspicuous blastocysts, rather than attempting to remove the parasite *in toto,* as described in Robinson (1958). Then, the larval cestodes were placed in Petri dishes with a 0.65% NaCl solution, counted and morphologically studied at the optical microscope (Leica DM2500). They were morphologically assigned to the order Trypanorhyncha, following the diagnostic keys proposed by Palm (2004). For the molecular identification, fragments of parasites were frozen at −20 °C until the molecular analysis has been performed.

2.2. Molecular identification

Total genomic DNA (gDNA) from each single cestode larva was extracted using *Quick*-gDNA™ Miniprep Kit (ZYMO RESEARCH), following the standard manufacturer-recommended protocol. DNA was quantified by a NanoDrop®TC1-E20 spectrophotometer (BioTek Synergy HT).

Plerocercoids and merocercoids were identified to the species level by the sequences analysis of the partial large subunit (lsrDNA) and the complete small subunit (ssrDNA) ribosomal RNA gene (Palm et al., 2009). The partial 1srDNA was amplified using the primers ZX-1 (5' – ACCCGCTGAATTTAAGCATAT – 3'; modified from Van der Auwera, Chapelle, & De Wachter, 1994) and 1500R (5' – GCTATCCTGAGGGAAACTTCG – 3′). The complete ssrDNA was amplified using primers WormA (5' - GCGAATGGCTCATTAAATCAG – 3′) and WormB (5' -CTTGTTACGACTTTTACTTCC – 3') (Littlewood & Olson, 2001). Both PCR reactions were carried out in a 25 μL volume containing 0.6 μL of each primer 10 mm, 2 μL of $MgCl_2$ 25 mm (Promega), 5 μL of 5 × buffer (Promega), 0.6 μL of dNTPs 10 mm (Promega), 0.2 μL of Go-Taq Polymerase (5U/μL) (Promega) and 2 μL of total DNA. PCR temperature conditions were the following: 94 °C for 3 min (initial denaturation), followed by 35 cycles at 94 °C for 30 s (denaturation), 53 °C for 30 s (annealing), 72 °C for 2 min (extension) and followed by post-amplification at 72 °C for 7 min.

2.3. Sequences analysis

The sequences identity was checked using the Basic Local Alignment Search Tool (Blast, www.ncbi.nih.gov/BLAST/). Sequences were then aligned and compared with the reference sequences available in the NCBI nucleotide (NT) database, using Clustal X software (Larkin et al., 2007).

For the phylogenetic analysis, the aligned data sets of ssrDNA and

1srDNA sequences, obtained in the present study, were concatenated by Sequence Matrix v.1.7.8 (Vaidya, Lohman, & Meier, 2011). Subsequently, JModeltest was used to determine the best-fit substitution model, as implemented with Akaike's Information Criterion (AIC) (Posada & Buckley, 2004). A Bayesian Inference (BI) was carried out by MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). The Bayesian posterior probability analysis was performed using the MCMC algorithm, with four chains, 0.2 as the temperature of heated chains, 2.000.000 generations, with a subsampling frequency of 500 and a burn-in fraction of 0.25. Posterior probabilities were estimated and used to assess support for each branch. Values with a 0.90 posterior probability were considered well-supported. The phylogenetic trees were rooted using *Pintneriella musculicola* Yamaguti, 1934 as outgroup.

2.4. Parameters of parasitic infection

The parameters of infection were estimated as prevalence (P, %) with 95% confidence limits (CI, Clopper-Pearson) (or adjusted Wald's for N > 1000), and mean abundance (MA) with standard deviation (±SD), following Bush, Lafferty, Lotz, and Shostak (1997). They were calculated using the Quantitative Parasitology (Qpweb) 3.0 software, implemented for the web (Reiczigel, Marozzi, Fabian, & Rózsa, 2019; Reiczigel & Rózsa, 2005).

3. Results

3.1. Morphological and molecular identification

A total of 1044 tapeworm larval forms were collected from the 36 examined silver scabbardfish (Fig. 1) (mean total length of 1298.57 ± 84.81 mm). These larvae were first morphologically referred to the order Trypanorhyncha. Then, the 46 plerocercoids recovered from the viscera were morphologically assigned to the genus *Hepatoxylon* (Family Sphyriocephalidae Pintner, 1913); while the 998 merocercoids recovered from the muscle were morphologically assigned to the Family Gymnorhynchidae Dollfus, 1935.

A subsample of the merocercoid (N = 100), and all plerocercoid larvae (N = 46) were identified to the species level by genetic/molecular markers. Specifically, the lsrDNA and ssrDNA sequences of the 100 merocercoids individuals matched at 100% with the sequences of *M. uncinatus* previously deposited in GenBank for the two gene loci (Accession Numbers DQ642908 and DQ642746) (Olson et al., 2010). At the intraspecific level, the sequences here obtained for the species *M. uncinatus* did not show different nucleotide positions from those previously deposited in GenBank at both lsrDNA and ssrDNA gene loci. Concerning the plerocercoid larvae, the 46 sequences of lsrDNA and ssrDNA here obtained, matched at 99–100% with the sequences of *H. trichiuri* previously deposited in GenBank by Olson et al. (2010) (Accession Numbers FJ572943 for lsrDNA, and FJ572907 for ssrDNA). However, at the gene locus lsrDNA all the sequences of *H. trichiuri* here obtained, showed a nucleotide variation at the position 94 (a T instead of a C), with respect to the sequence of the same species deposited in GenBank; while no variation in the ssrDNA sequences analysis was recorded when compared to the sequence available for the gene in GenBank.

Sequences obtained in the present study for the species *M. uncinatus* were deposited in GenBank (Accession Numbers MT823197-MT823198 (lsrDNA) and MT823193-MT823194 (ssrDNA)). Similarly, the sequences obtained for the species *H. trichiuri,* were deposited in GenBank (Accession Numbers MT823199-MT823200 for the gene lsrDNA, and MT823195-MT823196 for the gene ssrDNA).

The BI inferred from the concatenated sequences obtained at the lsrDNA and ssrDNA gene loci (Fig. 2) showed that all the individuals of *M. uncinatus* and *H. trichiuri* clustered, respectively, in two distinct and well-supported phylogenetic lineages, corresponding to the two cestode species. Each cluster also included the sequences previously deposited in GenBank for *M. uncinatus* and *H. trichiuri* (Fig. 2) at the studied gene loci.

3.2. Site and rate of infection with the two parasite species

The merocercoids of *M. uncinatus* were detected only in the muscle of the target fish species, while the *H. trichiuri* plerocercoids were found attached to the surface of the internal organs/mesenteries, and/or frequently free in the abdominal cavity.

The prevalence recorded for *M. uncinatus* was 100% (CI, 0.89–1.00), with a mean abundance of 28.81 ± 10.31. The frequency of distribution of merocercoids of *M. uncinatus*, observed in different portions of the fish muscles, is shown in Fig. 3. In the ventral region of the fish musculature, *M. uncinatus* showed higher value of relative proportion (64.50%), with respect to the dorsal one which showed a lower rate (35.50%) (Fig. 3).

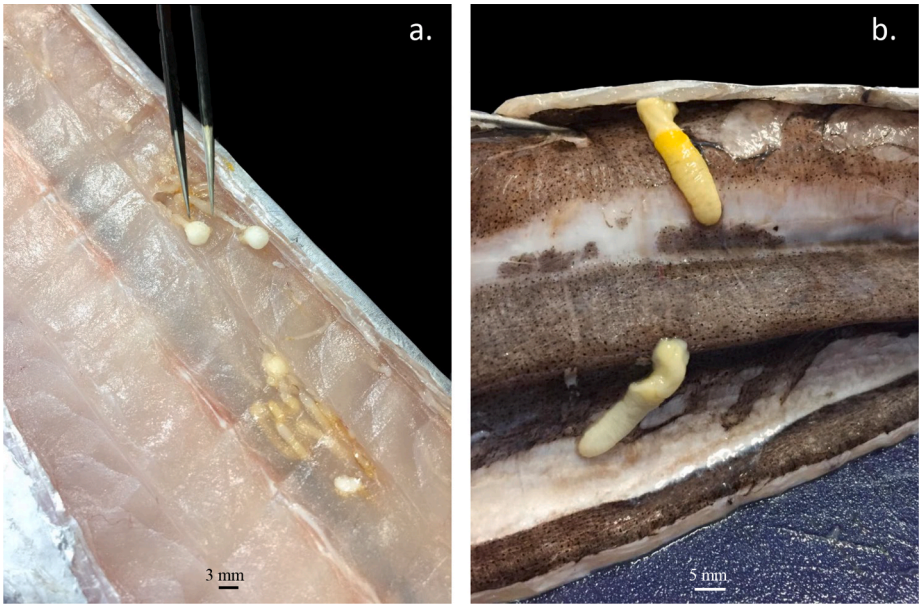

Fig. 1. Larval forms of Trypanorhyncha in the silver scabbardfish *L. caudatus* fished in Central Mediterranean Sea (off Malta coast). a) Merocercoid larvae of *Molicola uncinatus* located in the musculature; b) Plerocercoid larvae of *Hepatoxylon trichiuri* located in the mesenteries attached to musculature. Bar scale: a) 3 mm b) 5 mm.

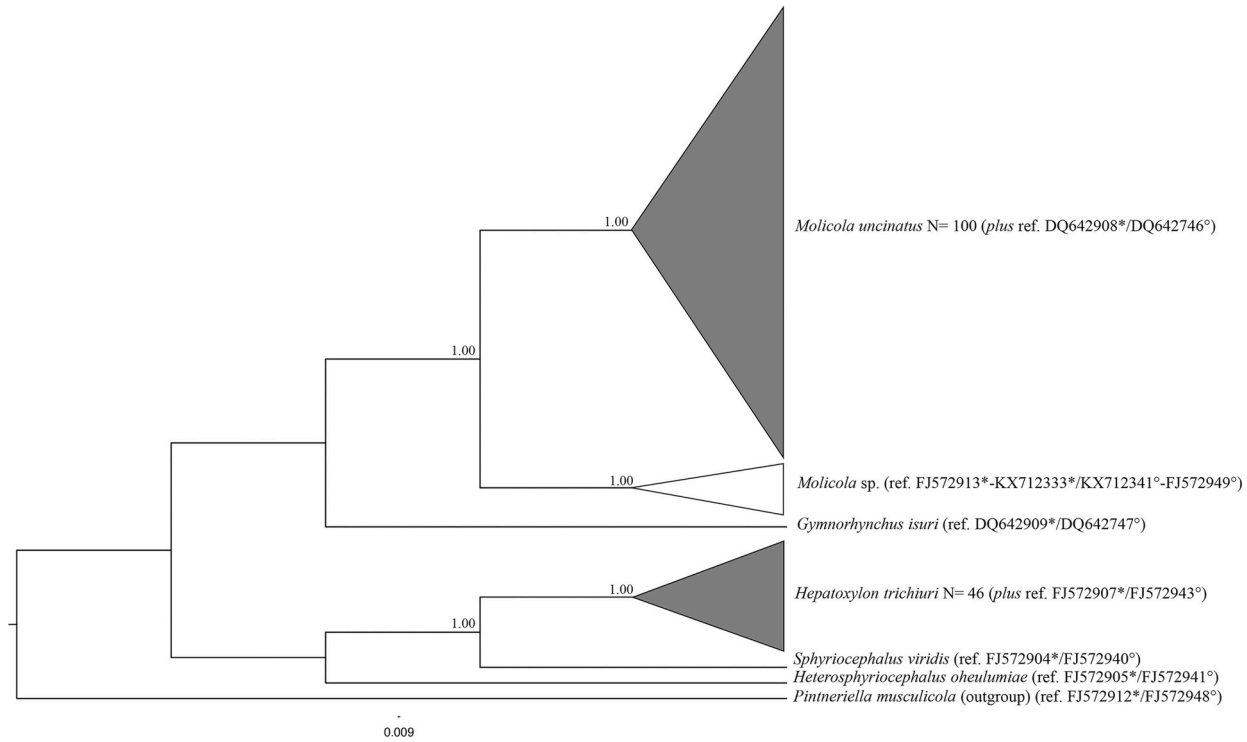

Fig. 2. Phylogenetic concatenated tree from Bayesian Inference (BI) based on 1srDNA and ssrDNA sequences of the specimens of *M. uncinatus* (N = 100) and *H. trichiuri* (N = 41) obtained in the present study, with respect to the sequences of the same species and other species of Gymnorhynchidae at the same gene loci available in GenBank (ssrDNA indicated with *, 1srDNA indicated with °). *Pintneriella musculicola* was used as outgroup (Olson et al., 2010).

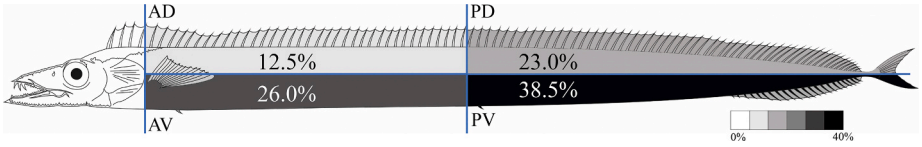

Fig. 3. Relative proportions of *Molicola uncinatus* larvae in different muscular portions of the silver scabbardfish, observed in the present study. Abbreviations: AD, anterior dorsal; AV, anterior ventral; PD, posterior dorsal; PV, posterior ventral (the four portions of the musculature follow Levsen & Karl, 2014).

As regards parasites located both in the dorsal and ventral area of the fish host, they showed a higher proportion in the caudal part of the fish body (61.50%), with respect to the cranial one (38.50%) (Fig. 3).

Finally, *H. trichiuri* plerocercoids showed a prevalence of 60% (CI, 0.42–0.76), and a mean abundance of 1.28 ± 1.60.

4. Discussion

The catches of the silver scabbardfish in some European countries has considerably increased in the last years, following a higher consumer's demand (EUMOFA, 2019). However, the parasite fauna of this fish species has been poorly investigated. To date, studies on the parasite infection of the silver scabbardfish from the Mediterranean Sea have documented the occurrence of zoonotic anisakid nematodes (i.e. *Anisakis* spp.) (Cammilleri et al., 2020; Levsen et al., 2018) and other parasites, such as *Kudoa thyrsites* (Gilchrist, 1924), responsible of *post-mortem* flesh liquefaction, with a detrimental effect on the fish product (Giulietti et al., 2019). Also, few records on the occurrence of the trypanorhynch tapeworms from the silver scabbardfish and/or other fish species of the Mediterranean Sea have been so far published (Abdelsalam et al., 2016; Muscolino, Giarratana, Giuffrida, & Panebianco, 2012; Tiralongo, Messina, Poidomani, Salvaggio, & Lombardo, 2020). These reports were only based on morphological analysis of merocercoids or plerocercoids detected in teleost fish species. Unfortunately, the morphological analysis of larval stages of trypanorhynch tapeworms often impedes the species identification (Palm, Walter, Schwerdtfeger, & Reimer, 1997). Indeed, the retraction of the tentacular apparatus inside the tentacular sheaths of the merocercoids makes difficult to refer the recovered specimens to the genus level; this happened also in the case of the specimens collected in the present work. Conversely, molecular systematics applied to these taxa allows the correct identification of several species, even at the larval forms, capable of infecting a wide range of intermediate and/or transport hosts (Abdelsalam et al., 2016; Olson et al., 2010; Santoro et al., 2018, Santoro, Palomba, Mattiucci, Osca, & Crocetta, 2020). Abdelsalam et al. (2016) identified, by molecular markers, plerocerci of *Callitetrarhynchus gracilis* (Rudolphi, 1819) in the little tunny (*Euthynnus alletteratus* Rafinesque, 1810), caught in the South-Eastern Mediterranean Sea. A massive infection with plerocerci of *Grillotia* (Guiart, 1927) was molecularly identified in an anglerfish by Santoro et al. (2018). In addition, the presence of *Gymnorhynchus isuri* Robinson, 1959 in *Mola mola* (Linnaeus, 1758) from the Mediterranean Sea was also genetically detected (Santoro et al., 2020).

The present study represents the first molecular identification of larval forms of *M. uncinatus* and *H. trichiuri* infecting a fish species from the Mediterranean Sea. In particular, the complete ssrDNA, in combination with partial 1srDNA, is a suitable set of molecular markers generally used in the Trypanorhyncha species identification. In fact, they resulted a reliable tool to assess the phylogenetic relationships among the species, including the metacestodes of the Families

Gymnorhynchidae and Sphyriocephalidae, found in the silver scabbardfish here examined. All the sequences obtained by specimens of *M. uncinatus* and *H. trichiuri* sequenced at ssrDNA and 1srDNA, clustered in two distinct and well-supported phylogenetic lineages, clearly indicating their belonging to two biological species. Interestingly, a low level of intraspecific genetic variation was observed in the two parasite species at both gene loci studied. Indeed, only in the species *H. trichiuri*, a different single nucleotide position at lsrDNA was found in our specimens with respect to the sequence of the same species obtained from Indonesia waters and sequenced by Olson et al. (2010). This seems to confirm previous findings about the low level of genetic variation and differentiation at the intraspecific level found in some trypanorhynch taxa, in spite of their interoceanic distribution (Palm et al., 2009). However, future genetic/molecular analysis of specimens of *H. trichiuri* collected in fish from other geographical areas will be useful to investigate the population genetic diversity and structure of this cosmopolitan parasite.

Data on the occurrence of these two larval species in silver scabbardfish from the Central Mediterranean Sea are also presented with a *focus* on the site of infection in the fish host. While Tiralongo et al. (2020) assigned plerocercoids found in the silver scabbardfish from the Mediterranean to the species *H. trichiuri*, the occurrence of *M. uncinatus* in this fish from the Mediterranean Sea represents a new host record for the parasite species, and it expands its known range of distribution. To date, larval stages of *M. uncinatus* have been previously recorded only in the musculature of snoek, *Thyrsites atun* (Euphrasen, 1791) from Cape Campbell, Cook Strait (Robinson, 1958), New Zealand waters (Palm, 2004) and from off the South Africa coast (Nunkoo, Reed, & Kerwath, 2016); in *M. mola* from Indonesia waters (Palm, 2004), and in *Xiphias gladius* from Sri Lanka (De Silva, Fernando, Ranatunga, & De Silva, 2017). Whereas plerocercoids of *H. trichiuri* were found in several fish species in all the world's oceans (Palm & Caira, 2008).

The sites of infection observed in silver scabbardfish, for the two detected parasite species, is in accordance with previous evidence (Oliva, 2001; Palm, 2004; Robinson, 1958; Tiralongo et al., 2020). The *H. trichiuri* plerocercoids were found attached to the surface of the coelomic organs/mesenteries, or free in the body cavity, as previously reported (Oliva, 2001; Palm, 2004; Tiralongo et al., 2020). While Mladineo (2006) detected the presence of the plerocercoids of *H. trichiuri* in the stomach mucosa of the bluefin tuna (*Thunnus thynnus* Linnaeus, 1758), no *H. trichiuri* plerocercoids were found in the gastric cavity of the silver scabbardfish examined in the present study. The infection with merocercoids of *M. uncinatus* occurred exclusively in the musculature of the fish host, showing the highest occurrence in the abdominal muscles (Fig. 3). These findings are consistent with those previously reported in another fish host species, i.e. the snoek (Robinson, 1958)).

The host specificity of fish parasites with respect to their definitive and intermediate/paratenic hosts might have implications for fish-food safety and quality aspects, in terms of their prevalence and abundance of infection in fish of commercial value. In this regard, *M. uncinatus* was considered as oioxenous parasite at its adult stage, being recorded exclusively in the digestive tract of the common thresher (*Alopias vulpinus*), while it is euryxenous at its larval stage, being so far found in the musculature of six fish species Palm & Caira, 2008). Whereas, *H. trichiuri* has been considered as euryxenous parasite, being found at adult stage in five species of pelagic sharks, and at plerocercoid stage in 86 species of intermediate/paratenic host species (Palm & Caira, 2008). Thus, considering the localization of *M. uncinatus,* with merocercoids infecting mostly the flesh of the fish host, and the wide range of hosts potentially infected with *H. trichiuri,* the two trypanorhynch species have direct implications on the fish-food quality aspects in fisheries.

Generally, trypanorhynch metacestodes are not considered as zoonotic parasites. The finding of a specimen of *H. trichiuri* larva eliminated with human faecal samples in South Africa (Heinz, 1954) and Mozambique (Fripp & Mason, 1983) was reported as the consequence of accidental ingestion, most likely a transeunt parasite. Indeed, in those cases, the presence of the parasite seemed to have not provoked any symptom or pathological consequence after human's exposure. A specimen of *Nybelinia surmenicola* was found adhered to the human soft palate from Japan (Kikuchi, Takenouchi, Kamiya, & Ozaki, 1981), and Peru (reported by Felizardo, Torres, Pinto, Gomes, & Knoff, 2010).

However, the rules of required freezing of fish (−20 °C, at least for 24h) reported in the Reg. CE 1276/2011, set to avoid risk of infection if consuming raw or undercooked fish, guarantees the killing of larvae of any metazoan parasite species. This process could nonetheless retain antigenic properties of the larval products (Pelayo et al., 2009). In this respect, it has been suggested that the Trypanorhyncha larvae, i.e. *Gymnorhynchus gigas* (Cuvier, 1817) Rudolphi, 1819 and *M. horridus*, invading the fish host musculature, could release proteins, which have been suggested to be responsible for allergic reactions in humans (Gòmez-Morales et al., 2008; Pelayo et al., 2009; Vázquez-López et al., 2001). Despite those proteins were not so far identified in the target metacestodes, however it is generally known that larval helminth secreted proteins, involved in the invasion of the host tissues, could be responsible for IgE-hypersensitization in the immune response of accidental human host. For instance, this aspect has been observed in other larval parasites infecting fish muscles, i.e. the zoonotic nematodes *Anisakis simplex* (s. s.) and *A. pegreffii* (Mattiucci et al., 2017Palomba et al., 2019, 2020).

Even if harmless to humans, a heavy parasitic load with larval stages of Trypanorhyncha, particularly in the edible part of the silver scabbardfish (as reported for merocercoids of *M. uncinatus* – Fig. 1), could compromise the appearance of the fish-food, making it repugnant and unattractive for the final consumer. The larval forms of parasites as *M. uncinatus* are intimately located *intra-vitam* in the fish musculature (Fig. 1). In addition, the infected flesh, surrounding each single larval stage of *M. uncinatus* - as observed in the present study (Fig. 1) - appeared spoiled and softened, probably due to the presence of the merocercoids and the proteolytic property of the proteins they likely release during the host's invasion.

As a consequence, according to Reg. CE 178/2002 and, in detail, the Reg. CE 853/2004, the heavily parasitized fish are considered unmarketable and must be discarded in the processing factories or in-service inspections, because the larval forms of parasites as *M. uncinatus* cannot be easily removed (Fig. 1). Indeed, a consistent parasitic burden in fish, can still adversely affect the quality and value of the seafood products, retaining a great potential to cause monetary losses to the local fisheries. This phenomenon has been recently documented, for instance, with the infection of adult nematodes of the raphidascarid *Hysterothylacium aduncum* hitting the Norwegian-Spanish cod industry (Bao, Cipriani, Giulietti, Drivenes, & Levsen, 2020).

Concerning the *H. trichiuri* plerocercoids, its negative impact on the fish quality can be less relevant, because it has been found attached to the surface of the internal organs/mesenteries, or free in the abdominal cavity of the fish host. Its presence is not directly related to edible part of the fish host; in fact, all parasites located in the body cavity and among visceral organs would be removed by eviscerating the fish before their selling, as reported in the Reg. CE 853/04.

In the present study, detailed estimates of the parasitic infection levels, even by specific site of infection in the fish host, were produced. Unfortunately, scanty reports with detailed infections' values of these parasites are available. Mladineo (2006) reported a prevalence value of 28.4 and mean abundance of 3.12 with larval stages of *H. trichiuri* in the bluefin tuna from the Adriatic Sea. The infection rate with *G. gigas* reported in the Mediterranean silver scabbardfish reached a higher value (P = 72.3) (Giarratana et al., 2014), similar to the one here observed for the load by *M. uncinatus*.

Despite the fish quality and the possible safety implications, these parasites, and their infection parameters, could be used as ecological indicators of the marine food-webs. Indeed, the completion of such complex life cycle as that of the cestode species here detected, requires the presence of several hosts at different levels of a marine food-web.

The life cycle of both parasites includes sharks as definitive hosts, which are apex predators of marine food-chains. The scabbardfish represents an intermediate/transport host, which accumulates metacestodes in its muscle and visceral body cavity during its lifespan by a trophic web pathway. The scabbardfish, in turn, is a suitable shark's prey, thus likely permitting the completion of the life cycles of the Trypanorhyncha species and contributing to the maintaining of their high parasitic density in the Central Mediterranean Sea waters. Indeed, even if the demographic data of the elasmobranch populations are scanty in the Mediterranean Sea, the Strait of Sicily close to the Malta coast has been described as an area hosting viable and large populations of sharks, probably due to the occurrence of large off-shore banks, which offer refuge and protection from the fishing activity (Di Lorenzo et al., 2020; Di Lorenzo, Sinerchia, & Colloca, 2017). Thus, this sea basin seems to represent a *hot spot* of biodiversity for a great number of shark species, some of which have indeed become rare or, at least, no longer present in other sectors of the Mediterranean Sea (De Maddalena & Heim, 2012; Saıdi, Bradai, Bouain, Guelorget & Capapé, 2005). The highest demography of the shark species so far observed in this marine basin with respect to the other Mediterranean area, could be also related to the ecotonal nature of the Strait of Sicily, considered a *crossroad* for the westward expansion of warm-temperate and tropical species from the Levantin basin (Di Lorenzo et al., 2017). Thus, the high density of larval stages of Trypanorhyncha here observed in the scabbardfish, a suitable shark's prey, seems to indicate the presence of a stable food-web pathway in this basin area, involving those hosts which, in turn, favours the completion of the life-cycle of these heteroxenous parasites. This seems to indicate that these tapeworm parasites could be used as an ecological indicator of the integrity of the food web in this marine ecosystem of the Mediterranean Sea. Similarly, other heteroxenous marine parasites, such as anisakid nematodes, having an indirect life-cycle involving marine organisms at different trophic level and depending by the demography of the definitive and intermediate/paratenic hosts, have been suggested to be used as biological indicators of stability of marine food-webs (Mattiucci et al., 2015; Mattiucci & Nascetti, 2008).

As a concluding remark, it would be underlined that when monitoring the infection dynamics of heteroxenous parasites affecting the fish quality and safety of fisheries, the marine ecosystem and its trophic-webs, where the life-cycle of those parasites takes place should be also taken into account, as a "driver" of the epidemiology in a specific fishing ground (Hudson, Dobson, & Lafferty, 2006; Mattiucci, Cipriani, Levsen, Paoletti, & Nascetti, 2018; Poulin, 2006). This wider ecological approach would be of help to the Food Business Operators (FBOs) to evaluate the quality and safety aspect of a fish product for human consumption from a "One-Health" perspective (Mattiucci et al., 2018).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Marialetizia Palomba: Conceptualization, Methodology, Writing - original draft, preparation, Writing - review & editing, have read and agreed to the published version of the manuscript. **Mario Santoro:** Conceptualization, Writing - review & editing, have read and agreed to the published version of the manuscript. **Renato Aco Alburqueque:** Conceptualization, Writing - review & editing, have, to the published version of the manuscript. **Paolo Cipriani:** Conceptualization, Writing - review & editing, have read and agreed to the published version of the manuscript. **Simonetta Mattiucci:** Conceptualization, Methodology, Writing - original draft, preparation, Writing - review & editing, Supervision, have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.
Please check the following as appropriate:

- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript
- The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

References

Abdelsalam, M., Abdel-Gaber, R., Mahmoud, M. A., Mahdy, O. A., Khafaga, N. I., & Warda, M. (2016). Morphological, molecular and pathological appraisal of *Callitetrarhynchus gracilis* plerocerci (Lacistorhynchidae) infecting Atlantic little tunny (*Euthynnus alletteratus*) in Southeastern Mediterranean. *Journal of Advanced Research, 7*(2), 317–326. https://doi.org/10.1016/j.jare.2015.07.004

Alvito, A., Bellodi, A., Cau, A., Moccia, D., Mulas, A., Palmas, F., et al. (2018). Amount and distribution of benthic marine litter along Sardinian fishing grounds (CW Mediterranean Sea). *Waste Management, 75*, 131–140. https://doi.org/10.1016/j.wasman.2018.02.026

Bao, M., Cipriani, P., Giulietti, L., Drivenes, N., & Levsen, A. (2020). Quality issues related to the presence of the fish parasitic nematode *Hysterothylacium aduncum* in export shipments of fresh Northeast Artic cod (*Gadus morhua*). *Food Control*. https://doi.org/10.1016/j.foodcont.2020.107724 (in press).

Bates, R. M. (1990). *A checklist of the Trypanorhyncha (Platyhelminthes: Cestoda) of the world (1935-1985). National museum of wales: Zoological series*.

Beveridge, I., & Campbell, R. A. (2013). A new species of *Grillotia* Guiart, 1927 (Cestoda: Trypanorhyncha) with redescriptions of congeners and new synonyms. *Systematic Parasitology, 85*(2), 99–116. https://doi.org/10.1007/s11230-013-9416-9

Bottari, T., Savoca, S., Mancuso, M., Capillo, G., Panarello, G., Bonsignore, M., et al. (2019). Plastics occurrence in the gastrointestinal tract of Zeus faber and Lepidopus caudatus from Tyrrhenian Sea. *Marine Pollution Bulletin, 146*, 408–416.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology, 83*(4), 575–583.

Cammilleri, G., Pulvirenti, A., Costa, A., Graci, S., Collura, R., Buscemi, M. D., et al. (2020). Seasonal trend of anisakidae infestation in South mediterranean bluefish. *Natural Product Research, 34*(1), 158–161. https://doi.org/10.1080/14786419.2019.1573232

Campbell, R. A., & Beveridge, I. (1994). Order Trypanorhyncha diesing, 1863. In L. F. Khalil, A. Jones, & R. A. Bray (Eds.), *Keys to the cestode parasites of vertebrates* (pp. 51–148). Wallingford: Commonwealth Agricultural Bureaux International.

De Maddalena, A., & Heim, W. (2012). *Mediterranean great white sharks. A comprehensive study including all recorded sightings*. North Carolina: McFarland & Company, Inc., Publishers.

De Silva, D. P. N., Fernando, H. S. D., Ranatunga, R. R. M. K. P., & De Silva, B. G. D. N. K. (2017). First record of plerocercoid larvae belong to the order Trypanorhyncha (Diesing 1863) isolated from swordfish (*Xiphias gladius*, Linnaeus 1758) captured off Sri Lanka. *Sri Lanka Journal of Aquatic Sciences, 22*, 67–70. https://doi.org/10.4038/sljas.v22i1.7518

Deardorff, T. L., Raybourne, R. B., & Mattis, T. E. (1984). Infections with trypanorhynch plerocerci (Cestoda) in Hawaiian fishes of commercial importance. *Sea Grant Quart, 6*, 1–6.

Demestre, M., Moli, B., Recases, L., & Sanches, M. (1993). Life history and fishery of *Lepidopus caudatus* (pisces: Trichiuridae) in the Catalan sea (northwestern mediterranean). *Marine Biology, 115*. https://doi.org/10.1007/BF00349382, 23–3.

Di Lorenzo, M., Sinerchia, M., & Colloca, F. (2017). the North sector of the Strait of sicily: A priority area for conservation in the Mediterranean Sea. *Hydrobiologia, 821*, 235–253. https://doi.org/10.1007/s10750-017-3389-7

Di Lorenzo, M., Vizzini, S., Signa, G., Andolina, C., Boscolo Palo, G., Gristina, M., et al. (2020). Ontogenetic trophic segregation between two threatened smooth-hound sharks in the Central Mediterranean Sea. *Scientific Reports, 10*(1), 11011. https://doi.org/10.1038/s41598-020-67858-x

Dias, F. J. E., São Clemente, S. C., Pinto, R. M., & Knoff, M. (2011). Anisakidae nematodes and Trypanorhyncha cestodes of hygienic importance infecting the king mackerel Scomberomorus cavalla (Osteichthyes: Scombridae) in Brazil. *Veterinary Parasitology, 175*(3–4), 351–355. https://doi.org/10.1016/j.vetpar.2010.10.014

D'Onghia, G., Mastrotaro, F., & Maiorano, P. (2000). Biology of silver scabbard fish, *Lepidopus caudatus* (trichiuridae), from the ionian sea (Eastern-Central mediterranean). *Cybium, 24*, 249–262.

EUMOFA. (2019). European market observatory for fisheries and aquaculture products. Retrieved from https://www.eumofa.eu/ Accessed November 3, 2020.
Felizardo, N. N., Torres, E. J. L., Pinto, R. M., Gomes, D. C., & Knoff, M. (2010). Cestodes of the flounder *Paralichthys isosceles* Jordan, 1890 (osteichthyes – paralichthyidae) from the state of rio de Janeiro, Brazil. *Neotropical Helminthology, 4*(2), 113–125.
Fisher, W., Bauchot, M. L., & Schneider, M. (1987). *Fiches FAO d'identification des espèces pour les besoins de la peche. Méditerranèe et mer Noire. Zone de peche 37*. Rome: FAO.
da Fonseca, M. C. G., de São Clemente, S. C., Felizardo, N. N., Gomes, D. C., & Knoff, M. (2012). Trypanorhyncha cestodes of hygienic-sanitary importance infecting flounders *Paralichthys patagonicus* Jordan, 1889 and *Xystreurys rasile* (Jordan, 1891) of the Neotropical region, Brazil. *Parasitology Research, 111*(2), 865–874. https://doi.org/10.1007/s00436-012-2912-z
Fripp, P. J., & Mason, P. R. (1983). Spurious human infection with a Trypanorhynchiid tapeworm. *South African Journal of Science, 79*, 473.
Giarratana, F., Muscolino, D., Beninati, C., Ziino, G., Giuffrida, A., Trapani, M., et al. (2014). *Gymnorhynchus gigas* in *Lepidopus caudatus* (actinopterygii: Perciformes: Trichiuridae): Prevalence and related effects on fish quality. *Czech Journal of Food Sciences, 32*, 320–325. https://doi.org/10.17221/330/2013-CJFS
Giulietti, L., Mattiucci, S., Paoletti, M., Grevskott, D., Bao, M., Cipriani, P., et al. (2019). Morphological and molecular identification of a new *Kudoa thyrsites* isolate in Mediterranean silver scabbardfish *Lepidopus caudatus*. *Diseases of Aquatic Organisms, 132*(2), 125–134. https://doi.org/10.3354/dao03316
Gòmez-Morales, M. A., Ludovisi, A., Giuffra, E., Manfredi, M. T., Piccolo, G., & Pozio, E. (2008). Allergenic activity of *Molicola horridus* (Cestoda, Trypanorhyncha), a cosmopolitan fish parasite, in a mouse model. *Veterinary Parasitology, 157*(3–4), 314–320. https://doi.org/10.1016/j.vetpar.2008.07.010
Guardone, L., Giusti, A., Bilska-Zajac, E., Malandra, R., Rózycki, M., & Armani, A. (2020). Molecular characterization of *clistobothrium* sp. viable plerocercoids in fresh longfin inshore squid (*Doryteuthis pealeii*) and implications for cephalopod inspection. *Pathogens, 9*(7), 596. https://doi.org/10.3390/pathogens9070596
Heinz, H. J. (1954). A case of tetrarhynchid (cestode) infection in man. *Revista Ecuatoriana de Entomologia y Parasitologia, 2*, 227–230.
Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution, 21*(7), 381–385. https://doi.org/10.1016/j.tree.2006.04.007
Iwamoto, T. (2015). *Lepidopus caudatus*. In *IUCN red list of threatened species*. https://doi.org/10.2305/IUCN.UK.2015-4.RLTS.T198721A42691759.en
Kikuchi, Y., Takenouchi, T., Kamiya, M., & Ozaki, H. (1981). Trypanorhynchiid cestodes larva found on the human palatine tonsil. *Japanese Journal of Parasitology, 30*, 497–499.
Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., et al. (2007). Clustal W and clustal X version 2.0. *Bioinformatics, 23*(21), 2947–2948. https://doi.org/10.1093/bioinformatics/btm404
Levsen, A., Cipriani, P., Mattiucci, S., Gay, M., Hastie, L., Mackenzie, K., et al. (2018). *Anisakis* species composition and infection characteristics in Atlantic mackerel, *Scomber scombrus*, from major European fishing grounds – reflecting changing fish host distribution and migration pattern. *Fisheries Research*, 112–121. https://doi.org/10.2016/j.fishres.2017.07.030
Levsen, A., & Karl, H. (2014). *Anisakis simplex* (sl) in grey gurnard (*Eutrigla gurnardus*) from the North sea: Food safety considerations in relation to fishing ground and distribution in the flesh. *Food Control, 36*, 15–19. https://doi.org/10.1016/j.foodcont.2013.07.006
Lima dos Santos, C. A. M., & Howgate, P. (2011). Fish borne zoonotic parasites and aquaculture: A review. *Aquaculture, 318*, 253–261. https://doi.org/10.1016/j.aquaculture.2011.05.046
Littlewood, D. T. J., & Olson, P. D. (2001). Small subunit rDNA and the Platyhelminthes: Signal, noise, conflict and compromise. In D. T. J. Littlewood, & R. A. Bray (Eds.), *Interrelationships of the Platyhelminthes"* (pp. 262–278). London: Taylor & Francis.
Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., & Nascetti, G. (2018). Molecular epidemiology of *Anisakis* and anisakiasis: An ecological and evolutionary road map. *Advances in Parasitology*, 93–263. https://doi.org/10.1016/bs.apar.2017.12.001
Mattiucci, S., Cipriani, P., Paoletti, M., Nardi, V., Santoro, M., Bellisario, B., et al. (2015). Temporal stability of parasite distribution and genetic variability values of *Contracaecum osculatum* sp. D and *C. osculatum* sp. E (Nematoda: Anisakidae) from fish of the Ross Sea (Antarctica). *Parasites and Wildlife, 4*, 356–367. https://doi.org/10.1016/j.ijppaw.2015.10.004
Mattiucci, S., & Nascetti, G. (2008). Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Advances in Parasitology, 66*, 47–148. https://doi.org/10.1016/S0065-308X(08)00202-9
Mattiucci, S., Colantoni, A., Crisafi, B., Mori-Ubaldini, F, Fazii, P., Nascetti, G., & Bruschi, F (2017). IgE sensitization to *Anisakis pegreffii* in Italy: Comparison of two methods for the diagnosis of allergic anisakiasis. *Parasite Immunology, 39*(7), e12440. https://doi.org/10.1111/pim.12440
Mladineo, I. (2006). *Hepatoxylon trichiuri* (Cestoda: Trypanorhyncha) plerocercoids in cage-reared northern bluefin tuna, *Thunnus thynnus* (Osteichthyes: Scombridae). *International Journal of Marine Science, 47*, 1.
Muscolino, D., Giarratana, F., Giuffrida, A., & Panebianco, A. (2012). Inspective investigation on swordfish (*Xiphias gladius*) frozen slices of commerce: Anatomical-histopatological findings. *Czech Journal of Food Sciences, 3*, 206–210. https://doi.org/10.17221/151/2011-CJFS
Nakamura, I., & Parin, N. V. (1993). FAO Species Catalogue. Snake mackerels and cutlassfishes of the world (families Gempylidae and Trichiuridae). An annotated and illustrated catalogue of the snake mackerels, snoeks, escolars, gemfishes, sackfishes, domine, oilfish, cutlassfishes, scabbardfishes, hairtails, and frostfishes known to date. *FAO Fisheries Synopsis, 15*, 136.
Nunkoo, M. A. I., Reed, C. C., & Kerwath, S. E. (2016). Community ecology of the metazoan parasites of snoek *Thyrsites atun* (Euphrasen, 1791) (Perciformes: Gempylidae) off South Africa. *African Journal of Marine Science, 38*, 363–371. https://doi.org/10.2989/1814232X.2016.1216892
Oliva, M. (2001). Metazoan parasites of *Macruronus magellanicus* from southern Chile as biological tags. *Journal of Fish Biology, 58*, 1617–1622.
Oliveira, J. V. F. D., Kuraiem, B. P., Fonseca, M. C. G. D., Gomes, D. C., & Knoff, M. (2019). Trypanorhynch cestodes parasitizing *Mugil liza* (Mugiliformes: Mugilidae) commercialized in the state of Rio de Janeiro, Brazil. *Revista Brasileira de Parasitologia Veterinaria, 28*(4), 773–778. https://doi.org/10.1590/S1984-29612019039
Olson, P. D., Caira, J. N., Jensen, K., Overstreet, R. M., Palm, H. W., & Beveridge, I. (2010). Evolution of the trypanorhynch tapeworms: Parasite phylogeny supports independent lineages of sharks and rays. *International Journal for Parasitology, 40*(2), 223–242. https://doi.org/10.1016/j.ijpara.2009.07.012
Palm, H. W. (1997). An alternative classification of trypanorhynch cestodes considering the tentacular armature as being of limited importance. *Systematic Parasitology, 37*, 81–92. https://doi.org/10.1023/A:1005765126294
Palm, H. W. (2004). *The Trypanorhyncha diesing, 1863*. Bogor: IPB-PKSPL Press.
Palm, H. W., & Caira, J. N. (2008). Host specificity of adult versus larval cestodes of the elasmobranch tapeworm order Trypanorhyncha. *International Journal for Parasitology, 38*(3–4), 381–388. https://doi.org/10.1016/j.ijpara.2007.08.011
Palm, H. W., Waeschenbach, A., Olson, P. D., & Littlewood, D. T. (2009). Molecular phylogeny and evolution of the Trypanorhyncha diesing, 1863 (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution, 52*(2), 351–367. https://doi.org/10.1016/j.ympev.2009.01.019
Palm, H. W., Walter, T., Schwerdtfeger, G., & Reimer, L. W. (1997). *Nybelinia* Poche, 1926 (Cestoda: Trypanorhyncha) from the Mozambique coast, with description of *N. beveridgei* sp. nov. and systematic consideration of the genus. *African Journal of Marine Science, 18*, 273–285.
Palomba, M., Cipriani, P., Giulietti, L., Levsen, A., Nascetti, G., & Mattiucci, S. (2020). Differences in gene expression profiles of seven target proteins in third-stage larvae of *Anisakis simplex* (sensu stricto) by sites of infection in blue whiting (*Micromesistius poutassou*). *Genes, 11*(5), 559. https://doi.org/10.3390/genes11050559
Palomba, M., Paoletti, M., Colantoni, A., Rughetti, A., Nascetti, G., & Mattiucci, S. (2019). Gene expression profiles of antigenic proteins of third stage larvae of the zoonotic nematode *Anisakis pegreffii* in response to temperature conditions. *Parasite, 26*, 52. https://doi.org/10.1051/parasite/2019055
Pelayo, V., García-Hernández, P., Puente, P., Rodero, M., & Cuébllar, C. (2009). Seroprevalence of anti-*Gymnorhynchus gigas* (Trypanorhyncha, Gymnorhynchidae) antibodies in a Spanish population. *The Journal of Parasitology, 95*(3), 778–780. https://doi.org/10.1645/GE-1894.1
Posada, D., & Buckley, T. R. (2004). Model selection and model averaging in phylogenetics: Advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology, 53*(5), 793–808. https://doi.org/10.1080/10635150490522304
Poulin, R. (2006). Variation in infection parameters among populations within parasite species: Intrinsic properties versus local factors. *International Journal for Parasitology, 36*(8), 877–885. https://doi.org/10.1016/j.ijpara.2006.02.021
Reiczigel, J., Marozzi, M., Fábián, I., & Rózsa, L. (2019). Biostatistics for parasitologists - a primer to quantitative Parasitology. *Trends in Parasitology, 35*(4), 277–281. https://doi.org/10.1016/j.pt.2019.01.003
Reiczigel, J., & Rózsa, L. (2005). *Quantitative Parasitology 3.0*. Budapest. Distributed by the authors.
Robinson, E. S. (1958). Records of cestodes from marine fishes of New Zealand. *Transactions of the Royal Society of New Zealand, 86*, 143–153.
Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics, 19*(12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
Rosa, A., Menezes, G., Melo, O., & Pinho, M. R. (2006). Weightlength relationships of 33 demersal fish species from Azores archipelago. *Fisheries Research, 80*, 329–332.
Samn, A. A., Metwally, K. M., Zeina, A. F., & Khalaf Allah, H. M. (2014). First occurrence of *Nerocila bivittata*: Parasitic isopods (skin shedders) on *Lithognathus mormyrus* (osteichthyes, sparidae) from abu qir bay, alexandria, Egypt. *The Journal of American Science, 10*, 171–197.
Santoro, M., Palomba, M., Mattiucci, S., Osca, D., & Crocetta, F. (2020). New parasite records for the sunfish *Mola mola* in the Mediterranean Sea and their potential use as biological tags for long-distance host migration. *Frontiers Veterinary Science, 7*, 579728. https://doi.org/10.3389/fvets.2020.579728
Santoro, M., Uberti, B. D., Corrado, F., Cutarelli, A., Iaccarino, D., Di Nocera, F., et al. (2018). *Grillotia* (cestoda: Trypanorhyncha) plerocerci in an anglerfish (*Lophius piscatorius*) from the tyrrhenian sea. *Parasitology Research, 117*(11), 3653–3658. https://doi.org/10.1007/s00436-018-6067-4
Saıdi, B., Bradaı, M. N., Bouaın, A., Guelorget, O., & Capapé, C. (2005). Capture of a pregnant female white shark, *Carcharodon carcharias* (Lamnidae) in the Gulf of Gabes (southern Tunisia, Central Mediterranean) with comments on oophagy in sharks. *Cybium, 29*, 303–307.
Tiralongo, F., Messina, G., Poidomani, S., Salvaggio, A., & Lombardo, B. M. (2020). Morphological analysis reveals the presence of *Hepatoxylon trichiuri* (holten, 1802) Bosc, 1811 (cestoda: Sphyriocephalidae) in *Lepidopus caudatus* (euphrasen, 1788) (pisces: Trichiuridae) from the Mediterranean Sea. *Microscopy Research and Technique, 83*(8), 949–952. https://doi.org/10.1002/jemt.2348917(11), 3653–3658.
Torre, M., Sicuro, B., & Kallianiotis, A. (2019). Diet of silver scabbardfish *Lepidopus caudatus* (euphrasen, 1788) in the northern aegean sea. *Cahiers de Biologie Marine, 60*, 31–40. https://doi.org/10.21411/CBM.A.4C45C5BD

Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics, 27*, 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Van der Auwera, G., Chapelle, S., & De Wachter, R. (1994). Structure of the large ribosomal subunit RNA of *Phytophthora megasperma*, and phylogeny of the oomycetes. *FEBS Letters, 338*(2), 133–136. https://doi.org/10.1016/0014-5793(94)80350-1

Vázquez-López, C., de Armas-Serra, C., Bernardina, W., & Rodríguez-Caabeiro, F. (2001). Oral inoculation with *Gymnorhynchus gigas* induces anti-parasite anapyhylactic antibody production in both mice and rats and adverse reactions in challenge mice. *International Journal of Food Microbiology, 64*(3), 307–315. https://doi.org/10.1016/s0168-1605(00)00477-3