Journal of
Fish Diseases

The hidden companion of non-native fishes in Northeast Atlantic waters

Journal:	*Journal of Fish Diseases*
Manuscript ID	Draft
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
Complete List of Authors:	Rodríguez, Helena; Instituto de Investigaciones Marinas de Vigo, ECOBIOMAR Bañón, Rafael; Xunta de Galicia Conselleria del Mar, Dirección Xeral de Desenvolvemento Pesqueiro; Instituto de Investigaciones Marinas de Vigo Ramilo, Andrea; Instituto de Investigaciones Marinas de Vigo, ECOBIOMAR
Keywords:	tropicalization, non-native fish, Hysterothylacium spp., Anisakis pegreffii, Cucullanus dodsworthi

The hidden companion of non-native fishes in Northeast Atlantic waters

Helena Rodríguez[1], Rafael Bañón[1,2], Andrea Ramilo[1]

[1]*Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, IIM-CSIC, Vigo, Pontevedra, Spain*
[2]*Servizo de Planificación, Dirección Xeral de Desenvolvemento Pesqueiro, Consellería do Mar,Xunta de Galicia, Santiago de Compostela, A Coruña, Spain*

Correspondence

Helena Rodríguez, *Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, IIM-CSIC, Vigo, Pontevedra, Spain*.
Email: *helenard@iim.csic.es*

Abstract

A tropicalisation phenomenon of the ichthyofauna has been described in the last decades in Galicia (north-eastern Atlantic), with increasing reports of tropical and subtropical fishes appearing northward of this distribution range. A search for parasites was carried out in the digestive tract of two specimens first captured in Galician waters: the Prickly puffer *Ephippion guttifer* (Tetraodontidae) and the African stripped grunt *Parapristipoma octolineatum* (Haemulidae). The parasitological examination of *E. guttifer* showed a high intensity of nematodes, belonging to three different genera: *Cucullanus* (Cucullanidae), *Hysterothylacium* (Raphidascaridae) and *Anisakis* (Anisakidae), the last one with demonstrated pathogenicity to humans. Molecular identification allowed the identification of *Anisakis pegreffii* and the first report for European waters of *Cucullanus dodsworthi*, *Hysterothylacium reliquens* and a new *Hysterothylacium* sp*. P. octolineatum* showed far lower level of parasitation, with only two *Hysterothylacium* larvae, genetically identified as *Hysterothylacium deardorffoverstreetorum*, being thus also a first report of this species for the Eastern Atlantic. The possible ecological impact of the occurrence of non-native fish species in a new area reach a different point if we consider that only two individual fish can harbor more of one hundred nematoda belonging to five different species.

Keywords: tropicalization, non-native fish, *Anisakis pegreffii*, *Hysterothylacium* spp., *Cucullanus dodsworthi*

1. INTRODUCTION

There are multiple evidences that the climate of the planet is changing, and one of the most patent consequences is the increasing of the average surface temperature in the seas.

Galicia is an autonomous region of Spain located in the north-western corner of the Iberian Peninsula (41°–43°N), in the northern boundary of the Iberian upwelling system. In this upwelling system, an increase in the SST of 0.68ºC between 1982 and 2006 was observed, but the prediction for the period 1960/1990–2070/2100 is between 1.4 and 2.4ºC (Philippart et al., 2011). Studies from Galicia region also show similar results: a rise of 0.24°C per decade has been observed in the Galician sea waters since 1974 (Gómez-Gesteira et al., 2011).

Oceanic changes in temperature due to global climate change are causing poleward shifts in the latitudinal abundance and distribution ranges of fish species, which may cause dramatic changes in assemblages and trophic webs and have been shown to affect ecosystems and fisheries (Horta e Costa et al., 2014). As consequence, a tropicalization of coastal fish communities has indeed been occurring in the NE Atlantic, including the Macaronesian archipelagos, the Mediterranean Sea, and the European continental shelves, from the Iberian Peninsula up to the North Sea (Afonso et al., 2013).

In Galician waters, this tropicalization phenomenon is also very apparent. In a revision of the marine fish fauna, a total of 17 African fish species have been found in Galicia, most of them recorded for the first time during the last decades (Bañón, Villegas-Ríos, Serrano, Mucientes, & Arronte, 2010) and this migration persists to the present day. Among them, there are several species whose finding represents the current northern distribution boundary in the Eastern Atlantic, such as *Seriola fasciata* (Bloch, 1793) (Bañón & Mucientes, 2009), *Fistularia petimba* (Lacepède, 1803) (Bañón & Sande, 2008) or *Lagocephalus laevigatus* (Linnaeus, 1766) (Bañón & Santás, 2011).

The Prickly puffer *Ephippion guttifer* (Bennett, 1831) is a western African tetraodontid species, ranging from Morocco to Angola, with sporadic intrusions in the western Mediterranean (Bañón, Alonso-Fernández, Barros-García, Rios, & De Carlos, 2018). It is a demersal fish species inhabiting shallow coastal and estuarine environments to depths of approximately 50 m. The African striped grunt *Parapristipoma octolineatum* (Valenciennes, 1833) is a haemulid demersal species that is found over sandy and rocky areas from two to 180 m depth, ranging from Portugal to Angola and the western Mediterranean (Carpenter & Johnson, 2016).

In spite of the continuous new arrivals of southern fishes, there is a lack of information on those species that could be introduced hidden and simultaneously: their parasites. In Galicia, and for extension in the Atlantic European, most studies of parasites are focused on commercially exploited shellfish, mainly bivalves, due to their economic importance and the mortalities they cause (Villalba et al., 2014). Studies of parasites in fish are less numerous and focused in fishery species (Sanmartin-Duran, Quinteiro, & Ubeira, 1989), including *Anisakis* infestation (Abollo, Gestal, & Pascual, 2001; Rodríguez, Abollo, González, & Pascual, 2018), but also in farmed fishes (Iglesias et al., 2001). The public concern about fish parasites increases when considering those which affect commercial species, devaluating their market price, or those which are responsible for seafood-associated infections.

This paper aims to describe the parasite fauna in non-native fishes detected in Galician waters, emphasizing the increased impact of the occurrence of some southern fish out of their habitual distribution range when we put the focus on their hidden companion.

2. MATERIAL AND METHODS

2.1. Host species

Two tropical fish species were caught alive for the first time in Galician waters, NW Spain. A male specimen of *E. guttifer* of 570 mm TL was caught on 10 January 2017 with trammel nets in the mouth of the Ría de Vigo, at 40° 42' 46.021" N, 8° 51' 24.998" W and at a depth of approximately five metres (Bañón et al., 2018). The second specimen was a female of *P. octolineatum* of 293 mm TL, caught by a spear fisherman in Punta Faxilda, in the mouth of Ría de Pontevedra, on 21 July 2018, at 42º24.907N, 8º53.208W and 7 m depth. Both specimens were deposited in the Museo de Historia Natural da Universidade de Santiago de Compostela (MHNUSC, Santiago de Compostela, Spain) with the collection number MHNUSC25103 for *E. guttifer* and MHNUSC 25124 for *P. octolineatum*.

2.2. Parasite detection and morphological characterization

Guts were dissected and examined under a dissecting microscope, and all the detected parasites were recovered and morphologically classified. Then, viscera were processed by peptic digestion for specific recovery of any remaining nematodes, following Llarena-Reino et al. (2013). Again, different morphological types of nematodes were collected separately. Data and samples were collected and coded in a BioBank platform, a certified service (ISO9001) that hosts a collection of biological samples and organized as a technical unit with defined quality criteria, order and destination, to ensure full traceability of samples and data.

A scanning electron microscope (SEM) study of an adult female of *Hysterothylacium* was also carried out. Adult female nematode identified as *Hysterothylacium* sp. isolated from the digestive tract, were washed in PBS. Ova and the central part of the body were used for molecular identification. The anterior and posterior ends of one of the worms were processed at the CACTI Electron Microscopy Service (University of Vigo) for SEM. The selected specimens were fixed in 1% glutaraldehyde in 0.01M PBS, pH 7.4, postfixed in OsO4 1% in cacodylate buffer 0.01M pH 7.4, dehydrated using an acetone series and then critical point dried. The specimens were coated with gold and examined using a FEI Quanta 200 scanning electron microscope in rough vacuum conditions at an accelerating voltage of 12.5 kV.

2.3.

Molecular identification of nematode parasites

Twenty four samples of those nematodes (23 from *E. guttifer* and one from *P. octolineatum*), including all different morphological types were then selected and processed for molecular identification. Genomic DNA purification of the nematode parasites was performed employing NucleoSpin Tissue Kit (Macherey-Nagel, Easton, PA), according to the manufacturers protocol for isolating genomic DNA from human or animal tissue and cultured cells. DNA quality and quantity was checked in a spectrophotometer Nanodrop® ND-2000 (Thermo Scientific). To identify the nematodes species, ITS rDNA region was amplified using the primers NC5/NC2 described by Zhu et al. (2000), as well as a SSU rDNA fragment using the primer 18SU467F/18SL1310R (Suzuki, Hoshino, Murakami, Takeyama & Cho, 2008) in one case. Reactions were performed in a total volume of 25 ml containing 1 µl of genomic DNA (10 ng), PCR buffer at 1x concentration, 0.3 µM primers, 0.2 mM nucleotides and 0.025 U. µl $^{-1}$ KAPA Taq DNA polymerase (KAPABIOSYSTEMS). The PCR products were separated on a 2% agarose gel in Tris acetate EDTA buffer, stained with Red Safe and scanned in a GelDoc XR documentation system (Bio-Rad Laboratories). PCR products were cleaned for sequencing using ExoProStar™ 1 Step (GE Healthcare, NJ, USA) for 15 min at 37 ◦C, followed by inactivation for 15 min at 80 ºC. Sequencing was performed in a specialised service (StabVida, Portugal) and the chromatograms were analysed using ChromasPro v.1.41 Technelysium Pty Ltd. All generated sequences were searched for identity using BLAST (Basic Local Alignment Search Tool) through web servers of the National Center for Biotechnology Information (USA). Phylogenetic analysis was performed with ITS sequences obtained in this study and with sequences of the genus *Hysterothylacium* and *Anisakis* availables in GenBank (Table 1). *Ascaridia columbae* (KF147909) was used as outgroup. Aligments was performed using Clustal W (Thompson, Higgins, & Gibson, 1994) included in MEGA 7 (Kumar, Stecher, & Tamura, 2016). Maximum-likelihood analysis (ML) implemented in MEGA 7 software was used to generate phylogenetic tree. Kimura 2-parameter model was found the most appropriate evolutionary model for the analysis. The ML tree was assessed by bootstrap analysis with 500 replicates.

3. RESULTS

3.1. Morphological features

Examination under a dissecting microscope and after artificial peptic digestion for parasitological analysis showed the presence of 116 nematoda in the visceral tract of *E. guttifer*. Two of them were female adults belonging to the genus *Hysterothylacium*, 111 were *Hysterothylacium* larvae, 1 was a *Cucullanus* sp. and 2 were L3 larvae of the genus

Anisakis. Different morphological features leading to this generic identification could be seen in Figure 1. Figure 1a shows the anterior extremity of a *Hysterothylacium* adult female with 3 lips, approximately equal in size, and with prominent lateral flanges. Figure 1b shows the tail tip, with numbers of small nodular protuberances. Figure 1c provides an image of the general aspect of a specimen of one of the *Hysterothylacium* larvae. Figure 1 also shows the characteristics boring tootht (bt, Figure 1d) and tail mucrom (m, Figure 1e) of a L3 larvae of the genus *Anisakis* and Figures 1f and 1g showed the characteristic muscular esophagus, expanded in width at both ends (Figure 1f) and the cephalic end (Figure 1g) of a *Cucullanus* nematode.

In *P. octolineatum,* only two nematode larvae were detected, and morphologically identified as belonging to the genus *Hysterothylacium* (Figures 1h, 1i and 1j), with some very distinctive features, such as tail mucron presence (m, Figures 1h and 1i).

3.2. Molecular identification

Blast search of ITS1-5.8S-ITS2 sequences obtained of the nematode parasites from *E. guttifer* showed the following results: 3 sequences (from ova and adults of *Hysterothylacium* sp.) were 99-100% similar to *Hysterothylacium reliquens* sequences deposited in GenBank; 18 sequences from the larval stages of the genus *Hysterothylacium* were 91-92% similar to those of *Hysterothylacium rigidum* and one of the samples morphologically identified as *Anisakis* sp. shared 100% nucleotide identity with *Anisakis pegreffii* sequences deposited in GenBank. The *Cucullanus* sp. nematode could not be identified with NC5/NC2 primers and then 18S sequence was obtained. Its sequence was 100% similar to that of *Cucullanus dodsworthi* from a checkered puffer *Sphoeroides testudineus* from Mexico (HQ241923).

Hysterothylacium larvae sequence from *P. octolineatum* was 100% similar to a *Hysterothylacium* sp. sequence from *Zenopsis conchifer* from Brazil (KU594488) and to *Hysterothylacium deardorffoverstreetorum* sequences from *Paralichthys isosceles,* also from Brazilian coast (JF730200).

The sequences reported in this study have been deposited in GenBank under accession numbers: KY781734-KY781736 (*H. reliquens*), KY781737 (*A. pegreffii*), MK039143-

MK039160 (*Hysterothylacium* sp. from *E. guttifer*), MK039161 (*H. deardorffoverstreetorum)* and MK045808 (*C. dodsworthi*).

An ITS tree constructed with a total of 423 sites and 43 sequences showed two clearly differentiate clades (Figure 2): one of them belonging to *Anisakis* sequences with bootstrap value of 99%, which included the *A. pegreffii* sequence obtained in this study grouped with other *A. pegreffii* sequences deposited in GenBank; and the other clade including *Hysterothylacium* sequences with bootstrap value of 92%. Within this clade, *H. reliquens* sequences from *E. gutiffer* were placed in a subclade with other *H. reliquens* sequences with bootstrap value of 99% whereas the 18 *Hysterothylacium* sequences similar to *H. rigidum* (91% similarity in BLAST) formed an only subclade with strong bootstrap value of 99%. The *Hysterothylacium* sequence obtained from *P. octolineatum* was grouped in the clade of *H. deardorffoverstreetorum* sequences with bootstrap value of 100%.

4. DISCUSSION

This study provides information about the parasitological condition of two non-native fishes reported recently for the first time in Galician waters (NW Spain) in order to give an insight of the potential risk of these occurrences as a vehicle for alien parasites in a new area.

Both the two host species are tropical species caught northward of its habitual distribution range in the eastern Atlantic, which supposes a new northern limit for *E. guttifer* (Bañón et al., 2018) and the second northernmost record for *P. octolineatum*, only further south than a specimen recently observed, but not examined, in the South of Bay of Biscay (Casamajor, 2016).

Ephippion guttifer showed a high parasitization by different nematodes in the digestive tract. Molecular identification allowed first reports of *A. pegreffii*, *C. dodsworthi H. reliquens* and a new *Hysterothylacium* sp. in this species. Previously, only copepod ectoparasites (Walter, 2015a, b), trematodes (Fischthal & Thomas, 1970) and mixosporidia (Kpatcha, 1994) were reported. Regarding other tetraodontid species, the presence of Anisakidae (*Anisakis* sp.) and Raphidascaridae, *Hysterothylacium aduncum* (Rudolphi, 1802) have been described in *Lagocephalus sceleratus* (Bakopoulos, Karoubali, & Diakou, 2017).

Parapristipoma octolineatum showed a very lower level of parasitation, with only two *Hysterothylacium* larvae, genetically identified as *H. deardorffoverstreetorum*. There are also scarce parasitological studies available about the parasitic fauna of *P. octolineatus*. To our knowledge, only protozoa belonging to microsporidia (Kpatcha, Diebakate, Faye, & Toguebaye, 1995) and coccidia (Diouf, 1993) have been found, together with metazoan trematodes (Aleshkina & Gaevskaya, 1985; Diagne et al., 2015, 2016). There are no records of nematode parasites in this species, although a high diversity of parasitic nematode have been described in other haemulids, including larvae of *Contracaecum* sp., *Pseudoterranova* sp., *Rhapidascaris* sp. and *Hysterothylacium* sp. (Chero et al., 2014; Moravec & Justine, 2017; Paschoal, Cezar, & Luque, 2015).

Nematodes belonging to the families Anisakidae and Raphidascaridae are widespread in fish population worldwide (Mattiucci & Nascetii, 2008; Mattiucci et al., 2018; Nadler et al., 2005). Only *E. guttifer* presented *Anisakis* larvae, but in a low number, molecularly identified as *A. pegreffii*. In European waters, that is the most prevalent species of the genus in the Mediterranean Sea, whereas *Anisakis simplex* is the most common one in North Atlantic waters with a sympatric area between both species from the Alborán Sea (Mediterranean Gibraltar area) to the Spanish Galician coast (Mattiucci et al., 2018).

With regards to *H. reliquens,* this species has been described in 25 fish species belonging to eight different orders, from Indian, western Atlantic and eastern Atlantic, off Morocco (Zhao et al., 2017). Therefore, this represents the first record of this parasite species from European waters and a northward extension of its geographic distribution range in the eastern Atlantic, through a translocation of its host species. The two individuals were live mature females and they were ovopositing when detected, and this constitutes an increased risk for parasite dissemination in the ecosystem. Moreover, a high number of *Hysterothylacium* larvae were detected in the digestive tract of *E. guttifer* and the molecular identification did not allow to find correspondence to sequences of any described species of the genus. Specific diagnosis was not possible as all the individuals were third stage larvae (as the lips were poorly or not developed).

Another species from the genus *Hysterothylacium*, *H. deardorffoverstreetorum*, was the only parasite detected in *P. octolineatum*. This species was first described parasitizing the flounder *Paralychthys isosceles* (Jordan, 1891) from Brazilian coast (Knoff et al., 2012) and it has also been found afterwards in many other Brazilian fishes (Di Azevedo & Iñiguez, 2018; Kuraiem et al., 2017; Silva et al., 2017). Therefore, this also represents a first record for this nematode species in the eastern Atlantic, from European waters. Although our L3 larvae fits morphologically and molecularly with *H. deardorffoverstreetorum*, described by Knoff et al. (2012) as a new species, this species has recently been considered species inquirenda due to its problematic description and diagnosis which are based only on larvae (Pantoja, Pereira, Santos, & Luque, 2016).

Among anisakids, *A. simplex*, *A. pegreffii* and *Pseudoterranova decipiens* are the main species responsible for anisakidosis and gastroallergic reactions in humans (Arizono, Yamada, Tegoshi, & Yoshikawa, 2012; Mattiucci et al., 2013). The Raphidascaridae genus *Hysterothylacium* has been commonly considered not pathogenic to humans, although recent data pointed to a case of invasive gastroallergic infection caused by the third stage larvae of *H. aduncum* (González-Amores, Clavijo-Frutos, Salas-Casanova, & Alcain-Martínez, 2015). Thus, in the case of *E. guttifer*, apart from its own toxicity derived from the presence of tetrodotoxin, the presence of zoonotic parasites could be an additional risk for human health. Although it has not been possible the examination of the edible part of the fish to detect nematode parasites, the presence of zoonotic nematodes in viscera and the demonstrated migration capacity from viscera to flesh of *A. pegreffii* (Cipriani et al., 2016) might pose a threat of human infection.

In the case of Cucullanidae, nematodes of genus *Cucullanus* comprise a large number of species that parasitize a variety of fresh, brackish, and marine fishes (Lanfranchi, Timi, & Sardella, 2004; Moravec & Justine, 2017; Moravec, Levron, & de Buron, 2011; Moravec & Scholz, 2017). Up to now, *C. dodsworthi* is the only species of this genus infecting tetraodontiforms, specifically the checkered puffer *Sphoeroides testudineus* (type host) from Bahia de Guanabara, Brazil (Barreto, 1922) and from Mexican waters off the Yucatán Peninsula (Mejía-Madrid & Aguirre-Macedo, 2011) as well as parasitizing *Lagocephalus laevigatus* from the eastern Atlantic coast of Africa (Campana-Rouget, 1957). It has also been reported from *Mugil cephalus* from Biscayne Bay (Florida) (Boucher, 1974; Skinner, 1975), although it was considered an accidental host (Mejía-Madrid & Aguirre-Macedo, 2011). So, the detection of *C. dodsworthi* in *E. guttifer* widens the host range of this parasite and represents the first record in European waters.

Climate change affects parasite species directly, enhancing transmission rates and the virulence, but also through changes in the distribution and abundance of their hosts (Palm, 2011). These changes could be even enhanced if we consider that introduction of only one fish individual in a new ecosystem can imply the introduction of a far higher number and species diversity (more than one hundred individuals and four different species in *E. guttifer*) when we consider the parasite fauna. Even, in the case of the prickly puffer, two of the parasites were completely mature females of *H. reliquens*, which started laying a huge amount of ova during parasitological examination. All the parasitic species and genus described in this paper has been found as parasites of different fish species, so we cannot discard that these species could find a suitable host between the local fish species.

The effect of these changes in parasite distribution through host species translocation has a good example looking at migrations through the Suez Canal, so-called Lessepsian migrations. Thus, the cornetfish *Fistularia commersonii* has spread right across the Mediterranean Sea along with its parasite *Allolepidapedon fistulariae* (and other worms) (Pais, Merella, Follesa, & Garipa, 2007). It is not yet known if these Lessepsian migrant parasites have spread into the open Atlantic Ocean (Bray, Diaz, & Cribb, 2016). With the opening in August 2015 of a new channel parallel to the old one, the exchange of fauna between the Red and Mediterranean Seas is bound to increase (Galil et al., 2015). Also, this migration has affected species of nematoda such as *Anisakis typica*, which have reached the Mediterranean Sea as a result of the migration of its intermediate/paratenic hosts from the Indian Ocean (Mattiucci et al., 2018). Lessepsian migrant may affect native fish hosts by potentially altering the dynamics of native and invasive parasite-host interactions via parasite release, parasite co-introduction and parasite acquisition (Boussellaa, Neifar, Goedknegt, & Thielges, 2018).

According a recent terminology, the parasites which have entered in a new area outside of their native range with an alien host species are defined as co-introduced parasites (Lymbery et al., 2014). This is in agreement with the new findings of *C. dodsworthi*, *H. reliquens*, *H. deardorffoverstreetorum* and a *Hysterothylacium* sp., although it is not always straightforward to determine whether a newly discovered parasite is alien or native to a region.

The combination of the oceanographic and topographic features makes Galician waters be very productive, able to support extensive costal fisheries and shellfish harvesting (Surís-Regueiro & Santiago, 2014). On the other hand, the introduction of parasite species in a new marine environment could affect negatively to native fauna but also can seriously endanger marine production in the area, causing important mortalities and economic losses, as have been probed in shellfishes (Cigarría & Elston, 1997; Ramilo et al., 2014; Villalba et al., 2014). Although this is the first attempt to investigate parasitic fauna introduced into European Atlantic waters by exotic fish species, more attention needs to be paid to how this parasitic fauna affects both the host species and the new ecosystem into which it is introduced.

ACKNOWLEDGEMENTS

We would like to thank Javier Tamame and José Antonio Durán for providing technical assistance. Also to Inés Pazos from CACTI Electron Microscopy Service (University of Vigo) for technical assistance in SEM. This study was by the Agreement between CSIC and Xunta de Galicia to analyse fisheries-dependent data from the monitoring program of small-scale fisheries in Galicia (Agreement No. 070401150009).

REFERENCES

Abollo, E., Gestal, C., & Pascual, S. (2001). *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. *Parasitology Research*, 87, 492-499. https://doi.org/10.1007/s004360100389

Afonso, P., Porteiro, F. M., Fontes, J., Tempera, F., Morato, T., Cardigos, F., & Santos, R. S. (2013). New and rare coastal fishes in the Azores islands: occasional events or tropicalization process?. *Journal of Fish Biology*, 83, 272-294. https://doi.org/10.1111/jfb.12162

Aleshkina, L. D., & Gaevskaya, A.V. (1985). Trematodes of fish from the Atlantic coast of Africa. *Nauchnye Doklady Vyssheĭ Shkoly, Biologicheskie Nauki*, 3, 35–40.

Arizono, N., Yamada, M., Tegoshi, T., & Yoshikawa, M. (2012). *Anisakis simplex* sensu stricto and *Anisakis pegreffii*: biological characteristics and pathogenetic potential in human anisakiasis. *Foodborne pathogens and disease*, 9, 517–521. https://doi.org/10.1089/fpd.2011.1076

Bakopoulos, V., Karoubali, I., & Diakou, A. (2017). Parasites of the Lessepsian invasive fish *Lagocephalus sceleratus* (Gmelin 1789) in the eastern Mediterranean Sea. *Journal of Natural History*, 51, 421–434. https://doi.org/10.1080/00222933.2017.1279690

Bañón, R., Alonso-Fernández, A., Barros-García, D., Rios, M. B., & De Carlos, A. (2018). Geographic range expansión of *Ephippion guttifer* (Tetraodontidae) in the Eastern Atlantic. *Journal of Fish Biology*, 93, 733–737. https://doi.org/10.1111/jfb.13761

Bañón, R. & Mucientes, G. R. (2009). First record of *Seriola fasciata* (Carangidae) from Galician waters (NW Spain). A new northernmost occurrence in the NE Atlantic. *Cybium*, 33, 247-248.

Bañón, R., & Sande, C. (2008). First record of the cornetfish *Fistularia petimba* (Syngnathiformes: Fistularidae) from Galician waters. A northernmost occurrence in the eastern Atlantic. *Journal of Applied Ichthyology*, 24, 106–107. https://doi.org/10.1111/j.1439-0426.2007.00918.x

Bañón, R., & Santás, V. (2011). First record of *Lagocephalus laevigatus* (Tetraodontiformes, Tetraodontidae) from Galician waters (north-west Spain), a northernmost occurrence in the north-east Atlantic Ocean. *Journal of Fish Biology*, 78, 1574–1578. https://doi.org/10.1111/j.1095-8649.2011.02935.x

Bañón, R., Villegas-Ríos, D., Serrano, A., Mucientes, G., & Arronte, J.C. (2010). Marine fishes from Galicia (NW Spain): an updated checklist. *Zootaxa*, 2667, 1−27. http://dx.doi.org/10.5281/zenodo.276366

Barreto, A. L. (1922). Revisao da familia Cucullanidae Barreto, 1916 (1). *Memorias do Instituto Oswaldo Cruz*, 14, 68-87.

Boucher, G. C. (1974). Parasites of the Checkered puffer, *Sphoeroides testudineus*, in Biscayne Bay, Florida, with an analysis of host-parasite relationships. M.S. Thesis. The University of Miami, Coral Gables, Florida, 69 p.

Boussellaa, W., Neifar, L., Goedknegt, M. A., & Thielges, D. W. (2018). Lessepsian migration and parasitism: richness, prevalence and intensity of parasites in the invasive fish *Sphyraena chrysotaenia* compared to its native congener *Sphyraena sphyraena* in Tunisian coastal waters. *PeerJ,* 6:e5558. https://doi.org/10.7717/peerj.5558

Bray, R. A., Diaz, P. E., & Cribb, T. H. (2016). Knowledge of marine fish trematodes of Atlantic and Eastern Pacific Oceans. *Systematic Parasitology*, 93, 223-235. https://doi.org/10.1007/s11230-016-9629-9

Campana-Rouget, Y. (1957). Parasites de Poissons de mer ouest-africains récoltés par J. Cadenat. Nématodes (4ème note). Sur quelques espèces de Cucullanidae. Révision de las sous-famille*. Bulletin IFAN*, *Série A*, 19, 417-473.

Carpenter, K. E., & Johnson, G. D. (2016). Haemulidae. In Carpenter, K. E. & De Angelis, N., (eds), *The living marine resources of the Eastern Central Atlantic*. Volume 4: *Bony fishes part 2 (Perciformes to Tetradontiformes) and Sea turtles* (pp. 2551-2564). FAO Species Identification Guide for Fishery Purposes, Rome, FAO.

Casamajor, M. N. (2016). First record of *Parapristipoma octolineatum* (Haemulidae) on the French Atlantic coast. *Cybium*, 40, 263-264.

Chero, J., Cruces, C., Iannacone, J., Saez, G., & Alvariño, L. (2014). Helminth parasites of *Anisotremus scapularis* (Tschudi, 1846) (Perciformes: Haemulidae) "Peruvian grunt" acquired at the Fishing Terminal of Villa Maria del Triunfo, Lima, Peru. *Neotropical Helmintrhology*, 8, 411-428.

Cigarría, J., & Elston, R. (1997). Independent introduction of *Bonamia ostreae*, a parasite of *Ostrea edulis*, to Spain. *Diseases of Aquatic Organisms*, 29, 157-158.

Cipriani, P., Acerra, V., Bellisario, B., Sbaraglia, G. L., Cheleschi, R., Nascetti, G., Mattiucci, S. (2016). Larval migration of the zoonotic parasite *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy, *Engraulis encrasicolus*: Implications to seafood safety. *Food Control*, 59, 148–157. https://doi.org/10.1016/j.foodcont.2015.04.043

Di Azevedo, M. I. N., & Iñiguez, A. M. (2018). Nematode parasites of commercially important fish from the southeast coast of Brazil: Morphological and genetic insight. International Journal of Food Microbiology, 267, 29-41.

Diagne, P. M., Bâ, C.T., Ndiaye, P. I., Bray, R. A., Marchand, B., & Quilichini, Y. (2016). Sperm ultrastructure of *Podocotyloides magnatestis* (Digenea, Opecoeloidea, Opecoelidae) a parasite of *Parapristipoma octolineatum* (Pisces, Teleostei). *Zoologischer Anzeiger*, 264, 56–63.

Diagne, P. M., Quilichini, Y., Bâ, C.T., Ndiaye, P. I., Dione, A., & Marchand, B. (2015). Ultrastructure of the spermatozoon of *Helicometroides atlanticus* (Digenea, Monorchiidae), an intestinal parasite of *Parapristipoma octolineatum* (Pisces, Teleostei) in Senegal. *Tissue Cell*, 47, 198–204.

Diouf, N. (1993). Etude taxonomique, ultrastructurale el biologique des Coccidies (Protozoaires, Apicomplexes) parasites de poissons des côtes sénégalaises. Thèse de Troisième Cycle de Biologie Animale. Université Cheikh Anta Diop de Dakar. Faculté des Sciences et Techniques, 157 pp.

Fischtal, J. H., & Thomas, J. D. (1970). Digenetic trematodes of marine fish from Ghana: Family Lepocreadiidae. *Journal of Helminthology*, 44, 365–386.

Galil, B., Boero, F., Campbell, M., Carlton, J., Cook, E., Fraschetti, S., Gollasch, S., Hewitt, C. L., Jelmert, A., Macpherson, E., Marchini, A., McKenzie, C., Minchin, D., Occhipinti-Ambrogi, A., Ojaveer, H., Olenin, S., Piraino, S., & Ruiz, G. (2015). 'Double trouble': the expansion of the Suez Canal and marine bioinvasions in the Mediterranean Sea. *Biological Invasions*, 17, 973-976. https://doi.org/10.1007/s10530-014-0778-y

Gómez-Gesteira, M., Gimeno, L., de Castro, M., Lorenzo, M. N., Álvarez, I., Nieto, R., Taboada, J. J., Crespo, A. J. C., Ramos, A. M., Iglesias, I., Gómez-Gesteira, J. L., Santo, F. E., Barriopedro, D., & Trigo, I. F. (2011). The state of climate in NW Iberia. *Climate Research*, 48, 109–144. https://doi.org/10.3354/cr00967

González-Amores, Y., Clavijo-Frutos, E., Salas-Casanova, C., & Alcain-Martínez, G. (2015). Direct parasitological diagnosis of infection with *Hysterothylacium aduncum* in a patient with epogastralgia. *Revista Española de Enfermedades Digestivas*, 107, 699–700.

Horta e Costa, B., Assis, J., Franco, G., Caselle, J. E., Erzini, K., Henriques, M., Gonçalves, E. J., & Caselle, J. E. (2014). Tropicalization of fish assemblages at temperate biogeographic transition zones. *Marine Ecology Progress Series*, 504, 241–252. https://doi.org/10.3354/meps10749

Iglesias, R., Paramá, A., Alvarez, M. F., Leiro, J., Fernández, J., & Sanmartín, M. L. (2001). *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain). *Diseases of Aquatic Organisms*, 46, 47-55. https://doi.org/10.3354/dao046047

Knoff, M., Nunes Felizardo, N., Mayo Iñiguez, A., Maldonado Jr. A., Torres, E. J. I., Magalhaes Pinto, R., & Correa Gomes, D. (2012). Genetic and morphological characterisation of a new species of the genus *Hysterothylacium* (Nematoda) from *Paralichthys isosceles* Jordan, 1890 (Pisces: Teleostei) of the Neotropical Region, state of Rio de Janeiro, Brazil. Memórias do *Instituto Oswaldo Cruz*, 107, 186-193.

Kpatcha, T. K. (1994). Recherches sur la faune myxosporidienne des poissons des côtes sénégalaises. Ph.D. thesis, Cheikh Anta Diop University, Dakar, 149 pp.

Kpatcha, T. K., Diebakate, C., Faye, N., & Toguebaye, B. S. (1995). Three new species of Myxosporean parasites of marine fishes from Senegal. *Journal of Islamic Academy of Sciences*, 8, 85-90.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870-1874. https://doi.org/10.1093/molbev/msw054

Kuraiem, B. P., Knoff, M., Felizardo, N. N., Menezes, R. C., Gomes, D. C., & de Sao Clemente, S. C. (2017). Histopathological changes induced by *Hysterothylacium deardorffoverstreetorum* larvae (Nematoda: Raphidascarididae) in *Priacanthus arenatus* Cuvier, 1829 (Actinopterygii). *Brazilian Journal of Veterinary Parasitology*, 26, 239-242.

Lanfranchi, A. L., Timi, J. T., & Sardella, N. H. (2004). *Cucullanus bonaerensis* n.sp. (Nematoda: Cucullanidae) parasitizing *Urophycis brasiliensis* (Pisces: Phycidae) from Argentinian waters. *Journal of Parasitology*, 90, 808-812. https://doi.org/10.1645/GE-3276

Lymbery, A. J., Morine, M., Kanani, H. J., Beatty, S. J., & Morgan, D. J. (2014). Co-invaders: The effects of alien parasites on native hosts. *International Journal for Parasitology: Parasites and Wildlife*, 3, 171-177. http://dx.doi.org/10.1016/j.ijppaw.2014.04.002.

Llarena-Reino, M., Piñeiro, C., Antonio, J., Outeiriño, L., Vello, C., González, A. F., & Pascual, S. (2013). Optimization of the pepsin digestion method for anisakids inspection in the fishing industry. *Veterinary Parasitology*, 191, 276–283. https://doi.org/10.1016/j.vetpar.2012.09.015

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., & Nascetti, G. (2018). Molecular epidemiology of *Anisakis* and anisakiasis: an ecological and evolutionary road map. *Advances in Parasitology*, 99, 93-263.

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. *Emerging Infectious Diseases*, 19, 496–499. https://doi.org/10.3201/eid1903.121017

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

Mejía-Madrid, H. H., & Aguirre-Macedo, M. L. (2011). Redescription and genetic characterization of *Cucullanus dodsworthi* (Nematoda: Cucullanidae) from the checkered puffer *Sphoeroides testudineus* (Pisces: Tetraodontiformes). *Journal of Parasitology*, 97, 695-706. https://doi.org/10.1645/GE-2664.1

Moravec, F., & Justine, J. L. (2017). Two new species of nematode parasites, *Cucullanus epinepheli* sp. n. (Cucullanidae) and *Procamallanus (Spirocamallanus) sinespinis* sp. n. (Camallanidae), from marine serranid and haemulid fishes off New Caledonia. *Folia Parasitologica*, 64: 011. https://doi.org/10.14411/fp.2017.011

Moravec, F., Levron, C., & de Buron, I. (2011). Morphology and taxonomic status of two little-known nematode species parasitizing North American fishes. *Journal of Parasitology*, 97, 297-304. https://doi.org/10.1645/GE-2651.1

Moravec, F., & Scholz, T. (2017). Some nematodes, including two new species, from freshwater fishes in the Sudan and Ethiopia. *Folia Parasitologica*, 64: 010. https://doi.org/10.14411/fp.2017.010

Nadler, S. A., D'Amelio, S., Dailey, M. D., Paggi, L., Siu, S., & Sakanari, J. A. (2005). Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova* and *Contracaecum* from northern Pacific marine mammals. *Journal of Parasitology*, 91, 1413–1429. https://doi.org/10.1645/GE-522R.1

Pais, A., Merella, P., Follesa M. C., & Garippa, G. (2007). Westward range expansion of the Lessepsian migrant *Fistularia commersonii* (Fistulariidae) in the Mediterranean Sea, with notes on its parasites. *Journal of Fish Biology*, 70, 269-277. https://doi.org/ 10.1111/j.1095-8649.2006.01302.x

Palm, H. W. (2011). Fish parasites as biological indicators in a changing world: can we monitor environmental impact and climate change? In: Mehlhorn H (ed), *Progress in Parasitology*. Springer Verlag, pp 223–250, https://doi.org/10.1007/978-3-642-21396-0_12

Pantoja, C. S., Pereira, F. B., Santos, C. P., & Luque, J. L. (2016). Morphology and molecular characterization hold hands: clarifying the taxonomy of *Hysterothylacium* (Nematoda: Anisakidae) larval forms. *Parasitology Research*, 115, 4353-4364. https://doi.org/10.1007/s00436-016-5221-0

Paschoal, F., Cezar, A. D., & Luque, J. L. (2015). Checklist of metazoan associated with grunts (Perciformes: Haemulidae) from the Neartic and Neotropical regions. *Check List*, 11, 1501. http://dx.doi.org/10.15560/11.1.1501

Philippart, C. J. M., Anadón, R., Danovaroc, R., Dippnerd, J. W., Drinkwater, K. F., Hawkins, S. J., Oguz, T., O'Sullivan, G., & Reid, P.C. (2011). Impacts of climate change on European marine ecosystems: observations, expectations and indicators. *Journal of Experimental Marine Biology and Ecology*, 400, 52–69. http://dx.doi.org/10.1016/j.jembe.2011.02.023

Ramilo, A., Iglesias, D., Abollo, E., González, M., Darriba, S., & Villalba, A. (2014). Infection of Manila clams *Ruditapes philippinarum* from Galicia (NW Spain) with a *Mikrocytos*-like parasite. *Diseases of Aquatic Organisms*, 110, 71-79. https://doi.org/10.3354/dao02737

Rodríguez, H., Abollo, E., González, A. F., & Pascual, S. (2018). Scoring the parasite risk in highly-valuable fish species from southern ICES areas. *Fisheries Research*, 202, 134-139. https://doi.org/10.1016/j.fishres.2017.06.019

Sanmartin-Duran, M. L., Quinteiro, P., & Ubeira, F. M. (1989). Nematode parasites of commercially important fish in NW Spain. *Diseases of Aquatic Organisms*, 7, 75-77.

Silva, A. M., Knoff, M., Felizardo, N. N., Gomes, D.C., & de Sao Clemente, S. C. (2017). Nematode and cestode larvae of hygienic-sanitary importance in *Lopholatilus villarii* (Actinopterygii) in the state of Rio de Janeiro, Brazil. *Bulletin of the Fisheries Institute, Sao Paulo*, 43, 385-398.

Skinner, R. (1975). Parasites of the stripped mullet, *Mugil cephalus*, from Biscayne Bay, Florida, with descriptions of a new genus and three new species of trematodes. *Bulletin of Marine Science*, 25, 318-345.

Surís-Regueiro, J. C., & Santiago, J. L. (2014). Characterization of fisheries dependence in Galicia (Spain). *Marine Policy*, 47, 99–109. https://doi.org/10.1016/j.marpol.2014.02.006

Suzuki, N., Hoshino, K., Murakami, K., Takeyama, H., & Cho, S. (2008). Molecular diet analysis of phyllosoma larvae of the Japanese spiny lobster *Palinurus japonicas* (Decapoda: Crustacea). *Marine Biotechnology*, 10, 49-55.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673-80.

Villalba, A., Iglesias, D., Ramilo, A., Darriba, S., Parada, J. M., No, E., Abollo, E., Molares, J., & Carballal, M. J. (2014). Cockle *Cerastoderma edule* fishery collapse in the Ría de Arousa (Galicia, NW Spain) associated with the protistan parasite *Marteilia cochillia*. *Diseases of Aquatic Organisms*, 109, 55-80. https://doi.org/10.3354/dao02723

Walter, T. C. (2015a). *Naobranchia hemiconiati* Nuñes-Ruivo, 1963. World of Copepods database. World Register of Marine Species. http://www.marinespecies.org/aphia.php?p=taxdetails&id=353650 (accessed 3 October 2017).

Walter, T. C. (2015b). *Caligus hemiconiati* Capart, 1941. World of Copepods database. World Register of Marine Species. http://www.marinespecies.org/aphia.php?p=taxdetails&id=349636 (accessed 3 October 2017).

Zhao, J. Y., Zhao, W. T., Ali, A. H., Chen, H. X., & Li, L. (2017). Morphological variability, ultrastructure and molecular characterisation of *Hysterothylacium reliquens* (Norris & Overstreet, 1975) (Nematoda: Raphidascaridae) from the oriental sole *Brachirus orientalis* (Bloch & Schneider) (Pleuronectiformis: Soleidae). *Parasitology International*, 66, 831–838. https://doi.org/10.1007/s00436-013-3720-9

Zhu, X., D'Amelio, S., Paggi, L., & Gasser, R. B. (2000). Assessing sequence variation in the internal transcribed spacers of ribosomal DNA within and among members of *Contracaecum osculatum* complex (Nematoda: Ascaridoidea:Anisakidae). *Parasitology Research*, 86, 677–683. https://doi.org/10.1007/PL00008551

FIGURE 1. Nematode parasites from the digestive tract of *E. guttifer* and *P. octolineatus*: scanning electron micrographs of an adult female of *H. reliquens* from *E. guttifer*, showing the cephalic extremity (a) and the tail tip (b); general aspect of the *Hysterothylacium* sp. larvae isolated from prickly puffer stomach (c); L3 larvae of the genus *Anisakis* isolated from *E. guttifer* stomach wall and identified by molecular analysis as *A. pegreffii*, showing the genus characteristics anterior boring tooth (d), and tail mucrom (e); *Cucullanus* nematode also from prickly puffer (genetically identified as *C. dodsworthi*), with their characteristic muscular esophagus, expanded in width at both ends (f) and an image of the cephalic end (g); *Hysterothylacium* larvae from *P. octolineatum* digestive tract (*H. deardorffoverstreetorum*) showing the tail mucron (h, i) and the cephalic end (j)
bt= boring tooth, m= tail mucrom.

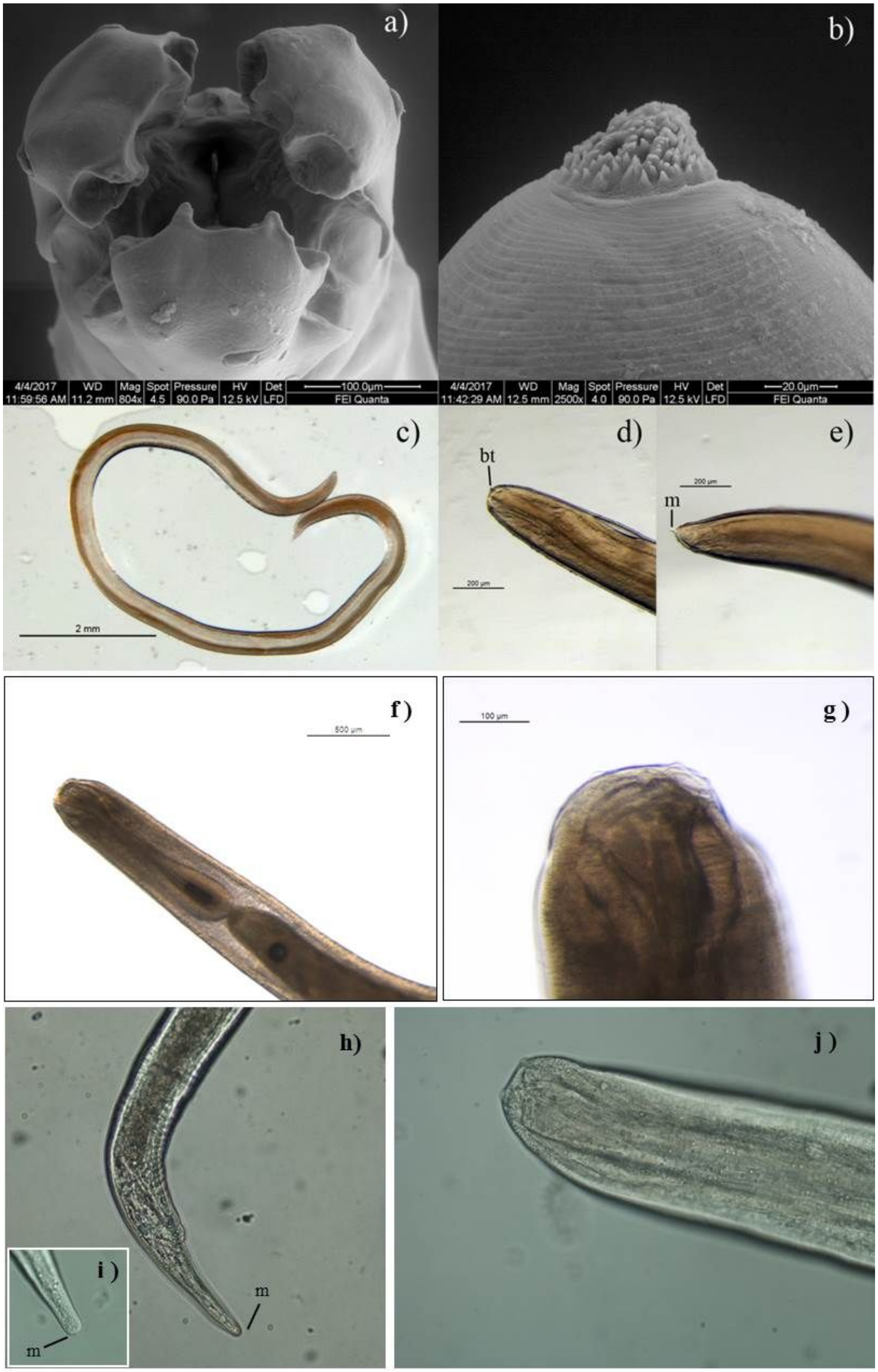
a)
4/4/2017 11:59:56 AM
WD 11.2 mm
Mag 804x
Spot 4.5
Pressure 90.0 Pa
HV 12.5 kV
Det LFD
100.0µm
FEI Quanta
b)
4/4/2017 11:42:29 AM
WD 12.5 mm
Mag 2500x
Spot 4.0
Pressure 90.0 Pa
HV 12.5 kV
Det LFD
20.0µm
FEI Quanta
c)
2 mm
d)
bt
200 µm
e)
200 µm
m
f)
500 µm
g)
100 µm
h)
m
i)
m
j)

FIGURE 2. Maximum-likelihood analysis showing the taxonomic position of the *Hysterothylacium* and *Anisakis* sequences obtained in this study. Numbers at branch nodes indicate bootstrap confidence values in percent. (*) Sequences obtained in this study.

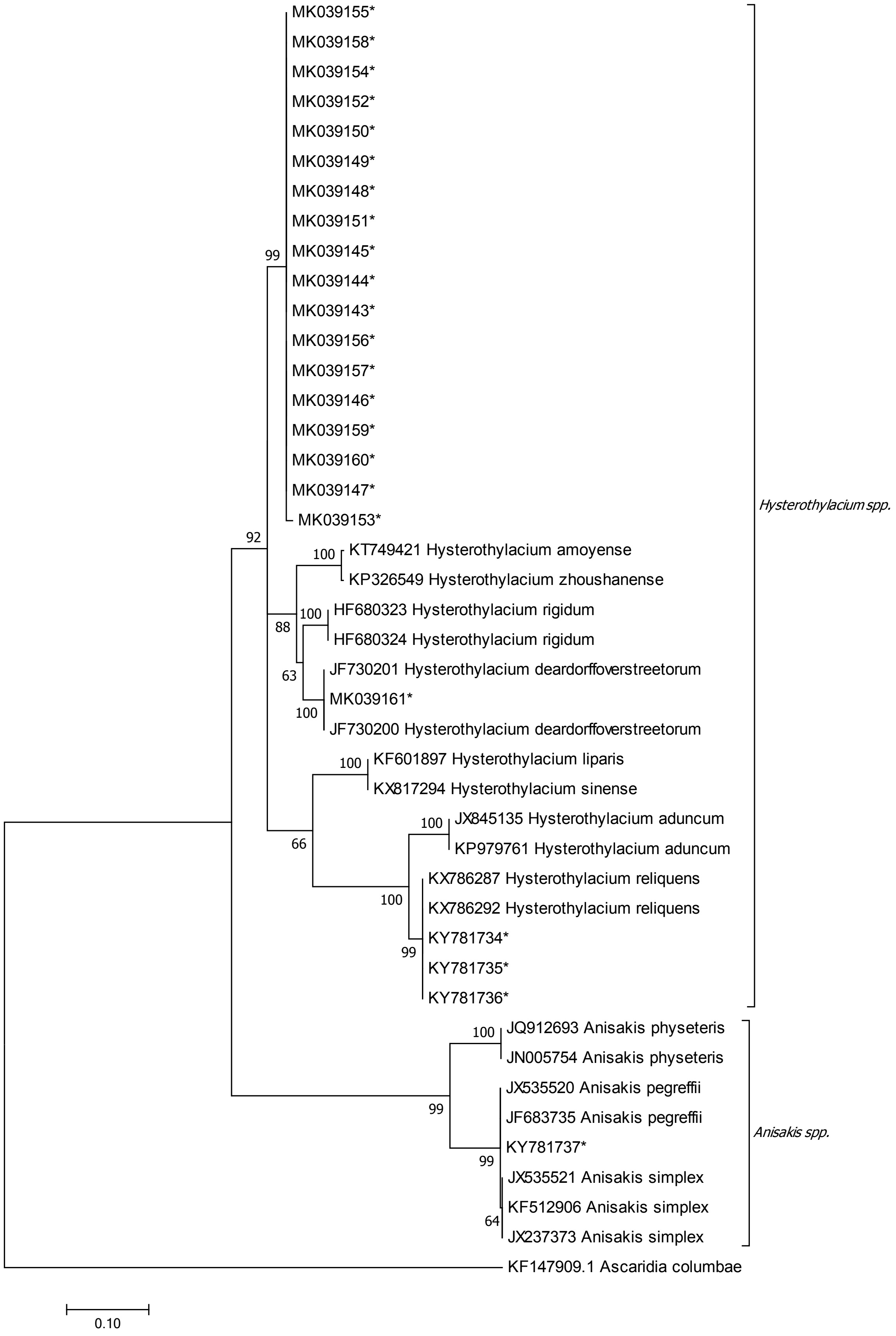
MK039155*
MK039158*
MK039154*
MK039152*
MK039150*
MK039149*
MK039148*
MK039151*
99
MK039145*
MK039144*
MK039143*
MK039156*
MK039157*
MK039146*
MK039159*
MK039160*
MK039147*
MK039153*
92
100
KT749421 Hysterothylacium amoyense
KP326549 Hysterothylacium zhoushanense
100
HF680323 Hysterothylacium rigidum
88
HF680324 Hysterothylacium rigidum
JF730201 Hysterothylacium deardorffoverstreetorum
63
MK039161*
100
JF730200 Hysterothylacium deardorffoverstreetorum
100
KF601897 Hysterothylacium liparis
KX817294 Hysterothylacium sinense
100
JX845135 Hysterothylacium aduncum
66
KP979761 Hysterothylacium aduncum
KX786287 Hysterothylacium reliquens
100
KX786292 Hysterothylacium reliquens
KY781734*
99
KY781735*
KY781736*
Hysterothylacium spp.
100
JQ912693 Anisakis physeteris
JN005754 Anisakis physeteris
JX535520 Anisakis pegreffii
99
JF683735 Anisakis pegreffii
KY781737*
99
JX535521 Anisakis simplex
KF512906 Anisakis simplex
64
JX237373 Anisakis simplex
Anisakis spp.
KF147909.1 Ascaridia columbae
0.10

TABLE 1. GenBank accession numbers of the sequences of rRNA ITS genes of the genus *Hysterothylacium* and *Anisakis* used in phylogenetic analysis

Species	GenBank accession	Host	Location
H. rigidum	HF680323 HF680324	*Lophius piscatorius*	Ireland
H. deardorffoverstreetorum	JF730200, JF730201	*Paralichthys isosceles*	Brazil
H. aduncum	JX845135	*Zoarces viviparus*	Denmark
	KP979761	*Engraulis encrasicolus*	Adriatic Sea
H. reliquens	KX786287, KX786292	*Brachirus orientalis*	Iraq
H. amoyense	KT749421	*Platycephalus indicus*	Iran
H. zhoushanense	KP326549	*Lepidotrigla japonica*	China
H. liparis	KF601897	*Liparis tanakae*	China
H. sinense	KX817294	*Conger myriaster*	China
A. physeteris	JQ912693	*Physeter macrocephalus*	Mediterranean Sea
	JN005754	*Pagellus bogaraveo*	Azores
A. pegreffii	JX535520	*Stenella coeruleoalba*	Mediterranean Sea
	JF683735	*Gadus macrocephalus*	South Korea
A. simplex	JX535521	*Balaenoptera acutorostrata*	Norwegian coast
	KF512906	*Merluccius merluccius*	Ireland
	JX237373	*Clupea harengus*	Denmark

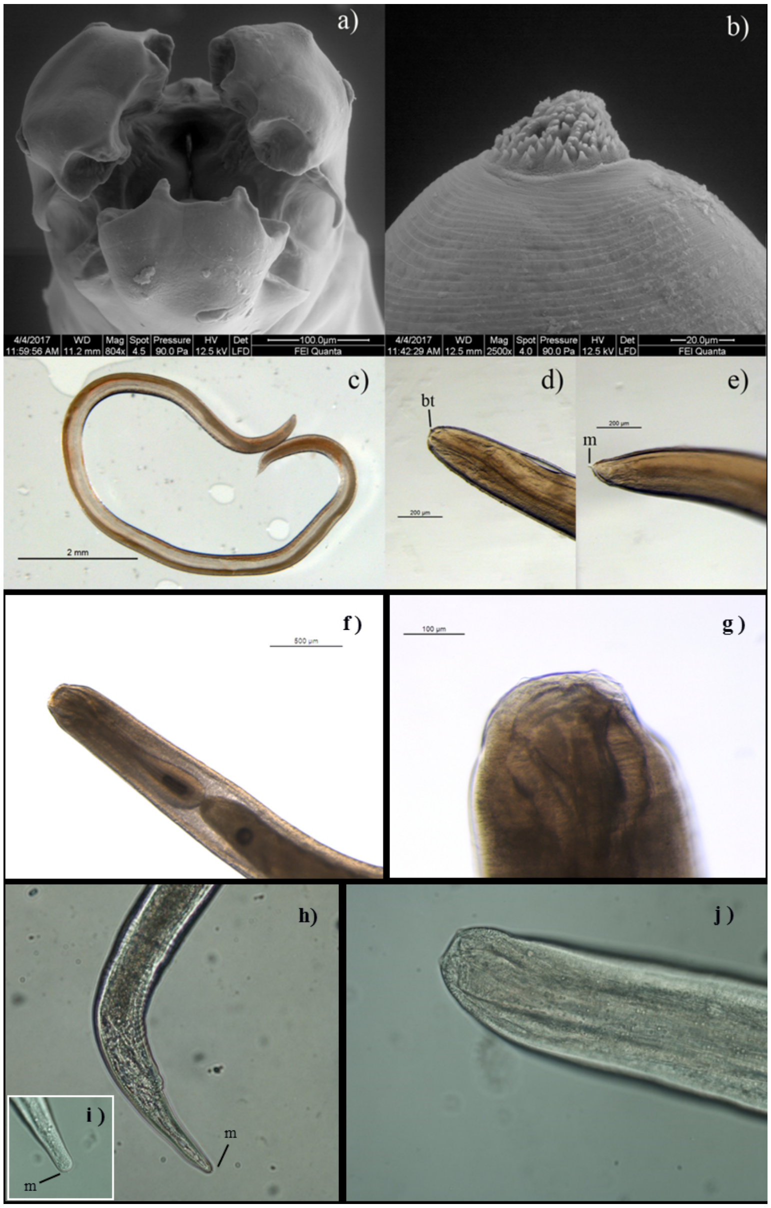

FIGURE 1. Nematode parasites from the digestive tract of E. guttifer and P. octolineatus: scanning electron micrographs of an adult female of H. reliquens from E. guttifer, showing the cephalic extremity (a) and the tail tip (b); general aspect of the Hysterothylacium sp. larvae isolated from prickly puffer stomach (c); L3 larvae of the genus Anisakis isolated from E. guttifer stomach wall and identified by molecular analysis as A. pegreffii, showing the genus characteristics anterior boring tooth (d), and tail mucrom (e); Cucullanus nematode also from prickly puffer (genetically identified as C. dodsworthi), with their characteristic muscular esophagus, expanded in width at both ends (f) and an image of the cephalic end (g); Hysterothylacium larvae from P. octolineatum digestive tract (H. deardorffoverstreetorum) showing the tail mucron (h, i) and the cephalic end (j)
bt= boring tooth, m= tail mucrom.

136x214mm (150 x 150 DPI)

FIGURE 2. Maximum-likelihood analysis showing the taxonomic position of the Hysterothylacium and Anisakis sequences obtained in this study. Numbers at branch nodes indicate bootstrap confidence values in percent. (*) Sequences obtained in this study.

TABLE 1. GenBank accession numbers of the sequences of rRNA ITS genes of the genus *Hysterothylacium* and *Anisakis* used in phylogenetic analysis

Species	GenBank accession	Host	Location
H. rigidum	HF680323 HF680324	*Lophius piscatorius*	Ireland
H. deardorffoverstreetorum	JF730200, JF730201	*Paralichthys isosceles*	Brazil
H. aduncum	JX845135	*Zoarces viviparus*	Denmark
	KP979761	*Engraulis encrasicolus*	Adriatic Sea
H. reliquens	KX786287, KX786292	*Brachirus orientalis*	Iraq
H. amoyense	KT749421	*Platycephalus indicus*	Iran
H. zhoushanense	KP326549	*Lepidotrigla japonica*	China
H. liparis	KF601897	*Liparis tanakae*	China
H. sinense	KX817294	*Conger myriaster*	China
A. physeteris	JQ912693	*Physeter macrocephalus*	Mediterranean Sea
	JN005754	*Pagellus bogaraveo*	Azores
A. pegreffii	JX535520	*Stenella coeruleoalba*	Mediterranean Sea
	JF683735	*Gadus macrocephalus*	South Korea
A. simplex	JX535521	*Balaenoptera acutorostrata*	Norwegian coast
	KF512906	*Merluccius merluccius*	Ireland
	JX237373	*Clupea harengus*	Denmark