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A molecular and epidemiological study of *Grillotia* (Cestoda: Trypanorhyncha) larval infection in *Etmopterus spinax* (Elasmobranchii: Squaliformes) in the Mediterranean Sea and Northeast Atlantic Ocean

Isbert Wolf [1, 2], Dallarés Sara [3, *], Grau Amalia [4, 5], Petrou Antonis [6], García-Ruiz Cristina [7], Guijarro Beatriz [8], Jung Armelle [9], Catanese Gaetano [4, 5, *]

[1] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), 27570 Bremerhaven, Germany
[2] Unidad de Zoología Marina, Institut Cavanilles de Biodiversitat I Biologia Evolutiva, Parc Científic, Universitat de València, PO Box 22085, València, 46071, Spain
[3] Unitat de Zoologia, Departament de Biologia Animal, Biologia Vegetal I Ecologia (BABVE)Campus de La UAB, 08193 Bellaterra (Cerdanyola Del Vallès), Barcelona, Spain
[4] LIMIA-IRFAP (Laboratorio de Investigaciones Marinas y Acuicultura) – Govern de Les Illes Balears, Av. Ing. Gabriel Roca, 69, 07157 Port D'Andratx (Illes Balears), Spain
[5] INAGEA (UIB)- Instituto de Investigaciones Agroambientales y de Economía Del Agua, Universidad de Las Islas Baleares, Carretera de Valldemossa, Km 7.5, 07122 Palma, Spain
[6] Enalia Physis Environmental Research Centre, 2, Acropoleos Str. Aglantzia 2101, Nicosia, Cyprus
[7] Centro Oceanográfico de Málaga, Instituto Español de Oceanografía (IEO), Consejo Superior de Investigaciones Científicas, Puerto Pesquero S/n, 29640 Fuengirola, Málaga, Spain
[8] Centro Oceanográfico de Baleares, Instituto Español de Oceanografía (IEO), Consejo Superior de Investigaciones Científicas, Moll de Ponent, S/n. 07015 Palma de Mallorca, Spain
[9] Des Requins et Des Hommes (DRDH) Bibliothèque La Pérouse Technopole Brest Iroise, 15 rue Dumont D'Urville, BP 70F, 29280 Plouzané, France

* Corresponding authors : Sara Dallarès, email address : Sara.Dallares@uab.cat ; Gaetano Catanese, email address : gcatanese@dgpesca.caib.es

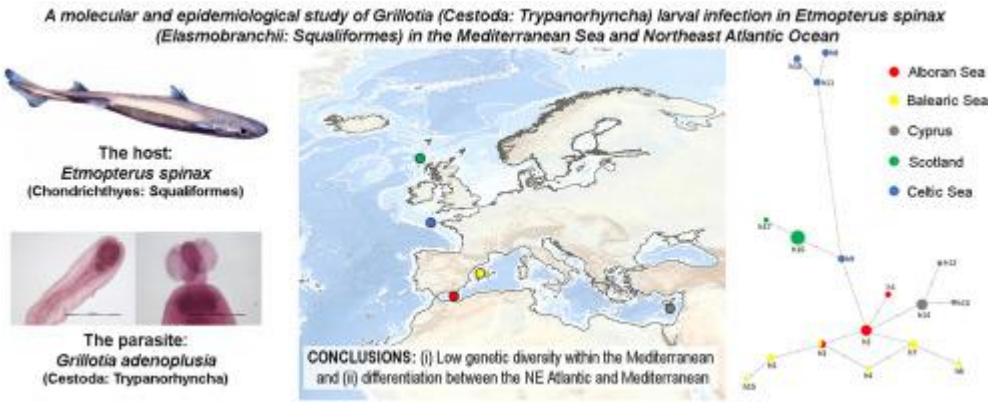
Abstract :

Amongst other factors, topographic features can influence the genetic variability among populations of marine organisms. This applies to host species but also to their parasites, which are poorly studied regarding this aspect, as well as with regard to their use as bioindicators. In the present work, the ribosomal DNA (28S rDNA) was used to assess genetic diversity of Grillotia (Cestoda, Trypanorhyncha) larvae in one of its paratenic hosts, namely Etmopterus spinax, across five different regions (off Scotland, Celtic, Alboran and Balearic Seas and off Cyprus) belonging to three major geographic areas (Northeast Atlantic, western and eastern Mediterranean). The obtained sequences revealed a total of 18 polymorphic sites and 17 haplotypes, as well as significant values of variance throughout the five different regions. Reconstructed phylogenetic trees highlighted that all Grillotia sp. sequences formed a monophyletic group, but divergent lineages split into different main clades which were in relation to the area of origin,

with a consistent cluster of sequences from the Atlantic Ocean, as well as another from the Eastern Mediterranean. In contrast, low genetic differentiation was observed between samples from Balearic and Alboran Seas, and with respect to Grillotia sp. larvae from the Gulf of Naples analysed in a previous study. Geographical differences in parasite infection descriptors (prevalence, abundance, and intensity) were assessed, revealing significant differences among the sampled regions.

The present study indicates that geographical distance and submarine barriers affect not only the connectivity of hosts but also their parasite infrapopulations by limiting interpopulation dispersal. It underlines the usefulness of parasites as biological tags for the study of susceptible and data-poor host species such as deep-water sharks and its potential implications for host population management and protection measures.

Graphical abstract



Highlights

► Cestode larvae of *Grillotia adenoplusia ex* its paratenic host *Etmopterus spinax*. ► RDNA (28S) revealed genetic diversity between NE Atlantic and Mediterranean regions. ► Consistent sequence cluster for the NE Atlantic and Eastern Mediterranean. ► Low genetic differentiation within Western Mediterranean. ► Limited interpopulation dispersal apply not only to hosts, but also to parasites.

Keywords : Etmopterus spinax, Grillotia, Mediterranean, Atlantic, Phylogeography, 28S rDNA

1. Introduction

Elasmobranchs represent an essential part of marine ecosystems ensuring their functioning as meso- and top-predators, which has also its implications to food security (Gubili et al., 2014; Heupel et al., 2014; Dulvy et al., 2021). Though, elasmobranchs are also part of one of the most threatened vertebrate class (Díaz et al., 2019) revealing high and increasing extinction risks due to human-induced threats, mostly overfishing, but also habitat loss, climate change and pollution (Dulvy et al., 2021).

The enhanced fishery impact in the deep-sea has raised concerns regarding the functioning of deep-sea ecosystems (Morato et al., 2006; Norse et al., 2012) and the protection of deep-water elasmobranchs. Like several other species of chondrichthyans, many deep-water elasmobranchs exhibit slow growth rates, late maturity, and low fecundity, resulting in extremely low rebound potentials and high susceptibility to fishing mortality (Simpfendorfer and Kyne, 2009). Despite these aspects, there is still a dearth of information on the ecology of deep-sea elasmobranchs (Gubili et al., 2016; Pinte et al., 2020), which also refers to migration and connectivity between populations and their discrimination (Neat et al., 2015; Gubili et al., 2016), such as is the case of the velvet belly lantern shark.

The velvet belly lantern shark, *Etmopterus spinax* (L. 1758), is a small-sized, bioluminescent, benthopelagic deep-water shark which occurs on the outer continental and insular shelves on upper to lower slopes at depths between 70 and 2000 m but usually at 200–500 m (Compagno, 1984). The geographical distribution of this shark species covers the eastern Atlantic Ocean from Iceland and Norway to southern Africa and the Mediterranean Sea (Coelho and Erzini, 2008). *Etmopterus spinax* feeds on crustaceans, small fishes, and cephalopods (Compagno, 1984) but diet differs among regions and ontogenic shifts let sharks being gradually become piscivorous with increased size (Neiva et al., 2006; Fanelli et al., 2009; Besnard et al., 2022). In general, available literature

indicate spatial differences in the importance of crustacean orders Decapoda and Euphausiacea between the Northeast Atlantic and Mediterranean Sea is partly based on environmental conditions such as topography and oceanography influencing the vertical stratification of prey and affecting its availability to predators, whereas preyed fish comprise meso-, bentho- and bathypelagic species of different families without a trend (e. g. Neiva et al., 2006; Fanelli et al., 2009; Preciado et al., 2009; Isbert et al., 2015). Overall, it is concluded that small benthopelagic sharks such as *E. spinax* are generalist feeders and represent mid to high trophic levels in the marine food webs, feeding often on dominant prey items (Neiva et al., 2006; Santoro et al., 2022). These shark species can occur in high abundances and act as prey for locally present larger predators (e. g. benthic sharks). All these aspects have implications on the parasite community of these small benthopelagic sharks.

Metazoan parasites are common inhabitants of marine ecosystems including many taxa being trophically transmitted within the food-webs among different trophic levels (Marcogliese and Cone, 1997; Marcogliese, 2002; 2005). Heteroxenous parasites exhibit complex life cycles where they reach their mature stage in the definitive host after having passed different intermediate/paratenic invertebrate and vertebrate hosts mostly *via* the food-web (Santoro et al., 2022). The diversity and abundance of available prey in a local ecosystem determines the abundance and richness of heteroxenous parasites in a certain fish host (Cirtwill et al., 2016). For instance, crustaceans are considered to play important roles as intermediate hosts of fish parasites (Marcogliese, 2002), and are involved in the life cycles of parasites such as cestodes.

This is the case for species of the cestode genus *Grillotia* (Lacisthorhynchidae) belonging to the order Trypanorhyncha which are commonly detected in teleosts (Beveridge and Campbell 2007). The life cycle includes a first (copepode) and a second intermediate host (schooling teleosts, cephalopods), in some species an additional paratenic/transport host may occur (larger fish, elasmobranch), and a definitive host (elasmobranch) (Palm, 2004; Dallarés et al., 2016). Species of *Grillotia* are cosmopolitan including the Mediterranean Sea (e. g. Özer et al., 2014; Dallarés et al., 2017a; Genç et al., 2018; Santoro et al., 2018; 2021) and the northeastern Atlantic Ocean (e. g.

MacKenzie, 1990; Palm and Schröder, 2001; Alvarez et al., 2006, Isbert unpubl. data) where second larval stage (plerocercus) were recorded in different organs and the muscle tissue of their second intermediate and paratenic hosts (teleosts, elasmobranchs). It is supposed that effects on host condition and host immune response is rather negligible while in cases of heavy infections with high abundances of plerocerci in the muscle tissue e. g. in the tail region, musculature could lose its functionality (Dallarés et al., 2017a; Santoro et al., 2018; 2021).

Morphological features characterising larvae and adults of Trypanorhyncha are two or four bothria, a tentacular apparatus with four retractile tentacles equipped with hooks (Palm et al., 2004; Rohde, 2005; Palm et al., 2009). The most recent revision of the genus *Grillotia* suggested that currently 18 species are assigned to this genus (Beveridge and Campbell, 2007; 2013). Three species have been recorded in the Mediterranean Sea (*G. adenoplusia* (Pintner, 1903) Palm, 2004, *G. erinaceus* (van Beneden, 1858) Guiart, 1927 and *Grillotia heptanchi* (Vaullegeard, 1899) Dollfus, 1942 (Paggi, 2008; Beveridge and Campbell, 2013; Dallarés, 2016)), whereas one of the formerly described species in this area, *G. scolecina* (Rudolphi, 1819), is considered as species *inquerida* (Beveridge and Campbell, 2013). Additionally, more recently two still unknown representatives of the genus *Grillotia* were recorded in the Mediterranean Sea from *Lophius piscatorus* and *Galeus melastomus* (Santoro et al. 2018; 2021). Molecular information exists for the latter both unknown taxa from the Mediterranean Sea, and three additional species *Grillotia pristiophori* Beveridge & Campbell 2001, *G. erinaceus*, and *G.* (*Christianella*) *yuniariae* Palm, 2004 from the North Atlantic and West Pacific (Palm et al. 2007; 2009). Up to date only two records of *Grillotia* in representatives of the family Etmopteridae (Palm et al., 2004 *G. amblyrhynchus ex*. *Etmopterus* sp.; Santoro et al., 2021 *Grillotia* sp. ex *E. spinax*) were published.

In the light of these aspects influencing the infection patterns of hosts described above, parasites can act as bioindicators. Parasites as natural biological tags can be used as powerful tools shedding light on different features of host life (Caira, 1990; Williams et al., 1992), and being recommended for studies specifically on deep-sea and rare marine species (MacKenzie and Abaunza, 1998). Amongst

other ecological and biological aspects, parasites can hint to separation or connectivity between fish populations, migration events or small-scale host movements (e. g. Grutter, 1998; MacKenzie and Abaunza, 1998; Mattiucci et al., 2015). These studies are essential for fisheries management and conservation efforts to understand potential population differentiation and therefore, for the identification of demographically independent fish populations (Gubili et al., 2016). Mattiucci et al. (2015) indicated that changes over the evolutionary timescale can be detected by fish population genetics, while parasitic bioindicators provide information on fish movements over smaller temporal and spatial scales. The authors stressed the usefulness of the phylogeographic analysis based on the same genes of fish host and biomarkers (parasites) for studies on fish population structure. In eukaryotic species, the nuclear ribosomal genes, which consist of several hundred tandemly repeated copies, represent potential candidates that can be easily applied in phylogenetic studies. The large subunit (such as 28S rDNA) nuclear rDNA gene is larger and shows many divergent domains in rates of evolution among phyla than does the small subunit (18S rDNA) (Hillis and Dixon, 1991). However, the multiple copies of ribosomal units are evolving in concert (Arnheim et al., 1983), that each copy of a unit is usually very similar to the other copies within individuals and species, even if the differences among species can accumulate rapidly (Hillis and Dixon, 1991). Although its level of variation is low compared with other molecular markers, the variability detected in this DNA region is useful for reconstructing relatively recent evolutionary events. In this context, the absence or reduction of gene flow among localities and the rapid concerted evolution of rDNA tend to homogenize allelic variation within individuals and consequently within populations, but variability among different populations can be pronounced. Therefore, the distribution of dominant haplotypes in conjunction with other morphological characters can delineate species boundaries among closely related species or can reveal clear geographic patterns and significant population discrimination within a species.

The objectives of the present work are, firstly, the description of the infection patterns of the cestode larvae assigned to the genus *Grillotia* in one of its paratenic hosts, namely *E. spinax*, caught

in different geographical regions in the Mediterranean Sea and the nearby Northeast Atlantic Ocean. Secondly, the assessment of the genetic diversity of this cestode across the sampled regions by means of the ribosomal DNA (28S rDNA). Additionally, a histological analysis of the infested musculature by *Grillotia* sp. has been conducted in *E. spinax* for the first time to assess the potential impacts imposed by the parasite on its host.

2. Materials and methods

2.1. Sampling

Between 2013 and 2017, we obtained a total of 419 specimens of *Etmopterus spinax* during hauls made at 50-800 m depth in five regions belonging to two predefined areas: Northeast Atlantic and Mediterranean Sea (western and eastern) (see Fig. 1, Tables 1 and 2). Part of the samples (273 specimens) from the Mediterranean Sea were obtained during the International Bottom Trawl Surveys (MEDITS) performed annually from June 2013 to June 2016 in the GSA 05 and GSA 01/02 (off Balearic Islands and Alboran Sea, respectively, Spain, western Mediterranean Sea), and in 2015 in the GSA 25 (off Cyprus, eastern Mediterranean Sea). The sampling scheme and gear (bottom trawl GOC 73, codend mesh size 20 mm) used in these cruises, as well as the sampling protocol for captures, were those applied throughout the Mediterranean within the framework of the MEDITS program (Bertrand et al., 2002). One *E. spinax* specimen was captured by local commercial fishing vessels off Blanes (Catalan coast, Spain) in 2018. Additionally, 92 specimens (not represented in Table 1 and 2) were obtained by observers on board of commercial trawlers fishing close to the Mallorca Island (Balearic Islands, Spain) in 2015 and 2017.

The samples from the Northeast Atlantic were obtained in the Celtic Sea in 2013 and in the Northern waters of Scotland in 2015. Samples from the Celtic Sea (28 specimens) were taken within the frame of the annual EVOHE (Evaluation des ressources halieutiques de l'ouest de l'Europe - Assessment of fisheries resources in Western Europe) campaigns by means of a trawling gear 40 m in length with vertical and horizontal openings of 3.5 m and 25 m, respectively

(IFREMER, 2015). Samples (26 specimens) from northern Scotland were obtained opportunistically from commercial trawlers.

Captured specimens of *E. spinax* were processed in different ways: (i) 367 specimens were frozen onboard (-25 °C) immediately after capture or, in the case of specimens obtained from commercial vessels, later in the laboratory. Later, sharks were thawed and morphometric data (total length (TL), total weight (TW), eviscerated weight (EW)) were recorded. The whole muscle tissue was analysed for the presence of cestode larvae by means of a stereomicroscope. Detected cestode larvae were collected, counted, and in many cases (see Table 2) preserved in 100% molecular grade ethanol for molecular analyses. This data was later used for the calculation of infection descriptors and for the assessment of the spatial infection patterns. (ii) 52 specimens obtained from commercial fisheries (November 2015, October/November 2017) were devoted to histological studies only and processed fresh. In this case, the tail was cut off at the level behind the second dorsal fin and immediately fixed in 10% neutral phosphate-buffered formalin.

2.2. Data treatment and analysis

Parasitological infection descriptors (prevalence, mean abundance, and mean intensity; see Bush et al., 1997) were calculated with the freely available software Quantitative Parasitology 3.0 (QP 3.0; accessed 23 September 2022).

Prior to statistical analyses, sharks TL was log-transformed to comply with normality and homoscedasticity requirements. A possible association between TL and individual parasite abundance was tested by a bivariate correlation.

Differences among the five sampled regions for sharks TL was tested by a general linear model (LM) with Student-Newman-Keuls post-hoc pairwise comparisons. In the case of parasite prevalence, abundance and intensity, geographical differences were tested using generalized linear models (GLM) setting host TL as covariate and selecting a logistic distribution for prevalence and a log-binomial distribution for abundance and intensity.

208 Statistical analyses were performed with the software PASW Statistics 18.

209

210 *2.3. Molecular analyses*

211 Total genomic DNA was extracted and purified using the Macherey-Nagel DNA Tissue extraction
212 kit following manufacturer's instructions. A fragment of the large subunit ribosomal DNA (28S
213 rDNA) gene was amplified using the primers LSU5/1500R and PCR conditions described by Olson
214 et al. (2003). We also designed a new pair of primers for a nested PCR: GrillF (5'
215 TTAGAGTCGGGTTGTTTGAGAATGC 3') and GrillR (5' CGAACAGACCCGTTGACAAGCAG
216 3'). PCR reactions were performed in a total volume of 20 ul containing: 10 ul of Kapa Taq Ready
217 mix (Sigma-Aldrich), 8.2 ul of sterile water, 0.4 ul of each primer (stock 20 Mmol) and 1 ul of DNA
218 at 50 ng/ul. Following an initial denaturation at 94◦C for 10 min, we run 35 cycles of 94◦C for 30 sec,
219 56◦C for 30 sec and 72◦C for 1.50 min, and a final extension step at 72◦C for 10 min. PCR products
220 were separated on 1 % agarose in TAE 1× buffer gels, stained with GelRed (Invitrogen) including a
221 molecular ladder size standard and visualized on an UV transilluminator.
222 Obtained amplicons were purified using a mi-PCR purification Kit (Metabion International,
223 Germany) following the manufacturer's instructions and bidirectionally sequenced at Secugen
224 service (www.secugen.es).
225 The obtained sequences of the 28S rDNA were aligned and edited using the BioEdit v7.2.5 software
226 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and MEGA 6.0 (Tamura et al. 2013). Number of
227 haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π) and the average number of
228 nucleotide differences (*k*) were computed with DNASP v. 6 (Rozas et al., 2017).
229 Sequences of species of the same genera or closely related taxa present in GenBank were included in
230 the analysis. We selected sequences based on the similarity detected by the Blast analysis and on the
231 comparable nucleotide length to those obtained in this work. Sequences of 12 *Grillotia* sp. and other
232 27 taxa available in GenBank were included in the phylogenetic tree, with *Horneliella annandalei*
233 species (order: Trypanorhyncha) as outgroup (Fig. 2; Table S1).

The sequences were then analyzed with JModelTest v2.1.7 (Darriba et al., 2012) using the Akaike Information Criterion (AIC; Posada and Buckley, 2004) to select the appropriate model of evolution, as a guide to determine the best-fit maximum likelihood model. Both Maximum likelihood (ML) and Bayesian Inference (BI) methods were adopted to reconstruct the phylogenetic relationships using MEGA 6.0, with 1000 bootstrap replicates, and MrBayes v. 3.2.1 (Huelsenbeck and Ronquist, 2001), respectively. A network based on the sequence data was constructed by NETWORK 4.6.1.1 (http://www.fluxus-engineering.com) using the Median-Joining Network approach (Bandelt et al., 1999) with default settings. Pairwise Nei's genetic distances (Nei, 1987) were calculated in MEGA 6.0. Hierarchical analysis of molecular variance (AMOVA) with F-statistics calculations were performed in ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Samples were grouped by area (Atlantic Ocean, western Mediterranean Sea and eastern Mediterranean Sea) and sampling sites (Alboran Sea, Balearic Sea, Celtic Sea, Cyprus and Scotland).

2.4. Histological assessment

Tails of the specimens fixed in 10% neutral phosphate-buffered formalin were processed as follows. The vertebral column was removed by performing a longitudinal dissection of both fixed symmetrical flanks. The skin was removed from the tail and transverse sections (3-4 mm thick) were cut off. Cross section samples of the dissected flanks were further dehydrated in an increasing ethanol gradient, cleared with Microclearing (X-free), embedded in paraffin wax, sectioned at 4 μm and stained with Mayer’s haematoxylin and eosin (MHE) for routine light-microscopy examination. Some additional sections were stained with Mayer’s haematoxylin and Light Green-Orange G-Acid fuchsine (MH-VOF) (Gutiérrez, 1967), or with Cajal-Gallego trichrome stain (Olivera-Bravo et al., 2022; Sanjai et al., 2017)—to differentiate collagen (blue) from muscle fibres (green)— or with Periodic Acid Schiff (PAS) for detection of neutral mucosubstances (Bancroft and Stevens, 1990).

3. Results

260 *3.1. Collection and infection site of larval Grillotia sp.*

261 Larval cestodes (plerocerci) were mostly removed from the tail muscle tissue close to the vertebrae.
262 Recovered *Grillotia* sp. larvae were encapsulated, appeared whitish and translucent, and ovoid in
263 shape. The scolex was invaginated in all cases, but glass plate compression between two petri
264 dishes revealed the presence of two bothria and a tentacular apparatus with invaginated tentacles.
265 Considering the body sites of infection, 98.9 % of the larvae were recorded in the tail musculature,
266 whereas the remaining 1.1 % were detected in the musculature close to the jaws or gills.

267

268 *3.2. Geographical trends on hosts size and parasite infection descriptors*

269 Sharks TL ranged between 8.0 and 57.2 cm and displayed significant differences among regions
270 (LM, $F_{(4, 362)}$=88.319, $p < 0.001$), with specimens from the Atlantic region being larger than those
271 from the Mediterranean area (all post-hoc pairwise comparison significant except that between the
272 Balearic Sea and Cyprus, see Table 2).
273 Parasite abundance was positively correlated with host TL (r_s=0.605, $p < 0.001$). Significant
274 differences among the five sampled regions were detected for prevalence, abundance and intensity
275 of *Grillotia* sp. (GZM, χ^2=54.714, $p < 0.001$; χ^2=190.406, $p < 0.001$ and χ^2=65.887, $p < 0.001$,
276 respectively). No interactions were found between regions and sharks TL ($p > 0.05$). Outcomes of
277 post-hoc pairwise comparisons are indicated in Table 2. In general, minimum, and maximum values
278 for *Grillotia* sp. infection descriptors were recorded in the Alboran Sea and off Scotland,
279 respectively.

280

281 *3.3. Molecular data*

282 The size of all the amplicons was of about 1.4 Kb. A total of 70 sequences of *Grillotia* sp. were
283 aligned. Analyzing the sequences, we found 18 polymorphic sites (1.28%) overall, including indels
284 and a total of 17 haplotypes throughout the different geographic areas were identified (Table 3; Table
285 S2). All haplotype sequences were deposited in GenBank under accession numbers: LC730478-

LC730494. The minimum and maximum number of haplotypes for each sampling region varied from 2 to 6, observed in Scotland and Balearic Sea respectively. One haplotype resulted also present both in Balearic Sea and in Alboran Sea sampling sites (Table 3; Fig. 3). The values of Haplotype diversity ranged from 0.2637 (Scotland) to 0.8309 (Balearic Sea), while the values of nucleotide diversity for all sampling regions were generally very low, varying from 0.0002 (Scotland) to 0.00185 (Celtic Sea).

The AMOVA analyses revealed significant values of variance (32.65%) among the areas (FCT=0.326; P<0.05), among the five sampling regions with 41.67% of variance (FSC=0.618; P<0.001) and within those regions with 25.67% of variance (FST=0.743; P<0.001) (Table 4).

The Akaike Information Criterion for the likelihood ratio test implemented in JModelTest software pointed to the GTR+G model as the best fit model of DNA sequence evolution.

Based on the sequences of the 28S rDNA region, the phylogenetic trees reconstructed using the ML and BI methods highlighted that all the sequences of *Grillotia* sp. formed a monophyletic group. However, within that group, divergent lineages split into different main clades supported by significant bootstrap values. The tree topologies obtained using the two different methods gave similar results (Fig. 2). In relation to the area of origin, a consistent cluster of sequences from the Atlantic Ocean (Scotland and Celtic Sea, comprising a subclade including only sequences from the Celtic Sea) was evident, as well as a group of sequences from Cyprus, all supported by significant bootstrap values. On the contrary, samples from the regions of Alboran and Balearic Sea were distributed in two different subclades, in which the sequences published by Santoro et al. (2021) from samples collected in the Tyrrhenian Sea (Italy) (accession numbers: MW838227-MW838233) were also included.

Likewise, the network analysis also revealed clusters with different geographical distribution patterns, and the haplotypes obtained in this study clustered in the groups present in the phylogenetic tree. The three dominant haplotypes, h16 (Scotland), h2 (Alboran) and h14 (Cyprus), occupy central positions, each being surrounded by several less frequent haplotypes (Fig. 3).

312

313 *3.4. Histological observations*

314 This is the first histological analysis of the musculature of *E. spinax* dealing with potential effects of
315 infestation by *Grillotia* sp. This analysis revealed parasite granulomas —formed by the encysted
316 parasite, an inner layer of macrophages and an outer layer of palisade-arranged epithelioid cells—
317 embedded in the caudal skeletal muscle tissue (Fig. 4A, B). These granulomas were similar in their
318 appearance in all sampled specimens regardless of the sampling area. The internal macrophage layer
319 was composed by large, round macrophages (in cases of apparently recent infestations) that appeared
320 increasingly flattened in more advanced stages of infestation. The outer layer of epithelioid cells was
321 made by a variable number of epithelioid layers (2 ─ >7). The encapsulation reaction consisted of a
322 layer of collagen fibers interspersed with fibroblasts (Fig. 4B); this capsule was only evident in
323 advanced stages of infestation. Necrosis of the adjacent tissue was not observed, although muscle
324 atrophy was present. The parasitic integument was formed by neutral mucosubstances, as well as the
325 reserve deposits of the larvae. These structures were stained in blue in Cajal-gallego's Trichrome.
326 The morphological aspects of the plerocerci observed under the stereomicroscope (e. g. presence of
327 two bothria and an invaginated tentacular apparatus) could also be confirmed by histological sections
328 (Fig. 4C-F).

329

330 **4. Discussion**

331 *4.1. Identification of Grillotia sp. detected in E. spinax*

332 Morphological identification to species level of larval trypanorhynch cestodes is possible by studying
333 hook morphology and distribution patterns on the tentacles (oncotaxis). In the present case, such
334 observations were not feasible because the tentacular apparatus was invaginated within the scolex in
335 all cases. However, unpublished molecular results based on 28S rDNA sequences have revealed
336 conspecificity between cestode larvae *ex E. spinax* from the Balearic Sea (for which sequences are
337 included in the present study) and larvae of *Grillotia* obtained from three other benthic shark species

collected in the same area (*Scyliorhinus canicula*, *G. melastomus* and *Centroscymnus coelolepis*) (unpubl. Data by Dallarés S.). The larvae infecting the musculature of these shark species were studied morphologically and identified as *G. adenoplusia* based on the oncotaxis (see Beveridge and Campbell, 2013). Therefore, and based on these results, we tentatively assign *Grillotia* larvae *ex E. spinax* included in the present study to the species *G. adenoplusia*. In the future, molecular characterization of adult specimens of this parasite will allow confirming its identity.

4.2. Infection patterns of G. adenoplusia in E. spinax

Being *Grillotia* a widely distributed cestode genus, and its plerocerci rather euryxenous, a high number of fish species can serve as intermediate or paratenic hosts in its life cycle (Palm, 2004). Regarding records of *Grillotia* plerocerci in *E. spinax*, former studies from the Mediterranean Sea have shown similar prevalence and mean abundance values with respect to present values, in the Gulf of Naples (N: 39, P%: 82.0, mA: 5.3, Santoro et al., 2021) or absence of plerocerci in case of the Balearic Sea, but based on a very low sample number (N: 11, Dallarés et al., 2017a). Up to date very few studies on the parasite communities of *E. spinax* have been conducted in the Northeast Atlantic, especially regarding complete necropsies including the analysis of the whole musculature. In relation to these studies, Klimpel at al. (2003) did not detect any *Grillotia* plerocerci in samples from South Norwegian waters, whereas *Grillotia* sp. could be among the trypanorhynch larvae infecting *E. spinax* individuals collected close to two different underwater features in northern and northwestern Spain (N:59, P%: 13.8, mA: 0.2, mI: 1.3, Isbert et al., 2015)

The present study shows that infection patterns of *Grillotia* plerocerci in the velvet belly lanternshark vary among regions within and between the three areas, namely western and eastern Mediterranean Sea and Atlantic Ocean. Previous studies and present data reveal that abundance of larval *Grillotia* sp. located in the muscle of transport hosts increases with host TL, as a result of plerocerci accumulation during host lifespan (Dallarés et al., 2017a, Santoro et al., 2021). However, despite this fact and the detected significant differences in host size among sampled regions, geographical

patterns detected for parasite descriptors are not likely explained by size distribution of the hosts examined. Indeed, in the present study larger shark specimens caught in the Atlantic regions were not necessarily more parasitised by this cestode than Mediterranean specimens (see Table 2). Accordingly, no interactions between host size and geographical regions were detected in the GLMs performed as part of the data analysis. Actually host length is not the only factor explaining parasite infection patterns in fishes, as also environmental conditions and the local trophic web affect the occurrence of parasites (Luque and Poulin, 2004) and these seem to be of more importance in the present case.

Different abiotic and biotic factors can affect parasite infection patterns in host species over spatial and temporal scales (Kuhn et al., 2016). Indeed, Kuhn et al. (2016) highlighted that abiotic factors are considered more relevant for the early life stages of parasites which are also linked to intermediate hosts of trophically transmitted cestodes. The occurrence, spatial distribution, and community composition of copepods in the marine environment, which are potential first intermediate hosts for this trypanorhynch, are influenced by currents, internal waves, and water masses with specific characteristics (Gómez et al., 2000; Molinero et al., 2009; Mohaghar et al., 2020; Hure et al., 2022). Underwater geomorphological features such as canyons and submarine mountains increase the sea bottom topography providing more complex habitats and affecting circulation of water masses. This can result in a longer retention period increasing abundance and diversity of marine taxa, such as potential parasite hosts, close to those underwater features (Ramírez-Amaro et al., 2015). Additionally, depth-related environmental conditions may result in variations observed in benthopelagic faunal assemblages (Cartes et al., 2013), favouring the aggregation of plankton organisms (e. g. crustaceans) and fishes in cases of increased turbidity (Macquart-Moulin and Patriti, 1996). For instance, *Grillotia* sp. abundance could be linked to an increased oxygen concentration since increased biomass of the potential first intermediate host (copepods) enhances under these conditions (Cartes et al., 2013; Dallarés et al., 2017b).

Regarding biotic factors affecting parasite infections, the occurrence and transmission of trophically transmitted heteroxenous parasites also depend on the availability of their other intermediate and the definitive hosts (Hudson et al., 2006; Gómez and Nichols, 2013). For example, far too low abundances of a key-host within the lifecycle of a parasite over time can result in its own disappearance (Mackenzie and Pert, 2018). At this respect, *E. spinax* seems not to be a key-host in the life cycle of *G. adenoplusia*, as infection patterns reveal rather low levels of infection in comparison to other demersal sharks (Dallarés et al., 2017b; Santoro et al., 2021). Plerocerci of this cestode species, as of most *Grillotia* spp., are rather euryxenous concerning their last intermediate host (Menoret and Ivanov, 2012). Therefore, even though in some regions, populations of the velvet belly lantern shark decrease owing to high fishing mortality (Coelho et al., 2010; Coelho et al., 2015), this may not affect the overall infection of *Grillotia* spp. within the local food webs. Other small benthic sharks hosting plerocerci of *G. adenoplusia* as evidenced by molecular identification (unpublished data) and based on comparable sample sizes (e. g. *G. melastomus* and *Scyliorhinus canicula* by Santoro et al., 2021, *G. melastomus* by Dallarés et al., 2017a) showed distinctly higher values for infection descriptors, especially regarding mean abundances (>84, 33 and >32, respectively). Those higher parasite loads by *Grillotia* sp. may indicate, that *G. melastomus* and *S. canicula* might be more appropriate intermediate hosts than *E. spinax*, which may be linked to their ecological characteristics. For instance, compared to a stronger pelagic behaviour in *E. spinax* a more benthic feeding habit described for *G. melastomus* and *S. canicula* influences the frequency of ingestion of infected prey (Carrassón et al., 1992; Fanelli et al., 2009; D'Iglio et al., 2021; Besnard et al., 2022). On the other hand, the presence or absence of the definitive host, in which adult parasites mate, reproduce and release offspring to the surrounding waters, affects the occurrence and infection parameters of those parasites in the local populations of intermediate hosts (McClelland, 2002; MacKenzie and Pert, 2018). Large pelagic and demersal sharks, rays and skates are identified as definitive hosts of *Grillotia* spp., and the bluntnose sixgill shark *Hexanchus griseus* (Bonnaterre, 1788) is known to be definitive host for *G. adenoplusia* (see Beveridge and Campbell, 2007; 2013).

While the scarce data available on its diet composition indicates that the bluntnose sixgill shark feeds on smaller sharks (Ebert, 1994; Kabasakal, 2004; Celona et al., 2005; Bizzarro et al., 2017) the contribution of small sharks potentially hosting larval *G. adenoplusia* to its diet in the Mediterranean Sea is unknown. The rarely detected kitefin shark, *Dalatias licha* (Bonnaterre, 1788) also preys on small demersal sharks such as the velvet belly lantern shark (Navarro et al., 2014; Barría et al., 2018; Mulas et al., 2021) and could thus be a potential definitive host for *G. adenoplusia*. Though, this has not been recorded up to date instead, *D. licha* has been listed as host of *G. heptanchi* and potentially other *Grillotia* spp. (Palm, 2004; Santoro et al., 2021).

4.3. Phylogeographical pattern of G. adenoplusia in E. spinax

Intraspecific divergence detected in 28S rDNA sequences was used to clarify the taxonomic status of the *Grillotia* species complex and the geographical variation among different areas. Ribosomal 18S and 28S genes are mostly used for phylogenetic studies at family and species levels, while population studies have generally focused on the spacer regions (Pereira and Baldwin, 2016). However, patterns of phylogenetic diversity using the 28S rDNA molecular marker have been described in marine organisms and its parasites (Aiken et al., 2007; Palacios-Abella et al., 2017; Redmond et al., 2011). The sequences of 28S rDNA obtained in this work showed that all analysed individuals were clearly identified as *Grillotia* sp. The hierarchical AMOVA analysis and phylogenetic reconstruction of *G. adenoplusia* carried out in this work showed a significant differentiation between the three different areas in which the cestode samples were taken (i.e. northeastern Atlantic, western and eastern Mediterranean), as well as among their populations. However, since the haplotypes of the western Mediterranean Sea have been separated into different clusters in the phylogenetic tree, they do not show a clear grouping related to the region of capture. In fact, effects of low genetic differentiation could be observed among the samples of the Alboran Sea, the Balearic Sea and the Tyrrhenian Sea, including in our analyses the sequences relative to different capture places obtained by other authors (*Grillotia* samples *ex G. melastomus*, *E. spinax* and *S. canicula* from the Gulf of Naples, Santoro et al., 2021). In contrast, the haplotypes of Cyprus samples (eastern Mediterranean Sea) were enclosed

441 in a separate clade, significantly different from the others, as well as the haplotypes of the Celtic Sea
442 and off Scotland. These differences appear to be related to areas separated by those considered to be
443 marine oceanographic barriers, particularly the Strait of Gibraltar and the Strait of Sicily (e. g. Pascual
444 et al., 2017; Sebastían et al., 2021). Oceanographic barriers can influence currents within water layers
445 and the circulation of water masses (Astraldi et al., 1999; Soto-Navarro et al., 2015) and consequently,
446 can affect the movement patterns of populations (e. g. Pascual et al., 2017; Čekovska et al., 2020,
447 Sefc et al., 2020). For instance, the Strait of Gibraltar eastward located thermal Almeria-Oran Front
448 (L'Helguen et al., 2002) is considered as an important discontinuity for genetic differences
449 ("phylogeographical break") between populations of the Atlantic Ocean and the Mediterranean Sea
450 (Patarnello et al., 2007). Reduction of genetic flow between the different basins disconnected by
451 impediments has been abundantly described for an array of marine organisms, indicating four main
452 geographical groups in the Atlantic, central Mediterranean, Aegean Sea, and Black Sea (Magoulas et
453 al., 2006; Patarnello et al., 2007). Other authors hint to 5 biogeographic districts solely in the
454 Mediterranean Sea based on different physical, chemical, and biological properties: eastern, central,
455 western, Adriatic Sea and Alboran Sea (Spanò and De Domenico, 2017). Genetic differences between
456 populations of different geographic areas are observed when populations in these areas remain mostly
457 isolated from each other and do not mix anymore (Strait of Gibraltar e. g. Comesaña et al., 2008;
458 Griffiths et al., 2011; Veríssimo et al., 2017; Strait of Sicily e. g. Viñas et al., 2010; Čekovska et al.,
459 2020; Tikochinski et al., 2020).
460 The molecular analyses of present samples showed their clustering into genetic groups that would
461 coincide with those described by Santoro et al. (2021) and add further information covering a larger
462 area within the Mediterranean Sea and regions from the Northeast Atlantic. It seems there may be an
463 inconsistency in the Mediterranean Sea, besides the Black Sea from where, unfortunately, no samples
464 were available. Haplotypes of *Grillotia* obtained in this work from samples of the western
465 Mediterranean were very similar to those from the Central Mediterranean described by Santoro et al.
466 (2021), indicating they form a panmictic unit in the western and Central Mediterranean in contrast to

467 the samples from the eastern Mediterranean. The genetic differences. detected among areas could be
468 a consequence of factors related to the behavioural characteristics (i.e. movement patterns) of the
469 hosts which are affected by local or regional environmental conditions. In general, the connectivity
470 and genetic population structure of marine and terrestrial parasites is highly affected by the mobility
471 and the general potential for dispersal of their hosts (Feis et al., 2015; Fraija-Fernández et al., 2017;
472 Tedesco et al., 2017). It is suggested that, compared to allogenic parasites with aquatic and
473 terrestrial/air-borne hosts, autogenic parasites transferred solely *via* aquatic hosts exhibit more
474 strongly structured populations (Feis et al., 2015). Though, the quoted authors indicated that few
475 studies have focused on marine environments and up to date studies on autogenic parasites often
476 referred to fragmented freshwater habitats. Feis et al. (2015) identified a strong genetic population
477 structure of an autogenic marine parasite, suggesting a limited connectivity between those
478 populations although a potential of high host dispersal was assumed. Additionally, Fraija-Fernández
479 et al. (2017) detected a patchy distribution of intermediate hosts and a limited mobility of definitive
480 hosts contributing to a significant ecological isolation of a digenean parasite.
481 Limited oceanographic barriers and less complex current systems may not affect migratory pathways
482 of hosts and consequently, do not prevent the mixing of parasite populations (Baldwin et al., 2011).
483 In contrast, more pronounced oceanographic barriers e. g. seamounts can affect regional circulation
484 patterns of water masses which can enhance regional higher primary production compared to
485 surroundings resulting in increased abundances of potential intermediate hosts (Leitner et al., 2010).
486 For instance, in the Mediterranean Sea copepods as an important component of the mesozooplankton
487 community and potential first intermediate hosts of cestodes reveal spatial and temporal differences
488 in their abundances (Siokou-Frangou et al., 2010). High density, abundance and/or biomass of
489 mesozooplankton is often associated with increased primary production events due to upwelling of
490 nutrient rich waters observed nearby hydrological features such as in the Alboran Sea close to the
491 Strait of Gibraltar and Almeria-Oran front (Siokou-Frangou et al., 2010). Additionally, a patchy

492 distribution of other intermediate and the definitive host can contribute to an ecological isolation of
493 marine parasites (Fraija-Fernández et al., 2017).
494 While genetic exchange between many teleost populations depends strongly on the larval dispersal
495 in their planktonic stage, several elasmobranchs, such as *E. spinax*, are viviparous with pups fully
496 developed to live in the benthic habitats. Some shark species are wide-ranging, but most lantern
497 sharks exhibit a more restricted distribution, including some which appear to be regional endemics
498 with a very narrow movement range (Ebert and Dando, 2021). Though, knowledge on movement and
499 migration patterns for many deep-sea sharks including *E. spinax* populations is scarce (e. g. Catarino
500 et al., 2015; McMillan et al., 2017). Currently available information hints to weak genetic
501 differentiation which indicates a certain kind of gene flow between shark populations even over large
502 distances (Straube et al., 2011; Catarino et al., 2015), which seems to apply also to *E. spinax* (Gubili
503 et al., 2016). Though, evidence based on molecular and stable isotope analyses indicate that
504 underwater features such as the Strait of Gibraltar are important bathymetric barriers for the
505 connectivity of *E. spinax* populations in the Northeast Atlantic and Mediterranean Sea (Gubili et al.,
506 2016; Besnard et al., 2022) and some authors supposed a limited dispersal behavior for this species
507 (Rees et al., 2019). In fact, using the control region sequence, a certain degree of genetic
508 differentiation between the Mediterranean and Atlantic *E. spinax* were detected, while no differences
509 were detected within the Mediterranean (Gubili et al., 2016).
510 The role of the definitive host in explaining the biogeographical patterns of parasites with indirect
511 life cycles can be even more important than that of intermediate or paratenic hosts. In this sense, a
512 highly vagile definitive host would be expected to disperse the parasite across geographical regions
513 much more than a definitive host with restricted movement capacity (Poulin et al., 2011). Related to
514 the present case, *Grillotia* species maturing in large sharks, which are likely to display higher
515 dispersal ability than the smaller intermediate or paratenic hosts involved in the life cycle of the
516 parasite, may display patterns of population structure that resemble those of their definitive hosts.
517 *Hexanchus griseus*, known definitive host for *G. adenoplusia*, as commented above, is globally

distributed across the continental shelves of tropical and temperate regions (Froese and Pauly, 2022). Contrary to what would be expected for a long-lived shark with a wide home range, genetic differentiation has been observed for this species between western and eastern Mediterranean regions, as also between the Mediterranean Sea and the North-East Atlantic Ocean (Vella and Vella, 2017). This is fully in accordance with present phylogeographical results based on sequences of the plerocerci recovered from just one of the many different paratenic hosts that seem to be involved in the life cycle of *G. adenoplusia*, and further highlights the importance of the definitive host in determining the geographical dispersal potential of marine heteroxenous parasites.

However, many factors are at play in the complex life cycles of parasites such as *Grillotia*. The geographical distribution patterns observed for species of this genus can be potentially shaped by the biological and ecological features of a wide array of intermediate, paratenic and even definitive hosts. As for the latter, the parasite fauna of large pelagic and demersal sharks is poorly characterized and there may be more than one definitive host species for *G. adenoplusia* apart from *H. griseus*. As has been reported in other heteroxenous parasites, patterns of parasite host-specificity can offset effects of host vagility (Thieltges et al., 2011). Therefore, interpretation of the observed biogeographical patterns might not be simple. Unravelling the intricate life histories of parasites will provide us with a better understanding of the patterns observed from the host individual scale to higher levels of complexity, like host populations and, ultimately, communities.

4.4. Histological assessment of G. adenoplusia in musculature of E. spinax

In the present study images of histological sections of the musculature of *E. spinax* enclosing the encysted stages of *G. adenoplusia* are presented for the first time. On the level of the host individual and based on the present histological analysis, we hypothesize that movements of *E. spinax* could be affected by a supposed reduced swimming ability, particularly in the small specimens, caused by the infection of this parasite. Our histological observations of the musculature in *E. spinax* tails exhibited a progressive formation of an outer, rigid collagen layer around the encysted parasite. Additionally,

544 especially in juveniles, the parasitic granulomas may occupy nearly half the volume on one flank of
545 the host's tail and the progressive flattening (atrophy) of the muscle fibers adjacent to them could
546 imply a loss of its functionality. This could imply an inadequate muscular contraction of the affected
547 body region with potential effects on the escape speed and/or the maneuverability of *E. spinax*, as has
548 been suggested by other authors (Dallarés et al., 2017a; Santoro et al., 2021).
549 It is supposed that smaller sharks outmaneuver attacking predators by performing short bursts and
550 sharp turns ('matador strategy') which limits the time for the predator to adjust its reaction (Blaxter
551 and Fuiman, 1990; Seamone et al., 2014). Since the suggested optimal attack strategy for larger
552 predators is approaching the prey from behind due to limited visual detection by the prey (Seamone
553 et al., 2014), escape success of the prey may depend strongly on the functionality of the tail and its
554 musculature, which might be negatively affected by infections of *Grillotia*.
555
556 **Conclusions**
557 Connectivity among fish populations, especially for non-target species of fisheries, is often
558 unknown. This applies to several elasmobranch species of which many are threatened due to diverse
559 anthropogenic impacts. Parasites, omnipresent in marine and terrestrial ecosystems, may indicate
560 connectivity or migration events in fish populations by their simple presence or spatial molecular
561 differences between infrapopulations obtained from hosts of different geographic areas.
562 The analysis of the infection patterns of complete parasite assemblages as bioindicators is
563 recommended as a tool for fish stock separation even at small spatial scales, with a specific focus on
564 permanent parasites and hosts not available for analyses in large numbers (George-Nascimiento 1996;
565 MacKenzie & Abaunza 2013; Levy et al. 2019). Nevertheless, as indicated by Poulin and Kamiya
566 (2013) single parasite species (e. g. representatives of anisakids) are regularly and successfully used
567 as reliable biological tags in studies on stock discrimination also associated with molecular
568 approaches in fishes (e. g. Mattiucci et al. 2008, 2015) and invertebrates (e. g. Pascual et al. 1996;
569 Tedesco et al. 2017).

The herein analysed cestode taxon *Grillotia* exhibited its usefulness as bioindicator as it revealed differences in its spatial infection patterns among the sampled regions and its 28S-rDNA sequences within Mediterranean regions and between the North-East Atlantic Ocean and Mediterranean Sea. The present study confirms previous research that submarine features can act as topographic barriers showing that also the connectivity of parasite infrapopulations can be affected due to limited interpopulation dispersal. This also indicates a spatial isolation of its host species which should have its implications for population management and protection measures. Future studies based on more sampling sites in a smaller spatial scale and combined with new molecular markers could provide more comprehensive results concerning the potential spatial isolation regarding single submarine features. It further underlines the usefulness of parasites as biological tags for host populations, and their potential application for the study of susceptible and data-poor species such as large-sized deep-water sharks, and that are difficult to sample due to their biological and ecological characteristics.

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Declaration of competing interest

None.

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Table 1. Region, survey/haul, month/year, coordinates, and depths of fishing surveys/hauls.

Region	Survey/haul	Month/year	Coordinates		Depth (m)
			Lat	Long	mean ± SD (range)
Balearic Sea	MEDITS GSA05	Jun 2013	39°07.16'N – 40°15.93'N	2°12.20'E – 4°31.24'E	643 ± 81 (497 – 737)
	MEDITS GSA05	Jun 2014	38°57.16'N – 40°06.81'N	2°10.92'E – 4°31.23'E	632 ± 82 (499 – 754)
	Com Fish EL	May 2015	39°10.72'N	2°34.17'E	602
	Com Fish JM	May 2015	39°38.13'N	3°44.15'E	558
	Com Fish EL	Jun 2015	39°01.49'N	2°40.47'E	551
	MEDITS GSA05	Jun 2015	39°06.93'N – 39°45.47'N	2°10.87'E – 4°31.38'E	646 ± 96 (513 – 748)
	MEDITS GSA05	Jun 2016	38°57.97'N – 40°16.00'N	2°03.20'E – 4°31.59'E	649 ± 78 (513 – 746)
	Com Fish BL	Jul 2018	41º28.25'N	2º48.63'E	335
Alboran Sea	MEDITS GSA01/02	Apr./May 2015	36°16.21'N – 35°58.84'N	2°11.94'E – 5°05.12'E	574 ± 174 (367 – 793)
Cyprus	MEDITS GSA25	Aug 2015	34°45.24'N – 34°38.38'N	33°29.97'E – 33°29.86'E	321 ± 309 (51 – 601)
Celtic Sea	NEATL (EVOHE)	Nov 2013	48°11.45'N	008°25.86'W	(412 – 496)
Scotland	NEATL (Com)	Jun 2015	59°53.02'N	006°33.14'W	500

Table 2. Region, survey/haul, month/year, number of shark individuals examined and mean total length (TL) followed by standard deviation (SD) and range of *Etmopterus spinax* sampled, as well as prevalence (P%), mean abundance (mA) and mean intensity (mI) of *Grillotia* sp. detected in the tail muscle tissue of *E. spinax*. Regional values for fish TL and parasite prevalence, abundance and intensity accompanied by different superscript capital letters indicate significant differences among regions. *indicates survey/hauls for which samples were used for molecular analysis.

Region	**Survey/haul**	**Month Year**	**No. host examined**	**Host TL (cm) mean ± SD (range)**	***Grillotia* sp. P%**	**mA ± SD**	**mI ± SD**
Balearic Sea	MEDITS GSA05	Jun 2013	37	19.3 ± 8.1 (8.0 - 41.8)	40.5	0.95 ± 1.45	2.33 ± 1.40
	MEDITS GSA05	Jun 2014	43	20.3 ± 6.1 (10.5 - 36.5)	62.8	1.60 ± 1.83	2.56 ± 1.69
	Com Fish EL*	May 2015	25	22.3 ± 5.7 (12.5 - 34.2)	76.0	1.70 ± 1.40	2.30 ± 1.20
	Com Fish JM	May 2015	4	29.2 ± 10.2 (18.6 - 28.7)	100.0	9.00 ± 7.86	9.00 ± 7.86
	Com Fish EL	Jun 2015	10	27.1 ± 5.7 (19.8 - 36.7)	80.0	2.90 ± 2.77	3.62 ± 2.62
	MEDITS GSA05*	Jun 2015	36	19.7 ± 6.7 (11.7 - 43.6)	72.2	1.69 ± 1.75	2.35 ± 1.75
	MEDITS GSA05*	Jun 2016	33	25.2 ± 7.7 (14.9 - 40.6)	81.8	2.75 ± 2.74	3.48 ± 2.64
	Com Fish BL*	Jul 2018	1	24.5 ± 0.0	100.0	–	–
	Total		189	21.7 ± 7.4 (8.0 – 43.6)^{B}	67.0^{B}	2.00 ± 2.50^{B}	2.90 ± 2.50^{A}
Alboran Sea	MEDITS GSA01/02*	Apr./May 2015	79	17.9 ± 6.6 (10.7 - 35.8)^{A}	24.0^{A}	0.50 ± 1.00^{A}	1.90 ± 1.20^{A}
Cyprus	MEDITS GSA25*	Aug 2015	45	20.2 ± 3.2 (14.0 - 25.4)^{B}	91.0^{BC}	7.00 ± 6.28^{C}	7.70 ± 6.2^{B}
Celtic Sea	NEATL (EVOHE)*	Nov 2013	28	48.0 ± 4.5 (40.5 - 57.2)^{C}	75.0^{BC}	2.39 ± 2.67^{B}	3.19 ± 2.64^{A}
Scotland	NEATL (Com)*	Jun 2015	26	36.6 ± 3.3 (31.3 - 42.8)^{D}	100.0^{C}	13.80 ± 6.82^{D}	13.80 ± 6.82^{C}

Table 3. Sampling regions and parameters: number of samples (N), number of haplotype (*h*), number of polymorphic sites (*S*), haplotype diversity (*Hd*), nucleotide diversity (π), and average number of nucleotide differences (k) were used to measure the DNA polymorphism.

	N	***h***	***S***	***Hd***	***π***	***k***
Alboran Sea	15	3	2	0.6	0.00052	0.68571
Balearic Sea	17	6	5	0.8309	0.00132	1.75000
Celtic Sea	12	4	7	0.8182	0.00185	2.45455
Cyprus	12	3	2	0.5455	0.00023	0.30303
Scotland	14	2	1	0.2637	0.0002	0.26374
Overall	70	17	18	0.9255	0.00256	3.39337

Table 4. AMOVA Analysis of molecular variance (AMOVA) results showing genetic variance for *Grillotia* sp. populations based on 28S rDNA sequence data.

Source of variation	**Sum of squares**	**Variance components**	**Percentage of variation**
Among areas	63.075	0.79297 Va	32.64907
Among regions within areas	30.458	1.01213 Vb	41.67242
Within regions	40.539	0.62367 Vc	25.67852
Total	134.071	2.42878	

Fixation Index	Va FCT= 0.32649	P=0.02248
	Vb FSC= 0.61874	P=0.0000
	Vc FST= 0.74321	P=0.0000

Figure captions

Figure 1. Origin of samples, *Grillotia* sp. larvae *ex Etmopterus spinax*, collected in five regions (red dots, from left to right: off Scotland, Celtic Sea, Alboran Sea, Balearic Sea, off Cyprus) in the Atlantic Ocean and Mediterranean Sea between 2013 and 2017.

Figure 2. Phylogenetic tree. The tree was rooted using *Hornelliella annandalei* as outgroup (not shown); Maximum likelihood bootstrap and Bayesian for posterior probabilities values >0.5, supporting non-collapsed clades, are indicated.

Figure 3. Median-Joining network of *Grillotia* sp. haplotypes. The sizes of the circles are proportional to frequencies of haplotypes.

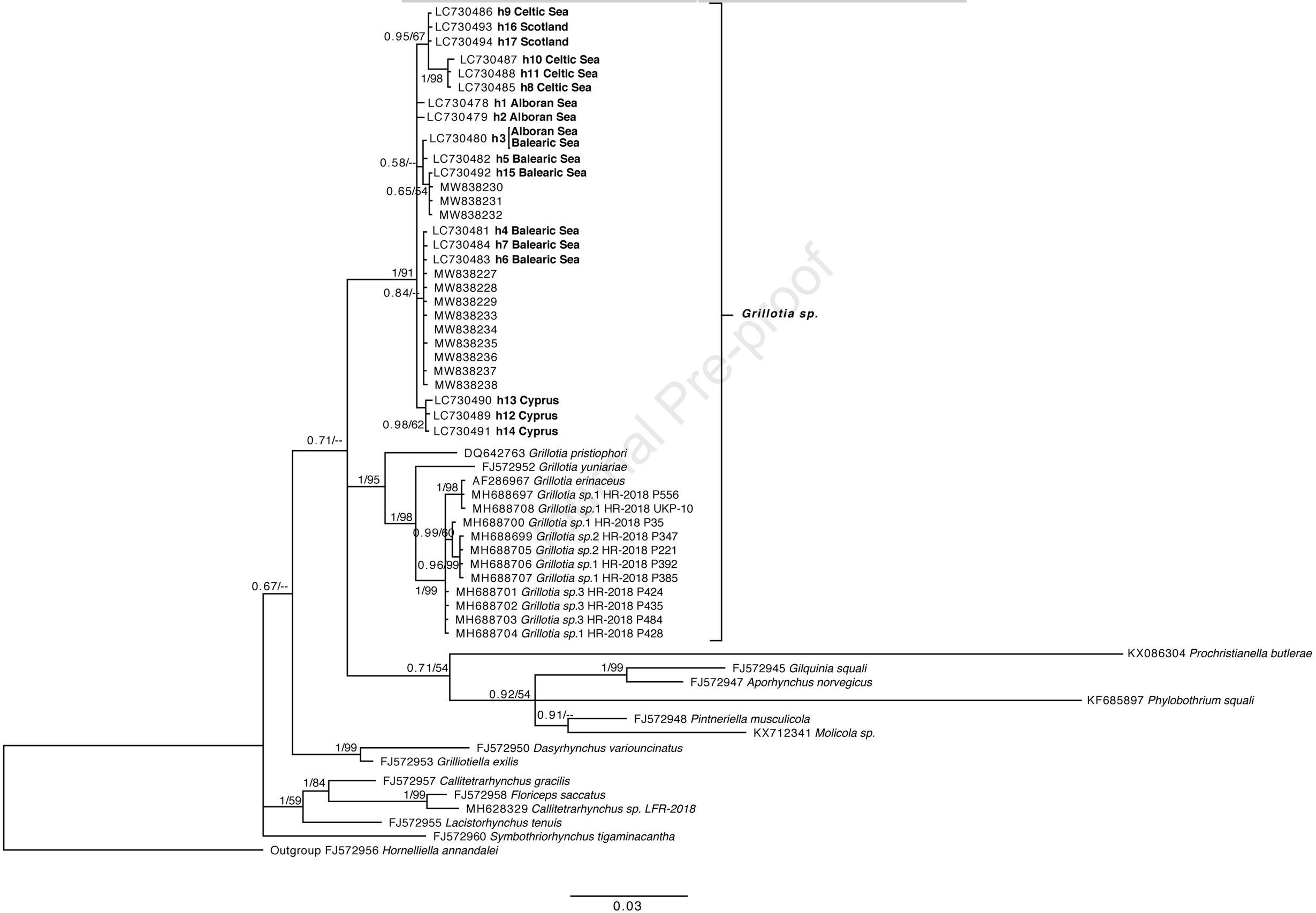
Figure 4. Encapsulated plerocerci of *Grillotia* sp. in *Etmopterus spinax* tail muscle tissue. A) The parasite is observed in the centre of the microphotograph (yellow asterisk) with the integument stained in blue, surrounded by a layer of macrophages. B) Detail of the granuloma envelope, showing the inner layer of macrophages (1), followed by a layer of epithelioid cells (2) and an outer layer of connective tissue interspersed with fibroblasts (3). Integument of parasite cyst (white asterisk). Cajal-Gallego's Trichrome stain. C-F: Micrographs of the apical part of a blastocyst *Grillotia* plerocercus ex. *Etmopterus spinax.* C) cross-section of the scolex with the "armature" of its tentacles (black arrow). D) Whole mount. E+F) Details of the tentacular apparatus, observing the bothria (white arrows) and the tentacular armature (black arrow). H/VOF staining. *Scale bars*: A, 200 µm; B, 50 µm; C, 200 µm; D, 500 µm; E 100 µm, F 50 µm.

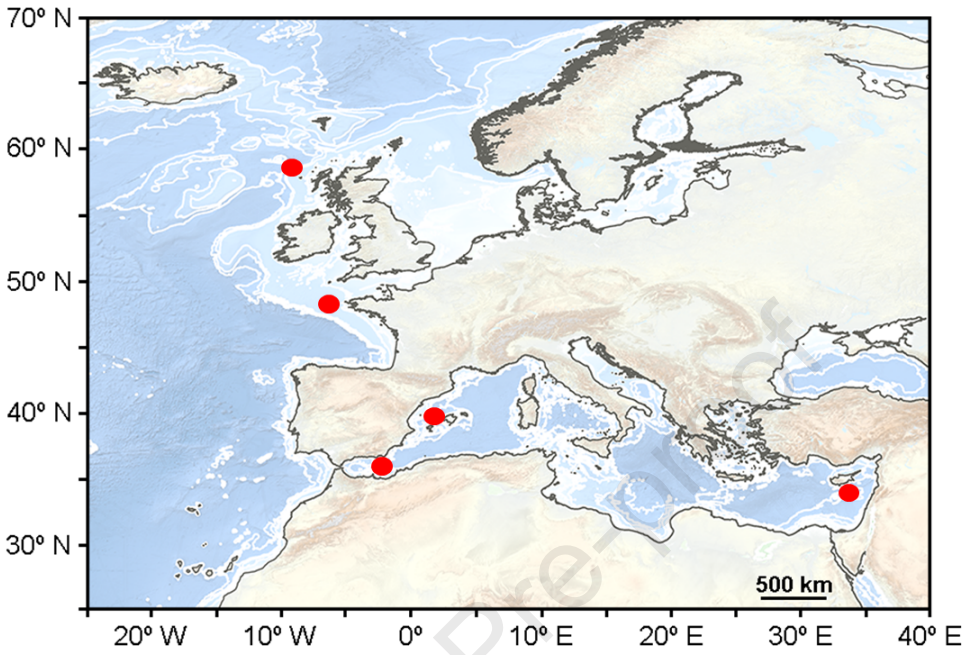
SUPPLEMENTARY MATERIAL

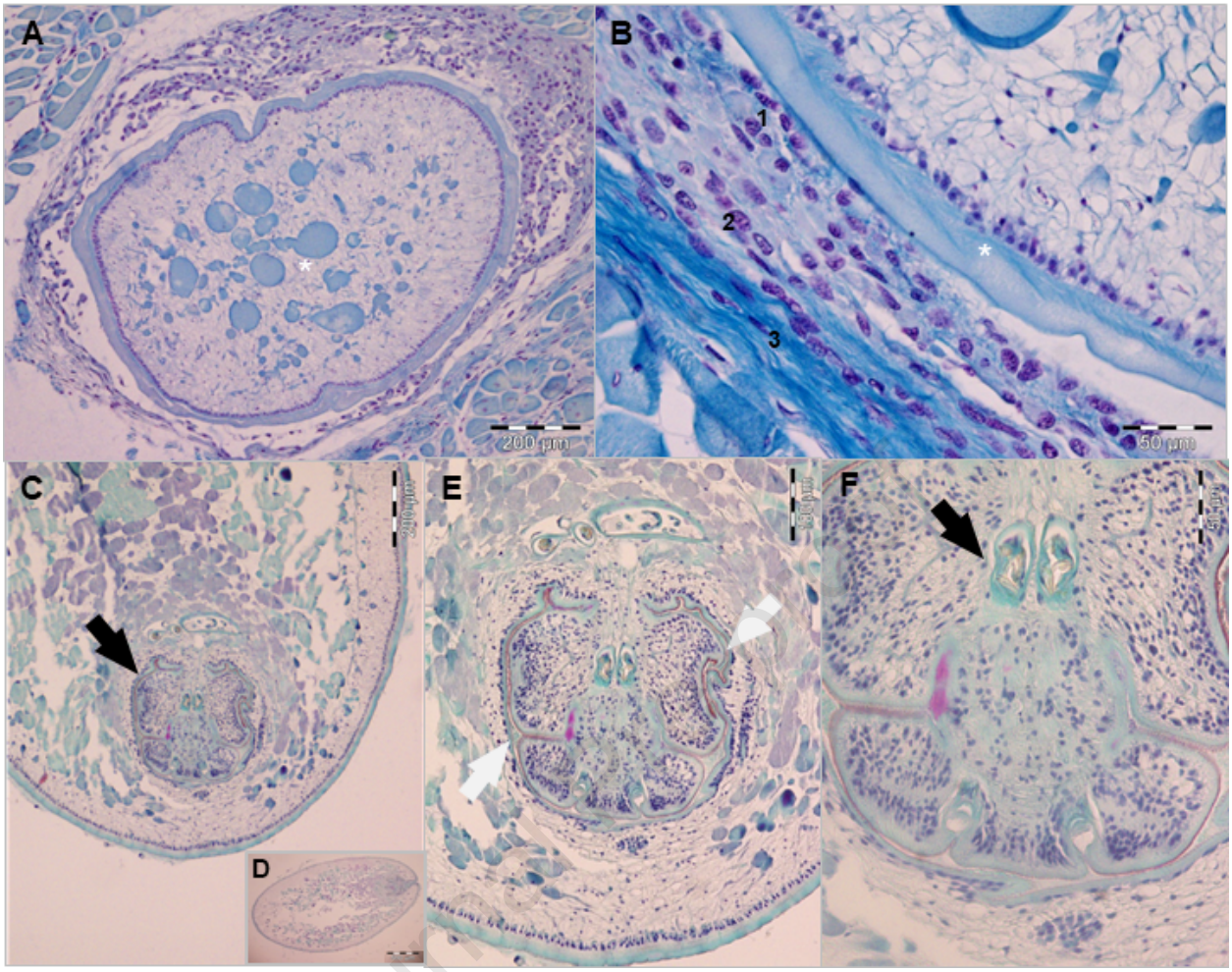
Table S1. Estimates of evolutionary divergence among 58 nucleotide sequences of cestodes. Analyses were conducted using the Tamura-Nei model genetic distance.

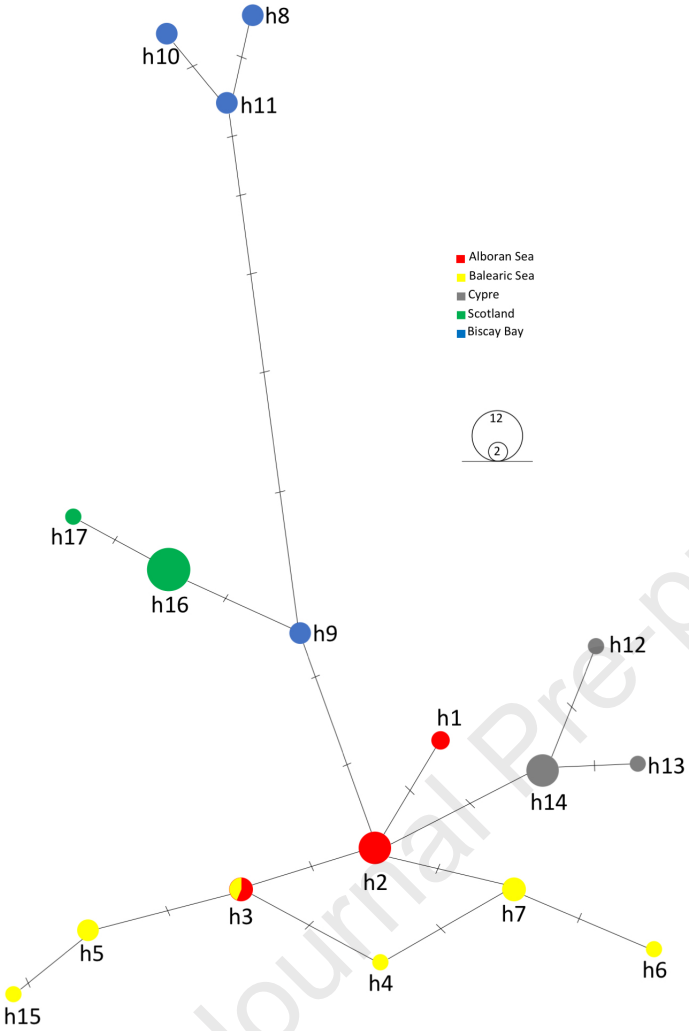
Table S2. Polymorphic sites that characterize the 17 haplotypes detected in the present study for the 28S rDNA sequences of *Grillotia* sp. Indels are indicated by hyphens while identical nucleotides with respect to haplotype 1 (h1) are indicated by dots.

Please see Excel files ("S1 Table" and "S2 Table")









Highlights:

- Cestode larvae of *Grillotia adenoplusia ex* its paratenic host *Etmopterus spinax*

- rDNA (28S) revealed genetic diversity between NE Atlantic and Mediterranean regions

- Consistent sequence cluster for the NE Atlantic and Eastern Mediterranean

- Low genetic differentiation within Western Mediterranean

- Limited interpopulation dispersal apply not only to hosts, but also to parasites

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: