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Twenty thousand parasites under the sea: a multidisciplinary approach to parasite communities of deep-dwelling fishes from the slopes of the Balearic Sea (NW Mediterranean)

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Tesis Doctoral

Twenty thousand parasites under the sea: a multidisciplinary approach to parasite communities of deep-dwelling fishes from the slopes of the Balearic Sea (NW Mediterranean)

Tesis doctoral presentada por Sara Maria Dallarés Villar para optar al título de Doctora en Acuicultura bajo la dirección de la Dra. Maite Carrassón López de Letona, del Dr. Francesc Padrós Bover y de la Dra. Montserrat Solé Rovira.

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A la niña que quería ser científica

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Cover: Drawings by the author of the fish species addressed in the present thesis.

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ABSTRACT

The Mediterranean deep-sea remains mostly unexplored and, specifically, the parasite communities of Mediterranean deep-dwelling fishes are largely unknown.

Parasites are known to be effective bioindicators. Because many of them are trophically-transmitted and show complex life cycles involving more than one host, parasites can reflect host feeding habits, trophic interactions and species composition of their ecosystems. Parasites can also reveal environmental changes of anthropogenic or natural origin, and respond to environmental conditions that may influence their own biology or their hosts' population dynamics. Furthermore, the differentiation of parasite communities across geographical gradients allows their use as discrimination tags of host populations.

Besides these applications, parasites are considered stressors to their hosts and, as such, would be expected to have an impact on fish general health condition, to alter stress markers (such as some enzymatic activities) or to induce histological alterations (such as variations of splenic or hepatic melano-macrophages) and pathological conditions.

Considering the poor existing knowledge on parasite assemblages and their dynamics in the Mediterranean deep-sea, the central purpose of the present thesis is to characterize the parasite communities infecting the following important deep-dwelling ichthyc species in the Balearic Sea: *Mora moro* (Risso, 1810), *Phycis blennoides* (Brünnich, 1768), *Galeus melastomus* Rafinesque, 1810, *Scyliorhinus canicula* (Linnaeus, 1758), *Etmopterus spinax* (Linnaeus, 1758) and *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864. Another main objective is to assess the responsiveness of these parasite communities to environmental gradients and variables, to host diet and trophic ecology and their possible impact on host health condition.

In the third chapter of the present thesis, the parasite community of the common mora *M. moro* (Gadiformes: Moridae) is described in two seasons and three localities of the Balearic Sea. This teleost shows a rich and abundant parasite fauna, with 18 different endoparasite taxa found, of which 17 constituted new host records. Significant differences were detected among groups resulting from the combination of the localities and seasons sampled, in turn related to different environmental variables, for Anisakidae gen. sp., *Anisakis* Type II and Tetraphyllidea fam. gen. sp.; therefore, these parasites were proposed as potentially useful biological tags for geographical discrimination of *M. moro* in the NW Mediterranean Sea. Detailed relationships were

found between parasite taxa and prey ingested (e.g. Anisakidae gen. sp. associated to meso-bathypelagic crustaceans, *Anisakis* Type I to benthopelagic squids). Most parasites were linked to samples with highest levels of near-bottom O_2 concentration, which is consistent with direct relationships found between near-bottom O_2 and zooplankton biomass in the Balearic Basin. Total parasite abundance and the abundance of Tetraphyllidea fam. gen. sp. showed significant positive relationships with acetylcholinesterase (AChE) activity, and the abundance of *Anisakis* Type II with lactate dehydrogenase (LDH) activity. Acetylcholinesterase activity was further associated with fish hepatosomatic index (HSI) and condition factor (K), and LDH activity with fish gonadosomatic index (GSI), K and total length (TL). Lactate dehydrogenase activity displayed differences among sampling groups. Splenic melano-macrophage centres and hepatic granulomas were not associated to fish parasite load. Positive relationships were found between the area of melano-macrophage centres and fish TL and LDH activity.

In the fourth chapter of the present thesis, the complete parasite community of *P. blennoides* (Gadiformes: Phycidae), at present unknown, is described in two bathymetric strata, four seasons and four localities of the Balearic Sea. A total of 20 different parasites were recovered, of which 11 constituted new host records. The most important parasites were the monogenean *Diclidophora phycidis,* the digeneans *Bathycreadium brayi* and *Lepidapedon* spp., the nematodes *Capillaria gracilis*, *Collarinema collaris*, *Cucullanus* sp. and *Hysterothylacium aduncum*, and the copepod *Clavella alata*. Overall, the parasite community of *P. blennoides* is characterized by high abundance, richness and diversity. Significant differences in the structure of the parasite community were detected between samples from < 1,000 and > 1,000 m depth and between samples from off the mainland and insular slopes. Significant seasonal and/or geographical differences were found for some specific parasites. Abundance of the nematode *C. collaris* was associated to high levels of turbidity and O_2 concentrations near the bottom. Abundances of *H. aduncum*, *D. phycidis*, *B. brayi* and *Lepidapedon* spp. were linked to high near-bottom temperature and salinity. Dietary analyses evidenced the role as potential intermediate hosts in parasite transmission by some prey (e.g. the teleost *Gaidropsarus biscayensis* for the cestode *Grillotia* cf. *erinaceus* and the nematodes *Anisakis* spp. or the euphausiid *Meganyctiphanes norvegica* for the acanthocephalan *Echinorhynchus* sp.). While the abundance of *B. brayi*, *C. collaris*, *Cucullanus* sp. and *Echinorhynchus* sp. was negatively linked to

AChE activity suggesting an inhibition of this enzyme as by infection-related stress, the abundance of *Echinorhynchus* sp. and *H. aduncum* correlated positively with lipid peroxidation levels, maybe due to oxidative stress linked to parasitism. Cysts of unknown etiology in fish gills were detected at higher prevalence than in any other fish from the same area. Number and area of hepatic macrophage centres seemed not significantly influenced by parasite infection levels.

In the fifth chapter of the present thesis, the parasite communities of *S. canicula* and *G. melastomus* (Carcharhiniformes: Scyliorhinidae) are described from the mainland slope of the Balearic sea at four seasons and two localities. For *S. canicula*, five parasites were recovered, of which the nematode *Proleptus obtusus* was the most important parasite in terms of prevalence and abundance. For *G. melastomus*, 13 parasites were recovered, of which the cestodes *Ditrachybothridium macrocephalum* and *Grillotia* sp. were the most frequent and abundant parasites. Overall, the parasite communities of both sharks were characterized by low mean richness and diversity, and high dominance. However, infracommunity structure and composition was significantly different between both species probably due to the consumption of different prey associated to their different bathymetric distributions. For *G. melastomus*, parasite infracommunity structure differed across seasons, with winter and spring samples grouping apart from summer and autumn ones, and between localities, with higher parasite burden in samples from off Besós than off Vilanova. The latter pattern is attributed to the vicinity of Besós to the Besós submarine canyon. *Ditrachybothridium macrocephalum* was more abundant in juvenile specimens of *G. melastomus* as a result of an ontogenic diet shift and *Grillotia* sp. accumulated in adult hosts, displaying higher abundance than in juveniles. The abundance of *Proleptus obtusus* was significantly higher in *S. canicula* than in *G. melastomus*, likely due to the higher consumption of reptantian decapods by the former. Monogenean parasites were associated to high turbidity and temperature levels, which are known to enhance monogenean infection and reproductive success. Cestodes of *G. melastomus* were linked to high turbidity and O_2 levels, which increase zooplankton biomass and thus favour the transmission of heteroxenous parasites

In the sixth chapter of the present thesis, the parasite communities of *G. melastomus*, *E. spinax* (Squaliformes: Etmopteridae) and *C. coelolepis* (Squaliformes: Somniosidae) are described between 400–2,200 m depth at two seasons and three localities off the mainland and insular slopes of the Balearic Sea. Environmental and fish biological,

parasitological, dietary, enzymatic and histological data were obtained for each specimen, and the relationships among them tested. For *G. melastomus*, *E. spinax* and *C. coelolepis* a total of 15, two and eight parasites were respectively recovered. The parasite community of *G. melastomus* is characterized by high abundance, richness and diversity, and the cestodes *D. macrocephalum* and *Grillotia adenoplusia* dominate the infracommunities of juvenile and adult specimens, respectively. A differentiation of parasite communities, linked to a diet shift, has been observed between ontogenic stages of this species. *Etmopterus spinax* displays a depauperate parasite community and that of *C. coelolepis*, described for the first time, shows moderate richness and diversity. Detailed parasite-prey relationships have been discussed and possible transmission pathways suggested for different parasites of the three hosts. Parasites were mostly related to high water turbidity and O_2 levels, which enhance zooplankton proliferation and could thus enhance parasite transmission. The nematodes *H. aduncum* and *P. obtusus* were linked to high salinity levels, as already reported by previous studies, which are associated to high biomass and diversity of benthic and benthopelagic crustaceans. A decrease of AChE activity and lower hepatosomatic index, possibly linked to infection-related stress, have been observed. Lesions associated to encapsulated larvae of *G. adenoplusia* have been observed in the muscle of *G. melastomus*, especially in the tail region, which can be indicative of the hunting strategy of its final host and may compromise the escape response of *G. melatomus* thus facilitating parasite transmission.

In the seventh chapter of the present thesis, new morphological, molecular and ecological data on the tapeworm *D. macrocephalum* based on specimens recovered from the blackmouth catshark *G. melastomus* in the western Mediterranean, are presented and discussed. A redescription of the plerocercus of this parasite, new data on juvenile and mature worms and the first description of the eggs, based on light microscopy and SEM observations, are presented. Molecular analyses based on 28S rDNA (domains D1–D3) sequences from plerocerci, immature and adult specimens revealed that they are conspecific with the specimens previously recovered from the North East Atlantic. Despite previous authors had considered that museum specimens identified as *D. macrocephalum* may represent more than one species, examination of type- and voucher material has yielded the conclusion that no relevant morphological differences exist among them or between them and the present material. Information on infection levels of *D. macrocephalum* in a large host sample (n = 170) is provided. This

parasite was more abundant in juvenile than in adult *G. melastomus* and from the middle slope with respect to the upper slope, probably related to ontogenetic and bathymetric diet shifts of its definitive host.

In the eighth chapter of the present thesis, the family Sphyriocephalidae Pintner, 1913, which comprises the genera *Hepatoxylon* Bosc, 1811, *Sphyriocephalus* Pintner, 1913 and *Heterosphyriocephalus* Palm, 2004, is revised from newly-collected and museum material. *Heterosphyriocephalus encarnae* n. sp. is described from the pelagic thresher, *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae) collected from the Pacific Ocean off Boca del Alamo, Mexico. This species can be readily distinguished from the rest of sphyriocephalids by its small size, low number of proglottids and long velum with a characteristically irregular and folded border, among other features. The tentacles show a distinctive basal armature, and a heteroacanthous typical metabasal armature with heteromorphous hooks. Redescriptions are provided for S*phyriocephalus tergestinus* and *Sphyriocephalus viridis* based on novel morphological data. Furthermore, *S. tergestinus* is allocated into *Heterosphyriocephalus* as *H. tergestinus* n. comb. based on new molecular data. New generic diagnoses are provided for *Sphyriocephalus* and *Heterosphyriocephalus*, as well as keys for the determination of genera and of species. Although the morphology of the genus *Hepatoxylon* is not addressed in the present study, the available sequence of its type-species has been incorporated in the phylogenetic analysis and its relationship to the other two genera of the family is discussed.

RESÚMEN

El Mar Mediterráneo profundo permanece en gran parte inexplorado, y, específicamente, las comunidades parásitas de los peces mediterráneos de aguas profundas son mayormente desconocidas.

Es sabido que los parásitos son efectivos bioindicadores. Debido a que muchos de ellos se transmiten por vía trófica y tienen complejos ciclos vitales que involucran a más de un hospedador, los parásitos pueden reflejar los hábitos alimentarios de sus hospedadores, interacciones tróficas y la composición de especies de sus ecosistemas. Los parásitos pueden también poner de manifiesto cambios ambientales de origen antropogénico o natural, y responder a condiciones ambientales que influyan en su propia biología o en las dinámicas poblacionales de sus hospedadores. Asimismo, la diferenciación de las comunidades de parásitos a lo largo de gradientes geográficos posibilita su uso como marcadores de discriminación de las poblaciones de hospedadores.

Al margen de estas aplicaciones, los parásitos se consideran agentes estresantes para sus hospedadores y, como tales, sería esperable que ejerzan un impacto en el estado general de salud de los peces, que alteren marcadores de estrés (como por ejemplo las actividades de algunas enzimas), o que induzcan alteraciones histológicas (como variaciones de melano-macrófagos esplénicos o hepáticos) y condiciones patológicas.

Teniendo en cuenta el poco conocimiento que se tiene de las comunidades parásitas y de sus dinámicas en el Mediterráneo profundo, el objetivo central de la presente tesis es caracterizar las comunidades parasitarias de las importantes especies ícticas de aguas profundas siguientes: *Mora moro* (Risso, 1810), *Phycis blennoides* (Brünnich, 1768), *Galeus melastomus* Rafinesque, 1810, *Scyliorhinus canicula* (Linnaeus, 1758), *Etmopterus spinax* (Linnaeus, 1758) y *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864. Otro propósito importante es evaluar la respuesta de estas comunidades a gradientes y variables ambientales, a la dieta y a la ecología trófica de los hospedadores y su posible impacto en el estado de salud de los estos últimos.

En el tercer capítulo de la presente tesis, la comunidad parasitaria de la mora común *M. moro* (Gadiformes: Moridae) se describe en dos estaciones y tres localidades del Mar Balear. Este teleósteo muestra un parasitofauna rica y abundante, con 18 endoparásitos hallados distintos, de los cuales 17 constituyen nuevas citas para este hospedador. Se detectaron diferencias significativas entre los grupos resultantes de la combinación de

las localidades y las estaciones muestreadas, asociadas a su vez a distintas variables ambientales, para Anisakidae gen. sp., *Anisakis* Tipo II y Tetraphyllidea fam. gen. sp.; por lo tanto, estos parásitos son sugeridos como marcadores biológicos útiles para la discriminación geográfica de *M. moro* en el NO del Mar Mediterráneo. Se hallaron relaciones detalladas entre taxones parásitos y presas ingeridas (e.g. Anisakidae gen. sp. Asociado a crustáceos meso-batipelágicos, *Anisakis* Tipo I a calamares bentopelágicos). La mayor parte de los parásitos se asociaron a muestras con máximos niveles de concentración de O_2 cerca del fondo, lo que es coherente con las relaciones directas halladas entre el O_2 cerca del fondo y la biomasa de zooplancton en la Cuenca Balear. La abundancia total de parásitos y la de Tetraphyllidea fam. gen. sp. mostraron relaciones positivas significativas con la actividad de la acetilcolinesterasa (ACe), y la abundancia de *Anisakis* Tipo II con la de la lactato deshidrogenasa (LDH). La actividad de la ACe estaba además asociada con el índice hepatosomático (IHS) y con el factor de condición (FC) de los peces, y la actividad de la LDH con el índice gonadosomático, el FC y la longitud total (LT) de los peces. La actividad de la LDH mostró diferencias entre grupos muestrales. Los centros melano-macrofácigos esplénicos y los granulomas hepáticos no se encontraron asociados a la carga parasitaria. Se hallaron relaciones positivas entre el área de los centros melano-macrofágicos y la LT y la actividad de la LDH de los peces.

En el cuarto capítulo de la presente tesis, la comunidad parásita completa de *P. blennoides* (Gadiformes: Phycidae), desconocida por ahora, se describe en dos estratos batimétricos, cuatro estaciones y cuatro localidades del Mar Balear. Se halló un total de 20 parásitos distintos, de los que 11 constituían nuevas citas para este hospedador. Los parásitos más importantes eran el monogeneo *Diclidophora phycidis*, los digeneos *Bathycreadium brayi* y *Lepidapedon* spp., los nematodos *Capillaria gracilis*, *Collarinema collaris*, *Cucullanus* sp. e *Hysterothylacium aduncum* y el copépodo *Clavella alata*. En conjunto, la comunidad parásita de *P. blennoides* se caracteriza por alta abundancia, riqueza y diversidad. Se detectaron diferencias significativas en la estructura de la comunidad parásita entre muestras de < 1,000 y > 1,000 m de profundidad y entre muestras procedentes de las vertientes continental e insular. Se hallaron diferencias estacionales y/o geográficas significativas para algunos parásitos concretos. La abundancia del nematodo *C. collaris* se asoció a niveles altos de turbidez y de concentraciones de O_2 cerca del fondo. Las abundancias de *H. aduncum*, *D. phycidis*, *B. brayi* y *Lepidapedon* spp. se asociaron a alta temperatura y salinidad cerca

del fondo. Los análisis de dieta evidenciaron el papel como hospedadores intermediarios en la transmisión de parásitos de algunas presas (e.g. el teleósteo *Gaidropsarus biscayense* para el cestodo *Grillotia* cf. *erinaceus* y los nematodos *Anisakis* spp. o el eufausiáceo *Meganyctiphanes norvegica* para el acantocéfalo *Echinorhynchus* sp.). Mientras que las abundancias de *B. brayi*, *C. collaris*, *Cucullanus* sp. y *Echinorhynchus* sp. se relacionaron negativamente con la actividad de la ACe sugiriendo la inhibición de esta enzima por el estrés asociado a las infecciones parásitos, las abundancias de *Echinorhynchus* sp. y *H. aduncum* se relacionaron positivamente con los niveles de peroxidación de lípidos, quizás debido a estrés oxidativo asociado a parasitismo. Se detectaron quistes de etiología desconocida en las branquias de los peces, a niveles de prevalencia superiores a los mostrados por cualquier otro pez de la misma área. El número y área de los centros macrofágicos hepáticos no parecieron significativamente afectados por los niveles de infección parasitaria.

En el quinto capítulo de la presente tesis, las comunidades parásitas de *S. canicula* y *G. melastomus* (Carcharhiniformes: Scyliorhinidae) se describen en la vertiente continental del Mar Balear en cuatro estaciones y dos localidades. Para *S. canicula*, se hallaron cinco parásitos, de los cuales el nematodo *Proleptus obtusus* era el parásito más abundante en términos de prevalencia y abundancia. Para *G. melastomus*, se hallaron 13 parásitos, de los cuales los cestodos *Ditrachybothridium macrocephalum* y *Grillotia* sp. eran los parásitos más frecuentes y abundantes. En conjunto, las comunidades parasitarias de ambos tiburones se caracterizaron por baja riqueza y diversidad medias, y por una alta dominancia. Sin embargo, la estructura y la composición de las infracomunidades eran significativamente distintas entre ambas especies debido al consumo de distintas presas asociadas a sus distintas distribuciones batimétricas. Para *G. melastomus*, la estructura de las infracomunidades parásitas difería entre estaciones, con las muestras de invierno y primavera agrupadas aparte de las de verano y otoño, y entre localidades, con mayor carga parasitaria en las muestras de Besós que en las de Vilanova. Este último patrón se atribuye a la vecindad de Besós al cañón submarino del mismo nombre. *Ditrachybothridium macrocephalum* era más abundante en juveniles de *G. melastomus* como resultado de un cambio ontogénico de dieta y *Grillotia* sp. se acumulaba en los hospedadores adultos, en los que mostró mayor abundancia que en juveniles. La abundancia de *P. obtusus* era significativamente mayor en *S. canicula* que en *G. melastomus*, probablemente debido al mayor consumo de decápodos reptantia por el primero. Los parásitos monogeneos se relacionaron con altos niveles de turbidez y

temperatura, que se sabe que estimulan la infección por monogeneos y su éxito reproductivo. Los cestodos de *G. melastomus* se asociaron a altos niveles de turbidez y de O_2, que aumentan la biomasa de zooplancton y favorecen por tanto la transmisión de los parásitos heteroxenos.

En el sexto capítulo de la presente tesis, las comunidades parásitas de *G. melastomus*, *E. spinax* (Squaliformes: Etmopteridae) y *C. coelolepis* (Squaliformes: Somniosidae) se describen entre 400–2,200 m de profundidad en dos estaciones y tres localidades de las vertientes continental e insular del Mar Balear. Datos ambientales y biológicos, parasitológicos, dietarios, enzimáticos e histológicos de los peces fueron obtenidos para cada espécimen, y las relaciones entre ellos fueron testadas. Para *G. melastomus*, *E. spinax* y *C. coelolepis* se hallaron un total de 15, dos y ocho parásitos, respectivamente. La comunidad parásita de *G. melastomus* se caracteriza por alta abundancia, riqueza y diversidad, y los cestodos *D. macrocephalum* y *Grillotia adenoplusia* dominan las infracomunidades de especímenes juveniles y adultos, respectivamente. Una diferenciación de las comunidades parásitas, asociada a un cambio de dieta, se observó entre estadíos ontogénicos de esta especie. *Etmopterus spinax* muestra una comunidad parásita depauperada y la de *C. coelolepis*, descrita por primera vez, muestra una riqueza y diversidad moderadas. Se han discutido relaciones detalladas entre parásitos y presas, y se han sugerido posibles vías de transmisión para distintos parásitos de los tres hospedadores. Los parásitos se relacionaron mayormente con altos niveles de turbidez y de O_2, que estimulan la proliferación del zooplancton y pueden por lo tanto promover la transmisión parasitaria. Los nematodos *H. aduncum* y *P. obtusus* se asociaron a altos niveles de salinidad, como ya había sido reportado por estudios anteriores, que a su vez de asocian a alta biomasa y diversidad de crustáceos bentónicos y bentopelágicos. Se observaron un descenso de la actividad de la ACe y un menor índice hepatosomático, posiblemente ligados a estrés asociado a las infecciones parasitarias. Se detectaron lesiones asociadas a larvas encapsuladas de *G. adenoplusia* en la musculatura de *G. melastomus*, especialmente en la región caudal, lo que podría comprometer la respuesta de huida del tiburón frente a depredadores y por tanto promover la transmisión del parásito.

En el séptimo capítulo de la presente tesis, se presentan y discuten nuevos datos morfológicos, moleculares y ecológicos del cestodo *D. macrocephalum* a partir de especímenes extraídos del tiburón bocanegra *G. melastomus* en el oeste del Mediterráneo. Se aportan una redescripción del plerocerco de este parásito, nuevos

datos de parásitos juveniles y adultos y la primera descripción de los huevos, en base a observaciones por microscopía óptica y SEM. Los análisis moleculares a partir de secuencias del ARNr del 28S (dominios D1–D3) de plerocercos y especímenes juveniles y adultos han revelado que éstos son conspecíficos con especímenes previamente encontrados en el noreste del Atlántico. A pesar de que autores previos habían considerado que los especímenes de museo identificados como *D. macrocephalum* podrían pertenecer a más de una especie, el análisis de material-tipo y de vouchers ha arrojado la conclusión de que no existen diferencias morfológicas relevantes entre los individuos de ese material, ni entre ellos y los del Mediterráneo. Se aporta información acerca de los niveles de infección de *D. macrocephalum* en una muestra amplia de hospedadores (n = 170). Este parásito era más abundante en hospedadores juveniles que en adultos y en el talud medio que en el talud superior, probablemente como consecuencia de cambios ontogénicos y batimétricos en la dieta de su hospedador definitivo.

En el octavo capítulo de la presente tesis, la familia Sphyriocephalidae Pintner, 1913, que comprende los géneros *Hepatoxylon* Bosc, 1811, *Sphyriocephalus* Pintner, 1913 y *Heterosphyriocephalus* Palm, 2004, se revisa a partir de material nuevo y de museo. Se describe la especie *Heterosphyriocephalus encarnae* n. sp. del tiburón zorro *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae), recolectada en el Océano Pacífico, en Boca del Alamo, México. Esta especie se puede distinguir fácilmente del resto de miembros de la familia por su pequeño tamaño, bajo número de proglótides y largo velo con un margen plegado y característicamente irregular, entre otras características. Los tentáculos muestran una armadura basal distintiva, y una armadura metabasal heteroacanta típica con ganchos heteromorfos. Se proporcionan redescripciones para *Sphyriocephalus tergestinus* y *Sphyriocephalus viridis* a partir de nuevos datos morfológicos. Además, S. tergestinus se asigna al género *Heterosphyriocephalus* como *H. tergestinus* n. comb. en base a nuevos datos moleculares. Se proporcionan nuevas diagnosis genéricas para *Sphyriocephalus* y *Heterosphyriocephalus*, así como claves para la determinación de géneros y especies. Aunque la morfología del género *Hepatoxylon* no se trata en el presente estudio, la secuencia disponible de su especie tipo se ha incorporado al análisis filogenético y su relación con los otros dos géneros de la familia se discute.

CHAPTER 1 - INTRODUCTION

INTRODUCTION

1.1. The Mediterranean deep-sea

The Mediterranean Sea is a semi-enclosed water body communicating with the Atlantic Ocean through the Strait of Gibraltar and with particular hydrographic, physical and biological features. It is a highly dynamic system and has an active overturning circulation, with one shallow and two deep circulation cells (the latter located in each of the two main basins) (Tanhua et al., 2013).

The Mediterranean mean depth is around 2,000 m and reaches its maximum value of 5102 m in the Matapan trench, in the Ionian Sea (eastern basin) (Türkay, 2004; Sardà et al., 2009). The deep-sea, usually considered to start at the break of the continental shelf, at around 200 m depth (Thistle, 2003; Sardà et al., 2004), comprises an approximate 83% of the total Mediterranean area (Türkay, 2004).

The Mediterranean Sea is a concentration basin with a negative hydrographic balance due to high evaporation rates, and consequently, salinity levels are high (about 38 psu in the western basin and 39 psu in the eastern one (Türkay, 2004)). Since the cold Atlantic deep waters cannot surpass the Gibraltar sill, Mediterranean deeper layers originate by the sinking of surface waters and, therefore, their temperature is also unusually high (*ca.* 13 ºC vs. 1-4ºC in oceanic waters) (Klein and Roether, 2004; Türkay, 2004). In consequence, many species adapted to the temperatures of Atlantic shallow waters have expanded their vertical distribution and colonized deeper habitats in the Mediterranean (Türkay, 2004), the so-called submergence effect (Ekman, 1953). Regarding to nutrient concentrations, the Mediterranean is an oligotrophic environment, mainly attributed to the antiestuarine water circulation at the Strait of Gibraltar, where the eastward flow of Atlantic nutrient-poor surface waters is compensated by a westward countercurrent of Mediterranean nutrient-rich deep waters (Huertas et al., 2012). Moreover, the high temperatures of Mediterranean waters enhance bacterial degradation of sinking organic particles, in such a way that most of them do not reach the seafloor (Stefanescu et al., 1993; Türkay, 2004). These factors are considered to account for the lesser richness and abundance of the deep-dwelling benthic fauna in the Mediterranean with respect to the Atlantic (Pérès, 1985; Stefanescu et al., 1992a; Türkay, 2004), as well as for the smaller body size and feeding rates often reported for Mediterranean deep-sea animals (Stefanescu et al., 1992b; Carrassón and Cartes, 2002; Carrassón and Matallanas, 2002).

The main habitats found in the Mediterranean deep-sea are the continental slopes and the abyssal plains (Türkay, 2004). Traditionally, the Mediterranean continental slope has been divided into three distinct regions: the upper slope (*ca.* between 200-800 m), the middle slope (*ca.* between 800-1,400 m) and the lower slope (*ca.* below 1,400 m) (Emig, 1997). Organic matter, mainly of terrestrial origin, is easily transported down the steep slopes (especially through submarine canyons) through cascading events of dense shelf waters (Foglini et al., 2016). As a result, benthic faunal communities are better sustained in the slope than in the abyssal depths: indeed, abundance, biomass and richness of deep-sea faunal assemblages generally decrease with depth, so that the richest communities are found in the upper and middle slopes while in the abyssal plains they remain comparatively sparse and depauperate (Cartes and Sardà, 1992; Stefanescu et al., 1993; Tecchio et al., 2011).

1.1.1. The study area: the Balearic Sea

The Balearic Sea is a subdivision of the Mediterranean Sea located on its western basin and delimited by the coastline of Spain and the Balearic Islands (IHO, 1953) (Fig. 1). The maximum depth in the Balearic Sea is of approximately 2400 m (Salat and Font, 1987) and its hydrographic characteristics are fairly homogeneous (Stefanescu et al., 1992a), with almost constant temperature and salinity values below 150 m (Stefanescu et al., 1993).

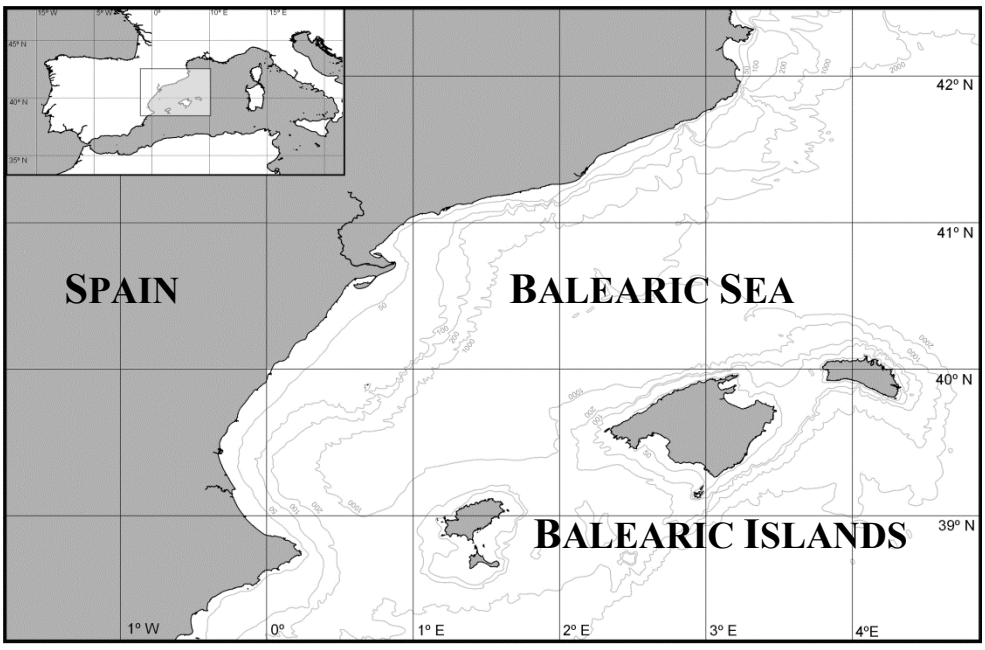

Figure 1. Map of the study area

However, different environmental and hydrographic conditions, in turn associated to different dynamics of trophic resources, are found between the mainland and the insular slopes. A complex system of submarine canyons extending from the Catalan coast crosses the mainland slope and transfers organic matter to the deep sea through cascading events (Sardá et al., 2004; Sanchez-Vidal et al., 2009; Duran et al., 2014). Water turbidity and O_2 concentration are known to favor the proliferation of invertebrate communities and are more elevated in the mainland slope (Cartes et al., 2013). As a result, enhanced biomass and faunal abundance and more complex trophic webs are found on the mainland with respect to the insular slope of the Balearic Sea (Cartes et al., 2009; Fanelli et al., 2013).

Inputs of organic material follow seasonal patterns, mainly associated to the stratification of the water column from spring to autumn, the late winter surface bloom of production and patterns of continental discharges through submarine canyons (Papiol et al., 2013, 2014; Rumolo et al., 2015). These are linked to the availability of food sources for the slope communities and determine their trophic, population and reproduction dynamics (Cartes et al., 2008; Papiol et al., 2014).

In addition, an heterogeneous depth-related nutrient distribution, alongside with different environmental conditions and fluxes of water masses, (e.g. the Levantine Intermediate water, characterized by low O_2 and high temperature and salinity levels (Aguzzi et al., 2013)), are found throughout the slopes (Puig and Palanques, 1998; Cartes et al., 2013). All these factors govern peaks of invertebrate populations (e.g. zooplankton) and are translated into important bathymetric changes regarding the composition and structure of deep-sea demersal faunal assemblages (Stefanescu et al., 1993; Cartes et al., 2004, 2009, 2013).

1.2. Importance of two large-sized gadiforms and four abundant chondrichthyans of the Mediterranean deep-sea

Among the deep-dwelling teleost species of the Balearic Sea, the common mora *Mora moro* (Risso, 1810) and the greater forkbeard *Phycis blennoides* (Brünnich, 1768) are species of large size that dominate, in terms of biomass, the ichthyofaunal assemblages found in the upper and middle slopes (Stefanescu et al., 1993; D'Onghia et al., 2004). Among the chondrichthyans inhabiting these same waters, the blackmouth catshark *Galeus melastomus* Rafinesque, 1810, the lesser spotted dogfish *Scyliorhynus canicula*

(Linnaeus, 1758), the velvet belly *Etmopterus spinax* (Linnaeus, 1758) and the Portuguese dogfish *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864 are the most important species, in terms of abundance, that can be found along the slopes (Massutí and Moranta, 2003).

1.2.1. The common mora, *Mora moro* (Risso, 1810)

Mora moro (Gadiformes: Moridae) (Fig. 2) is a worldwide-distributed species usually inhabiting depths below 1000 m in the Balearic sea, where it is one of the main contributors to biomass between 1000 and 1400 m (Stefanescu et al., 1992a; Froese and Pauly, 2016). While adults of *M. moro* live in close association with the sea-floor, juveniles have been suggested to have a pelagic existence (Stefanescu et al., 1992b). The diet of this species is mainly based on large epi- and suprabenthic prey, including crustaceans, cephalopods and fishes (Carrassón et al., 1997; Carrassón and Cartes, 2002). As suggested by its large size and high fecundity, it is a fish of high energy requirements (Gordon and Duncan, 1985), and its disappearance below 1400 m is likely a consequence of the scarcity of feeding resources in the lower slope (Carrassón et al., 1997). *Mora moro* is not a targeted species by commercial fisheries and its commercial importance is thus moderate (Froese and Pauly, 2016; IUCN, 2016).

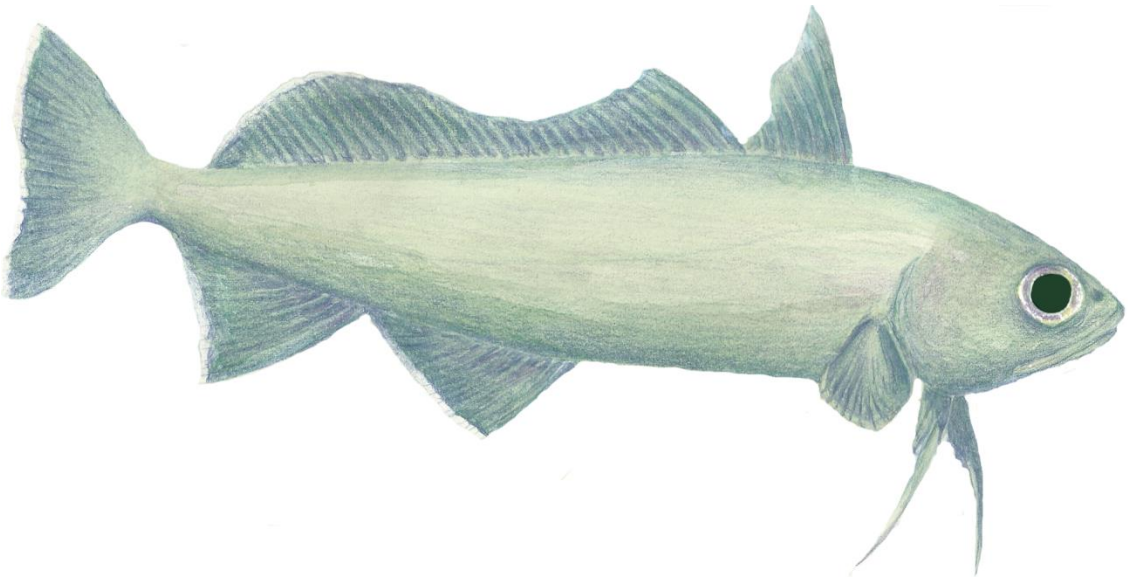

Figure 2. *Mora moro* (Risso, 1810)

1.2.2. The greater forkbeard, *Phycis blennoides* (Brünnich, 1768)

Phycis blennoides (Gadiformes: Phycidae) (Fig. 3) is a benthopelagic species distributed in the continental shelves and slopes of the northeastern Atlantic Ocean and the Mediterranean Sea (Froese and Pauly, 2016). In the Balearic Sea, it is a dominant species in the upper slope, although it has been found down to 1300 m depth (Stefanescu et al., 1992a; Cartes et al., 2004). Like *M. moro*, it is a large fish with high energetic demands (Carrassón and Cartes, 2002). Its diet is based on benthic prey, among which crustacean decapods are of major importance (MacPherson, 1978; Morte et al., 2002; Papiol et al., 2014). An ontogenic diet shift is known for this species, with young specimens feeding on small crustaceans (e.g. mysids, amphipods and isopods) and adult fishes consuming larger prey (e.g. natantia, reptantia, teleosts) (MacPherson, 1978; Morte et al., 2002). *Phycis blennoides* also shows an ontogenic migration, with juvenile specimens distributed up to 700 m depth and adults inhabiting deeper grounds (Massutí et al., 1996). This phenomenon is in all likelihood related to the different feeding habits of juvenile and adult specimens coupled with the bathymetric distribution of their respective prey (Massutí et al., 1996). It is a commercially exploited species in Atlantic and Mediterranean waters (Froese and Pauly, 2016).

Figure 3. *Phycis blennoides* (Brünnich, 1768)

1.2.3. The blackmouth catshark, *Galeus melastomus* Rafinesque, 1810

Galeus melastomus (Carcharhiniformes: Scyliorhinidae) (Fig. 4) inhabits the outer continental shelves and the slopes of the northeastern Atlantic Ocean and the Mediterranean Sea (Froese and Pauly, 2016). It is one of the most abundant sharks in the Balearic Sea (Massutí and Moranta, 2003) and the most important one in the upper and middle slopes in terms of abundance and biomass (Carrassón et al., 1992; Massutí and Moranta, 2003; D'Onghia et al., 2004). This small demersal species consumes benthic and benthopelagic fauna, among which natantian decapods, fishes and cephalopods are the most common prey (Carrassón et al., 1992; Valls et al., 2011). A marked ontogenic diet shift has been reported, with adults selecting large-sized prey such as cephalopods, fishes and large crustaceans, and juveniles consuming mostly small crustaceans, with cephalopods and fishes being of low importance (Carrassón et al., 1992). *Galeus melastomus* also shows an ontogenic migration, with youngest specimens being most abundant above a depth of 700 m and adults inhabiting the greatest depths of its bathymetric range, at depths free of trawling activity (Carrassón et al., 1992; Massutí and Moranta, 2003; Papiol et al., 2014). It is an important by-catch in the Balearic Sea (Carbonell et al., 2003), and adult specimens are commonly traded and consumed in some areas (Erzini et al., 2001; Abella and Serena, 2005).

Figure 4. *Galeus melastomus* Rafinesque, 1810

1.2.4. The lesser spotted dogfish, *Scyliorhynus canicula* (Linnaeus, 1758)

Scyliorhynus canicula (Carcharhiniformes: Scyliorhinidae) (Fig. 5) is a small shark distributed in the northeastern Atlantic ocean and the Mediterranean Sea (Capapé, 1997; Froese and Pauly, 2016). Alongside with *G. melastomus*, it is one of the most common sharks in the Balearic Sea (Massutí and Moranta, 2003). This species dominates the elasmobranch fauna of the continental shelf of the Balearic Sea, showing its peak of abundance around 100 m depth, although it can be also found in the upper slope down to 500 m (Massutí and Moranta, 2003). In the northwestern Mediterranean, the diet of *S. canicula* is fairly generalist and based on crustaceans, polychaetes and teleosts (Valls et al., 2011). A more diverse diet based on larger prey as body size increases has been reported, with juveniles consuming mainly crustaceans and adults switching to a diet with a higher proportion of teleosts (Valls et al., 2011). This diet shift is coupled with a bathymetric segregation of maturity stages: while juvenile specimens inhabit deeper grounds, adults are located in shallower waters (Massutí and Moranta, 2003; Valls et al., 2011). *Scyliorhinus canicula* is a very common by-catch in the northwestern Mediterranean (Carbonell et al., 2003), although it has commercial value in some Mediterranean regions (Capapé et al., 2008).

Figure 5. *Scyliorhinus canicula* (Linnaeus, 1758)

1.2.5. The velvet belly, *Etmopterus spinax* (Linnaeus, 1758)

Etmopterus spinax (Squaliformes: Etmopteridae) (Fig. 6) is distributed in the eastern Atlantic ocean and the Mediterranean Sea (Compagno et al., 2005). In the Mediterranean Sea, this small-sized shark shows its maximum peak of abundance between 800-1200 m (Stefanescu et al., 1992a; D'Onghia et al., 2004) and is one of the most abundant sharks below 1000 m (Carrassón et al., 1992). In the northwestern Mediterranean, *E. spinax* shows strongly pelagic habits and a poorly diversified diet essentially composed of cephalopods and teleosts, the former more abundant in adult specimens (Carrassón et al., 1992; Valls et al., 2011). It displays a differential bathymetric distribution according to size, with larger specimens located at deeper waters (Carrassón et al., 1992). Although it is captured as a by-catch by deep-water commercial fisheries, its commercial importance is very low (Coelho et al., 2015).

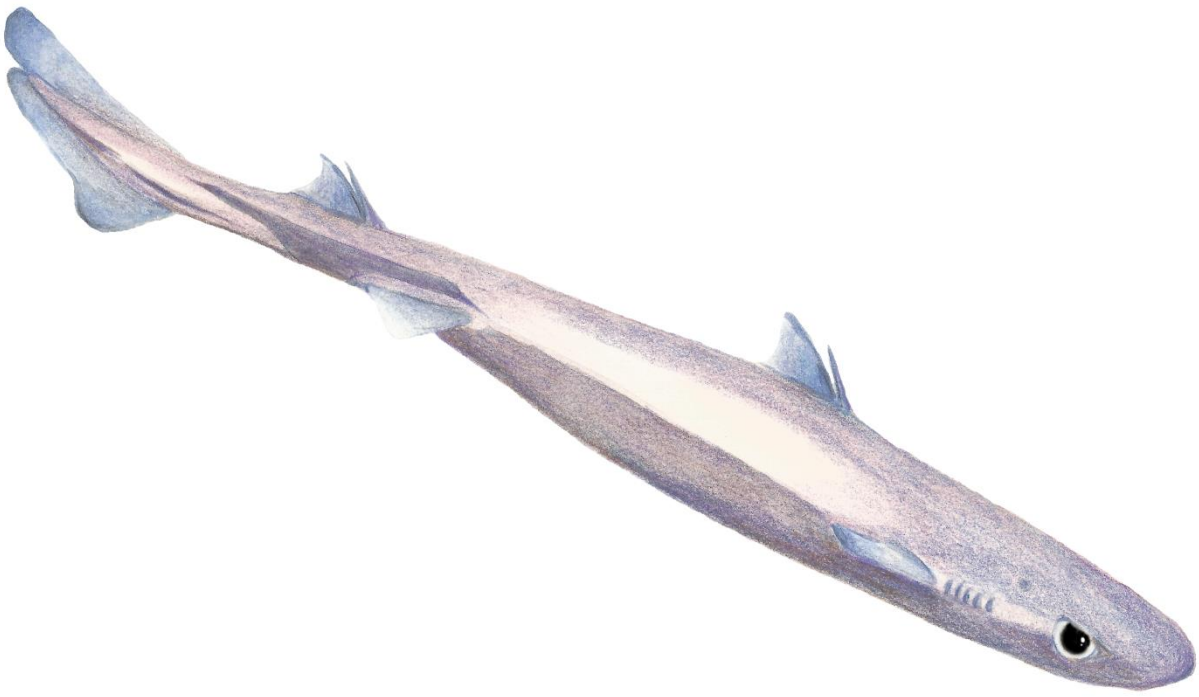

Figure 6. *Etmopterus spinax* (Linnaeus, 1758)

1.2.6. The portuguese dogfish, *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864

Centroscymnus coelolepis (Squaliformes: Somniosidae) (Fig. 7) is a bathydemersal shark inhabiting deep waters of the Atlantic, Indian and western Pacific oceans, as well as the Mediterranean Sea (Froese and Pauly, 2016). In the Mediterranean, this species inhabits deeper grounds than in the Atlantic or Pacific oceans (Carrassón et al., 1992; Bañón et al., 2006). Actually, in Mediterranean waters it is almost exclusively found on the lower slope (between *c.a.* 1500 and >2500 m depth), where it is the only abundant shark (Carrassón et al., 1992). *Centroscymnus coelolepis* has a diet characterized by poor diversity and based on nectobenthic prey (Carrassón et al., 1992; Carrassón and Cartes, 2002; Cartes et al., 2016), although moderate scavenging habits have also been reported (Carrassón et al., 1992; Cartes et al., 2016). Cephalopods are by far the preferred prey and, in a much lower proportion, decapod crustaceans and fishes are also consumed (Carrassón et al., 1992). This shark shows an important ontogenic diet shift: while cephalopods constitute practically the totality of prey in adult specimens, the diet of juveniles also includes natantian decapods in a good proportion (Carrassón et al., 1992). A possible trend in sexual and ontogenic segregation by depth has been suggested (Carrassón et al., 1992; Bañón et al., 2006), although available data are insufficient to confirm this hypothesis. Although its commercial value is generally low, in the northeast Atlantic (Portugal) it is moderately exploited and one of the most important commercial deep-dwelling sharks (Bañón et al., 2006; Froese and Pauly, 2016).

Figure 7. *Centroscymnus coelolepis* Barbosa du Bocage and de Brito Capello, 1864

1.3. Parasites in the deep-sea environment

The XIX century naturalist H. N. Moseley, on returning from the Challenger expedition, considered the most important early contribution to deep-sea biology, stated that 'the unhappy deep-sea animals have not escaped their parasites in their cold and gloomy retreat' (Moseley, 1880). This observation highlighted the ubiquitous nature of parasitism in the marine environment, which at that time was not as obvious as it may seem today. Actually, parasitism is so successful that has evolved in almost every phylum of the animal kingdom, as well as in many groups of plants, and the number of parasitic species outnumbers by far that of non-parasitic ones (Roberts and Janovy, 2009).

Sampling the deep is difficult and costly; these facts coupled with the huge extension of the deep-sea environment yield a scenario in which the vast majority of it remains unexplored (Levinton, 2011). As a consequence, there is still a great lack of knowledge on the parasites infecting deep-sea hosts, for which life cycles, host relationships, distribution and zoogeography are largely unknown (Bray, 2005). The fact that fishes recovered from deep waters often evert their stomach as a result of pressure changes during their capture and transport to the surface, with the subsequent loss of gut contents (parasites among them) (Bray et al., 1999), does not help.

In spite of these difficulties, important efforts have been undertaken to sample deep-sea parasite fauna. Manter (1934) carried out the first deep-sea parasitological survey in the Atlantic, with a special focus on digeneans. Campbell et al. (1980), Houston and Haedrich (1986), Zubchenko (1981), Gartner and Zwerner (1989) also carried out extensive samplings in north-Atlantic waters. These authors outlined the general patterns followed by deep-sea parasite populations and communities for the first time, and set the stage for the more recent parasitological studies. Comprehensive parasitological studies in the southern hemisphere have been comparatively neglected. The first detailed study on deep-sea fish parasites from this area, off southeastern Australia, was provided by Heath (1989). In the Mediterranean Sea, although several early parasitological citations exist (e.g. see Brian (1912), Pintner (1913), Guiart (1935)), no extensive surveys on deep-sea parasite fauna have been performed, and, in general, parasites of deep-waters in this region have been barely addressed.

From the above mentioned and subsequent studies, some patterns followed by fish parasite diversity and abundance as encroaching into deep waters have been elucidated.

As a general rule, meso- and bathypelagic fish harbour less diversity and abundance of parasites than benthic species (Campbell et al., 1980; Klimpel et al., 2009). In turn, since abundance and diversity of the parasite fauna tends to decrease with depth and with distance from the continental slope, benthic fish inhabiting shallower waters show higher helminth diversity than those dwelling in deeper ones, (Campbell et al., 1980). Concerning deep-sea fish living on the continental slopes, species living in or close to submarine canyons accumulate more parasites and more diversity than those living at equivalent depths outside them (Campbell et al., 1980).

For heteroxenous parasites (those needing more than one host to complete their life cycle), these trends are mostly correlated with the abundance of benthic organisms acting as intermediate hosts along vertical and horizontal gradients (Campbell et al., 1980; Marcogliese, 2002). In the case of monoxenous parasites (those needing a single host to complete their life cycle), such as monogeneans, density of host populations is considered to be the most determinant factor (Campbell et al., 1980).

The Digenea is recognized as the largest group of metazoan parasites found in the deep-sea, with approximately an 11% of digenean families reported in this environment (Bray 2004, 2005). According to Klimpel et al. (2009), who carried out an extensive survey on deep-sea metazoan fish parasites reported from all around the globe, approximately 39% of deep-sea parasites belong to Digenea, 17% to Crustacea, 14% to Cestoda, 9% to Monogenea, 6% to Nematoda, 5% to Acanthocephala and 1% to Hirudinea, with an average of 1.5 metazoan parasites per individual host. These authors also reported low host-specificity in many deep-sea parasites, attributed to the generalist feeding habits of many deep-sea fish and the comparative scarcity of adequate intermediate hosts with respect to shallower waters (Klimpel et al., 2009).

1.4. Parasites as indicators of fish biology

Parasites are widely used as effective biological indicators of different aspects of fish biology. Hosts feeding habits and trophic relationships, phylogeny, ontogenic shifts, population segregation, migratory patterns, exposition to pollutants and even ecosystem alterations can all be inferred by the study of their parasite fauna (Williams et al., 1992). Many trophically-transmitted parasites have complex life cycles involving two or more hosts, and if at least part of these hosts is known, parasites allow elucidating trophic interactions among them (Marcogliese, 2005). Inferring dietary habits of a given host

species by identifying individual parasites for which its previous hosts are known is a common approach (Bertrand et al., 2008). This is particularly useful when these components are unrecognizable or difficult to identify in gut contents (i.e. soft preys, such as gelatinous plankton). In a similar way, the identification of larval forms for which the subsequent or final hosts are known allows deducing which species prey onto the studied host (Valtonen et al., 2010). Host diet shifts are easily detected through parasitological studies, since the consumption of different intermediate hosts is corresponded with changes in the composition of the parasite assemblage (Münster et al., 2015). From a wider perspective, the structure of local food webs and the position that the studied host occupies within them can also be addressed (Marcogliese, 2005 and references therein; Valtonen et al., 2010; Culurgioni et al., 2015). In comparison with the analysis of stomach contents, which provides a snapshot of the recent diet composition, parasites can be used to make inferences about long-term feeding habits (Marcogliese, 2005; Knudsen et al., 2010). Actually, the combination of both approaches (short and long-term feeding habits inferred by stomach contents and parasites, respectively) is recommended to improve resolution in dietary and food web studies (Valtonen et al., 2010; Locke et al., 2013; Isbert et al., 2015)

The study of parasites can be also applied to reveal similarities among groups of hosts. For instance, host species phylogenetically closer tend to share more parasite species than unrelated species (Seifertová et al., 2008; Locke et al., 2013). Therefore, some light can be shed on dubious taxonomic relationships by the accurate determination of the parasites infecting the host species under consideration (Mateu et al., 2014). Ontogenic changes occurring throughout the lifespan of the hosts are also reflected by their parasite fauna in such a way that the latter changes as a function of host age (Timi et al., 2010a). As exposed above, ontogenic diet shifts, which modify the host exposure to parasites, are especially well reflected by parasite assemblages (Münster et al., 2015). Ecological convergence of groups of hosts (e.g. similar habitat use or same trophic level) seems to be an important predictor of parasite community resemblance as well (Poulin, 2010; Locke et al., 2013), in a similar way as geographical proximity (Locke et al., 2012). Conversely, similarity among parasite communities is expected to decay with geographical distance (Timi et al., 2010b). Actually, decreasing similarity among parasite assemblages should occur along any dimension that implies some sort of separation among them.

If baselines are set for the composition and structure of the parasite assemblage present in a given host, it is possible to detect alterations of such baselines as a consequence of environmental disturbances that have affected food chains and local food webs (Pérez-del-Olmo et al., 2007, 2009; Vidal-Martínez et al., 2010). Different studies have proved that effects of environmental impacts are aggravated for parasites with respect to their hosts, and that re-establishment of baseline levels takes longer for parasites too (Koch, 2004; Rohde, 2005). Therefore, parasite assemblages provide greater resolution than host assemblages when reflecting environmental conditions that may be negatively affecting the host populations.

At the individual level, parasites effectively serve as accumulation indicators (sentinels) of specific contaminants present in the environment (Nachev and Sures, 2016). Accumulation indicators are organisms able to concentrate in their tissues certain substances present in the environment, to significantly higher levels (Beeby, 2001). Some intestinal helminths, out of which acanthocephalans and tapeworms appear as the most promising ones, are able to concentrate metals in their tissues at concentrations several orders of magnitude higher that those present in the environment, and much more effectively than the tissues of their hosts (Sures and Reimann, 2003). This means that parasites easily allow the detection of some pollutants present in the environment in small concentrations, even below the detection limits for available techniques, which may not be revealed by analyzing the environment or host tissues. Parasites can even be more efficient in accumulating pollutants than established free-living sentinels, such as mussels (Sures et al., 1999; Sures and Reimann, 2003). In a similar way as described for environmental disturbances but on a different scale, parasites again magnify negative environmental conditions thus becoming more reliable as bioindicators than their own hosts.

One of the most important applications of establishing dissimilarities among parasite assemblages is discriminating distinct stocks of hosts. Different authors have discussed the criteria for determining whether a parasite can be considered a good tag of host populations (Kabata, 1963; Sindermann, 1983; MacKenzie, 1987; Williams et al., 1992; MacKenzie and Abaunza, 1998). The basic principle in which this procedure is based is that a fish becomes infected with a specific parasite when it enters the endemic region of that parasite (MacKenzie and Abaunza, 1998; MacKenzie, 2005). Therefore, fish populations living in different areas can be characterized on the basis of the parasite species that are infecting them, and that are associated to each area (Bertrand et al.,

2008; Marcogliese and Jacobson, 2015; Mateu et al., 2014). Additionally, it can be inferred that a fish has been within the endemic area of a given parasite at some moment in the past when it is found infected by such parasite outside that endemic area, which is the base of studies on host migration patterns (MacKenzie, 2005; Mele et al., 2016).

In some of the applications described above, parasites are sometimes segregated into different functional groups that are analyzed separately since they provide different kinds of information. For instance, when using parasites as biological tags to investigate the population structure of their hosts, parasite taxa can be classified either as temporary or permanent, depending on their life spans in the studied host (MacKenzie, 2005 and references therein). The history of the contrasted host populations can be different depending on the time scale of the parasites considered: temporary parasites inform on the recent history, and permanent parasites inform on the long-term history of their respective hosts (Lester, 1990). In a similar way, in studies dealing with environmental impacts on parasite assemblages a separation is often established between monoxenous parasites (those whose life cycle involve a single host, generally exoparasites) and heteroxenous parasites (those whose life cycle involves two or more hosts, generally endoparasites). Exoparasities are more resistant due to their continuous exposition to external conditions and their levels are expected to increase in a stressed environment, meanwhile endoparasites show higher susceptibility given their dependence on different intermediate hosts and the vulnerability of their free-swimming larval stages (Diamant et al., 1999; MacKenzie, 1999; Pérez-del-Olmo et al., 2007).

1.5. The nature of host-parasite interactions

Because of the intrinsic nature of parasitism, in which the parasite lives at the expense of its host and in close association with it, harm to the host and benefit to the parasite are always implicit (Esch and Fernández, 1993; Roberts and Janovy, 2009). However, harm is not easy to quantify and it is a rather relative term (Esch and Fernández, 1993). While harm by the parasite is sometimes evident (e.g. reduced fecundity of trematode-infected snails), in many other cases damage is undetectable (Goater et al., 2014).

The relationships between parasites and their hosts are quite intimate and a certain degree of metabolic dependence of the parasite on the host always exists (Roberts and Janovy, 2009). Therefore, parasites use host resources to support their own development and reproduction and can thus reduce host fitness, either directly by stealing host food

or energy reserves, or indirectly via the reallocation of limited host resources to defense and/or damage protection (Roberts and Janovy, 2009). The harm induced by the parasite can range from digging a hole in the skin of its host to stimulating an immune response, altering its behavior or stealing its food resources (Roberts and Janovy, 2009). Parasites are usually much smaller than their hosts and a single parasite may provoke a negligible impact. However, a host infected by a large number of individuals of this same parasite can undergo serious illness or even die. Similarly, a low parasite burden may have little impact on a healthy host, but the same burden infecting an unhealthy, starving host can have dramatic effects (Gunn and Pitt, 2012). Moreover, an enormous variation exists between species of parasites on the effects they may have on their hosts, since these effects depend not only on the overall parasite burden, but also on particularities of the parasite itself (e.g. specific kind of pathogenic action or site-specificity and related damage) and on host susceptibility (Goater et al., 2014).

The effects of parasite infection on the survival and fecundity of individual hosts are reflected at the population and the community levels. As long as parasites affect the survival and fecundity of individual hosts, they have the potential to reduce the size of their host populations (Goater et al., 2014). Anderson and May (1978) showed that this potential acts regulating hosts populations in a density-dependent way. As an example, the myxozoan *Myxobolus cerebralis* or the sea louse *Lepeophtheirus salmonis* can heavily reduce populations of infected salmonid fishes (Hoffman, 2011; Bateman et al., 2016).

At a higher scale, the effects of parasites on hosts' populations are translated into impacts on host communities and ecosystems. These impacts can be of crucial importance if the affected hosts are keystone species with vital ecological roles in the ecosystem. In such a case, parasites act as 'ecosystem engineers' due to their effects on biodiversity and their influence on the community and ecosystem structure (Goater et al., 2014).

CHAPTER 2 - OBJECTIVES

OBJECTIVES

Considering the poor existing knowledge on parasite assemblages and dynamics in the Mediterranean deep-sea, the central purpose of the present thesis is to characterize the parasite communities infecting important deep-dwelling ichthyc species of the Balearic Sea. Another main objective is to assess the responsiveness of these parasite communities to environmental gradients and variables, to host diet and trophic ecology and their possible impact on host health condition.

In order to fulfill these general goals, the following specific objectives are established:

1) To provide accurate descriptions of the parasite communities infecting the following deep-sea fishes in the Balearic Sea: *M. moro*, *P. blennoides*, *G. melastomus*, *S. canicula*, *E. spinax* and *C. coelolepis*.
2) To investigate whether variations in the structure and composition of such parasite communities and in the prevalence and abundance of individual parasites exist across temporal (i.e. different seasons), geographical (i.e. mainland vs. insular slopes and different sites within each) and bathymetric gradients.
3) To analyse the diet of the hosts addressed and elucidate how hosts feeding patterns influence the general structure and composition of parasite communities throughout the environmental gradients addressed and between host ontogenic stages.
4) To establish detailed parasite-prey relationships to reveal possible transmission pathways for individual parasites.
5) To test associations between individual parasite loads and environmental variables (i.e. temperature, salinity, O_2 concentration and turbidity levels of near-bottom water masses) to elucidate their influence on parasite abundance.
6) To determine activity levels of enzymes known to respond to different biological and stressing conditions (i.e. acetylcholinesterase, lactate dehydrogenase, citrate synthase) and lipid peroxidation levels in muscle of the hosts addressed and to relate them to parasite loads.
7) To analyse the relationship between general fish condition indices (i.e. condition factor, hepatosomatic and gonadosomatic indices) indicative of fish health status and their parasite burden.

8) To quantify the size and density of melano-macrophages found in spleen (in the case of teleosts) or liver (for elasmobranch species) sections, either isolated or forming centres, and to assess their variations as a function of parasite burden.
9) To carry out histopathological analyses of different organs (i.e. gills, liver, spleen, gonads and intestine) in order to detect microparasites, parasite-induced alterations and/or pathological conditions.
10) To address morphological, taxonomical or biological aspects of individual parasite species needing revision or additional studies that may arise during the study of the different parasite communities.

CHAPTER 3 - PARASITES OF THE DEEP-SEA FISH *MORA MORO* (RISSO, 1810) FROM THE NW MEDITERRANEAN SEA AND RELATIONSHIP WITH FISH DIET AND ENZYMATIC BIOMARKERS

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Parasites of the deep-sea fish *Mora moro* (Risso, 1810) from the NW Mediterranean Sea and relationship with fish diet and enzymatic biomarkers

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ABSTRACT

Specimens of *Mora moro* were collected in two seasons and three localities of the Balearic Sea (NW Mediterranean Sea) and parasitological, dietary (to prey-species level), enzymatic and histological data were obtained, alongside with environmental information (T, S, O_2). The relationships among fish parasite load, condition indices, diet, enzymatic activity of muscular acetylcholinesterase (AChE) and lactate dehydrogenase (LDH), intensity of splenic melano-macrophage centres (MMC) and hepatic granulomas were tested. *M. moro* showed a rich and abundant parasite fauna, and was a new host record for 17 out of the 18 different endoparasite taxa found. Significant differences were detected among locality-season groups, in turn related to different environmental variables, for Anisakidae gen. sp., *Anisakis* Type II and Tetraphyllidea fam. gen. sp.; thus, they are proposed as potentially useful as biological tags for geographical discrimination of *M. moro* in the NW Mediterranean Sea. Detailed relationships were found between parasite taxa and prey ingested (e.g. Anisakidae gen. sp. related with meso-bathypelagic crustaceans; *Anisakis* Type I with benthopelagic squids). Most parasites were linked to samples with highest levels of near-bottom O_2, which is consistent with direct relationships found between near-bottom O_2 and zooplankton biomass in the Balearic Basin. Total parasite abundance and the abundance of Tetraphyllidea fam. gen. sp. showed a significant relationship with the activity of AChE and the abundance of *Anisakis* Type II with LDH. AChE was associated with hepatosomatic index (HSI) and condition factor (K); LDH with gonadosomatic index (GSI), K and fish total length (TL). LDH activity showed differences among sampling groups. Splenic MMC and hepatic granulomas were not associated with fish parasite load. A positive relationship was found between MMC area and fish TL and LDH activity.

1. Introduction

The Mediterranean deep-sea has been grossly understudied (Pérès, 1985) until the last decades, when considerable effort has been directed to improving knowledge on structure and function of its systems (e.g. Fanelli et al., 2013a and references therein). However, even now most of the studies are sparse and further research is needed to reveal biological interactions and environmental forcing. Only through this understanding we will be able to protect and preserve this valuable and fragile ecosystem, where we find most of the extreme animal adaptations of the world. Within its ichthyofauna, the common mora, *Mora moro* (Risso, 1810) (Gadiformes, Moridae) is a cosmopolitan bathypelagic species of moderate commercial interest that displays a quiet behaviour strictly linked to the bottom, resting or moving on the seabed, often sheltering and feeding (D'Onghia et al., 2011). The species was first described from the Western basin of the Mediterranean Sea by Risso (1810) and presence has been more recently been confirmed in the eastern basin, the Ionian Sea (Mytilineou et al., 2005) and the Levantine Sea (Bilecenoglu et al., 2002). Its usual bathymetric distribution in the western Mediterranean ranges between 800 and 1500 m (Stefanescu et al., 1992a; D'Onghia et al., 2004). In the Catalan Sea, the family Moridae is the main contributor to biomass and one of the most important in terms of number of species below 1000 m (Stefanescu et al., 1992a; Fanelli et al., 2013a), and *M. moro* is one of the main contributors to biomass at depths of 1000–1400 m. Its rarefaction below this depth-range has been explained by the

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impossibility to satisfy its high energy requirements in an increasingly oligotrophic environment at higher depths (Stefanescu et al., 1992b; D'Onghia et al., 2004). A number of studies exist on *M. moro* bathymetric distribution and depth-related trends (Stefanescu et al., 1992a, 1992b; D'Onghia et al., 2004), ecological importance (Fanelli et al., 2013a), reproductive biology (Rotllant et al., 2002), bioaccumulation of pollutants and their detoxification systems (Solé et al., 2001; Koenig et al., 2013) as well as flesh properties and nutritional value (Rossano et al., 2005). Detailed knowledge on dietary habits of large specimens of *M. moro* below 1000 m is scarce and few data exist on prey ingested (Carrassón et al., 1997; Carrassón and Cartes, 2002), due to the evertion of the stomachs and subsequent loss of its content. To date no alternative analyses (e.g. intestine content analyses, lipidic biomarkers) have been attempted, and knowledge on its trophic position within food webs is restricted to the information given by stable isotope analyses (Polunin et al., 2001; Fanelli et al., 2013b).

Despite the broad range of issues assessed in relation to this species, information on its parasite fauna is very scarce and to the best of our knowledge no data exist on parasite communities in this species. To date, only a few studies reporting occasional presence of parasites in this fish have been published (Bray and Gibson, 1989, 1995; Waterman and Sin, 1991; Bruce et al., 1994; Bray et al., 1999; Dallarés et al., 2013). The usefulness of parasites as effect bioindicators has been reported repeatedly (Marcogliese, 2005; Blanar et al., 2009). Due to their frequently complex life cycles, involving hosts belonging to different trophic levels as well as free-living stages, parasites can provide information on trophic interactions, species composition and alterations of their ecosystems (Pérez-del-Olmo et al., 2009; Vidal-Martínez et al., 2010). Additionally, the use of parasites as biological markers for fish population discrimination has been increasing in importance since the mid-20th century and is based on the analysis of the parasite community with a subsequent selection of the most discriminating parasite species (Perdiguero-Alonso et al., 2008; Pérez-del-Olmo et al., 2010). Temporal patterns in parasite populations and their community structure have been associated to differential environmental conditions, but host biological features and availability are also known to strongly drive temporal variation of parasite assemblages (Fellis and Esch, 2004).

Although literature relating parasite infections with biochemical markers is scarce, evidence available indicates that parasites can interfere with the physiology of their hosts and modify their protection mechanisms and biomarker responses (e.g. enzymatic activities) in many ways (Frank et al., 2013). Acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) can provide information on peculiarities due to host ecology, diet, biology or phylogeny when the pollution gradient is particularly small (Solé et al., 2008, 2010). In recent years, the combined use of general stress markers that respond to natural (e.g. parasites) as well as anthropogenic stressors (e.g. contaminants) have been incorporated in ecotoxicological studies as a reflection of individual animal and ecosystem health (Marcogliese and Pietrock, 2011; Sures, 2008).

Parasite infections can also elicit a wide array of histological alterations of varying degrees of importance in its host (Feist and Longshaw, 2008). In this sense, frequency and size of aggregates of inflammatory cells, also known as melano-macrophage centres (MMC), are known to be influenced by health condition in fishes (Agius and Roberts, 1981), and could change depending on the parasite load. These kinds of effect markers are suitable indicators of stressful conditions because of their easy quantification and measurement in histological sections (Carrassón et al., 2008) and have never been assessed on *M. moro*.

The aim of this study is to provide, for the first time, information on the parasite communities from *M. moro* in the NW Mediterranean Sea at four locality-season combinations, and to assess their geographical differentiation within this area. In order to analyse the influence of natural variability on these communities, we compare parasite composition as a function of environmental gradients (i.e. the four different locality-season groups) and in relation to environmental variables such as temperature (*T*), salinity (*S*) and oxygen content of water masses, or prey ingested,

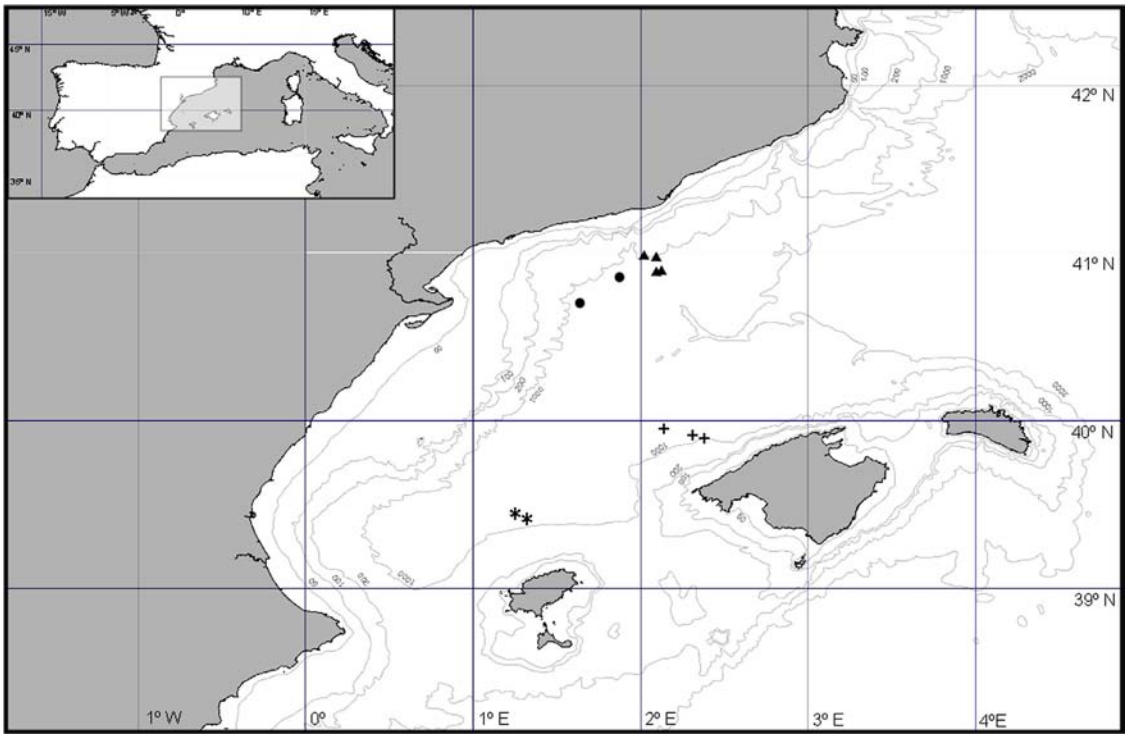

Fig. 1. Study area with sampling sites and seasons within the Barcelona and Balearic slopes. ▲ Barcelona summer; • Barcelona autumn; + Mallorca summer; *Ibiza autumn.

the last also giving clues on intermediate hosts. Biomarkers of exposure (AChE and LDH activities) and effect (splenic MMC number and area and histopathological alterations in gills, liver, spleen and gonads) were also assessed, to test possible relationships between them, the parasite load of the fish and their general condition.

2. Materials and methods

2.1. Sampling and study area

A total of 62 specimens of *M. moro* were collected in summer (July) 2010 and autumn (October) 2011 at depths between 1000 and 1400 m in western Mediterranean waters (Fig. 1). Samples were taken using a semi-balloon otter-trawl (OTSB 14) on the research vessel Garcia del Cid at three different locations of the continental slope (Barcelona) and insular slope of the Balearic Islands (Mallorca and Ibiza) (Table 1).

Environmental data (temperature (T) in °C, salinity (S) in psu, O_2 concentration and turbidity (voltage), the last representing organic and inorganic suspended material), were taken at 5 m above the sea-bottom by deployment of a CTD almost simultaneously (same data, same depth) to hauls performed to sample *M. moro* specimens.

Immediately upon capture, each fish was measured for records of total length (TL) in mm and total weight (TW) in g. A portion of the axial muscle was dissected and kept at −20 °C for biochemical analyses, and samples of right gills, liver, spleen and gonads were immediately fixed in 10% buffered formalin for histological assessment. The rest of the specimen was frozen at −20 °C for the parasitological study.

2.2. Parasitological examination

Once in the laboratory, fish were thawed and liver and gonads weighed and examined for the presence of parasites. External surfaces and mouth of each individual were checked for ectoparasites macroscopically and under stereomicroscope. All organs and musculature were carefully removed and inspected for endoparasites. Parasites collected were counted and preserved in 70% ethanol. Digeneans, cestodes and acanthocephalans were stained with iron acetocarmine, dehydrated through a graded ethanol series and examined as permanent mounts in Canada balsam. Nematodes were studied on temporary mounts in glycerine or saline solution. All parasites were identified to the lowest possible taxonomic level.

Table 1
Sampling data for *Mora moro*. Loc: locality, BS: Barcelona summer, BA: Barcelona autumn, MS: Mallorca summer, IA: Ibiza autumn, n=number of sampled specimens.

Trawl	Date	Depth (m)	Latitude (deg, min, N)	Longitude (deg, min, E)	Loc	n
A1–3	8/7/2010	1048	40° 58.06	2° 5.30	BS	6
A1–4	8/7/2010	1024	40° 58.69	2° 1.14	BS	10
A1–5	10/7/2010	1269	40° 54.35	2° 6.06	BS	2
A1–6	10/7/2010	1308	40° 53.85	2° 4.00	BS	2
A1–17	19/07/2010	1006	39° 52.39	2° 20.26	MS	7
A1–18	19/07/2010	1060	39° 53.37	2° 18.66	MS	4
A1–19	19/07/2010	1232	39° 55.16	2° 8.25	MS	3
A3–3	15/10/2011	1051	40° 50.80	1° 43.94	BA	8
A3–4	15/10/2011	1236	40° 41.96	1° 37.46	BA	8
A3–9	17/10/2011	1062	39° 23.12	1° 18.45	IA	6
A3–10	18/10/2011	1272	39° 25.31	1° 16.84	IA	6

2.3. Diet analysis

After examination of stomachs and intestines for the presence of parasites, all gut content was weighed to the nearest 0.001 g and prey were identified to the lowest possible taxonomic level under stereomicroscope. In three specimens, gut content was lost and could not be examined. In most of the fishes (56 out of 59 specimens) stomachs were everted and only intestine contents were analysed. Soft preys can be underestimated by analysing intestines, but even for soft animals such as polychaetes and siphonophores, hard structures (mandibles/setae and nectophores, respectively) can be found there, which allows prey quantification. These remains are often specific, i.e. allow identifying preys at genus or species levels, as it is the case of decapod mandibles or some crustacean appendages. Diet was considered in this study by prey number, to be directly comparable with parasitological indices analysed by Canonical Correspondence Analysis (CCA) (see below).

2.4. Biochemical determinations

A muscle portion, of about 0.3 g, was used for AChE and LDH determinations. The tissue was homogenised in a 50 mM buffer phosphate pH 7.4 in a 1:5 (w:v) ratio using a polytron® blender. The homogenate was centrifuged at 10,000g × 30 min and the supernatant (S10) was used for biochemical determinations. A range of six concentrations of acetylthiocholine iodide (ATC) from 0.05 to 10 mM were used to determine V_{max} and K_m of AChE. For AChE determination, the concentration of the substrate (ATC) selected was 1 mM, as described in Solé et al. (2010). AChE activities were assayed according to the principle of Ellman et al. (1961) at 405 nm. For the LDH determination, 150 µl of NADH solution in phosphate buffer was mixed with 25 µl of 1:40 diluted sample and 50 µl of pyruvate solution in each microplate well. LDH activity was given by the amount of pyruvate consumed due to NADH oxidation at 340 nm (Vassault, 1983).

In both determinations, reading was carried out in triplicate in a microplate reader (TECAN Infinte200) during 5 min at 25 °C. Activity was expressed in nmol/min/mg protein.

Total protein content in the S10 fraction was determined by Bradford (1976) method using bovine serum albumin as standard (BSA 0.1–1 mg/ml).

2.5. Histological and histopathological assessment

Fixed samples of gills, liver, spleen and gonads were embedded in paraffin, sectioned at 4 µm and stained with Haematoxylin and Eosin. When necessary, specific stains (Grocott and Ziehl-Neelsen) were carried out in some slides.

For splenic MMC analysis, three fields of view (0.23 mm^2/screen) were randomly selected from each section of the spleen at 200x of magnification and examined microscopically. Area and number of MMC were measured using a MicroComp Integrated Image Analysis System. A size discriminator was used to eliminate objects smaller than 11 µm^2. Area and number of MMC (SC and NC, respectively) per square millimetre were calculated for each field.

For liver granulomas and cysts of unknown etiology (CUEs), prevalence was calculated. In order to evaluate the intensity of the granulomas, three random quantitative counts of the number of granulomas were performed on each liver histological section at 100× of magnification (0.92 mm^2/screen). Mean intensity for each individual fish was calculated from the average intensity values per square millimeter (NG). In addition, in those cases with high intensity, the distribution of the lesions was classified as "extensive" (granulomas distributed uniformly throughout all the

examined tissue) or "aggregate" (granulomas affecting with high intensity only limited areas of the examined tissue).

2.6. Data analyses

To analyse the parasite fauna of *M. moro*, fish were grouped into two size categories, i.e. size 1 (TL $\leq$33 cm) and size 2 (TL $>$33 cm).

Parasitological terms such as prevalence (*P*) and mean abundance (MA) were calculated according to Bush et al. (1997) using data from all thawed specimens. Species with total $p>10\%$ are henceforth called common. The diversity of the parasite communities in individual fish was estimated using Brillouin's index (PRIMER v6; Anderson et al., 2008). Fish condition was assessed by condition factor: K calculated as $(TW/TL^3)\times 100$, hepatosomatic index: HSI calculated as (liver weight/TW) $\times$ 100 and gonadosomatic index (in females): GSI calculated as (gonad weight/TW) $\times$ 100. The numerical percentage of prey (%*N*) of *M. moro* and the frequency of occurrence of prey (%*F*) in intestines, intestine fullness (100 $\times$ intestine content weight/body weight, g), mean number of prey per specimen and prey richness (*S*), i.e. the number of different prey identified in the diet, was given as a function of: (i) the two seasons sampled (summer: July; autumn: October), and (ii) the three main areas explored (Continental slope: Barcelona; Insular slope: Mallorca and Ibiza). Analyses on MMC parameters (SC and NC) and liver granulomas intensity (NG) were performed using the average value of the three field measurements.

Possible effects of the factor locality-season (four categorical groups: Barcelona summer (BS), Barcelona autumn (BA), Mallorca summer (MS), Ibiza autumn (IA)) and fish size were tested for infracommunity parasite descriptors (mean species richness (MSR), total mean abundance (TMA), and mean diversity (Brillouin's index) (MD)), condition indices (K, GSI, HSI), enzymatic biomarkers activity (AChE, LDH), MMC parameters (SC and NC), number of liver granulomas (NG) and prevalence of CUEs, using General Linear Models (GLM) or Generalised Models (GZM) (only for TMA, NG and CUEs), with post-hoc pairwise comparisons. To comply for normality and homoscedasticity requirements, data from HSI, AChE and LDH were log-transformed. To visualise the patterns in parasite abundance in relation to the four categorical groups, a factorial correspondence analysis (FCA) was first applied on a data matrix comprising component population abundance of the 9 common parasite species. A hierarchical cluster analysis was simultaneously performed based on coordinates of the first two axis obtained in the FCA to define host groups clearly. Then, using individual fish as replicate samples, differences in abundance and prevalence among the parasite populations were tested using GZM for the factor locality-season and using fish TL as covariable (applying log-binomial model for abundance and logistic model for prevalence). A permutation multivariate analysis (PERMANOVA) was also performed using parasite infracommunities as replicate samples to test the null hypothesis of no differences in the parasite community structure between the samples off Barcelona from the two seasons and along the locality-season groups. Analyses were carried out using PERMANOVA+ for PRIMERv6 (Anderson et al., 2008) on Bray–Curtis similarity matrices derived from the logarithmic transformed ($\log(x+1)$) abundance data. Permutation *p*-values were obtained under unrestricted permutation of raw data (9999 permutations). A similarity percentages analysis (SIMPER) was carried out using parasite infracommunities as replicate samples to identify the parasite taxa that most contributed to the similarity/dissimilarity of infracommunities within/among the samples of the four locality-season groups.

Relationships between main parasites and prey in guts and between parasites and environmental variables were analysed by multivariate CCA (Ter Braak, 1986). CCA relates in this case the abundance of each parasite with each prey-species and with each environmental variable. It is probably the most widespread analysis used to assess the effect of environmental variability in a parameter (Ter Braak and Verdonschot, 1995). Arrows in CCA plots represent explanatory variables and are proportional in length to their importance on the explained variable (Ter Braak, 1986). CCA relates parasite abundance (using parasite infracommunity data) with number of prey ingested per *M. moro* specimen. Analyses were performed on 59 fish, after data were log-transformed. CCA were repeated considering the environmental, possible explanatory, variables (T, S, O_2 and turbidity).

GZM were carried out using AChE and LDH activities as covariates to explore the relationship between them and the fish individual total parasite abundance and the abundance of the three parasites that contributed most to the similarity/dissimilarity of infracommunities within/among locality-season categories according to the SIMPER results (Anisakidae gen. sp., *Anisakis* Type II and Tetraphyllidea fam. gen. sp.). GLM were performed to assess the relationship between the activities of both enzymes (AChE and LDH) and parasite diversity descriptors: infracommunity richness (*S*) and Brillouin's diversity index (MD), and between the former and fish TL and condition indices (K, HSI, GSI).

In the same way, GLM analyses were applied to verify whether parasite richness, total infracommunity abundance and the abundance in individual fish of the three most discriminating parasites, mentioned above, were influencing MMC parameters. GLM were also used to test the relationship between MMC parameters, TL, condition indices and enzymatic biomarkers activity. For hepatic granulomas (NG), GZM were applied to assess the relationship between them and parasite richness, total infracommunity abundance, the abundance of the three most discriminating parasites, TL and condition indices, enzymatic biomarkers activity and MMC parameters.

3. Results

3.1. Parasite composition and its relationships with biological and environmental parameters

Total length (TL) of the sampled fish ranged from 212 to 500 mm. All examined fish were infected by at least one parasite. A total of 4345 endoparasites belonging to 18 different taxa were identified: five digeneans, three cestodes, one acanthocephalan and nine nematodes (Table 2). Of these, the following 9 taxa were considered common ($p>10\%$): the digeneans *Lepidapedon desclersae* Bray and Gibson, 1995 and *Otodistomum* sp.; the larval cestodes Tetraphyllidea fam. gen. sp. and Trypanorhyncha fam. gen. sp. and the nematodes Anisakidae gen. sp., *Anisakis* Type I, *Anisakis* Type II, *Hysterothylacium aduncum* (Rudolphi, 1802) and *Capillostrongyloides morae* Gonzalez-Solís et al., 2014. Voucher material for the new host records (only parasites identified to species) is deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice (HCIP) with the catalogue numbers: *Echinorhynchus gadi:* A-7, *Bothriocephalus scorpii*: C-233, *Paraccacladium jamiesoni*: D-704, *Proctophantastes abyssorum*: D-705, *Oncophora melanocephala*: N-7, *H. aduncum*: N-147.

The parasites with the highest prevalence and abundance were the plerocercoids of tetraphyllidean cestodes known collectively as *Scolex pleuronectis* Müller, the anisakid nematode *Anisakis* Type II and a yet unidentified nematode belonging to the family Anisakidae. *L. desclersae* and *Otodistomum* sp. were the most prevalent and abundant digeneans.

Table 2
Developmental stage, location within host, prevalence (*P*%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Mora moro*. N: sample size of *M. moro*. Keys for developmental stages: A, adult; J, juvenile; L, larvae; Mt, metacercariae. Keys for locations within host: I, intestine; Li, liver; M, mesentery; PC, pyloric caeca; S, stomach; SW, stomach wall (encysted). Different superscript letters and numbers show significant differences in spatial/temporal assessment. Dashes indicate absence of the parasite.

N	Stage	Location	Barcelona slope				Balearic slope				Total	
			Barcelona summer (BS) 20		Barcelona autumn (BA) 16		Mallorca summer (MS) 14		Ibiza autumn (IA) 12		62	
			P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD
Digenea												
Bathycreadium sp.	J, A	I, PC	15	0.15 ± 0.37	6	0.13 ± 0.50	–	–	–	–	6	0.08 ± 0.33
Lepidapedon desclersae	J, A	I, PC	5^{1}	0.05 ± 0.22^{a}	25^{1}	0.50 ± 0.97^{a}	36^{1}	0.79 ± 1.31^{a}	25^{1}	0.58 ± 1.24^{a}	21	0.44 ± 0.99
Otodistomum sp.	Mt	SW	10^{1}	0.50 ± 1.67^{a}	13^{1}	0.13 ± 0.34^{a}	–	–	25^{1}	0.33 ± 0.65^{a}	11	0.26 ± 1.01
Paraccacladium jamiesoni	J	PC	5	0.05 ± 0.22	–	–	–	–	–	–	2	0.02 ± 0.13
Proctophantastes abyssorum	A	I	5	0.05 ± 0.22	–	–	–	–	–	–	2	0.02 ± 0.13
Cestoda												
Bothriocephalus scorpii	J	PC	5	0.05 ± 0.22	6	0.06 ± 0.25	7	0.07 ± 0.27	8	0.08 ± 0.29	6	0.06 ± 0.25
Tetraphyllidea fam. gen. sp.	L	S, I, PC	100^{1}	21.00 ± 32.55^{a}	94^{1}	26.44 ± 28.08^{a}	86^{1}	8.21 ± 9.95^{b}	100^{1}	87.83 ± 83.47^{c}	95	32.45 ± 50.91
Trypanorhyncha fam. gen. sp.	L	M, SW	60^{1}	1.00 ± 1.17^{a}	19^{2}	0.50 ± 1.21^{a}	7^{1}	0.29 ± 1.07^{a}	331^{2}	1.25 ± 3.41^{a}	32	0.76 ± 1.81
Acantocephala												
Echinorhynchus gadi	A	I	15	0.15 ± 0.37	13	0.25 ± 0.68	–	–	–	–	8	0.11 ± 0.41
Nematoda												
Anisakidae gen. sp.	L3	M, SW	90^{1}	9.65 ± 20.11^{a}	81^{1}	12.56 ± 19.01^{a}	432	0.64 ± 1.08^{b}	100^{3}	91.17 ± 107.42^{c}	79	24.15 ± 58.40
Anisakis Type I	L3	M	50^{1}	0.65 ± 0.74^{a}	38^{1}	0.44 ± 0.63^{a}	–	–	33^{1}	0.67 ± 1.23^{a}	32	0.45 ± 0.78
Anisakis Type II	L3	M, Li, SW	95^{1}	9.35 ± 10.82^{ab}	100^{1}	5.44 ± 3.65^{a}	931	15.29 ± 23.02^{b}	92^{1}	13.42 ± 11.76^{b}	95	10.47 ± 13.85
Capillostrongyloides morae	A	S, I, PC	30^{1}	0.45 ± 0.83^{a}	50^{1}	1.13 ± 1.36^{a}	–	–	8^{1}	0.25 ± 0.87^{a}	24	0.48 ± 0.99
Cucullanus sp.	L3	I	–	–	13	0.13 ± 0.34	–	–	–	–	3	0.03 ± 0.18
Hysterothylacium aduncum	L3, L4, A	S, I, PC	5^{1}	0.05 ± 0.22^{a}	13^{1}	0.19 ± 0.54^{a}	–	–	17^{1}	0.92 ± 2.87^{b}	8	0.24 ± 1.30
Hysterothylacium fabri	L3	M	–	–	–	–	–	–	8	0.08 ± 0.29	2	0.02 ± 0.13
Oncophora melanocephala	A	I, PC	5	0.05 ± 0.22	6	0.06 ± 0.25	–	–	–	–	3	0.03 ± 0.18
Raphidascaris sp.	L3	S	5	0.05 ± 0.22	–	–	–	–	–	–	2	0.02 ± 0.13

Parasitological descriptors (MSR, TMA, MD and MA (the latter for each taxon)), enzymatic activities (AChE, LDH) and histological parameters (SC, NC, NG and CUEs) showed no interaction between fish size and categorical groups (GLM/GZM, $p > 0.05$). No significant differences were observed among locality-season groups neither for K, HSI nor GSI (GLM, $p > 0.05$ in all cases).

Parasites mean species richness (MSR) and mean diversity (MD) were significantly lower in the summer sample from off Mallorca than in the other three categorical groups (GLM, $F_{(3,58)} = 8.978$, $p = 0.0001$ and $F_{(3,58)} = 6.37$, $p = 0.001$, respectively). Total mean abundance (TMA) was significantly higher in the autumn sample from off Ibiza than in the other groups (GZM, $\chi^2 = 28.855$, $p = 0.0001$) (Table 3).

Fig. 2 shows a plot of the first factorial plane of co-inertia analysis explaining 96.66% of the total variance, mainly on the first axis (84.58% of the total inertia) of the FCA carried out using component population data for the common parasite species. Component populations of four parasite species exhibited the strongest associations with the first FCA axis: *Anisakis* Type II, Anisakidae gen. sp., *H. aduncum* and *L. desclersae* ($\text{Cosine}^2 = 0.752$–0.988). Three more species were highly correlated with the second FCA axis: *C. morae*, *Anisakis* Type I and Tetraphyllidea fam. gen. sp. ($\text{Cosine}^2 = 0.777$–0.878). From FCA and cluster analysis (not shown) of the locality-season groups, three distinct assemblages of *M. moro* were established depending on their parasite load: group (A) Mallorca summer, group (B) Ibiza autumn and group (C) continental samples (Barcelona summer and Barcelona autumn).

Group A: The summer sample from off Mallorca was characterised by *Anisakis* Type II and *L. desclersae*, both reaching maximum abundances in this locality-season group. Significant differences in the abundance of *Anisakis* Type II were found among groups (GZM, $\chi^2 = 8.423$, $p = 0.038$) (Table 2). Fish size effect was found for Anisakis Type II, with higher abundance in larger fish (GZM, $\chi^2 = 19.777$, $p = 0.0001$).

Group B: The autumn sample from off Ibiza was characterised by Anisakidae gen. sp., Tetraphyllidea fam. gen. sp. and *H. aduncum*. All three showed highest significant abundances in this locality-season group (GZM, $\chi^2 = 96.174$, $p = 0.0001$; $\chi^2 = 34.788$, $p = 0.0001$ and $\chi^2 = 9.434$, $p = 0.009$, respectively). Anisakidae gen. sp. also showed the highest prevalence in this group (GZM, $\chi^2 = 8.631$, $p = 0.035$) (Table 2). Fish size effect was found for Anisakidae gen. sp. and Tetraphyllidea fam. gen. sp., with higher abundance in larger fish (GZM, $\chi^2 = 27.590$, $p = 0.0001$; $\chi^2 = 24.042$, $p = 0.0001$, respectively).

Group C: The group from off Barcelona was characterised by three parasite species: *Otodistomum* sp., *Anisakis* Type I and Trypanorhyncha fam. gen. sp. Trypanorhyncha fam. gen. sp. was significantly more prevalent in the summer sample from off Barcelona (GZM, $\chi^2 = 10.252$, $p = 0.017$) (Table 2). Fish size effect was found for Trypanorhyncha fam. gen. sp., with higher abundance in larger fish (GZM, $\chi^2 = 7.985$, $p = 0.005$).

The permutational multivariate analyses (PERMANOVA) carried out with the infracommunity samples showed no effect of the factor season in samples off Barcelona ($p_{(\text{perm})} > 0.05$) and a significant effect of the factor locality-season in all samples on the structure of parasite communities (Pseudo–$F_{(3,58)} = 4.9371$, $p_{(\text{perm})} = 0.0001$; 9925 unique permutations, all *post hoc* comparisons significant except those comparing continental samples). Therefore, the three formed groups matched with the ones obtained by means of the FCA.

The similarity percentages analysis (SIMPER) allowed the identification of the taxa that contributed most to the similarity/dissimilarity

Table 3
Means and standard deviations of total length (TL), condition factor (K), hepatosomatic index (HSI), gonadosomatic index (only for females) (GSI), Mean species richness (MSR), Total mean abundance (TMA), Mean diversity (MD), acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities, surface (SC) and number (NC) of melano-macrophage centres (MMC) and number (NG) of hepatic granulomas for each of the four categorical groups of *Mora moro*; N: sample size for *M. moro*; (*): number of females; SR: Species richness; CUEs: Prevalence (*P*%) for the cysts of unknown aetiology found in gills of *M. moro*. Different superscript letters show significant differences in spatial/temporal assessment.

	Barcelona slope		**Balearic slope**	
	Barcelona summer (BS)	**Barcelona autumn (BA)**	**Mallorca summer (MS)**	**Ibiza autumn (IA)**
N (*)	20 (8)	16 (7)	14 (5)	12 (8)
TL	34.74 ± 5.32^a	33.22 ± 3.31^a	34.99 ± 5.52^a	36.46 ± 5.03^a
K	0.83 ± 0.09^a	0.79 ± 0.09^a	0.86 ± 0.07^a	0.78 ± 0.06^a
HSI	4.14 ± 1.76^a	3.58 ± 2.19^a	4.19 ± 1.54^a	2.72 ± 1.30^a
GSI	0.60 ± 0.11^a	0.58 ± 0.26^a	0.66 ± 0.30^a	0.63 ± 0.17^a
SR	16	14	6	11
MSR	5.00 ± 1.41^a	4.75 ± 1.65^a	2.71 ± 0.91^b	4.50 ± 1.17^a
TMA	43.25 ± 60.79^a	47.94 ± 48.09^a	25.29 ± 31.11^a	196.58 ± 183.57^b
MD (Brillouin's Index)	0.94 ± 0.26^a	0.92 ± 0.26^a	0.59 ± 0.26^b	0.86 ± 0.18^a
AChE	24.65 ± 6.34^a	28.58 ± 11.81^a	22.01 ± 7.16^a	36.16 ± 21.57^a
LDH	2788 ± 1502^a	2390 ± 904^a	5173 ± 1084^b	2694 ± 1758^a
SC (μm^2 MMC/mm^2)	$144{,}285 \pm 60{,}175^a$	$191{,}555 \pm 100{,}291^a$	$164{,}880 \pm 106{,}502^a$	$114{,}421 \pm 52{,}378^a$
NC (N. of MMC/mm^2)	35.29 ± 10.96^a	34.67 ± 11.36^a	36.96 ± 9.66^a	31.88 ± 13.90^a
NG (N. of granulomas/mm^2)	0.85 ± 1.07^a	1.11 ± 2.27^a	1.38 ± 2.61^a	0.61 ± 0.75^a
CUEs	30.00^a	13.00^a	13.00^a	17.00^a

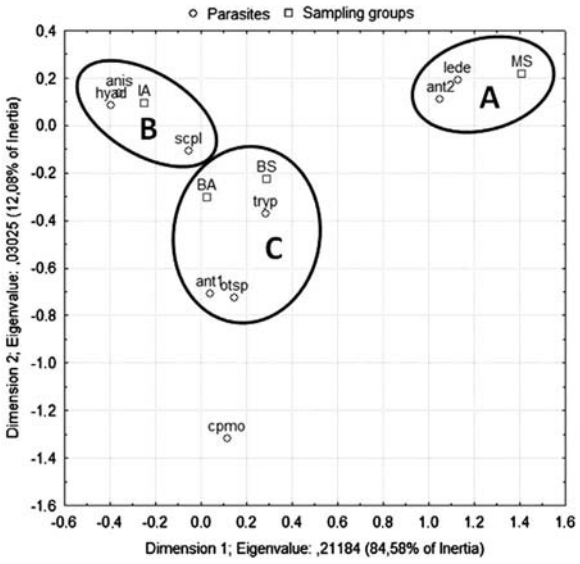

Fig. 2. Plot of the first factorial plane of co-inertia analysis of the factorial correspondence analysis (FCA) on component population data for the nine common parasites ($P > 10\%$) in *Mora moro*. A/B/C indicate the groups established in the parasite fauna description. Abbreviations for spatial/temporal groups: BS, Barcelona summer; BA, Barcelona autumn; MS, Mallorca summer; IA, Ibiza autumn. Abbreviations for species names: Anis, Anisakidae gen. sp.; Ant1, *Anisakis* Type I; Ant2, *Anisakis* Type II; Cpmo, *Capillostrongyloides morae*; Hyad, *Hysterothylacium aduncum*; Lede, *Lepidapedon desclersae*; Otsp, *Otodistomum* sp.; Scpl, larval tetraphyllideans (*Scolex pleuronectis*); Tryp, larval trypanorhynchs.

of infracommunities within/among the samples of the categorical groups. These species are: Tetraphyllidea fam. gen. sp., *Anisakis* Type II and Anisakidae gen. sp. (Table 4).

The CCA relating parasites and environmental variables (Fig. 3) accumulated 93.7% of the total variance and evidenced similar spatio-temporal patterns found in the FCA above. Thus, high near-bottom turbidity and *S* were linked to *H. aduncum* and Anisakidae gen. sp., coinciding with hauls performed in October 2011 in the Balearic slope (IA) (right-upper part of the plot). Similarly, *L. desclersae* and *Anisakis* Type II were associated to hauls performed over the slope of the Balearic Islands (left-upper part), but in summer (July) (MS). The major part of the parasites included in this CCA was linked to the mainland hauls strongly linked to high O_2 concentrations near the bottom (left-bottom part of the plot).

3.2. Fish diet and its relationships with parasite fauna

A total of 52 different prey-items (excluding some inorganic debris, e.g. plastics, scales and foraminiferans) were recorded in the 59 specimens analysed. A total of 359 prey were counted, mostly identified to genus/species level. Both meso-bathypelagic (shrimps: *Acanthephyra pelagica* or *Pasiphaea multidentata*, euphausiids: *Meganyctiphanes norvegica* and fish: myctophids or stomiids), benthopelagic (shrimps: *Aristeus antennatus*, squids: *Heteroteuthis dispar* and *Histioteuthis reversa* and some fish: *Gaidropsarus biscayensis*) and even some benthic prey (the crab *Monodaeus couchi*, flatfish like *Symphurus* sp. and foraminiferans) occurred in the diet of *M. moro*. Main prey (by number) accumulating 84% of prey are listed in Table 5 and were used for post-CCA analyses. Main prey (> 50% of diet) were, by decreasing order, the decapod shrimps *A. pelagica* (17.9% of prey) and *P. multidentata* (12.5%), the euphausiid *M. norvegica* (12.0%) and squids (other than *H. dispar*) (10.3%).

Both *%F* and prey number changed as a function of season for some main prey, the clearest tendency was the higher consumption of *A. pelagica* and squids in October, both over the Barcelona and Balearic slopes (Table 5).

The CCA relating main parasites and prey species explained 66.7% of the total variance by the first two axes (Fig. 4). Main relationships appearing in the right part of the plot associated *Otodistomum* sp. (otsp), *H. aduncum* (hyad) and Anisakidae gen. sp. (anis) with meso-bathypelagic crustaceans, such as *A. pelagica*, *P. multidentata* and *A. antennatus*; whereas in the upper part of the plot, *Anisakis* Type I and Trypanorhyncha coincided with benthopelagic squids and Myctophid fishes.

3.3. Enzymatic biomarkers and its relationships with parasite fauna and biological parameters

AChE and LDH mean activities (in nmol/min/mg prot) ranged from 22 to 36.2 and from 2390 to 5173, respectively (Table 3). AChE did not significantly differ throughout the four categorical groups contrasted, and for this enzyme V_{max} was 26.1 nmol/min/mg prot and K_m was 0.07 mM. By contrast, LDH activity in

Table 4
Mean similarity and dissimilarity for parasite infracommunities sampled within the four categorical groups in *Mora moro* and breakdown into contributions of individual parasite species. Only species contributing to more than 10% of the mean community similarity/dissimilarity within/among spatial/temporal groups are included. BS: Barcelona summer, BA: Barcelona autumn, MS: Mallorca summer, IA: Ibiza autumn.

Species/spatial/temporal/contrast	BS	BA	MS	IA	BS—BA	BS—MS	BS—IA	BA—MS	BA—IA	MS—IA
Mean similarity/dissimilarity (%)	**58.14**	**56.09**	**57.23**	**69.02**	**43.05**	**48.27**	**43.18**	**49.86**	**41.6**	**53.64**
Tetraphyllidea fam. gen. sp.	41.26	41.53	44.44	43.28	20.98	21.12	22.19	23.2	21.68	25.76
Anisakis Type II	28.88	29.34	48.01	19.43	14.62	21.81	15.02	18.06	13.88	15.54
Anisakidae gen. sp.	19.35	20.98	-	35.09	21.04	20.45	29.12	23.71	27.93	37.09
Cumulative contribution (%)	**89.49**	**91.85**	**92.45**	**97.8**	**56.63**	**63.38**	**66.33**	**64.97**	**63.5**	**78.39**

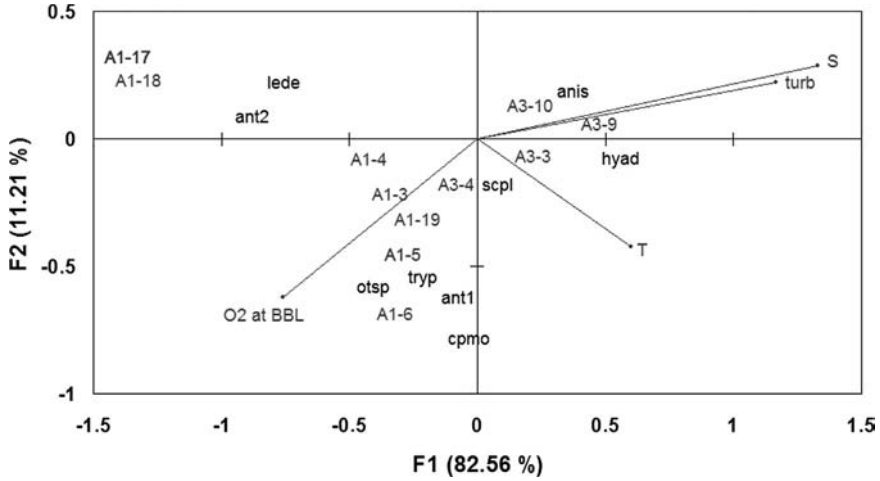

Fig. 3. Canonical correspondence analysis (CCA) showing relationships between the common parasites ($p > 10\%$) in *Mora moro* and environmental data. Abbreviations for parasites names: Anis, Anisakidae gen. sp.; Ant1, *Anisakis* Type I; Ant2, *Anisakis* Type II; Cpmo, *Capillostrongyloides morae*; Hyad, *Hysterothylacium aduncum*; Lede, *Lepidapedon desclersae*; Otsp, *Otodistomum* sp.; Scpl, larval tetraphyllideans (*Scolex pleuronectis*); Tryp, larval trypanorhynchs. Abbreviations for environmental variables: O2 at BBL, oxygen levels at the Benthic Boundary Layer; *S*, salinity; *T*, temperature; turb, turbidity. Trawl codes (A1–5, etc.) are defined in Table 1.

Table 5
Number (*n*), numerical percentage of prey (*%N*) and frequency of occurrence (*%F*) of the main prey-items in the 59 specimens of *Mora moro* analysed as a function of season and mainland/insular areas (Barcelona and Balearic slopes). Weights of food in intestines (Fullness, in g wet weight), mean number of prey per specimen and prey richness (*S*) are also given. * The Other category comprises a variety of secondary prey (e.g. siphonophores, polynoid polychaetes, isopods, copepods or pteropods).

n	**Barcelona slope**						**Balearic slope**					
	Barcelona summer (BS) 19			**Barcelona autumn (BA) 15**			**Mallorca summer (MS) 14**			**Ibiza autumn (IA) 11**		
	n	***%N***	***%F***	***n***	***%N***	***%F***	***n***	***%N***	***%F***	***n***	***%N***	***%F***
Amphipoda												
Lysianassidae	3	2.7	15.8	1	0.9	6.7	0	0	0	0	0	0
Hyperiidea	1	0.9	5.3	1	0.9	6.7	2	3.1	14.3	0	0	0
Euphausiacea												
Meganyctiphanes norvegica	28	25.2	57.9	16	14.3	53.3	7	10.9	28.6	0	0	0
Crustacea Decapoda												
Acanthephyra pelagica	4	3.6	21.1	31	27.7	86.7	8	12.5	42.9	35	48.6	100.0
Aristeus antennatus	2	1.8	10.5	6	5.4	40.0	4	6.3	28.6	6	8.3	45.5
Pasiphaea multidentata	15	13.5	73.7	9	8.0	60.0	10	15.6	71.4	14	19.4	81.8
Cephalopoda												
Teuthoidea	9	8.1	42.1	19	17.0	80.0	5	7.8	35.7	6	8.3	54.5
Heteroteuthis dispar	1	0.9	5.3	6	5.4	20.0	2	3.1	14.3	0	0	0
Osteychthyes												
Antonogadus megalokynodon	3	2.7	15.8	0	0	0	1	1.6	7.1	1	1.4	9.1
Notolepis rissoi	2	1.8	10.5	0	0	0	1	1.6	7.1	0	0	0
Myctophidae	6	5.4	21.1	2	1.8	13.3	1	1.6	7.1	2	2.8	18.2
Stomiiforme	5	4.5	26.3	1	0.9	6.7	5	7.8	35.7	1	1.4	9.1
Teleostei	13	11.7	52.6	9	8.0	60.0	6	9.4	42.9	4	5.6	36.4
Other*	19	17.1	89.5	11	9.8	66.7	12	18.8	71.4	3	4.2	27.3
Fullness (intestine)	0.0034			0.0077			0.0026			0.0095		
No. prey/specimen	5.8			7.5			4.6			6.5		
S (prey)	37			35			28			12		

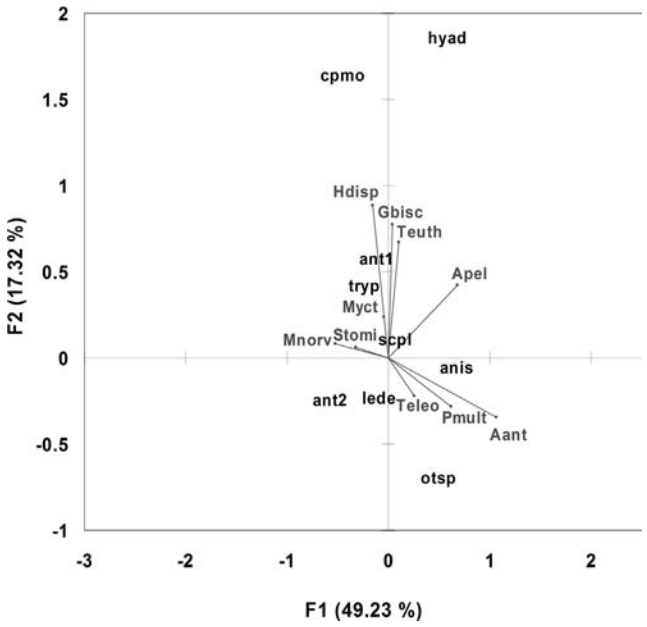

Fig. 4. Canonical correspondence analysis (CCA) showing relationships between the common parasites ($P > 10\%$) in *Mora moro* and main prey-items in the different stations sampled (A1-, A3-). Abbreviations for parasites names: Anis, Anisakidae gen. sp.; Ant1, *Anisakis* Type I; Ant2, *Anisakis* Type II; Cpmo, *Capillostrongyloides morae*; Hyad, *Hysterothylacium aduncum*; Lede, *Lepidapedon desclersae*; Otsp, *Otodistomum* sp.; Scpl, larval tetraphyllideans (*Scolex pleuronectis*); Tryp, larval trypanorhynchs. Abbreviations for prey names: Aant, *Aristeus antennatus;* Apel, *Acanthephyra pelagica*; Gbisc, *Gaidropsarus biscayensis*; Hdisp, *Heteroteuthis dispar;* Mnorv, *Meganyctiphanes norvegica;* Myct, Myctophidae; Pmult, *Pasiphaea multidentata;* Stomi, Stomiiformes; Teleo, Teleostei; Teuth, Teuthoidea.

fishes from off Mallorca in summer was significantly higher (GLM, $F_{(3,54)}=8.658$, $p=0.0001$) (Table 3). An association of AChE activity with parasite total mean abundance was found (GZM, $\chi^2=5.995$, $p=0.014$), and particularly with Tetraphyllidea fam. gen. sp. (GZM, $\chi^2=7.076$, $p=0.008$), similarly as between LDH activity and *Anisakis* Type II abundance (GZM, $\chi^2=6.446$, $p=0.011$). A negative relationship was observed between AChE and HSI and also between AChE and K (GLM, $F_{(1,57)}=14.989$, $p=0.0001$ and GLM, $F_{(1,57)}=11.802$, $p=0.001$, respectively). For LDH, a positive relationship was seen with GSI, K and TL (GLM, $F_{(1,56)}=4.389$, $p=0.041$; GLM, $F_{(1,56)}=4.417$, $p=0.04$ and GLM, $F_{(1,56)}=6.88$, $p=0.011$, respectively). No sex-related differences were detected for both enzymatic activities (GLM, $p > 0.05$).

3.4. Histological and histopathological assessment and its relationships with parasite fauna, enzymatic activities and biological parameters

The histological alterations were: (1) variations in splenic MMCs, (2) granulomas in liver and, occasionally, spleen and gonads, and (3) CUEs in gills.

MMC were distributed homogenously in splenic tissue of all specimens, and presented an irregular shape with variable size. Their average values for size and number ranged from 114.42 to 191.56 μm^2 of MMC/mm^2 and 31.88 to 36.96 MMC/mm^2, respectively (Table 3). There were no significant differences for either SC or NC among locality-season groups (GLM, $p > 0.05$ in all cases). There was no association between MMC parameters and parasite diversity descriptors or abundance (GLM, $p > 0.05$). Fish with larger size and higher LDH activity levels showed significant larger MMC (GLM, $F_{(1,48)}=4.429$, $p=0.041$ and GLM, $F_{(1,40)}=4.302$, $p=0.045$, respectively). The rest of fish condition indices and AChE activity were not related to MMC parameters (GLM, $p > 0.05$ in all cases).

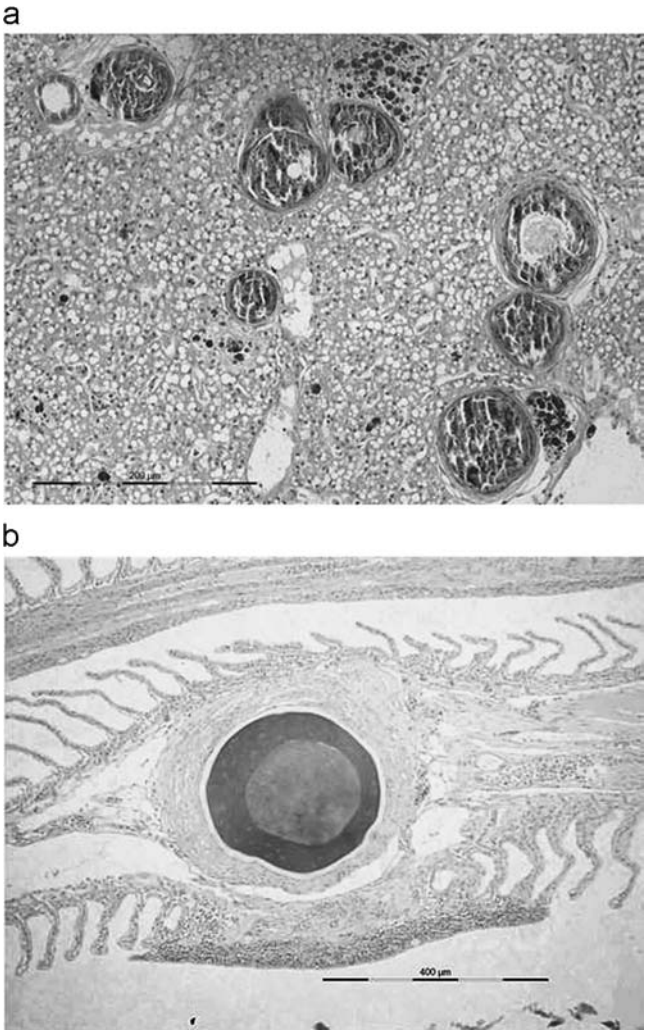

Fig. 5. (a) Granulomas in a HE-stained liver section from *Mora moro*. (b) Cyst of unknown aetiology in a HE-stained gill section from *Mora moro*.

Hepatic granulomas consisted of chronic inflammatory areas displaying a relatively common pattern: a calcified core, sometimes associated to melanin, surrounded by a layer of inflammatory cells, mainly macrophages, and fibroblasts (Fig. 5a). They were found at high prevalence and intensity in liver (81.82% and 0.61 to 1.38 granulomas/mm^2, respectively) (Table 3) and occasionally in spleen and gonad. Their distribution was generally extensive, although 63.64% of the fishes showing high intensity of granulomas displayed an aggregated distribution. Negative results for Grocott and Ziehl-Neelsen stains were obtained in all cases. There were no significant differences for NG among locality-season groups (GZM, $p > 0.05$). There was no relationship neither between NG and parasite abundance and diversity, fish condition indices, fish size, enzymatic biomarkers activity or MMC parameters (GZM, $p > 0.05$ in all cases).

CUEs were recognised as spherical nodules located in gill primary lamellae of about 400 μm in diameter. Microscopically, they were composed of three distinct layers of acellular material: a thin eosinophilic outer layer, an intermediate basophilic layer of varying width and an eosinophilic core also variable in size (Fig. 5b). They were found at a low total prevalence (18.18%) and number (on average, 0.18 CUEs for each right gill). There were no significant differences for CUEs prevalence among locality-season groups (GZM, $p > 0.05$).

4. Discussion

This is the first description of the parasite communities occurring in *M. moro* and of their temporal and geographical variations in the NW Mediterranean Sea. Moreover, the relationship between data on parasite communities and an extensive data set on fish diet, biomarkers of exposure and effect (i.e. enzymatic activities and histological alterations, respectively) and biological and environmental variables is addressed. With the exception of *L. desclersae*, all the parasites described in the present study are new host records. *M. moro* is characterised by a rich and abundant parasite fauna, consisting on 18 different endoparasite taxa, similar to the richness observed in other morids, such as *Lepidion lepidion* (12 different taxa) (unpubl. data), which shares niche in the sampled waters, or *Antimora rostrata* (21 different taxa) in the Atlantic Ocean (Campbell et al., 1980). An overlap of the parasite fauna of *M. moro* with other deep-sea generalised feeders is expected, as similarities in diet and habitat lead to similarities in parasite composition (Campbell et al., 1980). In fact, eight and seven parasite taxa are shared with *L. lepidion* and *Phycis blennoides*, respectively (Unpublished data of the ANTROMARE project).

In all likelihood, a varied diet both exploiting benthopelagic (even some benthic) prey and zooplankton (Relini Orsi, 1976; Carrassón et al., 1997; current results) could be the reason for the high parasite richness observed in *M. moro*. Parasite diversity and abundance are believed to be high in the near-bottom environment due to the higher availability of intermediate hosts in the benthos (Campbell et al., 1980; Marcogliese, 2002; Klimpel et al., 2010). In fact, fish species feeding in this realm are considered among the most infected teleosts (Campbell et al., 1980; Marcogliese, 2002), while mesopelagic fish (such as myctophids), whose diet is practically based on zooplankton (Fanelli et al., 2014), show much lower parasite richness (Klimpel et al., 2010). Zooplankton, however, experiences high turnovers, migrations and temporal changes in species succession (Sardou et al., 1996), which can enhance parasite transmission between habitats and thus, increase diversity, especially in the benthic boundary layer (Marcogliese, 2002). A diet involving both large invertebrates and fish, revealed in the present study, favours rich and abundant parasite communities due to the accumulation of the parasites from each prey and along the food chains on the host (Marcogliese, 2002). Active predation and large body size may also contribute to this pattern because they correlate with the range of prey exploited (Carrassón and Cartes, 2002). The high presence of larval and juvenile forms found in its parasite fauna is in accordance with stable isotope studies performed on *M. moro*, which reveal that this species is not among the deep-sea top predators (Polunin et al., 2001; Fanelli et al., 2013b).

Anisakid nematodes use crustaceans or other invertebrates as first intermediate hosts (Anderson, 2000). This is confirmed in the present work to species level for Anisakidae gen. sp., suggesting that both mesopelagic (*P. multidentata*, *A. pelagica*) and necto-benthic (*A. antennatus*) decapods may act as intermediate hosts for them. Especially interesting is the possible role of *A. antennatus*, a species of high commercial interest, with first-age recruits (age-sizes found in *M. moro* guts) distributed at >1000 m (Cartes and Demestre, 2003). *Anisakis* spp. are distributed worldwide and all representatives have heteroxenous life cycles involving an important and diverse array of invertebrate and/or vertebrate intermediate hosts, with cephalopods and fish acting as paratenic hosts (Abollo et al., 2001). *Anisakis simplex* also occur in other potential prey of *M. moro*, such as myctophids, in the Middle-Atlantic Ridge (Klimpel et al., 2010), and *Anisakis* type I (attributable to *A. simplex*) was also linked to myctophids in the CCA. According to present results, cephalopods (mainly benthopelagic squids as *H. dispar* and *H. reversa*) are also linked to *Anisakis* Type I found in *M. moro*. In contrast, *Anisakis* Type II (attributable to *Anisakis physeteris*), for which *M. moro* seems to be an important host, was not linked to any prey. *Mora moro* probably acts as paratenic host for L3 before anisakids are transmitted to the final host, likely to be another teleost (in the case of *H. aduncum*) or a marine mammal (in the case of *Anisakis* spp.) (Anderson, 2000). Cetaceans and pinnipeds are final hosts for *Anisakis* spp. (Klimpel et al., 2008), and seen the importance of these nematodes on *M. moro* parasite composition, this species could be part of the habitual diet of *Physeter macrocephalus* or other open-ocean cetaceans of the North-Western Mediterranean that feed on deep-sea fish (Evans and Hindell, 2004; Spitz et al., 2011). *H. aduncum* is a globally distributed generalist nematode that uses crustaceans as first and piscivorous fish as final hosts, and can also use either fish or invertebrates as intermediate paratenic hosts (Anderson, 2000; Klimpel et al., 2009). It has been rather related in our analyses to some fish (*G. biscayensis*) and benthopelagic squids prey of *M. moro*, which probably become infected by consuming crustaceans (*Histioteuthis* spp. consume mesopelagic crustaceans (Pasiphaeidae) as main prey Fanelli et al., 2012). Third and fourth-stage larvae and adult specimens were found in *M. moro*, thus indicated that it is a suitable final host for this nematode. A fish can act as paratenic or final host for *H. aduncum* depending on the size of the L3 in the first intermediate host, so that larvae moult into fourth-stage larvae in the intestinal lumen of the fish when they are longer than 3 mm (Køie, 1993). Prey linked to this parasite according to present results are big enough to host large L3, which can develop further in *M. moro*. *C. morae* is a recently described trichinelloid nematode species reported on two deep-sea Mediterranean fish (*L. lepidion* and *M. moro*) (González-Solís et al., 2014) and recently found also in *Alepocephalus rostratus* (unpublished data of ANTROMARE project). Although the life cycle of this genus is not known, the analyses carried out in the present study link *C. morae* to the same prey as *H. aduncum* (fish and cephalopods).

Lepidapedon is a speciose and generalistic digenean genus that has radiated predominantly in deep-sea, becoming the most common in this environment (Bray et al., 1999; Klimpel et al., 2009). Gastropods are first and ctenophores, chaetognaths, annelids, cnidarians, bivalves, gastropods and ophiuroids second intermediate hosts (Bray, 2001). The only possible intermediate hosts could be some polychaetes (Polynoidae) consumed accidentally by *M. moro*, although transmission via pelagic shrimps consumed by *M. moro* (e.g. Pasiphaeaidae), in turn preying on chaetognaths or cnidarians (Cartes, 1993), cannot be excluded. The cestodes Tetraphyllidea fam. gen. sp. and Trypanorhyncha fam. gen. sp. use elasmobranchs as final hosts (Klimpel et al., 2008). Tetraphyllideans are widespread and abundant in marine fish (Klimpel et al., 2009), and probably use *M. moro* as second intermediate host after infecting it through crustaceans or other fish present in its diet. They did not show a clear position in the CCA probably due to its linkage to many kinds of marine organisms (i.e. both invertebrates and fish). Trypanorhynchs may use *M. moro* as paratenic host, which could get infected in a similar way given that present results link them to teleosts and, less importantly, to different kinds of invertebrates.

The influence of fish size on parasite abundance can be explained depending on the microhabitat that each of these parasites inhabits within the host. The abundance of coelozoic parasites of the digestive tract, such as Tetraphyllidea fam. gen. sp., would be mainly determined by the feeding rates or dietary shifts of the host (Marcogliese, 2002), whereas histozoic parasites, such as Trypanorhyncha fam. gen. sp., Anisakidae gen. sp. and *Anisakis* Type II, would accumulate within the tissues throughout the life of their host (Cañás et al., 2010).

The higher abundance observed in sample from off Ibiza slope for Anisakidae gen. sp., tetraphyllideans and *H. aduncum*, is probably due to a differential availability of intermediate hosts between areas. Turbidity promotes an increase of the zooplanktonic and suprabenthic invertebrate communities, which are used as first intermediate hosts by many parasites, and it could be linked to the higher infection rates observed for these parasites in *M. moro* in autumn, when observed intestine fullness was also higher. The environmental analysis performed suggests that the majority of *M. moro* parasites (other than Anisakidae gen. sp., *H. aduncum* and Tetraphyllidea fam. gen. sp.) are linked to mainland (Barcelona slope) hauls, with maxima of near-bottom O_2. This is consistent with higher O_2 concentration found in mainland compared to insular areas in the Balearic Basin (Cartes et al., 2013). Higher O_2 concentration near the bottom enhances higher biomass of zooplankton at 1000–1300 m, higher food availability for *M. moro* and, likely, higher parasite infection via prey ingestion. Thus, it could account for the increase of parasite richness observed off Barcelona. The patterns observed at the component community and infracommunity levels were congruent, as the three groups obtained with the FCA correlated with the gradient of dissimilarity observed in SIMPER in most cases. All analyses revealed geographical variability mainly explained by the abundance patterns of Anisakidae gen. sp., *Anisakis* Type II and Tetraphyllidea fam. gen. sp. Based on the results of our GZM, Anisakidae gen. sp. and tetraphyllidean cestodes could allow differentiation between fish samples from off Barcelona, Mallorca and Ibiza, whereas *Anisakis* Type II might distinguish between samples from continental and insular slopes, especially in autumn.

General biomarkers of stress that respond to natural and anthropogenic stressors have been recommended in studies aiming to assess parasite influence on animal's health (Marcogliese and Pietrock, 2011). In this sense and under stressing conditions, activities of AChE in fish are inhibited while LDH are increased (Almeida et al., 2012a, 2012b). To date, very few studies have assessed the effect of parasitism on AChE and LDH activities in fish (see Liu et al., 2005; Podolska and Napierska, 2006).

Overall, the present results suggest that fish with signs of good health, as suggested by physiological markers, do host more abundant and diverse parasite fauna. Nevertheless, parasites are generally considered as stressors to their host, and a stress response with lower AChE and higher LDH activities could be expected. This pattern does not agree with the obtained results, the reason being either the infection threshold needed to compromise enzymatic activities was not reached or the healthy fish condition and the existence of a diverse parasite fauna are indicative of a good ecological status of their environment. A positive relationship has been found between LDH and TL in *M. moro*, in accordance with previous studies, which consider that LDH activity increases with body size to satisfy burst-swimming energetic demands (Almeida-Val et al., 2000). LDH fluctuations throughout the year have been linked to reproductive activity in several deep-sea fish (Koenig and Solé, 2014) and, in the present study, an association between LDH activity and female GSI supports the former association.

MMC are known to increase in size and frequency in conditions of environmental stress, infectious processes and destruction, recycling or storage of endogenous and exogenous materials, among others (Agius and Roberts, 2003). Since they play a major role as dumps for waste of spoiled or damaged cells, an increase of their size as the fish grows older and bigger, as observed in this study, is expected and has been also noted by other authors (Brown and George, 1985).

Granulomas can develop in different organs as a response to infective microorganisms (e.g. *Ichthyophonus* sp. Carreras-Aubets et al., 2011, mycobacteria Zerihun et al., 2012) or macroparasites (Dezfuli et al., 2013). Nevertheless, granulomas found in the present study were not associated with any of the mentioned agents and, in fact, did not seem to have an effect on fish health, as suggested by enzymatic markers. CUEs have been found at high prevalence in other fish species of the same locations, such as *P. blennoides* and *L. lepidion* (prevalences $>40\%$) (unpublished data), but they do not seem to be as common in *M. moro* specimens. A link between the presence of these structures and environmental contamination has been suggested in some studies (Munday and Brand, 1992; Carreras-Aubets et al., 2011) but has not been assessed in the present study. However, no conclusion should be taken due to the low prevalence for these cysts found in *M. moro*.

In summary, *M. moro* showed a rich and abundant parasite fauna, mainly as a consequence of its dietary habits, which include ichthyophagous habits and active predation. Except for *L. desclersae*, all parasites constitute new host records. Detailed parasite-prey relationships were found, increasing our understanding of the life cycles of the parasites assessed. Most parasites were favoured by high levels of near-bottom O_2 and associated zooplankton linked to mainland sites, which enhances biomass and parasite transmission. Furthermore, differential supply of organic material throughout the year affects zooplankton and benthopelagic communities and is suggested to determine the seasonal variability in most parasites. Anisakidae gen. sp., *Anisakis* Type II and Tetraphyllidea fam. gen. sp. abundance patterns might allow differentiation of populations of *M. moro* in the sampled area. A link between parasite load and enzymatic biomarkers was discussed.

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CHAPTER 4 - THE PARASITE COMMUNITY OF *PHYCIS BLENNOIDES* (BRÜNNICH, 1768) FROM THE BALEARIC SEA IN RELATION TO DIET, BIOCHEMICAL MARKERS, HISTOPATHOLOGY AND ENVIRONMENTAL VARIABLES

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The parasite community of *Phycis blennoides* (Brünnich, 1768) from the Balearic Sea in relation to diet, biochemical markers, histopathology and environmental variables

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ABSTRACT

The greater forkbeard *Phycis blennoides* is a benthopelagic fish distributed in the Mediterranean and NE Atlantic. The main goal of this study is to describe the complete parasite community of this species, which is at present unknown. A total of 188 specimens of *P. blennoides* were captured in the Balearic Sea (NW Mediterranean Sea) at 550–1250 m depth during the four seasons of 2007, in summer of 2010 and in summer and autumn of 2011 at five distinct localities off the mainland slope off Catalonia coasts and off the insular slope off the Balearic Islands. Environmental and fish biological, parasitological, dietary, enzymatic and histological data were obtained and the relationships among them tested. A total of 20 different parasites were recovered, of which 11 constitute new host records. The most important parasites were the monogenean *Diclidophora phycidis,* the digeneans *Bathycreadium brayi* and *Lepidapedon* spp., the nematodes *Capillaria gracilis*, *Collarinema collaris*, *Cucullanus* sp. and *Hysterothylacium aduncum*, and the copepod *Clavella alata*. Overall, the parasite community of *P. blennoides* was characterized by high abundance, richness and diversity. Significant differences in the structure of the parasite community were detected between samples from & $2lt;1000 to & $2gt;1000 m depth and between samples from off the mainland and insular slopes. Significant seasonal and/or geographical differences were found for some specific parasites. Abundance of the nematode *C. collaris* was associated to high levels of turbidity and O_2 concentrations near the bottom. Abundances of *H. aduncum*, *D. phycidis*, *B. brayi* and *Lepidapedon* spp. were linked to high near-bottom temperature and salinity. Dietary analyses evidenced the role as potential intermediate hosts in parasite transmission by some prey (e.g. the teleost *Gaidropsarus biscayensis* for the cestode *Grillotia* cf. *erinaceus* and the nematodes *Anisakis* spp. or the euphausiid *Meganyctiphanes norvegica* for the acanthocephalan *Echinorhynchus* sp.). While the abundance of *B. brayi*, *C. collaris*, *Cucullanus* sp. and *Echinorhynchus* sp. was negatively linked to acetylcholinesterase activity (AChE), the abundance of *Echinorhynchus* sp. and *H. aduncum* correlated positively with lipid peroxidation levels. Cysts of unknown etiology in fish gills were detected at higher prevalence than in any other fish from the same area. Number and area of hepatic macrophage centres varied significantly among seasonal and geographical groups and seemed not significantly influenced by parasite infection levels.

1. Introduction

The Greater forkbeard, *Phycis blennoides* (Brünnich, 1768) (Gadiformes: Phycidae) is a benthopelagic species distributed in the Mediterranean and North-Eastern Atlantic Ocean (Froese and Pauly, 2016). It inhabits sandy and muddy bottoms along the continental shelves and slopes at depths between few meters and 800 m (Cohen et al., 1990). In the Mediterranean Sea this species dominates the upper slope (D'Onghia et al., 2004; Fanelli et al., 2013), but it can be found up to 1300 m depth (Stefanescu et al., 1992; Massutí et al., 1996; Cartes et al., 2004). *P. blennoides* is typically a benthos feeder (Papiol et al., 2014), with a diet based on epibenthic-nectobenthic organims (MacPherson, 1978; Morte et al., 2002; Papiol et al., 2014). Diet gradually changes with fish size, so that older fish shift from smaller

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(e.g. mysids, amphipods and isopods) to larger prey, e.g. shrimps, crabs and especially teleosts (Morte et al., 2002). In parallel, younger fishes inhabit shallower waters (i.e. less than 700 m depth) and move down the slope to deeper waters with maturity (Massutí et al., 1996).

Interestingly, despite being an important target of the fishery industry in the Balearic Sea (Massutí et al., 1996), the parasite community of *P. blennoides* is poorly studied. A high number of studies have reported the presence of different parasite species infecting this fish in North Atlantic and Mediterranean waters for more than forty years (Lopez-Roman and Maillard, 1973; Paggi et al., 1975; Raibaut et al., 1998; Farjallah et al., 2006; Valero et al., 2006; Pérez-del-Olmo et al., 2014; Hassani and Kerfouf, 2015; Pulleiro-Potel et al., 2015, among others). However, most of these publications are mere citations or descriptions of a single parasite and only a few of them take into consideration different taxa. Furthermore, in this latter case they address only part of the community (e.g. Farjallah et al. (2006) and Valero et al. (2006) focus on anisakid nematodes).

Since each parasite species provides specific information related to its particular life cycle and biological traits, ecological studies that consider the entire parasite community are much needed as they provide a broader and integrated perspective than those focused on a single parasite (D'Amelio and Gerasi, 1997; Dzikovski et al., 2003; Marcogliese, 2005; Pérez-del-Olmo et al., 2007). This is especially true when the main research goal is to use the whole community as indicator of environmental health, local food webs and host biology.

Several studies have proved that parasite assemblages are effective indicators of environmental impact (Marcogliese, 2005; Vidal-Martínez et al., 2010 and references therein). Some of these works have highlighted the usefulness of considering different functional groups that respond differently to environmental stress; for example, monoxenous parasites (those whose life cycle involve a single host, normally ectoparasites) are better adapted for survival and their levels increase in a stressed environment, while heteroxenous parasites (those whose life cycle involves two or more different hosts, normally endoparasites) show higher vulnerability, which results in decreased richness and abundance-related parameters (Diamant et al., 1999; MacKenzie, 1999; Pérez-del-Olmo et al., 2007). Since the parasite community usually includes an array of species in most cases trophically transmitted, and each of them has a characteristic life cycle involving one or more intermediate hosts, the study of the whole assemblage provides an overview of the local food webs and any potential disturbances affecting them (Valtonen et al., 2010; Münster et al., 2015). Diet shifts (either ontogenic, seasonal or geographical), so common in many fishes, are coupled with changes in the structure and composition of their parasite community (Dallarés et al., 2014, 2015; Constenla et al., 2015; Münster et al., 2015; Pérez-i-García et al., 2015), and dissimilarities on parasite infracommunities (all parasites of all species infecting a single host) have been successfully used to discriminate host populations (MacKenzie and Abaunza, 2005; Timi et al., 2010a). Conversely, similar parasite assemblages can be effectively used to infer phylogenetic proximity among host species (Poulin, 2010; Locke et al., 2013), in the same way as they can reflect many other kind of affinities, either geographical (Locke et al., 2012 and references therein), ontogenic (Timi et al., 2010b) or ecological (Locke et al., 2013).

Morphometric indices are widely used for assessing the fitness and health status of fish stocks, as well as to evaluate the effects of stress and environmental pollution (Bolger and Connolly, 1989; Carreras-Aubets et al., 2011). However, more specific indicators of stress or pathological conditions, such as biochemical markers and histological changes, can reveal pathologies or alterations of different biological processes not revealed by the above mentioned morphometric indices (Garcia et al., 2000; Carrassón et al., 2008; Carreras-Aubets et al., 2011). In fact, the integrated use of different indicators that work at different biological levels is highly recommended to determine the impact of parasites in their fish hosts to a broad range of effects (Feist and Longshaw, 2008).

As far as we know, the effects of infection by different parasites in *P. blennoides* on its condition indices and health status have been barely addressed. Concerning condition indices, only Pulleiro-Potel et al. (2015) related infection levels of anisakids to fish condition factor. In relation to biochemical indicators, many studies conducted in NW Mediterranean waters have related the levels of a wide spectrum of enzymatic and biochemical markers in different tissues of *P. blennoides* to the concentration of environmental pollutants, biological parameters (i.e. sex, size or swimming capacity) and ecological variables (e.g. trophic level, feeding strategy, seasonality, etc.) (Garcia et al., 2000; Solé et al., 2006, 2008, 2009a, 2009b, 2010a, 2010b). However, the possible association between these biomarkers and parasitological infections has not been tested so far. Feist et al. (2015) published an histopathological assessment on several fish species in the NE Atlantic Ocean, including *P. blennoides,* to test the effect of anthropogenic contaminants; but in this case only liver and gonads were studied and with limited observations on parasite infections.

Since no studies considering the totality of parasites infecting *P. blennoides* have been published to date, the primary goal of the present study is to characterize the parasite community of the greater forkbeard in the Balearic Sea (NW Mediterranean Sea). Parasitological data are related to fish diet and environmental gradients (eight combinations of localities and seasons) and variables (i.e. temperature, turbidity, salinity and oxygen content of water masses) in order to infer possible pathways of trophic transmission for the parasites recovered and to reveal any geographical, seasonal and environmental variable-related trends on the host-parasite system. Moreover, an assessment of the impact of parasite infection on fish health is performed through different morphometric indices, biochemical markers (namely acetylcholinesterase, AChE and lactate dehydrogenase, LDH activities), lipid peroxidation (LP) levels and histological analysis of gills, liver, spleen and gonads, as well as their relationships.

2. Materials and methods

2.1. Study area and sampling collection

A total of 188 specimens of *P. blennoides* were captured in the Balearic Sea (NW Mediterranean Sea) using bottom trawls with 2 doors (the otter trawl semi balloon, OTSB 14, and commercial trawls) on board of two research vessels (García del Cid/Sarmiento de Gamboa) and of the boat Stella Maris III. Sixteen samplings (hauls) were performed along the four seasons of 2007 at depths ranging *ca* between 550 and 800 m, and 18 hauls were performed in summer of 2010 and summer and autumn of 2011 at two depth ranges *ca* 450–650 m and 1000–1250 m). Based on this, two depth strata were defined for post analyses (Depth 1: 450–800 m and Depth 2: & $2gt;1000 m) and five distinct localities, three off the mainland slope off Catalonia coasts (Besós, Vilanova and Tarragona) and two off the insular slope off the Balearic Islands (Mallorca and Ibiza) (Fig. 1) (see Table 1 for hauls data).

The following environmental parameters were measured at 5 m above the sea-bottom by deployment of a Seabird 25- CTD, used simultaneously to hauls: temperature (T) in °C, salinity (S) in psu, O_2 concentration (ml/l) and turbidity (voltage), the latter recording signals of both inorganic and organic suspended material.

Immediately upon capture, total length (TL) and total weight (TW) were taken for each fish. In a subsample of 110 fish, gills from the right side and a portion of liver, spleen and gonads were weighed and immediately fixed in 10% buffered formalin for histological purposes and a portion of axial musculature was dissected and frozen at −20 °C for biochemical analyses. The rest of the specimen, as well as the rest of the captured fish (n=78) were kept at −20 °C for parasitological studies.

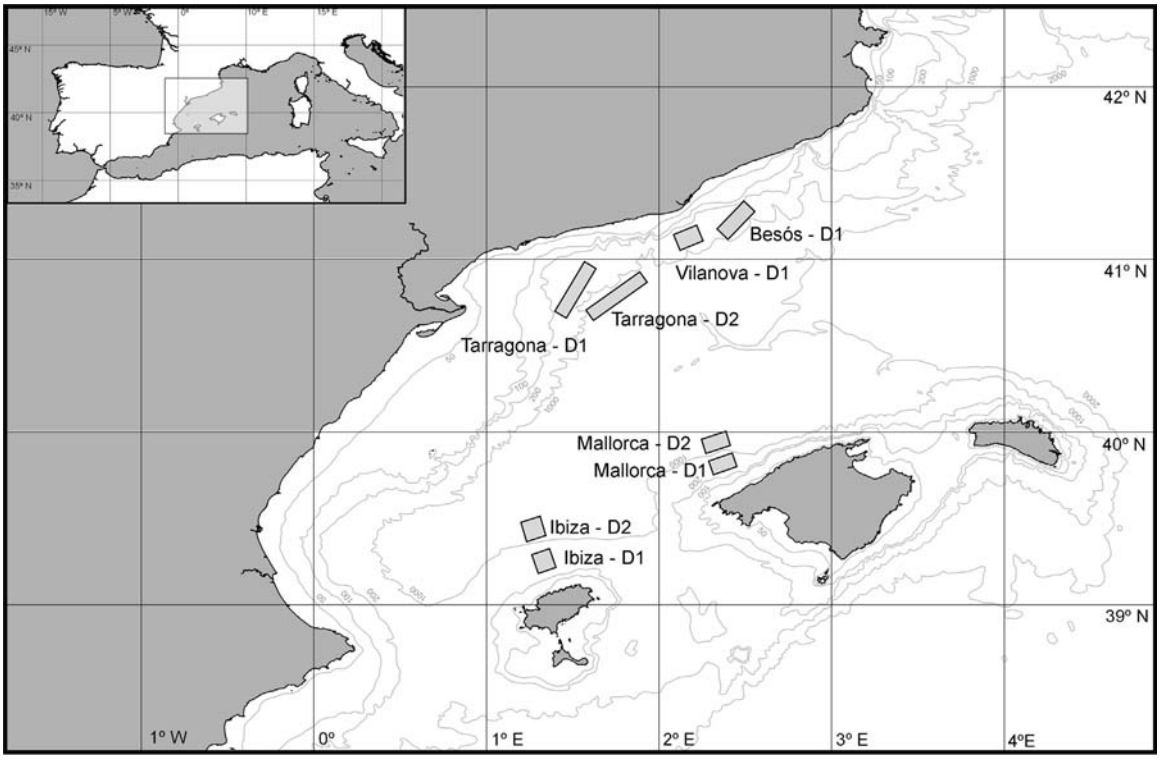

Fig. 1. Study area showing the sampling localities in the Balearic Sea. D1, depth 1 (400–1000 m depth); D2, depth 2 (& $2gt;1000 m depth).

Table 1
Sampling data of *Phycis blennoides*. N: number of specimens; T °C: temperature; S: salinity in PSU; O: oxygen concentration in ml/L; Tur: turbidity in V; OTSB: Semi-balloon otter-trawl; BOU: commercial fishing boat.

Locality-season group	Haul	Date (dd/mm/yy)	Depth (m)	Coordinates		n	Environmental variables			
				Latitude (N)	Longitude (E)		T °C	S (psu)	O (mL/L)	Turb (V)
Besós-winter	B1–BOU1	06/03/2007	651	41.24	2.46	14	13.29	38.53	3.93	0.15
	B1–OTSB2	25/02/2007	798	41.15	2.40	10	13.19	38.51	4.12	1.10
Besós-spring	B2–BOU3	11/05/2007	559	41.24	2.46	11	13.29	38.53	5.76	0.27
	B2–OTSB2	28/04/2007	650	41.17	2.38	8	13.29	38.53	5.76	0.27
	B2–OTSB4	29/04/2007	809	41.15	2.40	2	13.24	38.52	5.77	0.44
Besós-summer	B3–BOU7	19/07/2007	791	41.15	2.40	19	13.18	38.51	5.78	0.08
	B3–OTSB2	30/06/2007	663	41.17	2.39	5	13.32	38.54	5.76	0.03
	B3–OTSB3	30/06/2007	804	41.14	2.36	3	13.18	38.51	5.78	0.08
	B3–OTSB5	01/07/2007	671	41.24	2.49	1	13.32	38.54	5.76	0.03
Besós-autumn	B4–OTSB2	02/10/2007	668	41.17	2.45	2	13.39	38.55	8.22	0.47
	B4–OTSB3	02/10/2007	811	41.14	2.36	6	13.18	38.51	8.25	0.18
	B4–OTSB5	03/10/2007	660	41.24	2.46	1	13.39	38.55	8.22	0.47
	B4–OTSB6	03/10/2007	716	41.24	2.49	3	13.39	38.55	8.22	0.47
Vilanova-summer	B3–BOU1	26/06/2007	780	41.07	2.20	11	13.17	38.50	5.78	0.09
	B3–BOU2	26/06/2007	574	41.13	2.09	20	13.41	38.53	3.95	0.40
	B3–OTSB6	05/07/2007	662	41.09	2.18	4	13.41	38.53	3.95	0.40
Tarragona-summer	A2–OTSB1	18/06/2011	639	40.58	1.44	1	13.14	38.50	4.16	1.03
	A2–OTSB2	18/06/2011	646	40.57	1.44	3	13.14	38.50	4.16	1.03
	A2–OTSB3	18/06/2011	615	40.58	1.44	1	13.14	38.50	4.16	1.03
	A2–OTSB4	19/06/2011	627	40.91	1.58	2	13.12	38.50	4.14	0.24
	A2–OTSB5	19/06/2011	628	40.91	1.58	2	13.12	38.50	4.14	0.24
	A2–OTSB6	19/06/2011	648	40.91	1.58	3	13.12	38.50	4.14	0.24
	A2–OTSB7	20/06/2011	620	40.68	1.44	3	13.08	38.49	4.20	0.00
	A2–OTSB12	23/06/2011	1060	40.79	1.59	3	13.10	38.49	4.17	0.13
	A2–OTSB13	23/06/2011	1052	40.93	1.84	2	13.10	38.49	4.17	0.13
Tarragona-autumn	A3–OTSB3	15/10/2011	1051	40.85	1.73	4	13.11	38.49	4.21	0.24
	A3–OTSB4	15/10/2011	1236	40.70	1.62	1	13.12	38.48	4.25	0.28
Mallorca-summer	A1–OTSB15	17/07/2010	682	39.80	2.34	4	13.07	38.49	4.14	0.90
	A1–OTSB16	17/07/2010	457	39.78	2.36	8	13.09	38.50	4.12	0.07
	A1–OTSB17	19/07/2010	1006	39.87	2.34	5	13.06	38.48	4.33	0.08
Ibiza-autumn	A3–OTSB7	17/10/2011	508	39.19	1.31	3	13.07	38.49	4.10	0.38
	A3–OTSB8	17/10/2011	573	39.23	1.39	12	13.07	38.49	4.10	0.38
	A3–OTSB9	17/10/2011	1062	39.39	1.31	10	13.07	38.49	4.29	0.25
	A3–OTSB10	18/10/2011	1272	39.42	1.28	1	13.10	38.49	4.16	0.23

2.2. Parasitological survey

In the laboratory, fish were thawed and liver and gonads weighed prior to examination. For each fish, all external surfaces and buccal cavity were carefully inspected for ectoparasites to the naked eye and by means of a stereomicroscope; then all organs and musculature were checked for endoparasites using a stereomicroscope. Parasites were collected and preserved in 70% ethanol. For identification, monogeneans, digeneans and acanthocephalans were stained with iron acetocarmine, dehydrated through a graded ethanol series, cleared in clove oil or dimethyl phthalate and examined as permanent mounts in Canada balsam. Nematodes, copepods and everted tentacles of trypanorhynch cestodes were examined as semi-permanent mounts in pure glycerine. All parasites were identified to the lowest possible taxonomic level. Voucher material of the common parasites identified to species level has been deposited in the Helminthological Collection of the Universitat Autònoma de Barcelona (UABhc) under the accession numbers C20, C21, Co1, D1, M1, N1–N6.

2.3. Diet analysis

Guts (stomachs and intestines) of 100 individuals that were examined for the occurrence of parasites were further analysed for diet determination. Gut contents were individually weighed to the nearest 0.001 g and prey were identified to the lowest possible taxonomic level under a stereomicroscope. Not all specimens analysed for parasitology were analysed for diet due to the high number of specimens presenting everted stomachs and to the difficulty of counting prey, especially in intestines. Diet was considered by prey number, hence being directly comparable with parasitological indices used. In most fish with everted stomachs, especially large specimens (56 out of 100 specimens), only intestine contents were analysed. Soft prey can be underestimated by analysing intestines; however and in spite of this difficulty, small or soft animals (e.g. peracarid crustaceans or polychaetes) normally have hard specific structures (e.g. mandibles or telsons) that allow prey identification and quantification.

2.4. Biochemical determinations

A muscle portion of 0.3g was used for the measurement of AChE and LDH activities and LP levels. Muscle tissue was homogenised in a 50 mM buffer phosphate pH 7.4 in a 1:5 (weight:volume) ratio using a polytron® blender. The homogenate was then centrifuged at 10,000*g* ×30 min and the supernatant (S10) was used for the biochemical determinations.

For AChE assay, the concentration of substrate used (ATC) was 1 mM, as described in Solé et al. (2010a). AChE activities were determined according to Ellman et al. (1961) at 405 nm. For LDH assay, 150 µl of NADH solution (300 µM) in phosphate buffer was mixed with 25 µl of diluted samples (to maintain linearity in the measure) and 50 µl of pyruvate (4.5 µM) in each microplate well. LDH activity was given as the amount of pyruvate consumed due to NADH oxidation at 340 nm (Vassault, 1983). AChE and LDH activities were expressed in nmol/min/mg prot. For LP assay, 200 µl of S10 fraction were mixed with 650 µl of 1-methyl-2-phenylindole in acetonitrile:-methanol (3:1) and 150 µl of 37% HCl. After incubation at 45 °C×40 min, the reaction was stopped in ice and centrifuged at 10,000*g*×10 min. Reading was performed at 586 nm versus a standard solution of 1,1,3,3-tetramethoxypropane treated similarly. LP activity was expressed in nmol MDA (malondialdehyde)/g wet weight.

All readings were performed in triplicate at 25 °C in a 96-well plate using a Tecan Infinite 200 microplate reader. Total protein content in the S10 fraction was determined according to Bradford (1976) method using bovine serum albumin as standard (BSA 0.1–1 mg/ml).

2.5. Histological and histopathological processing and assessment

Fixed samples of gills, liver, spleen and gonads were processed for histology according to routine techniques; paraffin blocks were sectioned at 4 µm and stained with Haematoxylin and Eosin. Each slide was inspected microscopically for alterations, pathologies or the presence of parasites, which were identified according to their morphological characteristics when possible.

For the analysis of splenic macrophage centres (MC), three fields of view (0.24 mm^2/ screen) were randomly selected from each section of the spleen and examined microscopically at ×200. Area and number of MC on each field were measured using a Micro-Comp Integrated Image Analysis System.

2.6. Data analyses

Two size groups of hosts were established prior to data analyses, i.e. size 1 (TL≤20 cm) and size 2 (TL & $2gt;20 cm), approximately corresponding to immature and mature fishes, respectively (Rotllant et al., 2002).

Parasite prevalence (P) and abundance (MA) were calculated following Bush et al. (1997). Parasite species showing a total P & $2gt;10% are henceforth called common. The diversity and dominance of parasite infracommunities (i.e. all parasites of all species in an individual fish host) were estimated using Brillouin's index (PRIMER v6; Anderson et al., 2008) and Berger-Parker dominance index (calculated as the number of individuals of the most abundant parasite species divided by the total number of parasites in a given fish host) (B-P), respectively. Fish condition was assessed by Fulton's body condition factor (K, calculated as $(TW/TL^3)\times100$), the hepatosomatic index (HSI, calculated as (liver weight/TW)×100) and the gonadosomatic index (only considered for female fish) (GSI, calculated as (gonad weight/TW)×100). In histological sections, number (NC) and area (SC) of splenic MC per square millimetre were calculated for each field of view. Subsequent analyses involving SC and NC were carried out using the average value of the three field measurements. In the case of the cysts of unknown etiology (CUEs) found in gills, only prevalence was calculated.

To comply for normality and homoscedasticity requirements, HSI, GSI, AChE activity and LP levels were log-transformed and SC was square-root transformed prior to statistical analyses.

Significant relationships between fish TL and fish sex and condition indices were tested by means of General Linear Models (GLM).

Permutation multivariate analyses (PERMANOVA) were performed using parasite infracommunities as replicate samples to test separately the null hypotheses of no differences in the structure of the parasite infracommunities among host samples belonging to different localities, seasons and bathymetric ranges. Analyses were performed using PERMANOVA+ from PRIMERv6 (Anderson et al., 2008) on Bray-Curtis similarity matrices derived from logarithmically transformed (log(x+1)) abundance data. Permutation p-values were obtained under unrestricted permutation of raw data (9999 permutations). In view of the results obtained, subsequent analyses relating abundance and prevalence of parasite component populations with categorical groups were carried out separately for samples from depths 1 and 2.

A factorial correspondence analysis (FCA) was run using data from depth 1 in order to visualize the patterns of parasite abundance in relation to the groups of hosts belonging to the different combinations of the factors “locality” and “season” (Besós winter (BW1), Besós spring (BSp1), Besós summer (BSu1), Besós autumn (BA1), Vilanova summer (VSu1), Tarragona summer (TSu1), Mallorca summer (MSu1) and Ibiza autumn (IA1)). The analysis was applied on a data matrix including abundance data of the component populations of the 10 common parasite species. A replication of the FCA was not performed on data from depth 2 due to the low number of hosts present in three out of the four categorical groups (Tarragona summer (TSu2),

Tarragona autumn (TA2), Mallorca summer (MSu2) and Ibiza autumn (IA2)).

Then, using individual hosts as replicate samples, differences in abundance and prevalence of parasite component populations were tested using generalized models (GZM) for the factor "locality-season" using fish TL as covariate (log-binomial and logistic models were applied, respectively, for analyses on abundance and prevalence).

Possible effects of the factor "locality-season" were tested for infracommunity parasite descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (MD) and mean dominance (B-P)), fish TL, condition indices (K, HSI and GSI), levels of biochemical markers (AChE, LDH and LP), MC parameters (SC and NC) and prevalence of CUEs using GLM, with Student-Newman-Keuls post-hoc pairwise comparisons, or GZM (for TMA and prevalence of CUEs only).

Possible relationships between infracommunity parasite descriptors and abundance of common parasites with fish TL, condition indices, biochemical markers, MC parameters and prevalence of CUEs were explored by means of GLM and GZM (the latter for individual parasite abundance and abundance of common parasites).

Possible diet changes were explored by nMDS (non-parametric Multi-Dimensional Scaling), testing differences between groups based on the same factors adopted for parasite communities (i.e. "locality", "season" and "depth").

A matrix was generated including individuals for which parasitological and dietary information was available, and was further analysed by Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). To build this matrix, it is important that the number of explanatory variables (prey) is balanced with the number of objects (parasites in this case) in the data matrix. The diet of *Phycis blennoides* is highly diversified and the number of important prey contributing to diet is high. Therefore, in order to increase the number of objects, we included parasites with occurrences ≥3 in the matrix (e.g. *Anisakis* spp.), accumulating a total of 15 parasites. In such matrix, groups of individuals were considered as a function of locality, season and depth. Also, in order to increase the number of groups (cases) in the matrix, we sub-grouped individuals in hauls with high enough "*n*". This criterion was adopted in six hauls, in where males, females and undetermined specimens were grouped separately. CCA related in this case the abundance of main parasites (using infracommunity data) with prey found in guts. In CCA plots, arrows represent explanatory variables and are proportional in length to their importance on the explained variable. The same analysis was repeated in order to assess the relationships between main parasite abundance and environmental variables (T, S, O_2 and turbidity).

GLM were used to assess the relationship between biochemical markers and fish TL, sex and condition indices.

Finally, GLM and GZM were used to assess the relationship of SC, NC and CUEs with biochemical markers, fish TL, sex and condition indices, and of SC and NC with CUEs.

Fish TL was set as covariate in all analyses except those involving fish condition indices.

3. Results

3.1. Fish biological factors

Total length (TL) of the examined fish ranged from 11.3 to 56.2 cm and displayed significant differences between sexes (GLM, see Table 2 for values of statistical parameters), with females being larger than males (mean TL of 29.3 cm vs. 25.2 cm). TL varied significantly among categorical groups (GLM, $F_{(11,\ 176)}$=6.550, p & $2lt;0.001), with fish from depth 2 being larger than those of depth 1 (Table 3). K, HSI and GSI varied significantly as well among groups (GLM, $F_{(11,\ 176)}$=8.210, p & $2lt;0.001; $F_{(11,\ 176)}$=5.112, p & $2lt;0.001 and $F_{(10,\ 73)}$=3.329, p=0.001, respectively), GSI tending to be higher at depth 2, especially in fish from off Mallorca (Table 3).

3.2. Description of the parasite community

All fish were infected by at least one parasite. A total of 20 different parasite taxa were recovered: one monogenean, five digeneans, two cestodes, one acanthocephalan, nine nematodes, one copepod and one isopod, of which 11 parasites are recorded for the first time in *P. blennoides* (see Tables 4, 5).

Ten taxa were frequently found in the sampled fish and considered as "common" (total P & $2gt;10%): the monogenean *Diclidophora phycidis* (Parona and Perugia, 1889) (Mazocraeidea), the digeneans *Bathycreadium brayi* (Pérez-del-Olmo et al. 2014) (Allocreadioidea) and *Lepidapedon* spp. (Lepocreadioidea), the cestode *Grillotia* cf. *erinaceus* (van Beneden, 1858) Guiart, 1927 (Trypanorhyncha), the acanthocephalan *Echinorhynchus* sp. (Echinorhynchida), the nematodes *Capillaria gracilis* (Bellingham, 1840) (Trichinelloidea), *Collarinema collaris* (Petter, 1970) (Habronematoidea), *Cucullanus* sp. (Seuratoidea) and *Hysterothylacium aduncum* (Rudolphi, 1802) (Ascaridoidea) and the copepod *Clavella alata* Brian, 1909 (Siphonostomatoida). Amongst these, only one parasite (namely the cestode *G.* cf. *erinaceus*) was exclusively represented by larval stages.

The most abundant and prevalent parasites were the digeneans *B. brayi* and *Lepidapedon* spp. (total mean abundance (MA)=16.3 and 8.6; total P=80% and 77%, respectively) and the nematodes *C. collaris*, *Cucullanus* sp. and *H. aduncum* (MA=6.7, 4.2 and 2.4; total P=64%, 46% and 40%, respectively). The monogenean *D. phycidis*, the nematode *C. gracilis* and the copepod *C. alata* were not among the most abundant parasites, but showed high prevalence values of 44%, 49% and 41%, respectively.

The abundance of five parasites was significantly correlated with fish TL; while *D. phycidis*, *Echinorhynchus* sp. and *Cucullanus* sp. were more abundant in larger fish, *B. brayi*, *Lepidapedon* spp. and *H. aduncum* were more abundant in smaller hosts (GZM, Table 2). In view of this marked effect of host size on parasite abundance, fish TL was set as covariate in all analyses involving parasite populations.

The abundance of *Lepidapedon* spp. and *Echinorhynchus* sp. were positively correlated with K, and parasite total abundance displayed negative relationships with HSI and GSI, as also did the abundance of *D. phycidis*, *B. brayi*, *Echinorhynchus* sp., *C. collaris*, *Cucullanus* sp. and *H. aduncum* (GZM, Table 2). Finally, negative associations were found between GSI and the abundance of *B. brayi*, *Lepidapedon* spp., *C. collaris* and *H. aduncum* (GZM, Table 2).

Parasite richness and diversity showed a significant positive relationship with fish TL and a significant negative relationship with HSI, while Berger-Parker dominance index was negatively associated with fish TL (GLM, Table 2). No significant relationships were found between infracommunity parasite descriptors and K (GZM, GLM, Table 2).

3.3. Relationship between parasites and environmental parameters

The PERMANOVA analyses testing the effect of depth on parasite infracommunities between pairs belonging to the same locality-season category showed significant bathymetric differences in all cases (for the following comparisons: TSu1–TSu2, MSu1–MSu2, IA1–IA2; Pseudo-$F_{(1,\ 10)}$=8.5826, $p_{(perm)}$=0.001; 5770 unique permutations, Pseudo-$F_{(1,\ 15)}$=6.7324, $p_{(perm)}$=0.0003; 4963 unique permutations and Pseudo-$F_{(1,\ 24)}$=4.3545, $p_{(perm)}$=0.006; 9937 unique permutations, respectively). Therefore, the following analyses relating parasite component populations to the factor locality-season were performed treating the two depths separately.

Fig. 2 shows a plot of the first factorial plane of co-inertia analysis explaining 85.57% of the total variance, mainly on the first axis (61.04% of the total inertia) of the FCA using component population data of the common parasites for depth 1. Component populations of

Table 2
Values of statistical parameters (R^2 for general linear models (GLM), χ^2 for generalized models (GZM)) obtained in the different analyses performed to test possible relationships among individual parasitological descriptors (parasite richness, abundance, diversity (D) and Berger-Parker dominance index (B-P)), abundance of common parasites, fish total length (TL), sex and condition indices (condition factor (K), Hepatosomatic index (HSI), and female gonadosomatic index (GSI)), values of biochemical markers (acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities and lipid peroxidation (LP) levels), histopatological parameters (area (SC) and number (NC) of splenic macrophage centres (MC)) and cysts of unknown etiology (CUEs) found in gills). Variables with no significant relationships are not shown.

		TL	K	HSI	GSI	AChE	LDH	LP	NC	SC	CUEs[†]
Parasite richness	r	0.217**	–	**0.302*****	–	**0.421*****	–	–	–	0.243*	–
Total parasite abundance	χ^2	–	–	8.359**	9.229**	7.030**	–	–	–	–	–
Bathycreadium brayi	χ^2	5.761*	–	11.728**	**14.597*****	**12.723*****	10.775**	–	–	–	–
Clavella alata	χ^2	–	–	–	–	–	–	–	9.267**	–	–
Collarinema collaris	χ^2	–	–	20.180***	7.838**	**25.563*****	–	8.945**,[b]	–	–	–
***Cucullanus* sp.**	χ^2	**50.602*****	–	**20.073*****	–	**14.369*****	–	–	5.165*	10.598**	–
Diclidophora phycidis	χ^2	11.576**	–	**21.012*****	–	–	–	–	–	–	–
***Echynorhynchus* sp.**	χ^2	**60.475*****	5.438*	10.764**	–	**26.785*****	–	10.565**	–	–	–
Hysterothylacium aduncum	χ^2	**32.368*****	–	**21.200*****	**13.086*****	–	–	5.099*	–	–	–
***Lepidapedon* spp.**	χ^2	9.854**	8.448*	–	**22.031*****	9.823**,[a]	–	–	–	8.297**	–
D (Brillouin's index)	r	0.241**	–	**0.253*****	–	**0.366*****	–	–	0.230*	0.237*	–
B-P (Berger-Parker index)	r	0.195**	–	–	–	–	–	–	–	–	–
AChE	r	**0.681*****	0.321**	–	–						
LDH	r	0.389**	0.387**	–	–						
LP	r	–	–	–	–						
NC	r	**0.442*****	–	0.332**	–	0.276**	–	–			
SC	r	**0.634*****	–	**0.423*****	–	0.274*	–	–			
CUEs	χ^2	7.062**	–	–	–	–	–	–	3.941*	–	
Sex	r	**0.285*****	–	0.529*		–	–	–	–	–	–

[†] All analyses involving this variable were performed by GZM.
* p & $2lt;0.05.
** p & $2lt;0.01.
*** p & $2lt;0.001.
[a] result valid for fish from size 2 (TL & $2gt;20 cm).
[b] result valid for fish from size 1 (TL≤20 cm). Highly significant results are highlighted in bold.

three parasites showed the strongest associations with the first FCA axis: *C. collaris*, *B. brayi* and *G.* cf. *erinaceus* (Cosine^2=0.537–0.992). Two more species were highly correlated with the second FCA axis: *Lepidapedon* spp. and *C. gracilis* (Cosine^2=0.754 and 0.597, respectively). From FCA and cluster analyses (not shown) of the locality-season categories, three distinct assemblages of hosts were established depending on their parasite load: one including all host groups from off the mainland slope (A), another with hosts from off Mallorca in summer (B) and a third group including hosts from off Ibiza in autumn (C).

Group A: Host groups from the mainland slope were characterized by *D. phycidis*, *B. brayi, Echinorhynchus* sp., *Cucullanus* sp., *H. aduncum* and *C. alata*. *D. phycidis*, *Echinorhynchus* sp., *H. aduncum* and *C. alata* showed significant differences in abundance among locality-season groups (GZM, χ^2=30.767, p & $2lt;0.001; χ^2=15.057, p=0.01; χ^2=38.570, p & $2lt;0.001 and χ^2=16.322, p=0.022, respectively), with the two latter parasites being always more abundant in the mainland than in the insular slope (Table 4). *Echinorhynchus* sp. was only detected in the mainland slope. For *B. brayi* and *Cucullanus* sp., an interaction with fish size was found and differences among groups were therefore tested on both size-groups of hosts separately. *Bathycreadium brayi* showed significant differences among groups in both size groups of hosts (GZM, χ^2=57.325, p & $2lt;0.001 and χ^2=32.202, p & $2lt;0.001, respectively), with a tendency to be more abundant in the mainland than in the insular slope, especially in adult hosts. *Cucullanus* sp., showed significant differences in abundance in hosts of size 2 (GZM, χ^2=21.439, p=0.002), being more abundant in winter and summer. *Diclidophora phycidis*, *B. brayi*, *Cucullanus* sp. and *H. aduncum* also displayed significant differences in prevalence (GZM, χ^2=20.062, p=0.005; χ^2=17.787, p=0.013; χ^2=17.668, p=0.014 and χ^2=20.503, p=0.005, respectively) (Table 4).

Group B: The summer sample from off Mallorca was characterized by *Lepidapedon* spp. and *C. gracilis*. Both parasites showed significant differences in abundance among locality-season groups (GZM, χ^2=53.219, p & $2lt;0.001 and χ^2=17.731, p=0.013, respectively), *Lepidapedon* spp. being overall more abundant in spring and summer than in autumn and winter. *Lepidapedon* spp. also displayed differences in prevalence (GZM, χ^2=14.758, p=0.039) (Table 4).

Group C: The autumn sample from off Ibiza was characterized by *G.* cf. *erinaceus* and *C. collaris*, the former parasite reaching maximum abundance and prevalence in this group. For *C. collaris*, an interaction with fish size was found, so differences among locality-season groups were performed on both size-groups of hosts separately. Significant differences in the abundance of this parasite were detected among categorical groups both in hosts of size 1 and size 2 (GZM, χ^2=30.524, p & $2lt;0.001 and χ^2=64.160, p & $2lt;0.001, respectively), being more abundant in adult hosts from off Ibiza in autumn (Table 4).

In samples from depth 2, the abundance of *Lepidapedon* spp. and *Cucullanus* sp. displayed seasonal differences in the samples from off Tarragona (GZM, χ^2=11.171, p=0.004 and χ^2=16.164, p=0.001, respectively), the former with higher abundance in autumn while the latter in summer (Table 5). *Lepidapedon* spp. also showed geographical differences in autumn, with higher abundance in the samples from off Tarragona than in those from off Ibiza. *Echinorhynchus* sp. was significantly more abundant in the mainland than in the insular slope (GZM, χ^2=7.220, p=0.027). No significant differences in prevalence among categorical groups were detected for any parasite (GZM, p≥0.05 in all cases) (Table 5).

Significant differences were detected among categorical groups for parasite infracommunity descriptors (MSR, TMA, MD and B-P) (GZM, χ^2=56.012, p & $2lt;0.001; GLM, $F_{(11, 176)}$=5.311, p & $2lt;0.001; $F_{(11, 176)}$=5.506, p & $2lt;0.001 and $F_{(11, 176)}$=2.833, p=0.002, respectively) (Table 3). The three former descriptors displayed significant lower values in the samples from off Mallorca, while B-P was highest in the samples from off Mallorca and Ibiza at depth 1.

The CCA relating the abundance on common parasites with environmental variables (Fig. 3) explained 90.4% of the total variance. The nematode *C. collaris* was associated to high levels of turbidity and O_2 concentrations near the bottom, coinciding with most of the hauls from off Besòs. *Hysterothylacium aduncum* and, to a lesser degree, *D.*

Table 3
Means and standard deviations of fish total length (TL), condition factor (K), hepatosomatic index (HSI), female gonadosomatic index (GSI), parasitological descriptors (mean species richness (MSR), total mean abundance (TMA), mean diversity (MD) and Berger-Parker dominance index (B-P)), acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities, lipid peroxidation (LP) levels and surface (SC) and number (NC) of macrophage centres (MC); and prevalence of cysts of unknown etiology found in gills (CUEs) in the different categorical groups of *Phycis blennoides*. Abbreviations for categorical groups: for depth 1 (400–1000 m depth): BW1, Besós winter; BSp1, Besós spring; BSu1, Besós summer; BA1, Besós autumn; VSu1, Vilanova summer; TSu1, Tarragona summer; MSu1, Mallorca summer and IA1, Ibiza autumn; and for depth 2 (& $2gt;1000 m depth): TSu2, Tarragona summer; TA2, Tarragona autumn; MSu2, Mallorca summer and IA2, Ibiza autumn. N: sample size of *P. blennoides*; (*): number of females; SR: species richness. Different superscript letters show significant differences among categorical groups. Dashes indicate non available data.

	Mainland slope								**Insular slope**			
	BW1	**BSp1**	**BSu1**	**BA1**	**VSu1**	**TSu1**	**TSu2**	**TA2**	**MSu1**	**MSu2**	**IA1**	**IA2**
N(*)	24 (9)	21 (13)	28 (5)	12 (6)	35 (17)	15 (7)	5 (4)	5 (1)	12 (3)	5 (4)	15 (8)	11 (8)
TL	25.21 ± 7.91^{ABC}	25.62 ± 5.19^{ABC}	24.21 ± 8.20^{ABC}	26.24 ± 6.81^{ABC}	26.73 ± 5.44^{ABC}	21.73 ± 10.26^{AB}	41.58 ± 9.62^{E}	30.46 ± 5.89^{BCD}	17.59 ± 6.65^{A}	36.56 ± 7.90^{DE}	21.64 ± 8.02^{AB}	32.89 ± 7.95^{CD}
K	0.59 ± 0.07^{A}	0.66 ± 0.06^{ABC}	0.71 ± 0.10^{BC}	0.66 ± 0.04^{ABC}	0.72 ± 0.07^{C}	0.64 ± 0.07^{ABC}	0.61 ± 0.10^{AB}	0.62 ± 0.02^{ABC}	0.62 ± 0.10^{ABC}	0.60 ± 0.05^{A}	0.56 ± 0.11^{A}	0.64 ± 0.03^{ABC}
HSI	1.55 ± 0.66^{A}	2.97 ± 1.49^{ABC}	3.95 ± 1.98^{BC}	3.65 ± 1.38^{BC}	3.26 ± 1.90^{ABC}	3.98 ± 2.03^{BC}	2.28 ± 1.68^{AB}	2.77 ± 1.10^{ABC}	4.28 ± 1.04^{C}	2.16 ± 1.52^{AB}	2.80 ± 2.42^{ABC}	2.58 ± 1.98^{ABC}
GSI	0.13 ± 0.14^{AB}	0.07 ± 0.03^{A}	0.09 ± 0.03^{AB}	0.14 ± 0.08^{AB}	0.09 ± 0.04^{AB}	0.07 ± 0.03^{AB}	0.21 ± 0.15^{AB}	–	0.22 ± 0.13^{B}	0.22 ± 0.14^{B}	0.11 ± 0.06^{AB}	0.20 ± 0.14^{AB}
SR	8	10	8	8	10	8	7	7	5	3	7	7
MSR	6.08 ± 1.44^{A}	5.48 ± 2.14^{A}	5.00 ± 1.70^{AB}	4.58 ± 1.73^{AB}	5.83 ± 2.06^{A}	4.27 ± 1.79^{ABC}	6.20 ± 0.84^{A}	5.60 ± 1.95^{A}	2.92 ± 1.38^{BC}	2.40 ± 0.55^{C}	4.00 ± 1.60^{ABC}	5.09 ± 1.30^{AB}
TMA	51.83 ± 36.97^{AB}	72.52 ± 45.99^{A}	45.64 ± 30.81^{AB}	29.25 ± 22.77^{BC}	55.31 ± 34.48^{AB}	26.13 ± 17.71^{BC}	33.80 ± 47.44^{ABC}	30.20 ± 23.06^{ABC}	13.58 ± 12.31^{CD}	5.20 ± 2.68^{D}	28.47 ± 23.22^{C}	17.09 ± 7.42^{C}
MD	1.07 ± 0.26^{A}	0.99 ± 0.32^{A}	1.00 ± 0.28^{A}	0.85 ± 0.31^{AB}	1.03 ± 0.30^{A}	0.82 ± 0.41^{AB}	1.09 ± 0.32^{A}	1.15 ± 0.31^{A}	0.51 ± 0.38^{B}	0.49 ± 0.18^{B}	0.72 ± 0.30^{AB}	1.08 ± 0.24^{A}
B-P	0.51 ± 0.17^{ABC}	0.54 ± 0.14^{ABC}	0.53 ± 0.15^{ABC}	0.58 ± 0.17^{ABC}	0.54 ± 0.14^{ABC}	0.59 ± 0.20^{ABC}	0.55 ± 0.33^{ABC}	0.45 ± 0.08^{BC}	0.71 ± 0.22^{A}	0.62 ± 0.12^{ABC}	0.64 ± 0.17^{AB}	0.40 ± 0.12^{C}
AChE	37.01 ± 12.22^{BC}	35.81 ± 10.09^{BC}	–	51.74 ± 6.11^{BCDE}	40.47 ± 5.94^{BC}	47.31 ± 22.91^{BCD}	13.55 ± 6.81^{A}	28.92 ± 8.08^{B}	94.94 ± 43.90^{E}	80.45 ± 39.42^{DE}	76.58 ± 41.71^{CDE}	33.60 ± 17.00^{B}
LDH	–	–	–	–	–	2414 ± 2161^{AB}	1205 ± 635^{B}	3125 ± 718^{AB}	1792 ± 1668^{AB}	3585 ± 2060^{A}	1129 ± 919^{B}	2743 ± 1247^{AB}
LP	6.89 ± 1.27^{A}	–	–	–	–	1.49 ± 0.84^{B}	0.93 ± 0.43^{B}	0.92 ± 0.84^{B}	3.60 ± 2.13^{AB}	2.29 ± 0.74^{B}	1.26 ± 1.23^{B}	0.95 ± 1.70^{B}
SC	65328 ± 40,752^{ABC}	15,580 ± 13,454^{A}	19,544 ± 26,539^{A}	39,016 ± 49,619^{AB}	23,668 ± 46,643^{A}	35,104 ± 43,396^{A}	145,306 ± 35,206^{C}	8,509 ± 9237^{A}	15,479 ± 24,780^{A}	95,720 ± 28,369^{BC}	32,348 ± 23,760^{AB}	60,342 ± 55,303^{AB}
NC	29.78 ± 11.34^{AB}	14.18 ± 9.48^{AB}	15.19 ± 16.93^{AB}	23.84 ± 13.78^{AB}	15.60 ± 12.82^{AB}	21.27 ± 26.25^{AB}	38.85 ± 9.70^{B}	12.76 ± 9.26^{AB}	9.32 ± 13.25^{A}	32.05 ± 7.81^{AB}	18.26 ± 10.70^{AB}	18.91 ± 12.94^{AB}
CUEs	100.00^{A}	88.89^{A}	87.50^{A}	100.00^{A}	87.50^{A}	71.43^{A}	100.00^{A}	40.00^{A}	30.00^{A}	80.00^{A}	77.78^{A}	70.00^{A}

Table 4
Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Phycis blennoides* at depth 1 (400–1000 m depth). N: sample size of *P. blennoides*. *: New host record. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae. Abbreviations for locations within host: B, buccal cavity; G, gills; I, intestine; L, liver; M, mesenteries; PC, pyloric caeca; S, stomach; SW, stomach wall (encysted). Different superscript letters and numbers show significant differences among categorical groups in abundance and prevalence, respectively. Dashes indicate absence of the parasite.

	Stage	Location	Mainland slope												Insular slope			
			Besós winter		Besós spring		Besós summer		Besós autumn		Vilanova summer		Tarragona summer		Mallorca summer		Ibiza autumn	
N			24		21		28		12		35		15		12		15	
			P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD
Monogenea																		
Diclidophora phycidis	A	G	71^{1}	1.75 ± 1.96^{A}	62^{1}	2.48 ± 3.34^{A}	29^{2}	0.71 ± 1.65^{B}	33^{12}	0.50 ± 0.90^{B}	63^{1}	2.11 ± 2.49^{A}	13^{2}	0.40 ± 1.30^{B}	–	–	40^{12}	0.53 ± 0.74^{B}
Digenea																		
Bathycreadium brayi	J, A	S, I, PC	96^{1}		86^{12}		93^{1}		83^{13}		97^{1}		80^{13}		50^{3}		67^{23}	
Bathycreadium brayi - S1	J, A	S, I, PC		41.73 ± 23.29^{A}		21.00 ± 21.21^{AB}		24.80 ± 22.79^{A}		0.50 ± 0.71^{C}				4.29 ± 5.68^{AB}		2.38 ± 4.21^{C}		2.71 ± 2.93^{C}
Bathycreadium brayi - S2	J, A	S, I, PC		14.77 ± 18.79^{AB}		28.95 ± 29.57^{A}		16.09 ± 21.90^{AB}		15.40 ± 17.77^{AB}		25.49 ± 20.99^{A}		13.88 ± 16.15^{AB}		4.75 ± 7.54^{BC}		3.50 ± 3.51^{C}
Lepidapedon spp.	J, A	S, I, PC	71^{1}	3.67 ± 3.53^{AB}	90^{12}	16.19 ± 15.71^{C}	86^{12}	12.64 ± 13.82^{C}	58^{1}	5.67 ± 10.34^{AB}	97^{2}	14.34 ± 15.53^{C}	80^{12}	5.93 ± 5.15^{B}	92^{12}	7.67 ± 7.09^{BC}	60^{1}	2.60 ± 3.83^{A}
Podocotyle sp.*		I, PC	–	–	–	–	–	–	–	–	–	–	–	–	17	0.50 ± 1.45	–	–
Steringotrema sp.*		I	–	–	–	–	4	0.11 ± 0.57	–	–	–	–	–	–	–	–	–	–
Cestoda																		
Grillotia cf. *erinaceus**	L	L, M, S, SW	8^{1}	0.13 ± 0.45^{A}	5^{1}	0.05 ± 0.22^{A}	18^{1}	0.21 ± 0.50^{A}	17^{1}	0.25 ± 0.62^{A}	11^{1}	0.17 ± 0.51^{A}	–	–	17^{1}	0.17 ± 0.39^{A}	27^{1}	0.73 ± 2.05^{A}
Tetraphyllidea fam. gen. sp.*	L	I, PC	–	–	–	–	–	–	–	–	–	–	7	0.07 ± 0.26	25	0.25 ± 0.45	7	0.07 ± 0.26
Acanthocephala																		
Echinorhynchus sp.*	A	I, PC	38^{1}	1.50 ± 3.39^{A}	19^{1}	0.86 ± 2.41^{AB}	25^{1}	0.86 ± 2.51^{AB}	17^{1}	0.42 ± 1.00^{B}	20^{1}	0.40 ± 1.24^{B}	27^{1}	1.53 ± 3.25^{A}	–	–	–	–
Nematoda																		
Anisakis Type I	L3	L, M	–	–	–	–	4	0.04 ± 0.19	–	–	–	–	–	–	–	–	–	–
Anisakis Type II	L3	M, S, SW	4	0.04 ± 0.20	–	–	7	0.07 ± 0.26	–	–	–	–	–	–	–	–	7	0.07 ± 0.26
*Capillaria gracilis**	J, A	S, I, PC	54^{1}	1.17 ± 1.61^{A}	62^{1}	2.76 ± 3.25^{B}	46^{1}	1.79 ± 3.35^{AB}	67^{1}	1.58 ± 1.78^{AB}	49^{1}	1.54 ± 2.67^{AB}	13^{1}	0.13 ± 0.35^{C}	50^{1}	1.17 ± 1.80^{AB}	47^{1}	1.73 ± 3.90^{AB}
*Capillostrongyloides morae**	J, A	S, I	13	0.50 ± 1.72	–	–	–	–	–	–	–	–	13	0.60 ± 2.06	–	–	7	0.27 ± 1.03
*Collarinema collaris**	A	S, I, PC	71^{1}		95^{1}		71^{1}		83^{1}		60^{1}		53^{1}		–	–	67^{1}	
Collarinema collaris - S1	A	S, I, PC		11.73 ± 12.11^{A}		20.00 ± 9.90^{A}		0.20 ± 0.45^{B}		2.00 ± 0.00^{AB}				1.29 ± 2.98^{B}		–		2.43 ± 3.74^{B}
Collarinema collaris - S2	A	S, I, PC		5.85 ± 8.25^{B}		18.32 ± 25.04^{A}		8.22 ± 10.08^{B}		4.30 ± 6.67^{BC}		2.60 ± 4.72^{C}		3.63 ± 3.50^{BC}		–		31.13 ± 17.46^{A}
Cucullanus sp.	L3, L4, A	S, I, PC	54^{1}		43^{12}		25^{2}		17^{2}		63^{1}		33^{12}		83^{2}		40^{12}	
Cucullanus sp. - S1	L3, L4, A	S, I, PC		0.36 ± 0.67^{A}		0.50 ± 0.71^{A}		–		1.50 ± 2.12^{A}				–		0.13 ± 0.35^{A}		0.14 ± 0.38^{A}
Cucullanus sp. - S2	L3, L4, A	S, I, PC		2.85 ± 2.41^{A}		0.84 ± 1.46^{BC}		0.78 ± 1.68^{BC}		0.20 ± 0.63^{C}		1.97 ± 2.48^{A}		2.63 ± 4.34^{A}		–		1.63 ± 1.69^{AB}
Hysterothylacium aduncum	L3, L4, A	S, I, PC, M, L	75^{1}	4.25 ± 9.09^{A}	48^{12}	1.29 ± 2.70^{B}	36^{2}	2.18 ± 5.06^{B}	25^{2}	1.75 ± 4.59^{B}	69^{1}	5.60 ± 8.30^{A}	60^{12}	3.47 ± 10.41^{AB}	8^{2}	0.08 ± 0.29^{C}	–	–
Hysterothylacium fabri	L3	I, PC	4	0.04 ± 0.20	5	0.05 ± 0.22	–	–	–	–	3	0.06 ± 0.34	–	–	–	–	–	–
Raphidascaris sp.*	L3	I, PC	–	–	–	–	4	0.14 ± 0.76	–	–	3	0.03 ± 0.17	13	0.13 ± 0.35	–	–	–	–
Copepoda																		
Clavella alata	A	G	50^{1}	1.42 ± 1.89^{A}	29^{1}	0.95 ± 1.86^{AB}	54^{1}	1.82 ± 3.16^{A}	50^{1}	1.75 ± 2.34^{A}	46^{1}	0.97 ± 1.40^{AB}	33^{1}	0.53 ± 0.92^{B}	25^{1}	0.50 ± 1.00^{B}	27^{1}	0.47 ± 0.92^{B}
Isopoda																		

(continued on next page)

Table 4 *(continued)*

	Stage	Location	Mainland slope								Insular slope					
			Besós winter		Besós spring		Besós summer		Besós autumn		Vilanova summer		Tarragona summer		Mallorca summer		Ibiza autumn	
			P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD
N			24		21		28		12		35		15		12		15	
Cymothoidae gen. sp.*		B	–	–	–	–	–	–	–	–	3	0.03 ± 0.17	–	–	–	–	–	–

phycidis, *B. brayi* and *Lepidapedon* spp. were, in turn, linked to high near-bottom temperature and salinity, coinciding with hauls from off Vilanova, where these four parasites reached high abundance values (Table 4).

3.4. Relationship between parasites and fish diet

A total of 34 prey-groups (excluding items like plastics, scales and foraminiferans) were established grouping a total of 916 prey belonging to 81 different prey items (grouped in higher taxonomic levels) identified in guts, most of them to genus/species level (Table 6).

Diet was highly diversified, mainly based on benthic-benthopelagic prey but also including some pelagic prey. Among benthopelagic (suprabenthic) prey, peracarids were dominant, especially at the mainland localities, with the isopod *Munnopsurus atlanticus* reaching to 44–47% of prey in summer groups and the mysid *Boreomysis arctica* reaching to 40% of diet in autumn (Table 6). Natantian shrimps (mainly Pandalidae, *Plesionika* spp.) and some fish (being *Gaidropsarus biscayensis* the most abundant among those identified) were also important in number, increasing their contribution in prey mass (data not included). Among benthic (not swimming) prey, crabs/lobsters (e.g. *Monodaeus couchi*, *Calocaris macandreae*, *Munida iris*, *Munida tenuimana*) and polychates (e.g. Polynoidae, *Harmothoe* sp.) were dominant. In addition, some meso-bathypelagic prey (e.g. euphausiids like *Meganyctiphanes norvegica* or myctophid fishes) were also identified in *P. blennoides* guts (Table 6).

Based on the 10 dietary groups (columns) shown in Table 6, nMDS did not evidence any significant ordination of dietary groups (Stress=0.1, plot not shown) as a function of any of the 3 factors explored (i.e. locality, season and depth). Some dietary changes can, however, be interpreted as a function of insularity and of particular seasons. In fact, the comparison between insular and mainland groups (t=1.32, p=0.06) was close to the significance level, as also was the contrast between summer and autumn samples (t=1.45, p=0.06), although no general seasonal changes in diet were evidenced.

The CCA relating the common parasites and prey explained 56.4% of the constrained variance in the first two axes (Fig. 4). Main relationships arose between the two only important prey belonging to zooplankton (myctophid fishes and *M. norvegica*) and the nematodes *Anisakis* spp. (Type I and II) and *Cucullanus* sp. and, to a lesser extent, the acanthocephalan *Echinorhynchus* sp. in the right part of the plot. At the upper-right part, *G. biscayensis* and *Munida* spp. appeared related with the larval forms of the cestode *G.* cf. *erinaceus*. Such relationships were linked to specimens from hauls of more than 1000 m depth. At the left part of the plot, practically all the rest of the parasites were linked to suprabenthic-benthic prey (corresponding to hauls of less than 1000 m depth). Among peracarid crustaceans, those species with higher swimming capacity (e.g. *Natatolana borealis*, *B. arctica*) were rather grouped at the lower part of the plot, linked to the nematodes *C. collaris* and *C. morae*. In contrast, the digeneans *Lepidapedon* spp. were related to more epibenthic (less swimming) peracarids (Oedicerotidae) at the upper-left part of the plot. An additional observation arisen from the analysis is the “canyon effect”, with all individuals from hauls performed in submarine canyons grouped at the left-lower part of the plot and linked to benthic, mud-dwelling prey (e.g. the crabs/lobsters *M. couchi* and *C. macandreae*). These samples appear to be associated with the nematode *H. aduncum*. Note also that the two parasites with direct life cycle (i.e. *C. alata* and *D. phycidis*) appear at the central part of the plot not related to any prey, a logical outcome since they are not dependent on trophic transmission for infecting their hosts.

Diet/parasites of males/females/undetermined were very similar, since they appeared very close in the plot of the CCA, in those hauls where sex categories were separated (Fig. 4).

Table 5
Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Phycis blennoides* at depth 2 (& $2gt;1000 m depth). N: sample size of *P. blennoides*. *: New host record. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Mt, Metacercariae. Abbreviations for locations within host are defined in Table 4. Different superscript letters and numbers show significant differences among categorical groups in abundance and prevalence, respectively. Dashes indicate absence of the parasite.

	Stage	Location	Mainland slope	Insular slope						
			Tarragona summer	Tarragona autumn		Mallorca summer	Ibiza autumn			
N			**5**	**5**		**5**	**11**			
			P(%)	**MA ± SD**	**P(%)**	**MA ± SD**		**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**
Monogenea											
Diclidophora phycidis	A	G	60^{1}	0.60 ± 0.55^{A}	20^{1}	1.00 ± 2.24^{A}		60^{1}	0.80 ± 0.84^{A}	36^{1}	0.91 ± 1.81^{A}
Digenea											
Bathycreadium brayi	J, A	S, I, PC	40^{1}	3.80 ± 6.50^{A}	80^{1}	4.60 ± 5.98^{A}		–	–	36^{1}	1.36 ± 2.80^{A}
Lepidapedon spp.	J, A	S, I, PC	40^{1}	1.20 ± 1.79^{A}	100^{1}	7.20 ± 6.06^{B}		–	–	36^{1}	0.91 ± 1.81^{A}
Otodistomum sp.*	Mt	SW	20	0.20 ± 0.45	–	–		–	–	–	–
Cestoda											
Grillotia cf. *erinaceus**	L	L, M, S, SW	20^{1}	0.20 ± 0.45^{A}	60^{1}	0.60 ± 0.55^{A}		–	–	27^{1}	0.82 ± 1.60^{A}
Tetraphyllidea fam. gen. sp.*	L	I, PC	–	–	60	8.00 ± 10.68		–	–	18	0.27 ± 0.65
Acanthocephala											
Echinorhynchus sp.*	A	I	80^{1}	1.60 ± 1.14^{A}	20^{1}	2.00 ± 4.47^{A}		–	–	9^{1}	0.09 ± 0.30^{B}
Nematoda											
Anisakis Type I	L3	L, M	20	0.20 ± 0.45	–	–		20	0.20 ± 0.45	–	–
Anisakis Type II	L3	M, S, SW	60	0.60 ± 0.55	20	0.20 ± 0.45		20	0.40 ± 0.89	9	0.09 ± 0.30
*Capillaria gracilis**	J, A	S, I, PC	40^{1}	1.20 ± 2.17^{A}	60^{1}	1.00 ± 1.22^{A}		40^{1}	1.00 ± 1.41^{A}	55^{1}	1.64 ± 2.73^{A}
*Capillostrongyloides morae**	J, A	S, I	20	0.80 ± 1.79	–	–		–	–	73	4.18 ± 3.71
*Collarinema collaris**	A	S, I, PC	80^{1}	1.60 ± 1.82^{A}	40^{1}	0.60 ± 0.89^{A}		–	–	82^{1}	2.36 ± 2.16^{A}
Cucullanus sp.	L3, L4, A	S, I, PC	100^{1}	26.75 ± 47.50^{A}	60^{1}	2.80 ± 3.42^{B}		60^{1}	1.80 ± 2.05^{B}	91^{1}	3.64 ± 3.44^{B}
Copepoda											
Clavella alata	A	G	40^{1}	0.40 ± 0.55^{A}	40^{1}	2.20 ± 3.03^{A}		40^{1}	1.00 ± 1.73^{A}	36^{1}	0.82 ± 1.17^{A}

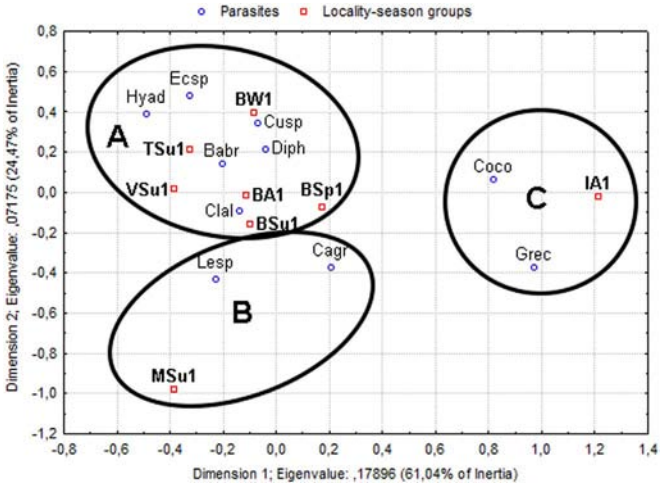

Fig. 2. Plot of the first factorial plane of co-inertia of the factorial correspondence analysis (FCA) performed using component population data of the ten common parasites (P & $2gt;10%) in *Phycis blennoides* at depth 1 (400–1000 m depth). A/B/C refers to the groups established in the description of the parasite fauna. Abbreviations for locality-season groups: BW1, Besós winter; BSp1, Besós spring; BSu1, Besós summer; BA1, Besós autumn; VSu1, Vilanova summer; TSu1, Tarragona summer; MSu1, Mallorca summer; IA1, Ibiza autumn. Abbreviations for parasite names: Babr, *Bathycreadium brayi*; Cagr, *Capillaria gracilis*; Clal, *Clavella alata*; Coco, *Collarinema collaris*; Cusp, *Cucullanus* sp.; Diph, *Diclidophora phycidis*; Ecsp, *Echinorhynchus* sp.; Grec, *Grillotia* cf. *erinaceus*; Hyad, *Hysterothylacium aduncum*; Lesp, *Lepidapedon* spp.

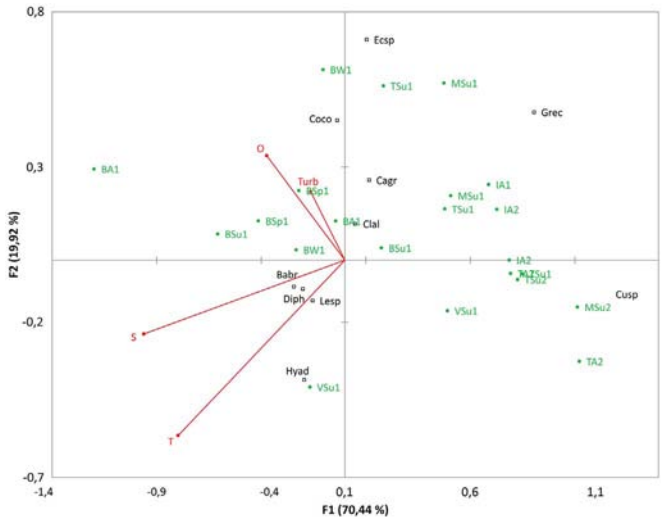

Fig. 3. Canonical correspondence analysis (CCA) showing relationships between the abundance of common parasites (prevalence & $2gt;10%) infecting *Phycis blennoides* and environmental data. Abbreviations for parasites names: Babr, *Bathycreadium brayi*; Cagr, *Capillaria gracilis*; Clal, *Clavella alata*; Coco, *Collarinema collaris*; Cusp, *Cucullanus* sp.; Diph, *Diclidophora phycidis*; Ecsp, *Echynorhynchus* sp.; Grec, *Grillotia* cf. *erinaceus*; Lesp, *Lepidapedon* spp. Abbreviations for environmental variables: O, oxygen concentration; S, salinity; T, temperature; Turb, turbidity; all measurements taken at 5 m above the sea-floor. Haul codes (BW1, etc.) are defined in Table 2.

3.5. Biochemical markers vs. parasites and host biological parameters

Acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) mean activities ranged from 13.55 to 94.94 and from 1129 to 3585 nmol/min/mg prot, respectively, and lipid peroxidation (LP) levels from 0.92 to 6.89 nmol MDA/g ww. All three biochemical markers displayed significant differences among locality-season groups; while AChE and LDH activity levels reached highest values in fish from off Mallorca and from off Mallorca at depth 2, respectively, LP levels were much higher in the samples from off Besós than in all other localities (GLM, $F_{(10, 89)}$=11.167, p & $2lt;0.001; $F_{(6, 59)}$=2.822, p=0.018 and $F_{(7, 62)}$=7.023, p & $2lt;0.001, respectively) (Table 3).

Significant negative associations were found between AChE activity

Table 6
Numerical percentage of prey of the main prey-items found in guts of the 100 *Phycis blennoides* examined in the different localities and season sampled in the mainland and insular slopes. N: sample size of *P. blennoides*. Abbreviations for categorical groups are defined in Table 3.

	Mainland slope							Insular slope		
	BW1	BSp1	BSu1	BA1	VSu1	TSu1	TA2	MSu1	MSu2	IA1
N	**19**	**17**	**14**	**4**	**14**	**6**	**4**	**12**	**5**	**5**
Polychaeta										
Harmothoe sp.	7.6	9.1	3.9	6.7	14.0	2.4	7.7	14.1	17.0	5.3
Other polychaeta (Nephthydae)	1.7	0.5	1.9	6.7	0.6	0	7.7	0	0	0
Bivalvia	0	0	0	0	0	0	0	0	3.8	0
Echinodermata	0.8	0.5	0	0	1.1	2.4	0	2.4	0	0
Copepoda										
Calanoidea	0.8	0	1.0	0	0.6	2.4	0	1.2	0	0
Amphipoda										
Bruzelia typica	0	0	0	0	0	2.4	0	1.2	0	0
Rhachotropis spp.	3.4	0.5	2.4	0	2.8	0	0	3.5	3.8	0
Oedicerotidae (*Bathymedon* sp.)	2.5	2.7	1.0	0	0	4.8	7.7	5.9	1.9	0
Other amphipoda (Lysianassidae)	0	0.5	0	0	0.6	0	7.7	1.2	1.9	5.3
Isopoda										
Cirolana borealis	11.0	9.1	6.3	6.7	4.5	9.5	0	0	0	5.3
Gnathia sp.	0.8	0.5	0.5	0	0	0	0	1.2	5.7	0
Munnopsurus atlanticus	18.6	39.0	46.9	6.7	44.1	4.8	7.7	14.1	9.4	5.3
Other Assellota (*Ilyarachna* sp.)	0.8	2.1	0	0	1.7	0	0	3.5	0	15.8
Cumacea	1.7	0	0	0	0.6	0	0	0	0	0
Mysida										
Boreomysis arctica	11.9	11.2	19.8	40.0	7.8	23.8	0	5.9	9.4	0
Pseudomma spp.	2.5	1.6	1.9	0	0.6	14.3	0	2.4	1.9	0
Other Mysida *(Erythops neapolitana)*	0	0	0	0	0	0	0	7.1	0	0
Euphausiacea										
Meganyctiphanes norvegica	0.8	0.5	0	0	0.6	2.4	0	0	0	0
Decapoda										
Alpheus spp.	0	1.1	0	0	0	0	0	3.5	0	0
Pandalina profunda	1.7	1.6	1.4	0	2.2	2.4	0	2.4	0	0
Plesionika spp.	0.8	1.6	0	0	1.1	0	15.4	2.4	0	0
Crangonidae	1.7	0	0	0	0.6	0	0	1.2	0	15.8
Processa spp.	0	0	0	0	0	0	0	2.4	1.9	0
Natantia	0.8	0.5	0	0	1.7	0	0	2.4	0	5.3
Calocaris macandreae	11.0	4.8	4.3	6.7	2.8	0	7.7	0	0	21.1
Goneplax rhomboides	0.8	0.5	0	0	1.1	0	0	0	0	0
Monodaeus couchi	5.9	7.5	3.4	13.3	7.8	4.8	0	0	0	10.5
Munida spp.	1.7	0.5	1.9	0	0.6	4.8	7.7	10.6	13.2	0
Brachyura	2.5	0.5	1.4	6.7	1.1	2.4	0	1.2	3.8	0
Cephalopoda	0	0.5	0	0	0	0	15.4	0	1.9	0
Pteropoda	0.8	0.5	1.0	6.7	0	0	0	2.4	1.9	0
Teleostei										
Gaidropsarus biscayensis	0.8	0.5	0	0	0	0	0	2.4	9.4	5.3
Myctophidae	0	0	0	0	0.6	2.4	0	0	5.7	5.3
Unidentified Teleostei	5.9	1.6	1.0	0	1.1	14.3	15.4	5.9	7.5	0
Total number of prey	118	187	208	15	179	42	13	85	53	16

and individual parasite richness, diversity, abundance and abundance of *B. brayi*, *C. collaris*, *Cucullanus* sp. and *Echinorhynchus* sp. (GZM, GLM, Table 2). For *Lepidapedon* spp., an interaction with fish size was found and the analysis was applied on both size-groups of hosts separately. A significant negative relationship was found between AChE activity levels and this parasite (GZM, Table 2) in fish from size 2. In contrast, LDH activity was positively correlated with the abundance of *B. brayi* (GZM, Table 2). LP levels were negatively related to the abundance of *C. collaris* in hosts of size 1 (an interaction with TL was found in this case) and positively associated to the abundance of *Echinorhynchus* sp. and *H. aduncum* (GZM, Table 2).

While AChE activity was negatively correlated with fish TL and K, LDH activity was positively associated to the same variables (GLM, Table 2).

3.6. Histological assessment and its relation with parasites, biochemical markers and host biological parameters

The presence of CUEs in gills was the most frequent histological alteration detected (Fig. 5a). Additionally, granulomas in liver and spleen, xenomas induced by microsporidian parasites in liver (Fig. 5b) and small regions of hyperplasia with lamellar fusion in gills were occasionally detected (P & $2lt;10%). CUEs appeared as spherical bodies of about 200–350 μm in diameter embedded within gill primary lamellae. Three distinct layers could be clearly appreciated microscopically: a thin, outer eosinophilic layer, an intermediate basophilic layer of variable thickness and an eosinophilic core, occasionally containing basophilic granules. These structures displayed a total prevalence of 76.67% and a total mean abundance of 1.97 CUEs for each right gill. Prevalence of CUEs showed a positive relationship with fish TL and NC (GZM, Table 2) and did not vary significantly among categorical groups (GZM, p & $2gt;0.05).

Macrophage centres (MC) were homogenously distributed throughout the splenic tissue of most of the specimens analysed (Fig. 5c). They were normally rounded to oval in shape, showed low levels of pigmentation and ranged in size and number from 8509 to 145,306 μm of MC/mm^2 and from 9.32 to 38.85 MC/mm^2, respectively (Table 3).

Significant differences among locality-season groups were detected for both parameters (GLM, $F_{(11,\ 66)}$=5.217, p & $2lt;0.001 and $F_{(11,}$

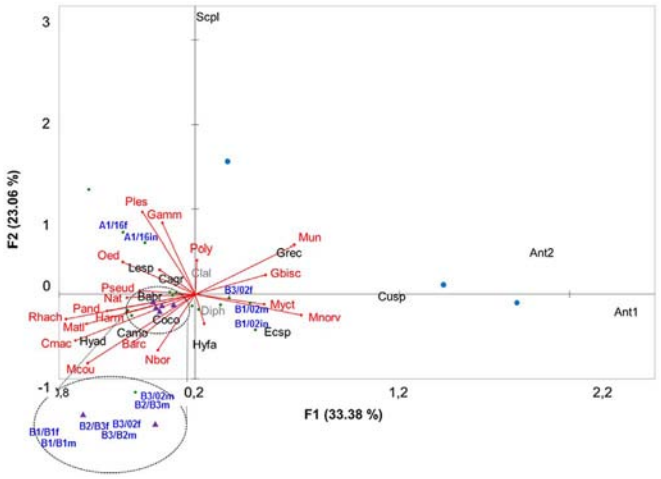

Fig. 4. Canonical correspondence analysis (CCA) showing relationships between the abundance of main parasites and main prey-items found in *Phycis blennoides*. Position of groups of specimens (hauls) analysed are represented by symbols: (•): hauls from depths & $2gt;1000 m; (•): hauls from 450 to 650 m depth; (▲): hauls collected into submarine canyons (450–650 m). When subgroups were formed within a haul, haul labels with sex of specimens analysed are included (f: females; m: males; in: indeterminate specimens). Hauls within the circle are extended at the lower-left part of the plot. Abbreviations for parasites names: Ant1, *Anisakis* Type I; Ant2, *Anisakis* Type II; Babr, *Bathycreadium brayi*; Cagr, *Capillaria gracilis*; Camo, Capillostrongyloides morae; Clal, *Clavella alata*; Coco, *Collarinema collaris*; Cusp, *Cucullanus* sp.; Diph, *Diclidophora phycidis*; Ecsp, *Echynorhynchus* sp.; Grec, *Grillotia* cf. *erinaceus*; Hyad, *Hysterothylacium aduncum*; Hyfa, *Hysterothylacium fabri*; Lesp, *Lepidapedon* spp. Abbreviations for prey names: Barc, *Boreomysis arctica*; Cmac, *Calocaris macandreae*; Gamm, Gammaridae; Gbisc, *Gaidropsarus biscayensis*; Harm, *Harmothoe* sp.; Matl, *Munnopsorus atlanticus*; Mcou, *Monodaeus couchi*; Mnorv, *Meganyctiphanes norvegica*; Mun, *Munida* spp.; Myct, Myctophidae; Nat, Natantia; Nbor, *Natatoloma borealis*; Oed, Oedicerotidae; Pand, Pandalidae; Ples, *Plesionika* spp.; Poly, Polynoidae; Pseud, *Pseudomma* spp.; Rhach, *Rhachotropis* spp.

$_{67)}$=2.293, p=0.019, respectively), both reaching highest values in samples from off Tarragona and Mallorca in summer at depth 2 (Table 3).

No link was detected between MC parameters and total parasite abundance (GZM, Table 2). However, surface of MC (SC) was positively correlated to *Cucullanus* sp. abundance and negatively correlated to the abundance of *Lepidapedon* spp. (GZM, Table 2). In turn, number of MC (NC) displayed a positive correlation with the abundance of *Cucullanus* sp. and a negative one with *C. alata* (GZM, Table 2). SC showed a positive relationship with parasite richness and diversity, and NC with diversity (GLM, Table 2).

SC and NC were negatively associated to HSI and AChE activity but positively to fish TL (GLM, Table 2).

4. Discussion

4.1. General features of the parasite community of P. blennoides

This is the first complete description of the parasite community of the greater forkbeard. Up to 17 different endoparasite taxa and three ectoparasites have been recovered, and the total mean parasite abundance considering the whole sample is of 44 parasites in each host, although individual total abundance reached up to 450 parasites. It is worth to note that total prevalence of infection is 100%.

As regards the metazoan parasite fauna encountered in congeneric species, Scott (1987) recovered 14 taxa in the intestinal tract of *Phycis chesteri* and Ternengo et al. (2009) reported seven taxa in *Phycis phycis*. Concerning the parasite assemblages reported from other phycids, 25 and 19 parasite taxa were recovered from the guts of *Urophycis tenuis* and *Urophycis chuss*, respectively, by Scott (1987), 15 taxa were reported from *U. tenuis* by Melendy et al. (2005) and 33 from *Urophycis brasiliensis* by Pereira et al. (2014). Present data thus suggest that *P. blennoides* is among the most parasite-rich species of its family.

In addition, specimens of *P. blennoides* examined in the present study were found in the deep-sea environment, where parasite burden tends to be low (Campbell et al., 1980). Campbell et al. (1980) surveyed the parasite assemblages of 52 deep-sea benthic fishes, which displayed a global prevalence of infection of 80% and an average abundance of 13 parasites per host. Parasite abundance and diversity are expected to decrease with depth and with distance from the continental slope, in such a way that the parasite burden in deep-water benthic fishes is lower than in shallow-water ones (Campbell et al., 1980; Marcogliese, 2002). This trend is a direct consequence of the variations in the horizontal patterns of distribution of free-living animals that can potentially act as intermediate hosts for the parasites (Marcogliese, 2002).

However, specimens of *P. blennoides* in the NW Mediterranean deep-sea host a parasite community characterized by high abundance, richness and diversity. The explanation for these features is in all likelihood related to feeding habits (Campbell et al., 1980). Generalist feeders that prey on a wide range of items are known to host more abundant, rich and diverse parasite faunas than fishes that show a restricted prey spectrum (Campbell et al., 1980; Marcogliese, 2002; Dallarés et al., ,2014; Pérez-i-García et al., 2015). Moreover, demersal species foraging on the sea bottom tend to host more diverse parasite faunas than pelagic fishes because of the greater availability of intermediate hosts (with a high proportion of crustaceans) in the benthic environment (Campbell et al., 1980; Marcogliese, 2002; Klimpel et al., 2010; Dallarés et al., 2014). In this sense, *P. blennoides* is an active predator whose diet includes a wide range of benthic/suprabenthic prey, mostly consisting of crustaceans and teleosts (Morte et al., 2002; present results), which most probably accounts for its diverse parasite community. Effectively, most of the parasites recov-

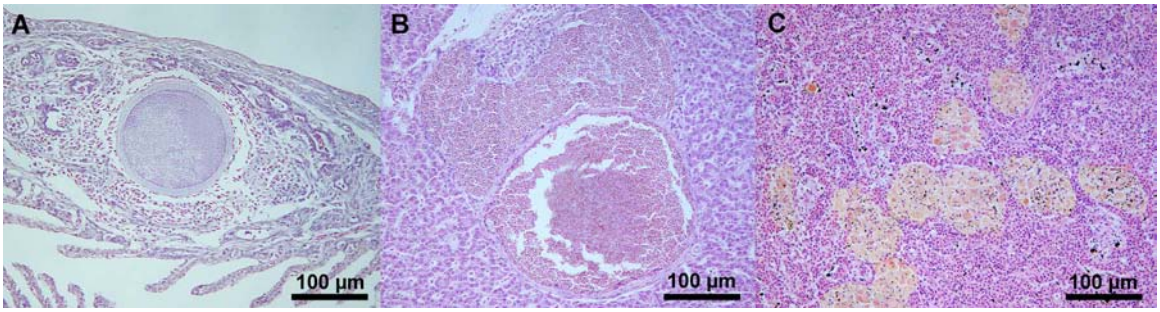

Fig. 5. Photomicrographs of histological pathologies and alterations found in histological sections of different organs of *Phycis blennoides*. A, Cyst of unknown etiology (CUE) in gills; B, Xenoma of an unidentified microsporidian parasite in liver. Note the presence of two sporophorous vesicles containing the parasite spores; C, Macrophage centres (MC) in splenic tissue.

ered in the present studies are associated to suprabenthic prey (Fig. 4). In a similar way, abundant and rich parasite communities have also been found in fishes from the same waters that share ecological features with *P. blennoides*, like the morids *Mora moro* (18 parasites, see Dallarés et al., 2014) and *Lepidion lepidion* (13 parasites, unpublished results), and the macrourid *Trachyrincus scabrus* (17 parasites, see Constenla et al., 2015); species also associated to the seafloor and characterized by a diverse diet based mainly on benthic and suprabenthic/benthopelagic prey. Furthermore, the composition of the parasite communities of these three species presents a high overlap with that of *P. blennoides*; 10 taxa are shared with *M. moro* and 11 with *L. lepidion* and *T. scabrus*.

4.2. Parasite community vs. environmental gradients and variables

Rich and diverse parasite faunas, like that of *P. blennoides*, are especially adequate for performing complex multivariate analyses, such as PERMANOVA, FCA or CCA (McKenzie and Abaunza, 2005). In this sense, the bathymetric change of the parasite infracommunities highlighted by the PERMANOVA analyses is associated to the ontogenic diet shift undergone by *P. blennoides* (Macpherson, 1978; Morte et al., 2002), in turn related to the different composition of the macroinvertebrate communities along the continental slope (Cartes et al., 2009, 2011). Moreover, the FCA showed a clear differentiation of parasite populations between the mainland and insular slopes (at & $2lt;1000 m depth). In general, parasite infracommunities were more abundant, rich, diverse and less dominated by a single species in the mainland (where most common parasites showed highest values of abundance and prevalence, see Table 4) than in the insular slope (Table 3). In all likelihood, this change is explained by the differential composition of benthopelagic faunal assemblages between both slopes due to different trophic and hydrographic conditions (e.g. trophic webs on mainland slope are more complex than on the insular slope) (Fanelli et al., 2013), which can lead, in turn, to different dynamics of parasite transmission and different composition of their communities. Furthermore, O_2 and turbidity levels near the bottom are higher in the mainland, coupled to higher availability of benthic prey linked to submarine canyons, than in the insular slope (Cartes et al., 2013; Dallarés et al., 2014, Fig. 3). These conditions favour more complex invertebrate communities that can enhance parasite transmission (Cartes et al., 2013; Dallarés et al., 2014).

4.3. Individual parasites vs. environmental gradients and variables

Prevalence and abundance of *D. phycidis* are likely related to environmental variables rather than to the host diet, given the direct life cycle of monogeneans. Abundance of *D. phycidis* was linked to high levels of salinity and temperature (Fig. 3), which can enhance egg hatching success and reduce time to maturity, overall accelerating the monogenean life cycle, as demonstrated by Brazenor and Hutson (2015). These conditions occurred in Besós and Vilanova hauls (Table 1), where prevalence and abundance of this parasite were the highest (Table 4). Despite the frequent presence of the monogenean *D. phycidis* on gills, no important lesions were observed in the affected organ, probably due to a weak attachment of these parasites to gill lamellae.

Lepidapedon is a big digenean genus and the most common in the deep-sea environment (Bray et al., 1999). Two distinct species of *Lepidapedon* have been identified in *P. blennoides* by molecular methods, namely *L. desclersae* and *L. guevarai* (unpublished results). However, due to the difficulty of discriminating both species based on morphology, all specimens of *Lepidapedon* recovered were treated as a single taxon. In contrast, *Bathycreadium*, a small genus composed by only four species (Pérez-del-Olmo et al., 2014), belongs to the large fish-infecting family Opecoelidae, mostly restricted to shallow waters but with some species encroaching into the deep-sea (Bray, 2004). Oligochaetes, fishes, cnidarians and especially crustaceans can act as intermediate hosts for *Bathycreadium* (Bray, 2005; Cribb, 2005). This parasite is associated to decapod crustaceans and polychaetes in the CCA relating parasites with prey (Fig. 4). Leiva et al. (2015) reported metacercariae of opecoelid digeneans in decapod crustaceans, and polychaetes are known intermediate hosts for some opecoelids (Cribb, 2005) and could be acting as transmitters of the parasite as well. As observed for *D. phycidis*, both digeneans were linked to high temperature and salinity levels (Fig. 3), parameters that have shown to stimulate trematode transmission (Mouritsen, 2002; Thieltges and Rick, 2006).

Cestodes of the genus *Grillotia* are among the most commonly found trypanorhynch cestodes, either as adults in elasmobranchs or as plerocerci in teleosts (Beveridge and Campbell, 2007). For all trypanorhynchs, copepods act as first intermediate hosts (Palm, 2004). In the case of *G. erinaceus*, teleosts (i.e. *P. blennoides*) act as second intermediate hosts, and piscivorous elasmobranchs, e.g. species of rajids harbour the adult cestodes (Palm, 2004; Beveridge and Campbell, 2007). In particular, *Raja clavata* and *Dipturus oxyrinchus* are known final hosts for this cestode in the Mediterranean Sea (Beveridge and Campbell, 2007) and both species prey on *P. blennoides* (Valls et al., 2011; Mulas et al., 2015). From our results, it is possible that *P. blennoides* becomes infected through feeding on *G. biscayensis* (Fig. 4), both species acting as intermediate hosts.

Adult acanthocephalans infect the intestines of all vertebrate groups, and use benthic crustaceans as intermediate hosts (Amin, 1998). The acanthocephalan *Echinorhynchus* sp. was linked to euphausiaceans (*M. norvegica*), which probably transmit this parasite to *P. blennoides*, since they have been described as intermediate hosts for some acanthocephalans (Gregori et al., 2012, 2013). The fact that both *Echinorhynchus* sp. and *M. norvegica* were almost and totally absent from the Insular slope (Tables 4–6) reinforces our assumption.

Anisakid nematodes of the genus *Hysterothylacium* are specifically found in the gut of fishes and its most common species, *H. aduncum*, is a widely distributed and generalist species that uses crustaceans as first and different invertebrates and fishes as second intermediate and final hosts (Anderson, 2000). Isopods, amphipods and mysids, to which it appears linked in the CCA, can act as intermediate hosts for *H. aduncum* (Koie, 1993; Klimpel and Rückert, 2005). The preference that younger fishes show for these three groups of crustaceans (Morte et al., 2002) can explain the higher abundance of *H. aduncum* in smaller fishes. The association with decapods in the CCA points to a possible transmission of this parasite through these crustaceans. This species has been associated to higher temperatures (Fig. 3). Although some authors have shown that this factor can modulate the life cycle of nematodes (De Meester et al., 2015), there are very few studies assessing its effect. *Collarinema collaris* is linked to mysids, isopods and decapods (*M. couchi*) in the CCA (Fig. 4). Leiva et al. (2015) found larval stages of nematodes of this family (i.e. Cystidicolidae) in decapod crustaceans. Although the first two groups have not yet been confirmed as hosts for larval stages of cystidicolid nematodes, they could be suggested as such. *Collarinema collaris* has been further associated to high levels of O_2 and turbidity (Fig. 3), which could favour the proliferation of its intermediate hosts, e.g. close to/into canyons. Nematodes of the genus *Cucullanus* infecting *P. blennoides* are known to belong to two distinct species based on molecular and morphological studies (unpublished results). One of them is *C. cirratus*, as identified by Hassani and Kerfouf (2015), whereas the second species remains presently unknown. In view of the difficulty in discriminating them with the available data, we have considered both species as a single taxon, in the same way as *Lepidapedon* spp. Koie (2000) described the life cycle of *C. cirratus* infecting Atlantic cod *Gadus morhua*. According to this author, third-stage larvae emerge from the eggs and are directly infective to teleosts (although they might previously use copepods as transport hosts), which may act either as intermediate of definitive hosts. Present results link this parasite to myctophid fishes, which

could be acting as intermediate hosts, and to larger fishes, that consume more teleosts than juvenile specimens (Morte et al., 2002).

In general, main prey-parasite relationships derived from the CCA are in agreement with what is known about parasites biology and life cycles (see above). Moreover, the two parasites with direct life cycles (i.e. *C. alata* and *D. phycidis*), not dependent on trophic transmission for infecting hosts, were not related to any prey in such analysis, which points to a good consistency of the outcomes of the CCA.

Some of the prey-parasite relationships found coincide with relationships found in previous studies, e.g. on *M. moro* (Dallarés et al., 2014, Fig. 4). This would be the case of the nematode Anisakis Type I and, to a lesser extent, Anisakis Type II, linked to *G. biscayiensis* and *M. norvegica* in *P. blennoides*, in the same way as for *M. moro* (Dallarés et al., 2014). These coincidences reinforce the presumable role of intermediate hosts by such prey for deep-sea fish.

4.4. Parasites vs fish condition

The negative associations between fish hepatosomatic or gonadosomatic indices and parasitological descriptors (i.e. total abundance, abundance of six parasites, richness and diversity for HSI and abundance of three parasites for GSI) could be attributed to a detrimental effect of the parasites on fish health or sexual development. Since a clear cause-effect relationship is really difficult to be established when dealing with indices that relate to many different factors, as is the case, the opposite interpretation, i.e. the higher susceptibility to parasitation of fishes with lower condition (or health), could be also possible. Hirazawa et al. (2016) reported impaired liver and kidney function in the greater amberjack *Seriola dumerili* infected with the monogenean *Neobenedenia girellae* while Heins and Baker (2008) detected prevented or delayed gamete production in the three-spine stickleback *Gasterosteus aculeatus* infected with the tapeworm *Schistocephalus* sp. However, while in some cases fish condition indices clearly vary as a function of parasitological infections, several examples exist in the literature where there is little or no relation at all between both factors (Masson et al., 2015; Constenla et al., 2015; Dallarés et al., 2014; Pérez-i-García et al., 2015). These contradictory results point to the high variability that exists regarding the outcomes of parasitological infections and its dependence on multiple factors, as highlighted by Heins and Baker (2008).

4.5. Biochemical markers (AChE, LDH and LP) vs. parasites and fish size

In relation to the biochemical markers addressed, acetylcholinesterase is an important enzyme involved in nerve impulse transmission (Podolska et al., 2014), lactate dehydrogenase, which has never been reported in *P. blennoides,* is a glycolytic enzyme involved in anaerobic metabolism (Koenig and Solé, 2014) and lipid peroxidation levels reflect the action of reactive oxygen species (ROS) over biological lipids (Solé et al., 2010a). Natural stressors, such as parasites, are known to modify the physiology of their hosts (Frank et al., 2013) and thus could alter the levels of the biomarkers selected in the present study. The few studies addressing the relationship between parasites and enzymatic biomarkers have yielded contradictory results: while some authors have found no effect of parasite infections on AChE and LDH activities (Podolska and Napierska, 2006; Dallarés et al., 2014) or LP levels (Pérez-i-García et al., 2015), others have detected positive (Gupta and Agarwal, 1985; Belló et al., 2000; Pérez-i-García et al., 2015) or negative (Pérez-i-García et al., 2015) associations between parasites and some of the afore mentioned markers. The consistently negative associations detected in the present study between AChE activity and different parasite descriptors of infracommunities and of individual parasite species could indicate, as expected, an inhibition of AChE induced by the infection-related stress. Pérez-i-García et al. (2015) reported similar results in *Alepocephalus rostratus* in our study area, but Gupta and Agarwal (1985) found the opposite trend in *Colisa lalia*. The positive relationship found between LP levels and the abundance of *Echinorhynchus* sp. and *H. aduncum* could point to oxidative stress induced by parasitism, as reported by Belló et al. (2000) in a freshwater fish species.

Lower AChE activity in larger fishes, as observed in the present study, has been repeatedly documented (Flammarion et al., 2002; Pathiratne et al., 2008; Koenig and Solé, 2014) and explained on the basis of a proportionality of AChE activity to the muscular cell surface, which is in turn proportional to fish total length. Likewise, LDH activity is known to be positively related to fish total length (Almeida-Val et al., 2000; Dallarés et al., 2014; present results), which serves to comply with burst-swimming energetic demands, which increase with the age of the fish (Almeida-Val et al., 2000).

4.6. Histological assessment vs. parasites and fish condition

Cysts of unknown etiology are frequently found structures embedded in the gill primary lamellae of gadiform fishes (Munday and Brand, 1992). The prevalence of such cysts in *P. blennoides* (i.e. more than 75%) is much higher than that recorded to date in any fishes from the same area (Carreras-Aubets et al., 2011; Dallarés et al., 2014; Constenla et al., 2015), which ranges between 5.71% and 34.78%. The higher number of CUEs in larger specimens points to an accumulation of these structures throughout the lifespan of the fish, the explanation for which is not yet known.

Macrophage centres are aggregates of inflammatory, often pigmented, cells usually present in different organs of fishes (Carrassón et al., 2008). Although they are commonly used in fishes as indicators of exposure to environmental pollution, they also change in response to pathologies, physiology and biological factors (Agius and Roberts, 1981; Carrassón et al., 2008; Borucinska et al., 2009). They act as sink for physiological waste, thus increasing in number and size throughout the lifespan of the fish, as evidenced by present results and other authors (Brown and George, 1985; Dallarés et al., 2014). If, as commented above, a low hepatosomatic index could suggest a worse health condition of the fish, either provoked by parasitological infections or by any other factor, we hypothesize that the association detected between lower HSI and higher values of MC parameters (i.e. surface and number) could be reflecting an increased metabolic effort to face damage induced by the stressing agent. However, attributing high values of MC parameters to parasites might be too adventurous. Although parasites can induce damage to their host, a previous study also testing a possible effect of parasites on MCs found no relationship between both factors in *M. moro* (Dallarés et al., 2014) and present results are not conclusive. Therefore, it seems that damage induced by parasites (at low infection levels and without especially harmful effects) do not alter significantly MC parameters, and that there might be other agents that determine changes in MCs.

Microsporidean parasites can infect different tissues of a wide array of hosts (from arthropods and molluscs to all kinds of vertebrate groups), and can cause serious disease in wild and cultured fish (Lom and Dyková, 2005). In the present case, however, prevalence and intensity of infection by these parasites was altogether negligible, and we thus consider that microsporideans do not have a significant negative effect on the health of *P. blennoides*. In the same way, although the presence of certain nematode species in the examined fish was relatively high (Tables 4 and 5), very few parasites of this group (essentially a few specimens of *Anisakis* spp. and *H. aduncum*) were detected in the liver during sections inspection or in organ squashes (personal observations). This observation can be associated with the very low prevalence and intensity of granulomas or other inflammatory responses detected in the liver during the histopathological study (P & $2lt;7%) and overall suggests that there is no relevant effect of these parasites on hepatic functions.

5. Conclusions

In conclusion, the parasite community of *P. blennoides* is characterized by high abundance, richness and diversity. The feeding habits of this species (active predation of a wide range of benthic/suprabenthic prey) are the main factor accounting for such characteristics. The observed bathymetric change of the parasite infracommunities (below vs. over 1000 m depth) is associated to the ontogenic diet shift undergone by *P. blennoides*, in turn related to the different composition of the macroinvertebrate communities along the continental slope. Moreover, such infracommunities were more abundant, rich, diverse and less dominated by a single species in the mainland than in the insular slope, which is explained by the different trophic and hydrographic conditions between both areas that favour more complex invertebrate communities that can enhance parasite transmission in the mainland slope. Abundance patterns of individual parasites have been linked to different environmental variables and/or main prey consumed by the host, confirming transmission patterns already known in some cases and suggesting new ways of infection in some others. The negative associations detected between acetylcholinesterase activity and different parasite descriptors of infracommunities and of individual parasite species point to an inhibition of this enzyme likely due to infection-related stress. Similarly, the positive relationship found between lipid peroxidation levels and the abundance of two parasites could be indicative of oxidative stress induced by parasitism. The outcomes of the histological assessment revealed very high prevalence and abundance of CUEs in fish gills and suggest that, in general, parasite infection damage does not alter significantly splenic MC parameters.

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Conflict of interest

The authors of the present work declare that they have no conflict of interest.

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CHAPTER 5 - PARASITE COMMUNITIES OF *SCYLIORHINUS CANICULA* (L., 1758) AND *GALEUS MELASTOMUS* RAFINESQUE, 1810 (ELASMOBRANCHII) FROM THE NW MEDITERRANEAN SEA, ASSESSING THE INFLUENCE OF SEASONALITY AND ENVIRONMENTAL VARIABLES

Parasite communities of *Scyliorhinus canicula* (L., 1758) and *Galeus melastomus* Rafinesque, 1810 (Elasmobranchii) from the NW Mediterranean Sea, assessing the influence of seasonality and environmental variables

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Short running title: Parasite communities of *Scyliorhinus canicula* and *Galeus melastomus* from the NW Mediterranean Sea

SUMMARY

The parasite communities of *Scyliorhinus canicula* and *Galeus melastomus* are described for the first time in the NW Mediterranean. Their seasonal and geographical variations, as well as their relationship with environmental and fish biological data were tested. Overall, the parasite communities of both sharks were characterized by low mean richness and diversity, and high dominance. However, infracommunity structure and composition was significantly different between both species probably due to the consumption of different prey associated to their different bathymetric distributions. For *G. melastomus*, parasite infracommunity structure and the abundance of some parasites differed across seasons and/or localities as a result of different dynamics of the populations of intermediate hosts in turn linked to different environmental conditions. *Ditrachybothridium macrocephalum* was more abundant in juvenile specimens of *G. melastomus* as a result of ontogenic diet shifts and *Grillotia* sp. accumulated in adult hosts, displaying higher abundance than in juveniles. The abundance of *Proleptus obtusus* was significantly higher in *S. canicula* than in *G. melastomus*, likely due to the

higher consumption of reptantian decapods by the former. Monogenean parasites of *S. canicula* both hosts were associated to high turbidity and temperature levels, which are known to enhance monogenean infection and reproductive success. Cestodes of *G. melastomus* were linked to high turbidity and O_2 levels, which increase zooplankton biomass and thus favour the transmission of heteroxenous parasites

Keywords: *Scyliorhinus canicula*, *Galeus melastomus*, Parasites, Communities, Seasonality, Mediterranean

INTRODUCTION

Elasmobranchs are of great relevance for the human being from an ecological, commercial, economic, conservationist and cultural point of view. Sharks and their relatives play a significant role as top predators in marine food webs, for which they are key components in their habitats, where they regulate trophic interactions and community composition (Stevens, 2000; Ruiz et al. 2016). Unfortunately, they are a main target of the fishing industry: the global market of shark products (which includes meat, fins, and other shark-derived commercial goods, such as liver oil, cartilage or skin) declares nearly 1 billion USD per year (Dent and Clarke, 2015). Such a high commercial importance has led to the overexploitation of these interesting animals, raising concerns about their vulnerability, conservation status and population decline (Stevens, 2000; Gross, 2014; Dent and Clarke, 2015). Of the approximately one thousand elasmobranch species included in the IUCN Red List of threatened species, nearly 20% hold one of the three endangered categories (IUCN, 2016), and approximately 44% are categorized as "data deficient", which suggests that the real percentage of threatened species of this group can be actually higher. Among the factors that make elasmobranchs particularly vulnerable to population mining, their life-cycle strategy, characterized by slow growth, late sexual maturity and low fecundity, is of major relevance (Stevens, 2000). Sharks are, additionally, objects of great social interest and cultural significance, largely influenced by movies and the popular literature (Carrier et al. 2004).

However, in spite of their relevancy and justified research interest, there is still little knowledge on many aspects of sharks biology and ecology. A good example is the

composition and dynamics of shark parasite communities, which have largely been neglected. Despite an increasing effort during the last years in describing and studying shark parasites, most publications either consist in taxonomic or phylogenetic studies (e.g. Caira et al. 2013, 2014; Dallarés et al. 2017) or deal with specific parasite groups, such as cestodes, or with the parasites found in a particular microhabitat, such as the spiral valve (e.g. Alarcos et al. 2006; Randhawa, 2012; Gračan et al. 2016).

While a large number of publications deal with teleost parasite communities, these have been barely addressed for shark species (the following list covers virtually all the existing literature on shark parasite communities: Henderson and Dunne, 1998; Moore, 2001; Henderson et al. 2002; Klimpel et al. 2003; Chambers, 2008; Isbert et al. 2015). In addition, these studies have been conducted in Northeastern Atlantic waters in almost all cases, and are completely absent from the Mediterranean Sea.

The spotted dogfish *Scyliorhinus canicula* (L., 1758) (Carcharhiniformes: Scyliorhinidae) and the blackmouth catshark *Galeus melastomus* Rafinesque, 1810 (Carcharhiniformes: Scyliorhinidae) are the two most abundant sharks in the Balearic Sea (NW Mediterranean) (Massutí and Moranta, 2003). These small demersal sharks are an important by-catch in northwestern Mediterranean waters (Carbonell et al. 2003) and both have shown to be negatively affected by trawling activity (Carbonell et al. 2003; Dimech et al. 2012). Furthermore, *S. canicula* has been suggested as an indicator of fishing pressure in the surroundings of Majorca Island, in the Balearic Sea (Carbonell et al. 2003).

Galeus melastomus shows a very wide bathymetric range, being the most important shark in the upper and middle slopes (i.e. 400–800 m and 800–1,400 m, approximately) in terms of abundance and biomass (Carrassón et al. 1992; Massutí and Moranta, 2003; D'Onghia et al. 2004). In contrast, the spotted dogfish *Scyliorhinus canicula* (L., 1758) (Carcharhiniformes: Scyliorhinidae) reaches its maximum abundance around 100 m depth, dominating the elasmobranch fauna of the continental shelf, although it can be found in the upper slope down to 500 m (Massutí and Moranta, 2003).

The bathymetric distribution of juveniles and adults also differs for both species: while *G. melastomus* follows the deeper-bigger rule, with adults distributed in deeper waters than juveniles, *S. canicula* follows the opposite trend, with adults distributed above 100 m and juveniles below this depth (Carrassón et al. 1992; Massutí and Moranta, 2003).

Regarding their feeding biology, both species are generalistic predators and occasional scavengers that consume mainly benthic and benthopelagic prey (Carrassón et al. 1992;

Valls et al. 2011; Mnrasi et al. 2012). Although *G. melastomus* shows a more specialized diet than *S. canicula*, they can show a high diet overlap in the Balearic Sea, mainly in the upper slope (Valls et al. 2011). Furthermore, both sharks show similar ontogenic diet shifts, with adults consuming larger prey, such as cephalopods, teleosts and large crustaceans, than juveniles, for which smaller crustaceans like mysids, amphipods or euphausiids are the main target (Carrassón et al. 1992; Valls et al. 2011).
The metazoan parasite community of *S. canicula* was already described in the northeastern Atlantic Ocean by Henderson and Dunne (1998) and Moore (2001). In the case of *G. melastomus,* although several records of different parasites exist (Pintner, 1899, 1930; Sproston, 1946; Brinkmann, 1988; Euzet et al. 1993; Raibaut et al. 1998 Dallarés et al. 2015, among others), these come mainly from northern Atlantic waters.
Therefore, and given the high importance of *S. canicula* and *G. melastomus* in the NW Mediterranean marine ecosystems, the main aim of present study is to describe the parasite communities of both species in this area. Seasonal variability throughout the year (and geographical differentiation between two localities in the case of *G. melastomus*) is tested for parasite community descriptors and for the abundance of the most frequent parasites. The influence of environmental variables (namely temperature, salinity, turbidity, O_2 content of water masses and phytoplankton concentration) on the abundance patterns of individual parasites is further addressed. Finally, differences on the parasite community composition and structure between the two sharks addressed are assessed and discussed.

MATERIALS AND METHODS

Sampling area and specimen collection

A total of 41 specimens of *S. canicula* and 159 of *G. melastomus* was collected during 2007 at the continental slope of the Balearic Sea off the mouth of the River Besòs (Barcelona) (seasonally) and off Vilanova (only in summer). The samples were obtained using a semi-balloon otter trawl (OTSB) and a commercial fishing gear (BOU) at depths comprised between 53–68 m for *S. canicula* and between 549–809 m for *G. melastomus* (Table 1). On board, fish were freshly frozen at –20 ºC for further parasitological examination.

Simultaneously to sampling hauls, measures of temperature (T) in ºC, salinity (S) in psu, O_2 content of water masses in ml/l, turbidity (voltage) and phytoplankton pigment concentration (Chla) were obtained at 5 m above the bottom by deployment of a CTD profiler (Table 1).

Table 1. Sampling data for *Galeus melastomus* and *Scyliorhinus canicula*. n: number of individuals captured in each haul, Lat.: Latitude, Long.: Longitude, G. mel.: *G. melastomus*, S. can.: *S. canicula*. Environmental variables: TºC: temperature, S: salinity, O_2: oxygen concentration, Turb: turbidity, Chla: chlorophyll a concentration.

Locality-season group	Haul	Date	Depth	Coordinates		n	N	Environmental variables				
		(dd/mm/yy)	(m)	Lat. (N)	Long. (E)	G. mel.	S. can.	TºC	S (psu)	O_2 (mL/L)	Turb (V)	Chla
Besós-Winter	B1–BOU1	06/03/2007	651	41.24	2.46	25		13,29	38,53	3,93	0,15	0,05
	B1–BOU2	06/03/2007	784	41.15	2.40	28		13,19	38,51	4,12	1,10	0,05
	B1–BOU3	14/03/2007	53	41.41	2.29		1	13.64	38.22	4.88	0.35	1.02
	B1–BOU4	14/03/2007	63	41.42	2.38		6	13.64	38.22	4.88	0.35	1.02
Besós-Spring	B2–OTSB2	28/04/2007	650	41.17	2.38	5		13.29	38.53	5.76	0.27	0.02
	B2–OTSB3	28/04/2007	797	41.15	2.41	5		13.24	38.52	5.77	0.44	0.02
	B2–OTSB4	29/04/2007	809	41.15	2.40	3		13.24	38.52	5.77	0.44	0.02
	B2–OTSB5	29/04/2007	661	41.24	2.48	1		13.29	38.53	5.76	0.27	0.02
	B2–BOU1	09/05/2007	66	41.38	2.34		3	13.63	38.08	5.74	0.07	0.22
	B2–BOU2	09/05/2007	67	41.39	2.34		6	13.63	38.08	5.74	0.07	0.22
	B2–BOU3	11/05/2007	559	41.24	2.46	12		13.29	38.53	5.76	0.27	0.02
	B2–BOU4	11/05/2007	785	41.15	2.40	3		13.24	38.52	5.77	0.44	0.02
Besós-Summer	B3–OTSB5	01/07/2007	671	41.24	2.49	5		13.32	38.54	5.76	0.03	0.02
	B3–BOU3	18/07/2007	66	41.38	2.34		7	14.80	38.04	5.60	0.02	0.54
	B3–BOU4	18/07/2007	66	41.38	2.33		4	14.80	38.04	5.60	0.02	0.54
	B3–BOU5	18/07/2007	68	41.38	2.35		2	14.80	38.04	5.60	0.02	0.54
	B3–BOU6	19/07/2007	561	41.24	2.46	19		13.32	38.54	5.76	0.03	0.02
	B3–BOU7	19/07/2007	791	41.15	2.40	10		13.18	38.51	5.78	0.08	0.02
Besós-Autumn	B4–OTSB3	02/10/2007	811	41.14	2.36	2		13.18	38.51	8.25	0.18	0.02
	B4–OTSB6	03/10/2007	716	41.24	2.49	4		13.39	38.55	8.22	0.47	0.02
	B4–BOU1	13/11/2007	60	41.39	2.34		2	17.00	38.03	7.67	0.83	0.77
	B4–BOU2	13/11/2007	60	41.36	2.33		2	17.00	38.03	7.67	0.83	0.77
	B4–BOU3	13/11/2007	60	41.38	2.34		8	17.00	38.03	7.67	0.83	0.77
	B4–BOU4	28/12/2007	549	41.24	2.45	2		13.39	38.55	8.22	0.47	0.03
	B4–BOU5	28/12/2007	791	41.15	2.40	13		13.18	38.51	8.25	0.18	0.03
Vilanova-Summer	B3–OTSB6	05/07/2007	662	41.09	2.18	4		13.41	38.53	3.95	0.40	0.01
	B3–OTSB7	05/07/2007	803	41.07	2.21	2		13.17	38.50	5.78	0.09	0.02
	B3–BOU1	25/06/2007	780	41.07	2.20	16		13.17	38.50	5.78	0.09	0.02
Total number of specimens sampled						159	41					

Parasitological study

In the laboratory and prior to dissection, total length (TL) in mm and total weight (TW) in g were obtained for each fish. Subsequently, external surfaces and gills were examined for the presence of ectoparasites. All internal organs were dissected out and examined separately for endoparasites under stereomicrocope. In the case of *S. canicula*, body musculature was also examined under stereomicroscope by compression between two glass plates. All metazoan parasites collected were preserved in 70% ethanol. Monogeneans, digeneans and cestodes were stained with iron acetocarmine and examined as permanent mounts in Canada balsam. Nematode larvae and crustaceans were observed as temporary mounts in saline solution. All parasites were identified to the lowest possible taxonomic level.

Data analyses

For *G. melastomus*, two size-based groups of hosts were defined, corresponding to immature (TL<34 cm for males and TL<40cm for females) and mature specimens (TL≥34 cm for males and TL≥40 cm for females) (Capapé and Zaouali, 1977).
Ecological terms used for parasite populations and communities follow Bush et al. (1997): prevalence (P%) was calculated as the proportion of hosts in each sample infected by a given parasite and mean abundance (MA) as the total number of parasites found in a particular host species divided by the total number of hosts of such species. For both species, parasite species displaying a P%>10% in at least one seasonal/geographical group were considered not accidental and are henceforth called common (see indications in Table 2). Parasite infrapopulations and infracommunities (i.e., all parasites of a given species in an individual fish and all infrapopulations in an individual fish, respectively) were used as replicate samples in the analyses. Infracommunity richness, abundance, diversity and dominance were calculated, the two latter based on parasite abundance, using Brillouin's Index (PRIMER v6; Anderson et al. 2008) and Berger-Parker dominance index (calculated as the number of individuals of the most abundant parasite species in a given host divided by the total number of parasites found in such host), respectively. Fish condition was assessed by the condition factor (K, calculated as $TW \times 100 / TL^3$), and the hepatosomatic index (HSI, calculated as liver weight (g) × 100 / TW).

Spearman rank correlation (r_s) tests were applied in order to assess the association of parasite infracommunity parameters (i.e. richness, abundance, diversity and dominance) with host TL, and condition parameters (i.e. K and HSI). The same tests were used to test the relationship between the abundance of common parasites of both hosts and the same fish biological factors.

Kruskal-Wallis tests and generalized linear model (GZM) analyses were performed to test the differences on parasite infracommunity parameters and on the prevalence and abundance of common parasites (using fish TL as covariate), respectively, among seasons. Similarly, general linear models (GLM) followed by post hoc tests using Bonferroni correction were carried out to assess differences among seasons on fish TL and condition parameters for both species. The same analyses were repeated using matched seasonal samples (i.e. summer) of *G. melastomus* collected in Besós and Vilanova in order to test geographical variability in parasite infracommunity parameters, the prevalence and abundance of common parasites and in fish TL and condition parameters.

Permutational multivariate analyses of variance (PERMANOVA) were carried out using abundance data of parasite infracommunities to test differences in the parasite community structure among the four seasons sampled off Besós for the two species of sharks, and between matched seasonal samples from off Besós and Vilanova for *G. melastomus*. Such analyses were applied using PERMANOVA+ for PRIMERv6 (Anderson et al. 2008) on Bray-Curtis similarity matrices generated from the logarithmically transformed (log (X+1)) abundance data, and permutation p-values were obtained under unrestricted permutation of raw data (9999 perms).

Canonical correspondence analyses (CCA) were used to relate the abundance of the common parasites found in the two species of sharks with environmental variables (Ter Braak, 1986). In CCA plots, arrows represent explanatory variables and are proportional in length to their importance on the explained variable.

A non-parametric Multi-Dimensional Scaling (MDS) was applied on infracommunity data of *S. canicula* and *G. melastomus* in order to visualize the ordination of parasite infracommunities of the two distinct hosts. Then, using the samples collected from off Besós, T-Student tests (assuming non-equal variances) were applied to test differences on infracommunity richness, diversity and dominance between *S. canicula* and *G. melastomus*, and GZM were used to test differences on infracommunity abundance and on the overall abundance of the shared parasites between both hosts.

RESULTS

The parasite community of S. canicula

The parasite community of *S. canicula* included a total of five parasite species comprising three nematodes, one monogenean and one cestode (Table 2). *Hysterothylacium aduncum* (Rudolphi, 1802) constitutes a new host record for this species. The nematode *Proleptus obtusus* Dujardin, 1845 was, by far, the most important parasite in terms of prevalence and abundance. All sharks examined were infected by at least one parasite.

Table 2. Overall prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Galeus melastomus* and *Scyliorhinus canicula*. N: sample size for each host, [a] species considered common in *G. melastomus*, [b] species considered common in *S. canicula*. Dashes indicate absence of the parasite.

	Galeus melastomus		***Scyliorhinus canicula***	
N	**159**		**41**	
	P%	**MA ± SD**	**P%**	**MA ± SD**
Monogenea				
Erpocotyle sp.[a]	5.7	0.07 ± 0.30	–	–
Leptocotyle minor	1.3	0.01 ± 0.11	–	–
Hexabothrium appendiculatum[b]	–	–	17.1	0.46 ± 1.33
Digenea				
Otodistomum cestoides	1.9	0.02 ± 0.14	–	–
Accacoeliidae gen. sp. (met)	1.3	0.01 ± 0.11	–	–
Cestoda				
Ditrachybothridium macrocephalum[a]	17.1	0.53 ± 2.43	–	–
Grillotia sp. [a]	14.6	0.21 ± 0.60	–	–
Sphyriocephalus viridis	1.9	0.03 ± 0.26	–	–
Nybelinia lingualis[b]	–	–	2.4	0.02 ± 0.16
Nematoda				
Dychelyne (Cucullanellus) sp.	0.6	0.01 ± 0.08	–	–
Piscicapillaria baylisi	5.7	0.06 ± 0.23	–	–
Anisakis type II (sensu Berland, 1961)[b]	2.5	0.03 ± 0.16	7.3	0.10 ± 0.37
Hysterothylacium aduncum[b]	1.9	0.02 ± 0.14	7.3	0.07 ± 0.26
Proleptus obtusus [a,b]	5.1	0.06 ± 0.26	100	49.37 ± 37.76
Copepoda				
Eudactylina sp.	0.6	0.01 ± 0.08	–	–

No relationship was detected between any of the infracommunity parameters or the abundance of common parasites and fish TL, K or HSI ($p>0.05$ in all cases).

Kruskal-Wallis tests and GZMs revealed no seasonal differences for infracommunity parameters or for the abundance or prevalence of common parasites ($p>0.05$ in all cases) (Tables 3 and 4). Regarding fish biological factors, only K showed significant seasonal variations ($F_{(3, 37)}= 6.293$, $p= 0.001$) being higher in summer and autumn than in the other two seasons (Table 3).

The PERMANOVA applied on parasite infracommunities showed no seasonal effect on the structure of such communities ($p_{(perm)}>0.05$).

The CCA relating common parasites of *S. canicula* and environmental variables accumulated 99.8% of the total variance (Fig. 1). The abundance of the monogenean *Hexabothrium appendiculatum* (Kuhn, 1829) was strongly associated to high near-bottom turbidity and, to a lesser extent, temperature and O_2 concentration, while the nematode *H. aduncum* was associated to high salinity levels.

Table 3. Means and standar deviations of fish biological factors and parasite infracommunity descriptors across the seasons and localities sampled for *Scyliorhinus canicula* and *Galeus melastomus*. Different superscript letters and numbers show significant differences among seasons and between localities, respectively. S1: size 1, S2: size 2.

Host	***Scyliorhinus canicula***				***Galeus melastomus***				
Locality-season group	**Besós winter**	**Besós spring**	**Besós summer**	**Besós autumn**	**Besós winter**	**Besós spring**	**Besós summer**	**Besós autumn**	**Vilanova summer**
Total length	42.14 ± 2.73^{a}	42.88 ± 1.15^{a}	42.22 ± 4.78^{a}	43.62 ± 2.26^{a}	32.16 ± 9.54^{ab}	28.73 ± 14.50^{a}	36.95 ± 13.09^{b1}	46.37 ± 8.68^{c}	36.95 ± 11.94^{1}
Condition factor	0.26 ± 0.03^{a}	0.29 ± 0.06^{ab}	0.34 ± 0.06^{b}	0.34 ± 0.02^{b}	0.26 ± 0.07^{a}	0.27 ± 0.06^{a}	0.23 ± 0.03^{b1}	0.27 ± 0.04^{a}	0.21 ± 0.03^{2}
Hepatosomatic index	8.61 ± 3.36^{a}	7.76 ± 2.38^{a}	8.06 ± 2.87^{a}	5.74 ± 2.17^{a}	3.69 ± 1.00^{a}	4.27 ± 2.40^{a}	4.40 ± 1.96^{a1}	5.84 ± 1.43^{b}	4.86 ± 1.92^{1}
Total parasite richness	4	4	3	3	11	5	7	8	2
Infracommunity richness	1.43 ± 0.54^{a}	1.44 ± 1.01^{a}	1.38 ± 0.65^{a}	1.17 ± 0.39^{a}	0.70 ± 0.72^{a}	0.57 ± 0.69^{a}	0.59 ± 0.78^{a1}	0.90 ± 0.70^{a}	0.14 ± 0.35^{2}
Infracommunity abundance	29.14 ± 22.00^{a}	80.56 ± 59.93^{a}	48.00 ± 29.37^{a}	41.50 ± 18.53^{a}	0.87 ± 1.02	2.29 ± 5.41	0.76 ± 1.16^{1}	1.14 ± 1.06	0.18 ± 0.50^{2}
Infracommunity abndance – S1					0.71 ± 0.98^{a}	3.00 ± 6.12^{b}	0.58 ± 1.26^{a}	1.00 ± 0.82^{ab}	0.08 ± 0.28
Infracommunity abundance – S2					1.42 ± 1.00^{a}	0.14 ± 0.38^{a}	1.00 ± 1.00^{a}	1.18 ± 1.13^{a}	0.33 ± 0.71
Infracommunity diversity (Brillouin's index)	0.09 ± 0.12^{a}	0.03 ± 0.06^{a}	0.07 ± 0.11^{a}	0.04 ± 0.11^{a}	0.10 ± 0.16^{a}	0.08 ± 0.15^{a}	0.11 ± 0.20^{a1}	0.09 ± 0.16^{a}	0.00 ± 0.00^{1}
Infracommunity dominance (Berger-Parker's index)	0.96 ± 0.06^{a}	0.99 ± 0.01^{a}	0.97 ± 0.05^{a}	0.99 ± 0.04^{a}	0.88 ± 0.21^{a}	0.88 ± 0.22^{a}	0.89 ± 0.19^{a1}	0.89 ± 0.19^{a}	1.00 ± 0.00^{1}

Table 4. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Scyliorhinus canicula* across the seasons sampled. N: sample size for each group. Different superscript letters and numbers show significant differences across seasons for MA and P% of common parasites, respectively. Dashes indicate absence of the parasite.

	Besós winter		**Besós spring**		**Besós summer**		**Besós autumn**	
N	**7**		**9**		**13**		**12**	
	P(%)	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**
Monogenea								
Hexabothrium appendiculatum	14.3[1]	0.29 ± 0.76[a]	11.1[1]	0.22 ± 0.67[a]	30.8[1]	0.62 ± 1.19[a]	8.3[1]	0.58 ± 2.02[a]
Cestoda								
Nybelinia lingualis	14.3	0.14 ± 0.38	–	–	–	–	–	–
Nematoda								
Hysterothylacium aduncum	14.3[1]	0.14 ± 0.38[a]	22.2[1]	0.22 ± 0.44[a]	–	–	–	–
Anisakis type II	–	–	11.1[1]	0.22 ± 0.67[a]	7.7[1]	0.08 ± 0.28[a]	8.3[1]	0.08 ± 0.29[a]
Proleptus obtusus	100.0[1]	28.57 ± 22.04 [a]	100.0[1]	79.89 ± 58.76 [a]	100.0[1]	47.31 ± 29.42 [a]	100.0[1]	40.83 ± 18.52 [a]

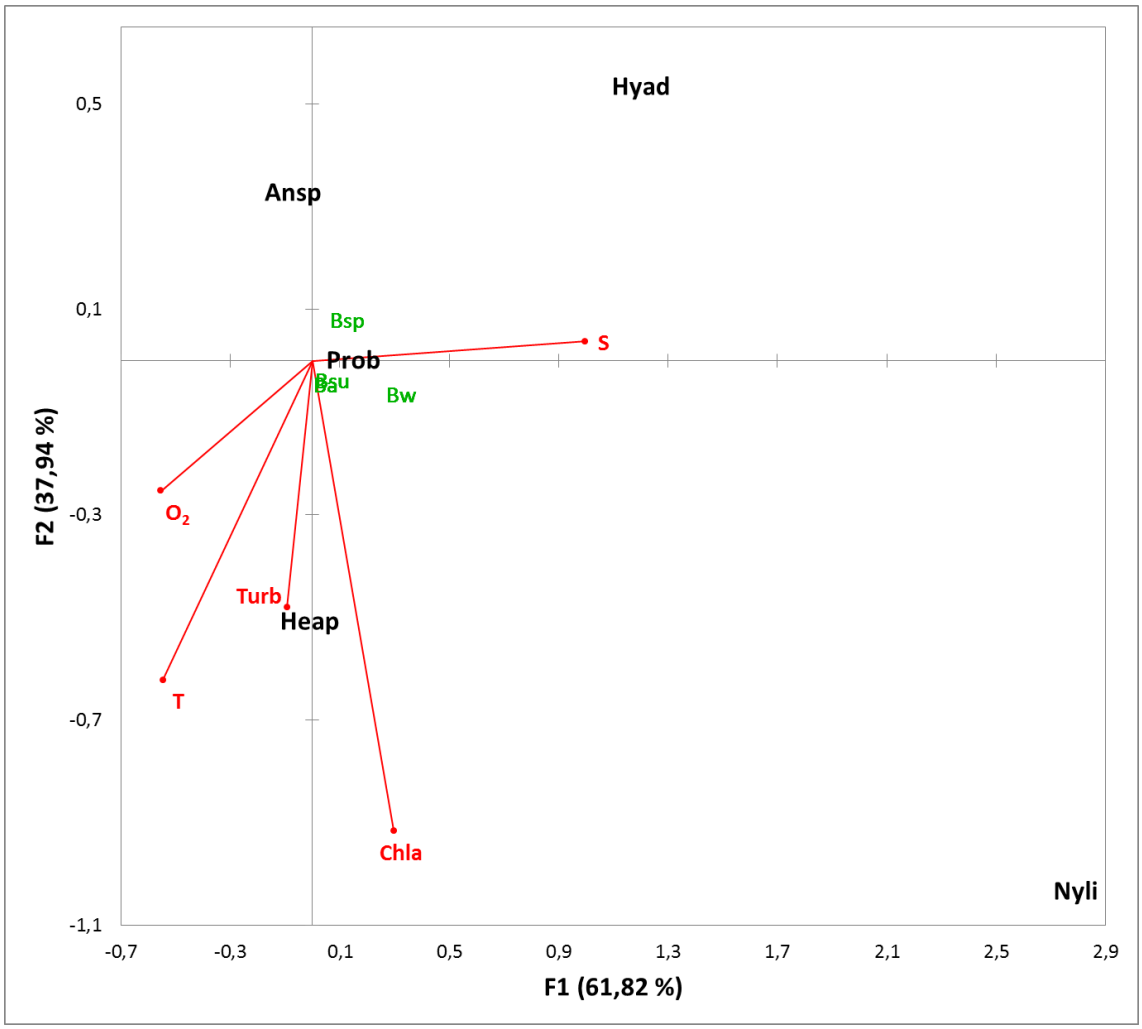

Figure 1. Canonical correspondence analysis (CCA) showing associations between the common parasites of *Scyliorhinus canicula* and environmental variables. Abbreviations for parasite names: Ansp, *Anisakis* Type II; Heap, *Hexabothrium appendiculatum*; Hyad, *Hysterothylacium aduncum*; Nyli, *Nybelinia lingualis*; Prob, *Proleptus obtusus*. Abbreviations for locality-season groups: Ba, Besós autumn; Bsp, Besós spring; Bsu, Besós summer; Bw, Besós winter. Abbreviations for environmental variables: Chla, chlorophyll a concentration; O_2, oxygen concentration; S, salinity; T, temperature; Turb, turbidity.

The parasite community of G. melastomus

A total of 13 parasite species comprising five nematodes, three cestodes, two monogeneans, two digeneans and one copepod were recovered from the specimens of *G. melastomus* examined (Table 2). Of these, ten species constitute new host records for this host: *Erpocotyle* sp., *Leptocotyle minor* (Monticelli, 1888), *Otodistomum cestoides* (Van Beneden, 1871) Odhner, 1911, Accacoelidae gen. sp., *Grillotia* sp., *H. aduncum*, *Anisakis* sp. ascribed to morphotype *Anisakis* Type II *sensu* Berland (1961), *P. obtusus, Piscicapillaria baylisi* Moravec, 1987 and *Cucullanus* sp.

The most frequent parasites were the cestodes *Ditrachybothridium macrocephalum* Rees, 1959 and *Grillotia* sp. Overall prevalence of infection in *G. melastomus* was 46%. Infracommunity richness and the abundance of the cestodes *D. macrocephalum* and *Grillotia* sp. were significantly correlated with fish TL (r_s= 0.17, p= 0.04; r_s= -0.16, p= 0.04 and r_s= 0.27, p= 0.001, respectively), with the abundance of *D. macrocephalum* being higher in juvenile sharks and infracommunity richness and the abundance of *Grillotia* sp. reaching higher values in adult hosts. Furthermore, the abundance of *D. macrocephalum* and *P. obtusus* showed significant positive and negative relationships, respectively, with K (r_s= 0.19, p= 0.019 and r_s= -0.23, p= 0.004, respectively), and the abundance of *D. macrocephalum* and *Grillotia* sp. were negatively and positively related, respectively, to HSI (r_s= -0.28, p= 0.004 and r_s= 0.20, p= 0.042, respectively). Parasite infracommunity descriptors were not significantly related to K or HSI (p>0.05 in all cases).

The abundance of *Grillotia* sp. showed significant differences among seasons, being maximum in summer and autumn (χ^2= 10.643, p= 0.014). In the cases of infracommunity mean abundance and abundance of *D. macrocephalum* interactions were found between the factor season and fish TL (χ^2= 16.478, p= 0.001 and χ^2=11.386, p= 0.003, respectively) and the analyses were thus repeated considering the two size-groups of hosts separately. Infracommunity mean abundance and abundance of *D. macrocephalum* displayed significant seasonal variability in juvenile sharks, (χ^2=21.737, p<0.001 and χ^2= 22.870, p<0.001, respectively), both being highest in spring than in the rest of seasons (Tables 3 and 5).

No seasonal differences were detected for infracommunity richness, diversity or dominance, or for the prevalence of common parasites (p>0.05 in all cases).

Concerning geographical variability, infracommunity richness and abundance were significantly higher in samples from off Besós than in those from off Vilanova in summer (χ^2= 6.031, p= 0.014 and χ^2= 5.679, p= 0.017, respectively) (Table 3). No significant differences were detected between localities for abundance or prevalence values of common parasites, although *Erpocotyle* sp., and *P. obtusus* were absent from the samples from off Vilanova (Table 5).

Table 5. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Galeus melastomus* across the seasons and localities sampled. N: sample size for each group, S1: size 1, S2: size 2. Different superscript letters and numbers show significant differences across seasons for MA and P% of common parasites, respectively. Dashes indicate absence of the parasite.

	Besós winter		**Besós spring**		**Besós summer**		**Besós autumn**		**Vilanova summer**	
N	**53**		**29**		**34**		**21**		**22**	
	P(%)	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**	**P(%)**	**MA ± SD**
Monogenea										
Erpocotyle sp.	5.7[1]	0.08 ± 0.33[a]	3.6[1]	0.04 ± 0.19[a]	2.9[1]	0.03 ± 0.17[a]	19.1[1]	0.24 ± 0.54[a]	–	–
Leptocotyle minor	1.9	0.02 ± 0.14	–	–	2.9	0.03 ± 0.17	–	–	–	–
Digenea										
Otodistomum cestoides	3.8	0.04 ± 0.19	–	–	–	–	4.8	0.05 ± 0.22	–	–
Accacoelidae gen. sp.	1.9	0.02 ± 0.14	3.6	0.04 ± 0.19	–	–	–	–	–	–
Cestoda										
Ditrachybothridium macrocephalum	28.3[1]	0.43 ± 0.84	32.1[1]	2.04 ± 5.47	–	–	14.3[1]	0.14 ± 0.36	–	–
Ditrachybothridium macrocephalum-S1		0.44 ± 0.84[a]		2.71 ± 6.20[b]		–		–		–
Ditrachybothridium macrocephalum-S2		0.42 ± 0.90[a]		–		–		0.18 ± 0.39[a]		–
Sphyriocephalus viridis	–	–	–	–	2.9	0.09 ± 0.51	9.5	0.10 ± 0.30	–	–
Grillotia sp.	7.6[1]	0.08 ± 0.27[a]	10.7[1]	0.11 ± 0.32[ab]	23.5[1]	0.32 ± 0.68[b]	28.6[1]	0.48 ± 0.87[b]	9.1	0.14 ± 0.47
Nematoda										
Hysterothylacium aduncum	1.9	0.02 ± 0.14	7.1	0.07 ± 0.26	–	–	–	–	–	–
Anisakis type II	5.7	0.06 ± 0.23	–	–	2.9	0.03 ± 0.17	–	–	–	–
Proleptus obtusus	3.8[1]	0.04 ± 0.19[a]	–	–	14.7[1]	0.18 ± 0.46[a]	4.8[1]	0.05 ± 0.22[a]	–	–
Piscicapillaria baylisi	7.6	0.08 ± 0.27	–	–	8.8	0.09 ± 0.29	4.8	0.05 ± 0.22	4.6	0.05 ± 0.21
Dychelyne (*Cucullanellus*) sp.	–	–	–	–	–	–	4.8	0.05 ± 0.22	–	–
Copepoda										
Eudactylina sp.	1.9	0.02 ± 0.14	–	–	–	–	–	–	–	–

Fish TL and HSI were significantly lower in autumn samples than in the rest of seasons ($F_{(3, 132)}$= 10.937, p<0.001 and $F_{(3, 96)}$= 5.446, p= 0.002, respectively), while fish K reached minimum values in summer ($F_{(3, 130)}$= 3.861, p= 0.011). Among fish biological descriptors, only K showed significant differences between localities ($F_{(1, 54)}$= 6.845, p= 0.012), being higher in fishes from off Besós than in those from off Vilanova (Table 3).

The PERMANOVA analyses applied on infracommunity abundance data revealed significant differences in the structure of parasite infracommunities among the four seasons sampled off Besós (Pseudo-$F_{(3, 67)}$= 3.1495, $p_{(perm)}$<0.001, unique perms= 9,928). Post-hoc pairwise comparisons separated winter and spring samples, which grouped together, from summer and autumn ones, which grouped together as well. In contrast, no geographical differences between infracommunities collected off Besós and Vilanova in summer were detected ($p_{(perm)}$> 0.05).

The CCA relating common parasites of *G. melastomus* and environmental variables accumulated 95.0% of the total variance (Fig. 2). The abundance of the cestode *D. macrocephalum* was associated to high near-bottom turbidity coinciding with some hauls from off Besós in winter and spring. The parasites *Erpocotyle* sp. and *Grillotia* sp. were linked to high O_2 concentration, partly associated to hauls from off Besós in autumn. Finally, the nematode *P. obtusus* was associated to high levels of salinity and temperature, in this case associated to hauls from off Besós, although of any particular season.

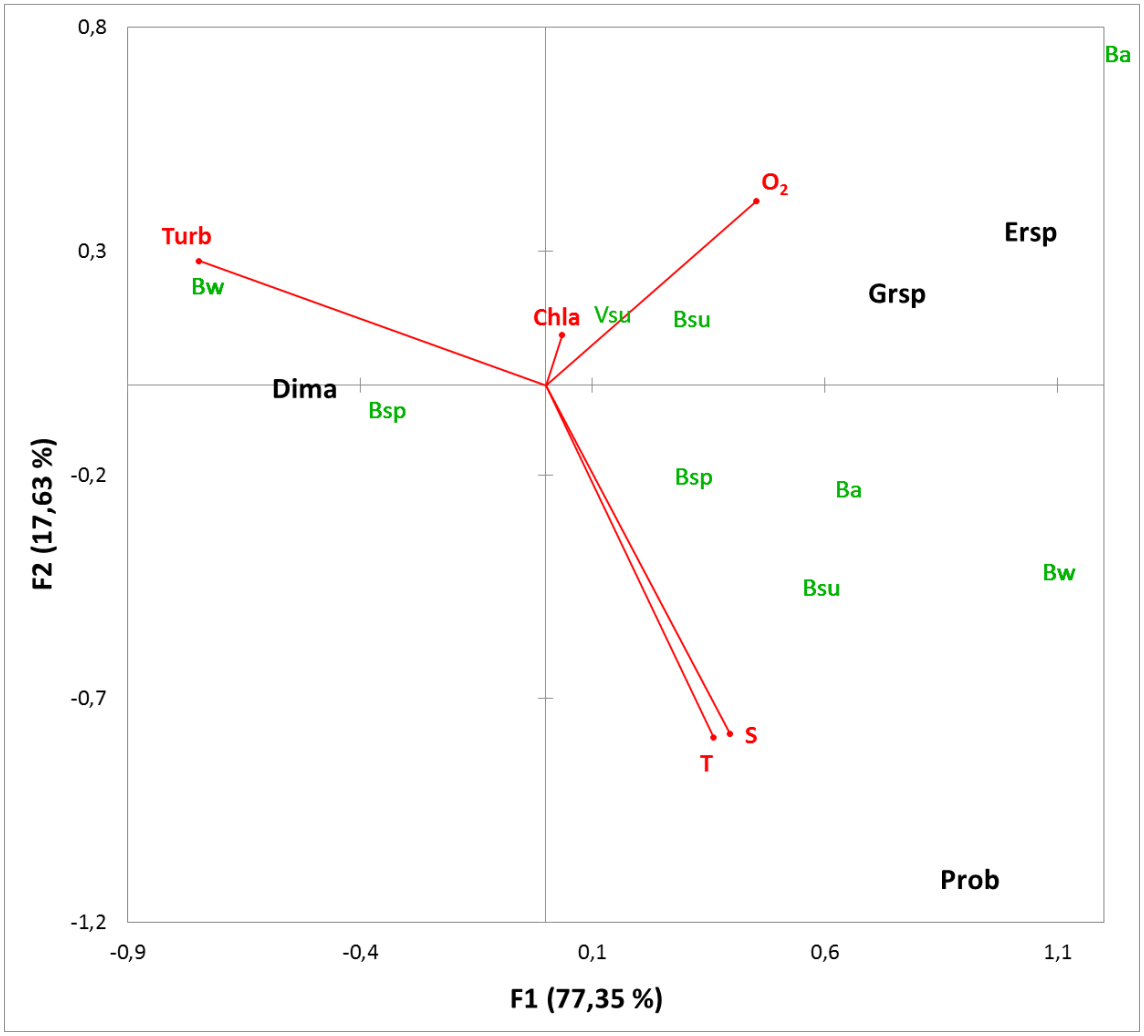

Figure 2. Canonical correspondence analysis (CCA) showing associations between the common parasites of *Galeus melastomus* and environmental variables. Abbreviations for parasite names: Dima, *Ditrachybothridium macrocephalum*; Ersp, *Erpocotyle* sp.; Grsp, *Grillotia* sp.; Prob, *Proleptus obtusus*. Abbreviations for locality-season groups: Ba, Besós autumn; Bsp, Besós spring; Bsu, Besós summer; Bw, Besós winter; Vsu, Vilanova summer. Abbreviations for environmental variables: Chla, chlorophyll a concentration; O_2, oxygen concentration; S, salinity; T, temperature; Turb, turbidity.

Comparison between the two sharks addressed

The MDS providing an ordination of parasite infracommunities of both hosts evidenced a clear differentiation between samples of *S. canicula* and *G. melastomus* (Stress= 0.04, Fig. 3).

Overall parasite infracommunity richness and dominance were significantly higher in *S. canicula* than in *G. melastomus* (t= -5.540, p<0.001 and t= -3.851, p<0.001, respectively), as also were total infracommunity abundance and the abundance of the nematode *P. obtusus* (χ^2= 365.441, p<0.001 and χ^2= 305.229, p<0.001, respectively, see Table 2). Infracommunity diversity and the abundance of the other two shared parasites (i.e. *H. aduncum* and *Anisakis* Type II) showed no significantly different values between hosts (p>0.05 in both cases).

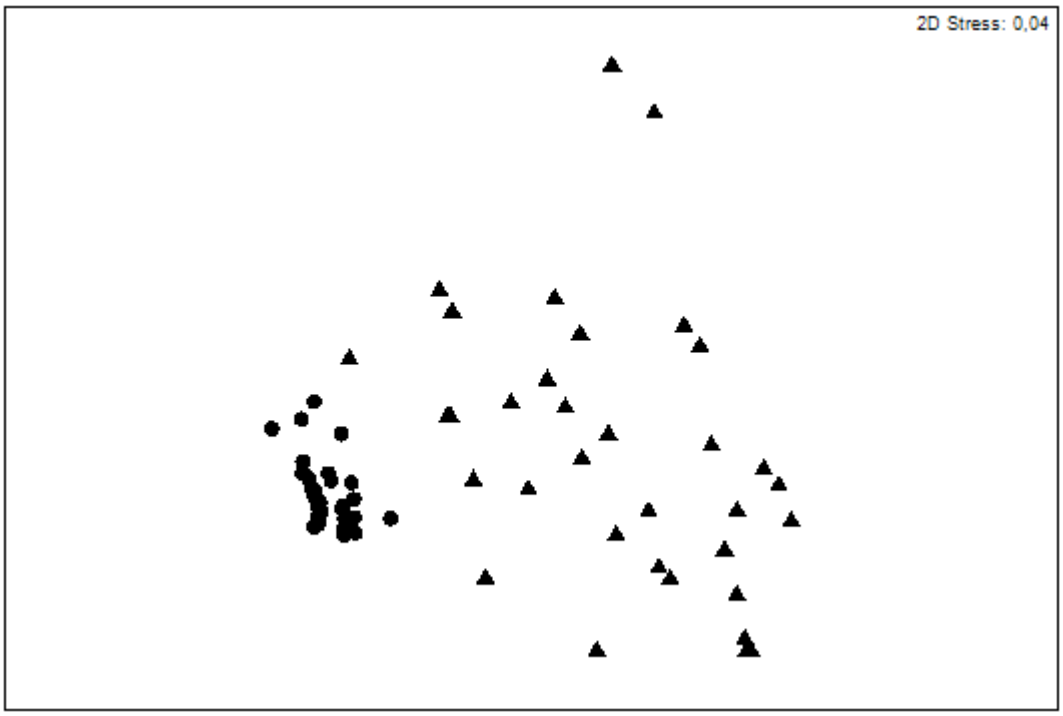

Figure 3. Non-parametric Multi-dimensional scaling (MDS) showing ordination of infracommunities of *Scyliorhinus canicula* (●) and *Galeus melastomus* (▲).

DISCUSSION

The present study represents the first attempt to describe the complete parasite communities of shark species in the Mediterranean Sea.

In the NW Mediterranean, *S. canicula* is characterized by an impoverished parasite community, displaying low richness and diversity and strongly dominated by a single species (i.e. the nematode *P. obtusus*). *Galeus melastomus*, for which the parasite community is described herein for the first time, shows in comparison markedly higher total parasite richness (13 vs. 5 parasite taxa), although parasite infracommunities are still depauperate, with lower mean richness and similar diversity and dominance values than *S. canicula*.

In spite of these similarities, structure of infracommunities is rather different between both species, as evidenced by their distinct ordination in the MDS. While infracommunities of *S. canicula* grouped together reflecting a high degree of similarity, those corresponding to *G. melastomus* appeared scattered throughout the plot, indicating a much higher heterogeneity among them. Actually, although values of Berger-Parker's dominance index were high in both species, in *G. melastomus* the dominant parasite was not consistently the same in all infracommunities, and relative abundances of the different parasites were generally modest. In contrast, in *S. canicula* the nematode *P. obtusus* showed very high abundance levels compared to the rest of parasite species, clearly dominating all infracommunities and displaying a homogenizing effect. Such effect was also appreciated in the CCA plot relating *S. canicula* parasite abundance levels with environmental variables, where all seasons appeared centralized, close to *P. obtusus*, since this parasite characterizes all seasonal groups in this host.

These differences in the parasite community composition and structure of both hosts are likely due to their different dietary habits (Carrassón et al. 1992; Valls et al. 2011; Mnrasi et al. 2012), largely influenced by the different availability of benthopelagic prey assemblages in the continental shelf (i.e. sampling depth of *S. canicula*) and in the upper slope (i.e. sampling depth of *G. melastomus*). Variations in the benthopelagic faunal assemblages occur along depth gradients in response to different environmental conditions (Cartes et al. 2006, 2013) and lead to different transmission dynamics for trophically-transmitted parasites and, therefore, to different composition of parasite communities.

The low mean richness and diversity, and high dominance of infracommunities observed for *S. canicula* and, to a lesser extent, for *G. melastomus,* have also been reported from other small-sized sharks from different areas. According to Isbert et al. (2015), infracommunities of *Etmopterus spinax* (L.) from the NE Atlantic were also characterized by low mean richness and high dominance values. Moore (2001) and Henderson et al. (2002) also reported depauperate infracommunities in *S. canicula* and *Squalus acanthias* L., respectively, from the NE Atlantic as well.

As far as we are concerned, available studies on shark parasite communities have focused on small-sized sharks from North-Atlantic waters, in which the total number of parasite taxa is usually low. Henderson and Dunne (1998) found five different parasites in *S. canicula*, Chambers (2008) reported eight parasites from *Centroscyllium fabricii* (Reinhardt), Henderson et al. (2002) recovered 10 parasites from *S. acanthias* and Moore (2001) and Isbert et al. (2015) found 11 different taxa in *S. canicula* and *E. spinax*, respectively. In larger, although still small-sized sharks, Palm and Schröeder (2001) reported six parasites from *Heptranchias perlo* (Bonnaterre), three from *Deania calcea* (Lowe), seven from *Deania profundorum* (Smith and Radcliffe) and nine from *Deania histricosa* Garman. Isbert et al. (2015) made the suggestion that poor parasite faunas, with low richness and diversity and high dominance values could represent features of small sharks, which would be to some extent supported by present results. However, there are few data available for comparison and the suggested trends could be a characteristic pattern of sharks in general, regardless of their size.

Different studies on teleost parasite communities have repeatedly reported lower parasite richness in Mediterranean with respect to Atlantic populations (e.g. Pérez-del-Olmo et al. 2009; Mattiucci et al. 2014; Constenla et al. 2015). A smaller fish size, lower food consumption and lower biomass and abundance of animal communities in the Mediterranean have been suggested as possible explanations for such pattern (see Constenla et al. 2015 and references therein). Actually, maximum size of *S. canicula* in the Mediterranean is lower than elsewhere (Compagno, 1984), and a similar pattern has been observed for Mediterranean specimens of *G. melastomus* with respect to their Atlantic counterparts (Compagno, 1984; Carrassón et al. 1992; present results). However, results available to date are far from sufficient to conclude whether elasmobranch parasite communities are more diverse and abundant in Atlantic than in Mediterranean waters. Parasitological data for *S. canicula* are scarce (only Henderson and Dunne (1998) and Moore (2001) have analysed the parasite community of this

shark in the Atlantic, and present results constitute the first report from the Mediterranean) and the range of variability reported for its total number of parasites is wide and thus not feasible for comparison. In the case of *G. melastomus*, present results represent the first description of its parasite community, and comparative data are thus completely absent. Undoubtedly, additional parasitological studies in sharks from Mediterranean and Atlantic waters will open the possibility to test if the same trend observed in teleosts is applicable to shark parasite communities.

Fish condition indices vary in response to multiple factors and clear relationships between them and parasitological infections are usually difficult to determine (Heins and Baker, 2008). Parasites can be detrimental to their hosts and consequently alter condition indices, but fish hosts with reduced health condition may show higher susceptibility to parasite infections (Dallarés et al. 2016). It is also possible that parasite infections do not reach the threshold needed to affect condition indices or even that healthier fishes harbour more abundant and rich parasite communities, as suggested by Dallarés et al. (2014). In any case, unless the impact of a given parasite on host fitness is strong (i.e. Heins and Baker (2008) detected heavily reduced reproductive fitness in the three-spine stickleblack *Gasterosteus aculeatus* infected with the cestode *Schistocephalus* sp.), observations are often inconsistent, and must therefore be carefully considered.

In this sense, the contrary associations detected in *G. melastomus* between the parasites *D. macrocephalum* and *P. obtusus* and fish condition factor, or between the cestodes *D. macrocephalum* and *Grillotia* sp. and fish hepatosomatic index, do not allow stating any general trend. Such relationships, observed for a few individual parasites only, may reflect variations in parasite loads in response to specific biological aspects of their host (i.e. dietary trends) or environmental patterns that are coupled with variations in fish condition parameters, instead of an impact of parasites on fish health.

These observations, alongside with the absence of noticeable effects of the parasite load on *S. canicula* general condition, point to a negligible repercussion of the parasite burden in general fish condition indices in the two sharks addressed.

In the present study, seasonal variations of the parasite burden of the two species of sharks addressed are assessed throughout the whole length of the year for the first time.

In the case of *S. canicula*, the absence of significant differences among seasons either for the abundance or prevalence of the different parasites recovered or for infracommunity descriptors and structure might be attributed to the low number of hosts

available for each season group. Further studies with increased number of specimens are needed in order to confirm the observed lack of seasonal patterns.

The much lower infracommunity richness and abundance observed in Vilanova with respect to Besós samples of *G. melastomus* could be possibly explained by the vicinity of the Besós submarine canyon to the latter locality. Submarine canyons, formed as a result of river discharge (the Besós River in this case), favour aggregation of zooplankton and more complex invertebrate communities (Macquart-Moulin and Patriti, 1996; Rumolo et al. 2015), which can presumably enhance parasite transmission. A similar pattern has been observed in the case of the parasite communities of the teleost *Phycis blennoides* (Brünnich) in the same area, where the more abundant, rich and diverse parasite composition of samples collected off the mainland vs. the insular slope in the Balearic basin are partly explained by the higher availability of benthic prey linked to submarine canyons in samples off the mainland slope (Dallarés et al. 2016).

Of the total number of parasites recovered from *S. canicula* and *G. melastomus*, the digenean *O. cestoides* (Plagiorchiida: Azygiidae) and the nematode *P. baylisi* (Enoplida: Capillariidae) are reported from the Mediterranean Sea for the first time and thus constitute new geographic records. While *O. cestoides* had been recovered in North-Atlantic and Pacific waters (Gibson and Bray, 1997), *P. baylisi* was only previously known from the NE Atlantic (Moravec, 1987).

Parasites with direct life cycles, such as monogeneans, are subjected to environmental rather than to biotic factors, in contrast with trophically-transmitted parasites. Although the response of larval and adult monogeneans to environmental variables has been repeatedly documented (Kearn, 1993 and references therein; Raymond et al. 2006; Marchiori et al. 2015), few studies have addressed the response of these parasites to O_2 levels, for which this relationship is not well understood yet. Monni and Cognetti-Varriale (2002) and Raymond et al. (2006) found negative correlations between monogenean prevalence and abundance and oxygen concentration, either explained by toleration to hypoxia allowing less competition with other parasites or by an enhanced antibody response of the host towards the parasite. These results contrast with the opposite trend observed in the present study, in which *Erpocotyle* sp. recovered from *G. melastomus* was linked to high O_2 concentration partly associated to hauls from off Besós in autumn, where the prevalence and abundance of this parasite were highest. *Hexabothrium appendiculatum* was also linked to high O_2 levels, to a lesser extent though, in samples of *S. canicula*. The response to environmental factors may vary

depending on the biology and/or ecology of the parasite, of its host and on the relationship between them. Although infection levels by both monogeneans were low and the obtained results should thus be taken with caution, an enhancement of monogenean reproduction and infection success in an environment with higher O_2 levels, as has been documented for other invertebrates (Cheung et al. 2008), could be suggested. Contrary to O_2 levels, effects of higher temperature and/or turbidity levels in increasing monogenean infection success have been demonstrated (Skinner, 1982; Brazenor and Hutson, 2015) and are consistent with the association observed between *H. appendiculatum* and these environmental parameters in *S. canicula*. While higher temperatures can enhance monogenean hatching success and reduce time to maturity (Brazenor and Hutson, 2015), aquatic environments with increased turbidity due to high levels of suspended materials can provoke irritation and inflammation of gill filaments accompanied by an inhibition of fish defense mechanisms, increasing their susceptibility to infection by gill parasites, such as monogeneans (Skinner, 1982; Moles and Wade, 2001; Madi and Ueta, 2009).

Ditrachybothridium macrocephalum uses *G. melastomus* as definitive host (Dallarés et al. 2015), where it was the most prevalent and abundant parasite. No complete life-cycle is known for any cestode of the order Diphyllidea, but Tyler (2006) hypothesized that these cestodes use two invertebrate intermediate hosts (a filter-feeding crustacean as first and a shrimp or crab as second) before reaching the elasmobranch final host where they will develop into adults. Among the known prey of *G. melastomus* (Carrassón et al. 1992), amphipods and different decapods have been found to host larval stages of diphyllideans (see Bray and Olson (2004) and references therein). The higher abundance of *D. macrocephalum* in juvenile compared to adult sharks is in all likelihood related to an ontogenetic diet shift of this host, as already highlighted by Dallarés et al. (2015). According to Carrassón et al. (1992) the decapod *C. macandreae* is an important prey in the sampled area, and its presence in guts decreases with age and depth. The rest of decapods increase in importance with age and amphipods are only relevant below 1,000 m. Therefore, *C. macandreae* could be a transmitter for this parasite. The lower rate of infection by this parasite in adult sharks further suggests either a reduced lifespan of these cestodes in their definitive host, or that sharks develop an immunitary response towards the parasites, as already pointed out by Tyler (2006).

Ditrachybothridium macrocephalum was found linked to high near-bottom turbidity levels, which, according to Cartes et al. (2013), suggests more food availability for

zooplankton. The consequent increase in biomass of these crustaceans enhances aggregation of benthopelagic fish (Cartes et al. 2013) and probably stimulates parasite transmission. The close association observed between water turbidity and Besós winter samples in the CCA plot (further corroborated by Rumolo et al. (2015) with the environmental data recorded in the present study) suggests that the transmission of larval stages of *D. macrocephalum* in their invertebrate hosts is enhanced in winter. The parasites must reach their final host with some temporal delay, which can explain the maximum abundance levels attained by this parasite in spring samples of *G. melastomus*.

Grillotia sp. is one of the most frequent genera recovered from fishes within the cestode order Trypanorhyncha (Beveridge and Campbell, 2007). Although copepods act as first intermediate hosts for trypanorhynchs (Palm, 2004), these are not abundant in the diet of *G. melastomus* and other prey, such as teleosts, in turn preying on copepods, could be the transmitters of the parasite. Its higher abundance in adult sharks responds to the accumulation of the larval forms of the parasite until the host is consumed by a larger predator, where the plerocerci excyst and develop into the adult form. The kitefin shark *Dalatias licha*, which is known to prey on *G. melastomus* in the NW Mediterranean Sea (Navarro et al. 2014), or *Hexanchus griseus*, which preys upon smaller sharks (Ebert, 1994), could be potential final hosts for the *Grillotia* specimens recovered.

The association observed between the abundance of *Grillotia* sp. and high levels of O_2 is in accordance with what is known about the life cycle of this parasite. High O_2 levels are known to enhance copepod biomass (Keister et al. 2000; Moon et al. 2006; Cartes et al. 2013), and specifically Cartes et al. (2013) found a significant association between O_2 levels in the benthic boundary layer (i.e. the layer of water immediately above the sea-floor) and copepod biomass in the Balearic Sea. These crustaceans are first intermediate hosts for trypanorhynch cestodes, as commented above, and higher copepod biomass likely favours parasite transmission to the subsequent hosts, *G. melastomus* among them. Oxygen levels off Besós at ca. 700 m depth increase in winter and spring according to Cartes et al. (2011), and maximum abundance values of *Grillotia* sp. in *G. melastomus* were observed in summer and autumn, which might be explained by the time needed by the parasite to reach higher trophic levels, in a similar way as suggested for *D. macrocephalum*.

The different abundance patterns across seasons displayed by *D. macrocephalum* and *Grillotia* sp., in turn associated to the temporal dynamics of the populations of their

intermediate hosts (as explained above), likely explain the differences in the structure of parasite infracommunities of this host across the distinct periods of the year, which was further evidenced by the results of the PERMANOVA analysis.

Proleptus obtusus was the preferential parasite of *S. canicula*. The presence of this nematode in all the examined sharks in the Mediterranean Sea is consistent with the previous results in the Atlantic by Moore (2001). Although very few information is available about the life cycle of physalopterid nematodes, *P. obtusus* is known to have a two-host life cycle, with sharks being final and crustaceans intermediate hosts. In the marine environment, larval forms of this nematode have been recovered from the decapods *Carcinus maenas*, *Eupagurus bernhardus*, *Pachygrapsus marmoratus* and *Hyas araneus* (Moravec, 2007 and references therein). Accordingly, Valls et al. (2011) reported reptantian decapods as the most important prey of *S. canicula* on the continental shelf off the slope of the Balearic Islands (NW Mediterranean Sea) (36% IRI). Therefore, the great importance of reptantian decapods in the diet of *S. canicula* is in accordance with the high prevalence and abundance of *P. obtusus* found in the present and in previous studies (Henderson and Dunne, 1998; Moore, 2001). In the case of *G. melastomus*, Carrassón et al. (1992) found that the reptantian decapod *Calocaris macandreae*, although important in juveniles, is only a casual prey in adult sharks at 371–667 m off the continental slope of the Balearic Sea. Valls et al. (2011) also reported that reptantian decapods seem to be of minor importance at depths between 500–750 m in the slope of the Balearic Islands. Hence, the low prevalence and abundance values attained by this parasite in *G. melastomus* are likely due to the low presence of such crustaceans in its diet.

Although no seasonal patterns have been observed for *P. obtusus* in any of the two hosts addressed, the abundance of this parasite has been associated to high temperature and salinity levels in the CCA performed with data of *G. melastomus*. In contrast, *P. obtusus* was not linked to any specific environmental variable in the CCA with data from *S. canicula*, probably because, within this host, this parasite generally reached maximum abundances in hauls with low values of the environmental parameters addressed (see Tables 1 and 4). This inconsistency, coupled with the low abundance of *P. obtusus* in samples of *G. melastomus*, make us reluctant to provide an explanation for the abundance patterns of this parasite based on the environmental variables addressed.

In a similar way as *P. obtusus* in *G. melastomus*, the raphidascaridid nematode *H. aduncum* appeared also linked to high water salinities in present samples of *S. canicula*.

This trend was also observed in the teleost *P. blennoides* off the same waters (Dallarés et al. 2016). In spite of these coincidendes, the abundance of *H. aduncum* in *S. canicula* was low and more consistent results should be obtained before any generalizations are made.

In conclusion, the parasite communities of *S. canicula* and *G. melastomus* in the NW Mediterranean Sea are characterized by low infracommunity richness and diversity, and high dominance. However, significant differences exist in the infracommunity structure and composition between both species, likely due to different feeding habits in turn influenced by differential availability of benthopelagic prey assemblages along the distinct depth ranges inhabited by these two sharks. In general, the parasite faunas of *S. canicula* and *G. melastomus* are comparable to those reported from other small-sized sharks from different areas. Seasonal and geographical variability has been observed in the parasite community of *G. melastomus*, with different parasite composition in winter and spring with respect to summer and autumn and with higher parasite burden in samples from off Besós than off Vilanova, probably due to the vicinity of the Besós submarine canyon to the latter locality. Possible intermediate hosts have been suggested for the more frequent parasites with heteroxenous life cycles based on parasite abundance patterns, the existing knowledge on their life cycles and, when available, previous dietary studies conducted in the sampled area.

Different environmental variables have been linked to the abundance of some parasites, mainly near-bottom turbidity and temperature levels to monogeneans, as these parameters enhance infection and reproductive success of these parasites, and O_2 and turbidity levels, which are known to enhance zooplankton biomass and thus favour parasite transmission, to heteroxenous parasites.

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CHAPTER 6 - THE PARASITE COMMUNITY OF *GALEUS MELASTOMUS* RAFINESQUE, 1810 AND TWO ADDITIONAL SHARKS FROM THE NW MEDITERRANEAN DEEP-SEA IN RELATION TO FEEDING ECOLOGY AND HEALTH CONDITION OF THE HOST AND ENVIRONMENTAL GRADIENTS AND VARIABLES

The parasite community of *Galeus melastomus* Rafinesque, 1810 and two additional sharks from the NW Mediterranean deep-sea in relation to feeding ecology and health condition of the host and environmental gradients and variables

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Abstract: The parasite communities of sharks have been largely neglected despite the ecological importance and vulnerability of this group of fish. The main goal of the present study is to describe the parasite communities of three deep-dwelling shark species in the NW Mediterranean. A total of 120 specimens of *Galeus melastomus*, 11 *Etmopterus spinax* and 10 *Centroscymnus coelolepis* were captured at 400–2,200 m depth at two seasons and three localities off the mainland and insular slopes of the Balearic Sea. Environmental and fish biological, parasitological, dietary, enzymatic and histological data were obtained for each specimen, and the relationships among them tested. For *G. melastomus*, *E. spinax* and *C. coelolepis* a total of 15, two and eight parasite species were respectively recovered. The parasite community of *G. melastomus* is characterized by high abundance, richness and diversity, and the cestodes *Ditrachybothridium macrocephalum* and *Grillotia adenoplusia* dominate the infracommunities of juvenile and adult specimens, respectively. A differentiation of parasite communities, linked to a diet shift, has been observed between ontogenic stages of this species. *E. spinax* displays a depauperate parasite community, and that of *C. coelolepis*, described for the first time, shows moderate richness and diversity. Detailed parasite-prey relationships have been discussed and possible transmission pathways suggested for the three hosts. Parasites were mostly related to high water turbidity and O_2 levels, which enhance zooplankton proliferation and could thus enhance parasite

transmission. The nematodes *Hysterothylacium aduncum* and *Proleptus obtusus* were linked to high salinity levels, as already reported by previous studies, which are associated to high biomass and diversity of benthic and benthopelagic crustaceans. A decrease of acetylcholinesterase activity and lower hepatosomatic index, possibly linked to infection-related stress, have been observed. Lesions associated to encapsulated larvae of *G. adenoplusia* have been observed in the muscle of *G. melastomus*, especially in the tail region, which can be indicative of the hunting strategy of its final host and may compromise the escape response of *G. melatomus* thus facilitating parasite transmission.

Keywords: *Galeus melastomus*, *Etmopterus spinax*, *Centroscymnus coelolepis*, Parasites, Mediterranean, Deep-sea

1. Introduction

From the late 1960s, bottom-trawl fisheries have progressively spread into deeper grounds as fishing resources became scarce in the continental shelf and pelagic waters (Morato et al., 2006; Norse et al., 2012). This global trend has raised concerns on the status and future perspectives of deep-water fishes (Morato et al., 2006), and chiefly on the still poorly understood deep-dwelling elasmobranchs (Carbonell et al., 2003), whose biological and reproductive characteristics make them very susceptible to population mining (Stevens et al., 2000). Because of their important role as top predators and modellers of the structure and dynamics of marine ecosystems (Stevens et al., 2000), the understanding of their vulnerability and exact role in deep-sea habitats is essential.

In the Balearic Sea, the blackmouth catshark *Galeus melastomus* Rafinesque, 1810 (Carcharhiniformes: Scyliorhinidae) is the most relevant shark in the upper and middle slopes (c.a. 400–800 m and 800–1,400 m, respectively) in terms of abundance and biomass (Carrassón et al., 1992; D'Onghia et al., 2004). Another frequent species, the lanternshark *Etmopterus spinax* (Linnaeus, 1758) (Squaliformes: Etmopteridae) shows its peak of abundance within the same depth range (Stefanescu et al., 1992; D'Onghia et al., 2004). In contrast, the sleeper shark *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello, 1864 (Squaliformes: Somniosidae) represents the most abundant selachian in the lower slope (below c.a. 1,400 m) (Carrassón et al., 1992; Moranta et al., 1998; D'Onghia et al., 2004). It is the only abundant shark and one of the most

important contributors to biomass below 2,000 m (Carrassón et al., 1992; Stefanescu et al., 1993; D'Onghia et al., 2004).

Galeus melastomus shows a rather diversified diet, with crustaceans and fishes being preferential prey, while that of *E. spinax* and *C. coelolepis* is largely based on cephalopods (Carrassón et al., 1992). Marked ontogenic diet shifts are known for the three species (Carrassón et al., 1992).

Although none of these species is currently endangered, *G. melastomus* and *E. spinax* are an important by-catch in the sampled area (Carbonell et al., 2003) and *C. coelolepis* bears the status of "Near threatened" according to The Red List of the IUCN (IUCN, 2016).

In spite of being often disregarded, parasites are an important part of all ecosystems (actually, parasite organisms are believed to outnumber free-living species (Price, 1980)) and are informative on many different aspects of their habitats and hosts (Williams et al., 1992). Many parasites use more than one host to complete their life cycle, and can be effectively used to infer dietary habits and trophic interactions of their hosts (Valtonen et al., 2010; Münster et al., 2015). Furthermore, parasites can reflect host phylogenetic relationships (Locke et al., 2013), respond to environmental impacts (Pérez-del-Olmo et al., 2007, 2009a) and their use as discriminators of fish populations has been widely recommended (MacKenzie and Abaunza, 1998). For all these reasons, recent studies have stressed that parasites should be incorporated into food webs (Lafferty et al., 2008) and ecotoxicological studies (Marcogliese et al., 2009), among others.

While the parasite assemblage infecting *G. melastomus* in the NW Mediterranean Sea is fairly well-known (Dallarés et al., in press), very few parasite data is available for *C. coelolepis* and *E. spinax* in this area (Guiart, 1935; Euzet, 1959). For the two latter species, a relevant number of single parasite records exist from the Atlantic Ocean (among others, Guiart, 1935; Gibson and Bray, 1977; Pascoe, 1987; Bates, 1990; Caira and Pickering, 2013 for *C. coelolepis* and Pintner, 1930; Williams, 1959; Hennemann, 1985; Noever et al., 2010; Caira and Pickering, 2013 for *E. spinax*) and two studies have addressed the parasite community of *E. spinax* in Atlantic waters (Klimpel et al., 2003 (only for juvenile specimens); Isbert et al., 2015).

Parasite-host relationships are mainly characterized by damage inflicted to the host by the parasite, which would be expected to alter stress markers or induce histological alterations. In this sense, enzymatic activities respond in a natural way to biological

factors such as size, sex or swimming behaviour (Drazen and Seibel, 2007; Koenig and Solé, 2014), but have also proved to be effective biomarkers of ecosystem alterations or stressing conditions in fishes (Chatterjee et al., 2010). Effects of fish parasites on enzymatic markers have barely been addressed and have never been attempted in sharks. Furthermore, the few existing studies in teleosts have yielded contradicting results (Podolska and Napierska, 2006; Pérez-i-García et al., 2015; Dallarés et al., 2014, 2016). Actually, information on enzymatic activities of deep-dwelling chondrichthyans is overall scarce and, in relation to the species addressed in the present work, only *G. melastomus* and *E. spinax* have received attention (Totland et al., 1978; Fänge et al., 1979; Solé et al., 2008, 2010), while *C. coelolepis* remains essentially unstudied regarding this aspect.

In a similar way, histopathological changes in different organs and systems have been used as markers of fish health status and as indicators of environmental changes (Carrassón et al., 2008; Carreras-Aubets et al., 2011; Fricke et al., 2012). Quantitative variations in numbers of melano-macrophages (MM) or MM aggregates are also known to be driven by physiological changes, pathological conditions or environmental pollution in teleosts (see Agius and Roberts, 2003 and references therein; Carrassón et al., 2008). In the last years, these structures have been characterized in a few shark species (Borucinska et al., 2009), but data are still extremely limited for this group and lacking for the three species addressed in the present study.

The aim of this study is, first and foremost, to carry out the first complete description of the parasite communities of *G. melastomus, E. spinax* and *C. coelolepis* in the Balearic basin. Secondly, ontogenic and environmental variability (eight distinct locality-season-depth combinations) on the composition of such parasite community is assessed for *G. melastomus*. With the aim of explaining infection patterns of the different parasites, their associations to environmental variables (water temperature, salinity, oxygen content and turbidity) and to the abundance of host prey identified in guts are tested. In order to assess the possible effects of parasite load on health condition of the hosts addressed, relationships between parasite abundance and fish general condition indices, activity of enzymatic biomarkers and density of hepatic MM (only for *G. melastomus*) are explored. Moreover, the presence of parasite-induced histological alterations and pathological conditions is evaluated in different organs.

2. Materials and methods

2.1. Sampling area and specimen collection

A total of 120 specimens of *G. melastomus*, 11 *E. spinax* and 10 *C. coelolepis* were captured in summer (July) 2010, summer (June) 2011 and autumn (October) 2011 at 400–2,200 m depth in the Balearic Sea (north-western Mediterranean Sea) (Fig. 1). Hauls were carried out on board of the research vessels Garcia del Cid and Sarmiento de Gamboa using a semi-balloon otter-trawl (OTSB 14) at three different localities: one off the mainland slope (Barcelona) and two off the slope of the Balearic Islands (Mallorca and Ibiza) (Fig. 1, Table 1).

Environmental data: temperature (T) in ºC, salinity (S) in psu, O_2 concentration in ml/l and turbidity (voltage), the latter representing organic and inorganic suspended material, were taken at 5 m above the sea-bottom by deployment of a CTD simultaneously (same data, same depth) to fish samplings (Table 1).

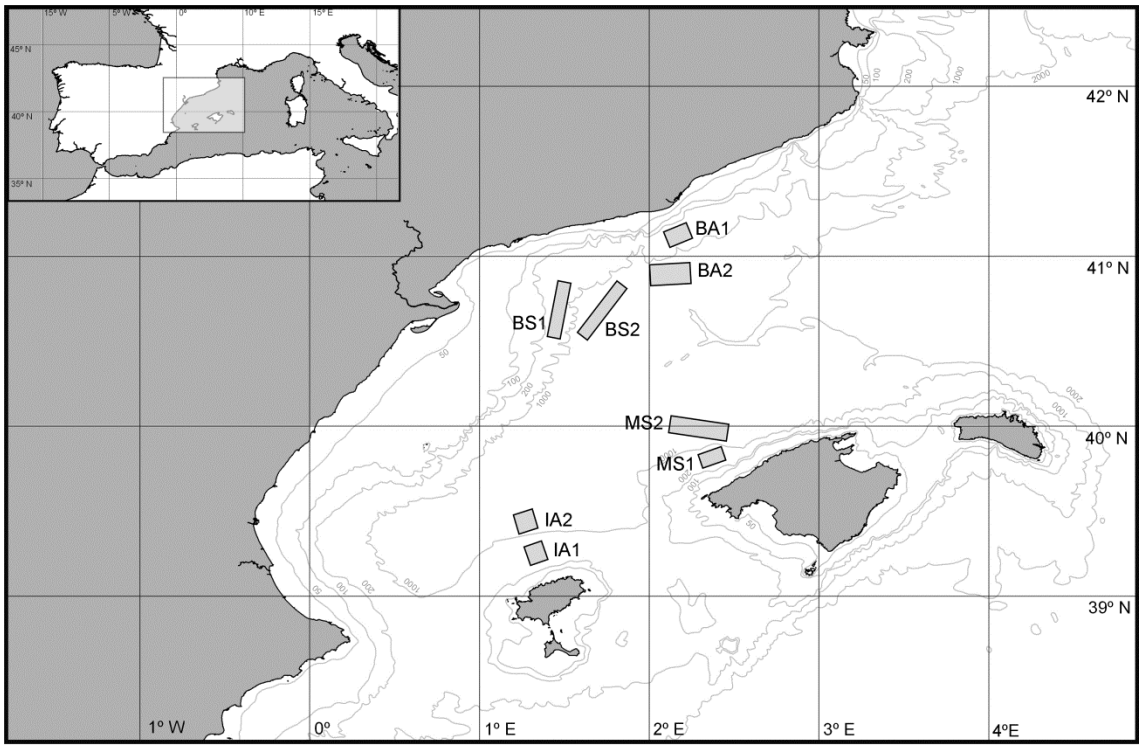

Figure 1. Study area showing the sampling localities in the Balearic Sea. BA1, Barcelona autumn at depth 1; BA2, Barcelona autumn at depth 2; BS1, Barcelona summer at depth 1; BS2, Barcelona summer at depth 2; IA1, Ibiza autumn at depth 1; IA2, Ibiza autumn at depth 2; MS1, Mallorca summer at depth 1; MS2, Mallorca summer at depth 2. Depth 1 (upper slope): 400–1,000 m; depth 2 (middle and lower slopes): > 1,000 m.

Table 1. Sampling data of the three species of sharks addressed. n: number of specimens; T ºC: temperature; S: salinity in psu; O: oxygen concentration in ml/L; Turb: Turbidity in V. Abbreviations for locality-season-depth groups (habitats): BS2: Barcelona summer, depth 2; MS2: Mallorca summer, depth 2; MS1: Mallorca summer, depth 1; BS1: Barcelona summer, depth 1; BA1: Barcelona autumn, depth 1; BA2: Barcelona autumn, depth 2; IA1: Ibiza autumn, depth 1; IA2: Ibiza autumn, depth 2.

Habitat	Haul	Date	Depth (m)	Coordinates		n			Environmental variables			
				Latitude (deg, min, N)	Longitude (deg, min, E)	*G. melastomus*	*C. coelolepis*	*E. spinax*	T ºC	S (psu)	O (mL/L)	Turb (V)
BS2	A1–3	08/07/2010	1,048	40º 58.06	2º 5.30	5			13.08	38.48	4.41	0.06
BS2	A1–4	08/07/2010	1,024	40º 58.69	2º 1.14	9			13.20	38.51	4.18	0.06
BS2	A1–6	10/07/2010	1,308	40º 53.85	2º 4.00	3			13.08	38.48	4.41	0.06
MS2	A1–13	16/07/2010	2,057	40º 38.82	3º 6.70		1		13.20	38.48	4.35	0.05
MS2	A1–14	16/07/2010	2,194	40º 37.59	3º 27.82		1		13.22	38.48	4.34	0.06
MS1	A1–15	17/07/2010	682	39º 48.24	2º 20.55	9			13.07	38.49	4.14	0.90
MS1	A1–16	17/07/2010	457	39º 46.73	2º 21.85	5			13.09	38.50	4.12	0.07
MS2	A1–17	19/07/2010	1,006	39º 52.39	2º 20.26	5			13.06	38.48	4.33	0.08
MS2	A1–18	19/07/2010	1,006	39º 53.37	2º 18.66	15			13.06	38.48	4.33	0.08
MS2	A1–19	19/07/2010	1,232	39º 55.16	2º 8.25	1	1		13.08	38.48	4.38	0.07
BS2	A1–24	22/07/2010	2,197	41º 4.31	3º 16.74		2		13.22	38.48	4.34	0.06
BS1	A2–1	18/06/2011	639	40º 34.50	1º 26.51	3			13.14	38.50	4.16	1.03
BS1	A2–2	18/06/2011	646	40º 34.45	1º 26.44	1			13.14	38.50	4.16	1.03
BS1	A2–4	19/06/2011	627	40º 54.40	1º 34.61	3			13.12	38.50	4.14	0.24
BS1	A2–5	19/06/2011	628	40º 54.72	1º 34.80	1			13.12	38.50	4.14	0.24
BS1	A2–6	19/06/2011	648	40º 54.32	1º 34.83	3			13.12	38.50	4.14	0.24
BS1	A2–8	20/06/2011	632	40º 40.86	1º 26.44	3			13.08	38.49	4.20	0.00
BS2	A2–11	23/06/2011	1,043	40º 51.97	1º 44.29	4			13.10	38.49	4.17	0.13
BS2	A2–12	23/06/2011	1,06	40º 47.23	1º 35.24	4			13.10	38.49	4.17	0.13
BS2	A2–13	23/06/2011	1,052	40º 55.86	1º 50.32	1			13.10	38.49	4.17	0.13
BA1	A3–1	14/10/2011	661	41º 5.88	2º 13.34	8			13.28	38.54	3.91	0.33
BA1	A3–2	14/10/2011	581	41º 7.85	2º 5.32	9			13.38	38.55	3.86	0.64
BA2	A3–3	15/10/2011	1,051	40º 50.80	1º 43.94	2			13.11	38.49	4.21	0.24
BA2	A3–4	15/10/2011	1,236	40º 41.96	1º 37.46	12		2	13.12	38.48	4.25	0.28
BA2	A3–6	16/10/2011	1,751	40º 9.65	2º 0.23		4		13.18	38.49	4.32	0.21
IA1	A3–7	17/10/2011	508	39º 11.63	1º 18.88	1		3	13.07	38.49	4.10	0.38
IA1	A3–8	17/10/2011	508	39º 13.92	1º 23.52	4		6	13.07	38.49	4.10	0.38
IA2	A3–9	17/10/2011	573	39º 13.92	1º 23.52	1			13.07	38.49	4.29	0.25
IA2	A3–10	18/10/2011	1,272	39º 25.31	1º 16.84	8			13.10	38.49	4.16	0.23
IA2	A3–11	19/10/2011	1,626	39º 56.20	1º 37.91		1		13.15	38.49	4.31	0.20

Immediately upon capture, records of total length (TL) in mm and total weight (TW) in g were obtained for each fish. A portion of the axial muscle was dissected out and kept at –20 ºC for biochemical purposes, and samples of right gills, liver, spleen, gonads and spiral valve (the latter for *G. melastomus* only) were immediately fixed in 10% buffered formalin for histological analyses. The rest of the specimen was frozen at –20 ºC for parasitological examination.

2.2. Parasitological study

Once in the laboratory, fish were thawed and external surfaces, buccal cavity and olfactory organs were examined macroscopically and under stereomicroscope in search of ectoparasites. All organs and musculature were carefully removed and inspected for endoparasites. Liver and gonads were weighed prior to examination. Parasites collected were counted and preserved in 70% ethanol. Nematodes, copepods and everted tentacles of trypanorhynch cestodes were studied as temporary mounts in glycerine. Cestodes were stained with iron acetocarmine, dehydrated through a graded ethanol series, cleared in clove oil and examined as permanent mounts in Canada balsam. Molecular analyses were also performed to confirm the identity of the cestode *Ditrachybothridium macrocephalum* Rees, 1959 (see Dallarés et al. (2015) for methodological details). All parasites were identified to the lowest possible taxonomic level. Nomenclature of tapeworm larval stages follows Chervy (2002).

The following voucher material was deposited in the Helminthological Collection of the Universitat Autònoma de Barcelona (UABhc) under the following accession numbers: *G. melastomus*: C22, 23 (*D. macrocephalum*), C24 (*Grillotia adenoplusia* (Pintner, 1903) Palm, 2004), N7 (*Proleptus obtusus* Dujardin, 1845), N8 (*Piscicapillaria baylisi* Moravec, 1987) and Co3 (*Eudactylina vilelai* Nuñes-Ruivo, 1956); *C. coelolepis*: C25 (*Clistobothrium* sp.) and C26 (*Monorygma* sp.). Material of *Sphyriocephalus viridis* (Wagener, 1854) from present specimens of *G. melastomus* and *C. coelolepis* were deposited in the same collection in a previous study (see Dallarés et al., 2017).

2.3. Diet analysis

Stomach contents of 104 specimens of *G. melastomus* previously examined for the occurrence of parasites were further analysed for diet determination. For each fish, prey recovered in guts were identified to the lowest possible taxonomic level using a stereomicroscope, counted and weighed to the nearest 0.001 g. Diet was considered by

prey number, hence being directly comparable with parasitological indices. In previous articles (see Dallarés et al., 2014, 2016) details on methodological limitations, e.g. difficulties in identifying and counting soft animals, have been provided. In parallel, stomach contents of *E. spinax* and *C. coelolepis*, rare at the usual depth range inhabited by *G. melastomus*, were analysed. Results of nine specimens of *E. spinax* and seven of *C. coelolepis* were considered for further prey-parasite analyses (see below). In the case of *C. coelolepis*, some prey items (e.g. carrion) were identified by means of molecular techniques (see Cartes et al., 2016).

2.4. Enzymatic determinations

A muscle portion of about 0.3 g was used for acetylcholinesterase (AChE), lactate dehydrogenase (LDH) and citrate synthase (CS) determinations. The tissue was homogenised in a 50 mM buffer phosphate (pH 7.4) in a 1:5 (weight:volume) ratio using a polytron® blender. The homogenate was centrifuged at 10,000 g × 30 min and the supernatant (S10) was used for biochemical determinations.

A range of six concentrations of acetylthiocholine iodide (ATC) from 0.05 to 10 mM was used to determine kinetic constants such as V_{max} and K_m of AChE in each species and catalytic efficiency measured as V_{max}/K_m. For AChE determination, the concentration of the substrate (ATC) selected was 1 mM, as described in Solé et al. (2010). Acetylcholinesterase activity was assayed according to the principle of Ellman et al. (1961) at 405 nm. Lactate dehydrogenase activity was determined at 340 nm according to the method developed by Vassault (1983), modified to 96-well microplate format, with the reactive quantities described in Dallarés et al. (2014) and using 1:40, 1:80 (for samples from *G. melastomus*) or 1:100 (for samples from *C. coelolepis* and *E. spinax*) diluted original sample. For CS assay, the protocol used was based on Childress and Somero (1979) at 412 nm and final conditions were: 0.1mM of dithiobis-nitrobenzoic acid solution in 50 mM Tris-HCl buffer (pH 8) were mixed with 25 µl of 1:5 diluted (for samples from *G. melastomus*) or undiluted sample (for samples from *C. coelolepis* and *E. spinax*), 0.1 mM of acetyl CoA and 0.5 mM of oxaloacetate in each microplate well.

In all determinations, reading was performed in triplicate in a microplate reader (TECAN Infinite 200) during 5 min at 25 ºC. Activity was expressed in nmol/min/mg protein.

Total protein content in the S10 fraction was determined by the Bradford (1976) method using bovine serum albumin as standard (BSA 0.05–0.5 mg/ml).

2.5. Histological assessment

Fixed samples of gills, liver, spleen, gonads and spiral valve (the latter for *G. melastomus* only) from the three elasmobranch species were embedded in paraffin, sectioned at 4 µm, stained with Haematoxylin and Eosin and examined microscopically. A quantitative analysis of melano-macrophages (MM) in liver sections of *G. melastomus* was carried out using a MicroComp Integrated Image Analysis System. Melano-macrophages were counted in five randomly-selected fields of view from each liver section at 100× of magnification (0.92 mm^2/screen), and MM/mm^2 was calculated for each field.

Protist parasites (i.e. intestinal coccidians) were only detected in histological sections. In this case, prevalence and intensity were calculated. Intensity was determined by counting the number of oocysts in five randomly-selected fields of view on each section of the spiral valve of the infected sharks at 400× of magnification (0.06 mm^2/screen), and was expressed as number of oocysts (OC) per square millimetre. Only developing and mature oocysts which could be clearly identified were considered.

2.6. Data Analyses

Two distinct size-groups for specimens of *G. melastomus*, i.e. size 1 (TL < 34 cm for males and TL < 40 cm for females) and size 2 (TL ≥ 34 cm for males and TL ≥ 40 cm for females), corresponding to juvenile and adult sharks (Capapé and Zaouali, 1977), were established prior to analyses.

Parasitological terms such as prevalence (P) and mean abundance (MA) were calculated following Bush et al. (1997) and using data from all specimens. Parasite taxa with total P ≥ 8% within any size-group of *G. melastomus* and > 30% in the total sample of *E. spinax* and *C. coelolepis* were considered not-accidental and are henceforth called common (P threshold was increased in the two latter species due to the low number of specimens available). Diversity and dominance of parasite infracommunities (i.e. all parasites of all taxa in an individual fish) were estimated using Brillouin's diversity index (PRIMER v6; Anderson et al., 2008) and Berger-Parker dominance index (B-P, calculated as the number of individuals of the most abundant parasite species divided by the total number of parasites in a given fish host), respectively. Fish condition was

assessed by condition factor (K, calculated as (TW/TL^3) × 100), hepatosomatic index (HSI, calculated as (liver weight/TW) × 100) and gonadosomatic index (GSI, calculated as (gonad weight/TW) × 100). In all tests, GSI was not considered for juvenile sharks and was addressed separately for adult females and males. Analyses involving MM/mm^2 and OC/mm^2 from *G. melastomus* were performed using the average value of the five field-measurements calculated for each section.

2.6.1. Galeus melastomus

Firstly, a non-parametric Multi-Dimensional Scaling (MDS) was applied on infracommunity data using the factor "size" in order to visualize the ordination of parasite infracommunities of *G. melastomus* with respect to the maturity stage of their host. A permutation multivariate analysis (PERMANOVA) was also carried out using infracommunities as replicate samples in order to test an age-related parasitological differentiation. Both analyses were applied on a Bray-Curtis similarity matrix derived from logarithmically transformed (log(x+1)) abundance data (PRIMER v6; Anderson et al., 2008). For the PERMANOVA analysis, permutation p-values were obtained under unrestricted permutation of raw data (9,999 permutations). Generalized Models (GZM) were applied to test possible associations between fish TL or condition indices (K, HSI and GSI) (set as covariates) and individual total parasite abundance, abundance of common parasites and parasite richness. Spearman rank correlation tests were used to assess relationships between fish TL or condition indices and diversity and dominance of parasite infracommunities. In view of the results obtained in the analyses above described, the description of the parasite community of *G. melastomus* was performed in the two size-groups of hosts separately.

Differences among the eight locality-season-depth groups (each of the following subdivided into depths 1 (i.e. upper slope: 400–1,000 m) and 2 (i.e. middle and lower slopes: 1,000–2,200 m): Barcelona summer (BS1 and BS2), Barcelona autumn (BA1 and BA2), Mallorca summer (MS1 and MS2) and Ibiza autumn (IA1 and IA2)) were tested for infracommunity parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (Brillouin's index) (MD) and mean dominance (Berger-Parker's index) (B-P)), fish TL and condition indices (K, HSI and GSI), enzymatic activity levels (AChE, LDH and CS), density of hepatic melano-macrophages (MM/mm^2) and intensity of intestinal coccidia (OC/mm^2) of *G. melastomus* by means of General Linear Models (GLM) followed by post hoc tests,

GZM (for TMA and MSR) or Kruskal-Wallis tests (for TL, MD, B-P and OC/mm^2). Differences among groups on parasitological descriptors were tested in the two size-groups of hosts separately. In all contrasts among categorical groups, only groups with n ≥ 5 host specimens were considered. No differences were tested for male GSI data because only one group showed n ≥ 5. Enzymatic and MM data were log-transformed prior to analyses to comply for normality and homoscedasticity requirements.

In order to visualize the parasite abundance patterns in relation to the categorical groups, factorial correspondence analyses (FCA) were applied on data matrices containing component population (i.e. all parasites of the same taxa and developmental stage in a particular group of hosts) abundance data of the common parasites in juvenile and adult sharks separately. Hierarchical cluster analyses were simultaneously performed based on the coordinates of the first two axes obtained in the corresponding FCA to define host groups clearly. In both cases, all-zero samples were removed. Then, using individual fish as replicate samples, differences in MA and P of the common parasites of each size-group of hosts were tested across locality-season-depth groups by GZM (applying the log-binomial model for abundance and the logistic model for prevalence).

Possible relationships between the most abundant parasites and prey were analysed by Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). A matrix was generated including individuals for which parasitological and dietary information was available. To build this matrix, parasitological and dietary information were grouped by haul in the case of *G. melastomus*, and exceptionally, when the number of specimens per haul was low, some hauls were grouped. Grouped hauls (i.e. A201/02/08 and A204/06) included specimens caught at the same locality, season and depth. Number of specimens analysed per haul (or group of hauls) ranged between four and 15. Bearing in mind that *G. melastomus* hosts a moderate parasite richness and diversity, four specimens were considered representative enough of the diet of this species for our objectives. Parasites with occurrences ≥ 2 were included in the analysis, accumulating a total of 10 parasites (and 15 prey) in the CCA matrix. The canonical correspondence analysis related in this case the abundance of main parasites (using infracommunity data) with prey found in guts. In CCA plots, arrows represent explanatory variables and are proportional in length to their importance on the explained variable. The same analysis was repeated in order to assess the relationships between the abundance of main parasites and environmental variables (T, S, O_2 and turbidity).

Enzymatic activity values and MM/mm^2 were used as covariates to assess their possible association with individual total parasite abundance, abundance of common parasites and parasite richness by means of GZM. The relationship of enzymatic activity values and MM/mm^2 with individual parasite diversity and dominance was assessed by Spearman rank correlation tests.

The correlations between log-transformed enzymatic activity levels and fish TL, condition indices and MM/mm^2 were tested by means of GLM. In a similar way, possible associations between log-transformed MM data and fish TL and condition indices were assessed by GLM.

Possible associations between OC/mm^2 and fish TL, condition indices (GSI was considered only for females, due to the low number of male sharks infected by intestinal coccidians), enzymatic activity levels and MM/mm^2 were tested by Spearman Rank correlation tests.

2.6.2. Etmopterus spinax and Centroscymnus coelolepis

Parasite infracommunity richness, diversity and dominance data of *E. spinax* were not used in the data analyses due to their almost uniform value in all host specimens. Gonadosomatic index was not considered for *E. spinax* because most specimens were juveniles, nor for males of *C. coelolepis* due to low sample numbers.

Nine specimens of *E. spinax* and seven *C. coelolepis* were included in the CCA relating the most important parasites with prey found in guts (described above, see section 2.6.1.). The two assemblages of *C. coelolepis* were grouped as a function of season (A1, A3) including hauls from different depths (between 1,626–2,224 m). For *E. spinax* only the group A3/4, containing three sharks, was finally included in the CCA, since the parasitological diversity and abundance of the rest of specimens was very low. Parasite abundance was much higher in the specimens of the group A3/4, although parasite diversity was also low. Actually, the diet of *E. spinax* at > 1,000 m, where the specimens of the group A3/4 were sampled, is known to be also poorly diversified, exclusively based on cephalopods (Carrassón et al., 1992, Cartes et al., 2016).

The same specimens of *E. spinax* and *C. coelolepis* were included in the CCA assessing relationships between the abundance of main parasites and environmental variables.

Fish TL, condition indices and enzymatic activity values were set as covariates to assess their relationship with individual total parasite abundance, the abundance of common parasites and parasite richness in the case of *C. coelolepis*, and with the abundance of

larval tetraphyllidean cestodes (Tetraphyllidea fam. gen. sp., collectively known as *Scolex pleuronectis* Müller, 1788) in the case of *E. spinax* by means of GZM. Total parasite abundance data of *E. spinax* were not used due to the meaningless contribution of the only parasite apart from Tetraphyllidea fam. gen. sp. (i.e. *Aporhynchus norvegicus* (Olsson, 1868)).

Spearman Rank correlation tests were applied to test the association of fish TL and condition indices with individual parasite diversity and dominance of *C. coelolepis*. General linear model analyses were used to test the relationship between square-root-transformed enzymatic activity levels of both species and fish individual parasite diversity and dominance (for *C. coelolepis* only), fish TL and condition indices.

3. Results

Unless otherwise stated, no interaction was found between fish TL and factors or covariates tested in any of the above described analyses.

3.1. Fish biological factors

For *G. melastomus*, TL of the sampled fish ranged between 100 and 610 mm. Significant differences among groups for fish TL (Kruskal-Wallis, χ^2= 36.382, p< 0.001) and K (GLM, $F_{(4,\ 38)}$= 3.108, p= 0.026) were detected, with fish from off Mallorca in summer at depth 1 showing lower values (Table 2). No differences among groups were detected for HSI or GSI ($p > 0.05$).

For *E. spinax* and *C. coelolepis*, TL of the sampled fish ranged between 152 and 435 mm and between 243 and 660 mm, respectively.

Table 2. Means and standard deviations of fish total length (TL), condition factor (K), hepatosomatic index (HSI), adults gonadosomatic index (GSI), acetylcholinesterase (AChE), lactate dehydrogenase (LDH) and citrate synthase (CS) activities and density of hepatic melano-macrophages (MM/mm^2) in the different categorical groups of *Galeus melastomus*. N: sample size of *G. melastomus*; (*): number of females. Different superscript letters show significant differences among categorical groups. Dashes indicate non-available data.

	Mainland slope				**Insular slope**			
	Barcelona summer		**Barcelona autumn**		**Mallorca summer**		**Ibiza autumn**	
	400-1,000 m	**1,000-1,400 m**	**400-1,000 m**	**1,000-1,400 m**	**400-1,000 m**	**1,000-1,400 m**	**400-1,000 m**	**1,000-1,400 m**
N(*)	14(8)	26(23)	17(8)	14(13)	14(12)	21(21)	5(2)	9(7)
TL	38.24 ± 19.60^{A}	45.65 ± 13.39^{A}	34.00 ± 17.92^{A}	38.40 ± 19.52^{A}	13.60 ± 3.38^{B}	43.15 ± 13.53^{A}	39.98 ± 16.97^{A}	39.38 ± 19.37^{A}
K	0.29 ± 0.06^{A}	0.29 ± 0.03^{A}	0.27 ± 0.04^{A}	0.29 ± 0.07^{A}	0.23 ± 0.03^{B}	0.28 ± 0.03^{A}	0.29 ± 0.04^{A}	0.27 ± 0.02^{A}
HSI	5.20 ± 3.10^{A}	4.44 ± 1.77^{A}	3.97 ± 1.41^{A}	3.57 ± 1.38^{A}	4.34 ± 2.27^{A}	5.05 ± 1.25^{A}	4.20 ± 1.31^{A}	3.60 ± 1.80^{A}
GSI (females)	4.28 ± 2.83^{A}	2.45 ± 1.97^{A}	2.54 ± 0.90^{A}	2.26 ± 2.19^{A}	–	2.87 ± 2.07^{A}	2.00 ± 2.55^{A}	2.50 ± 2.00^{A}
GSI (males)	2.04 ± 0.50	0.99 ± 0.78	0.91 ± 0.36	–	–	1.13 ± 1.20	1.76	2.08
AChE	22.84 ± 19.79^{AB}	10.72 ± 8.11^{B}	22.91 ± 16.15^{AB}	32.55 ± 27.14^{A}	26.73 ± 4.69	12.33 ± 5.95^{B}	18.31 ± 8.07	18.85 ± 8.92^{AB}
LDH	$1{,}728 \pm 445^{BC}$	$1{,}330 \pm 397^{C}$	$1{,}860 \pm 676^{BC}$	$2{,}925 \pm 1{,}298^{A}$	$1{,}437 \pm 46$	$1{,}483 \pm 328^{C}$	$1{,}628 \pm 517$	$2{,}398 \pm 889^{AB}$
CS	24.19 ± 6.01^{C}	24.51 ± 7.15^{C}	30.92 ± 17.92^{C}	64.68 ± 31.54^{A}	18.70 ± 0.37	34.91 ± 12.88^{BC}	32.58 ± 13.28	59.40 ± 37.19^{AB}
MM/mm^2	138.89 ± 171.97^{A}	187.95 ± 170.71^{A}	–	344.96 ± 251.31^{A}	34.11 ± 20.00^{A}	112.41 ± 96.62^{A}	91.00 ± 19.82	383.18 ± 358.53^{A}

3.2. Composition of the parasite communities and their relationship with fish biological parameters and environmental gradients and variables

3.2.1. Galeus melastomus

Globally, percentage of uninfected sharks was 10.83%. In the parasitized specimens, a total of 15 different metazoan parasite taxa were recovered: one monogenean, one digenean, five cestodes, seven nematodes and one copepod (Tables 3 and 4). Of these, the cestodes *G. adenoplusia* and Tetraphyllidea fam. gen. sp., and the nematodes Anisakidae gen. sp. and *Collarinema collaris* (Petter, 1970) constitute new host records. The MDS providing an ordination of parasite infracommunities with respect to host size evidenced a differentiation of such communities between juvenile and adult specimens of *G. melastomus* (Stress= 0.06, Fig. 2). The PERMANOVA analysis testing the effect of host size on the composition of parasite infracommunities showed a significant difference between juvenile and adult sharks on such composition (Pseudo-$F_{(1,\ 104)}$= 42.715, $p_{(perm)}$= 0.0001; 9,955 unique permutations).

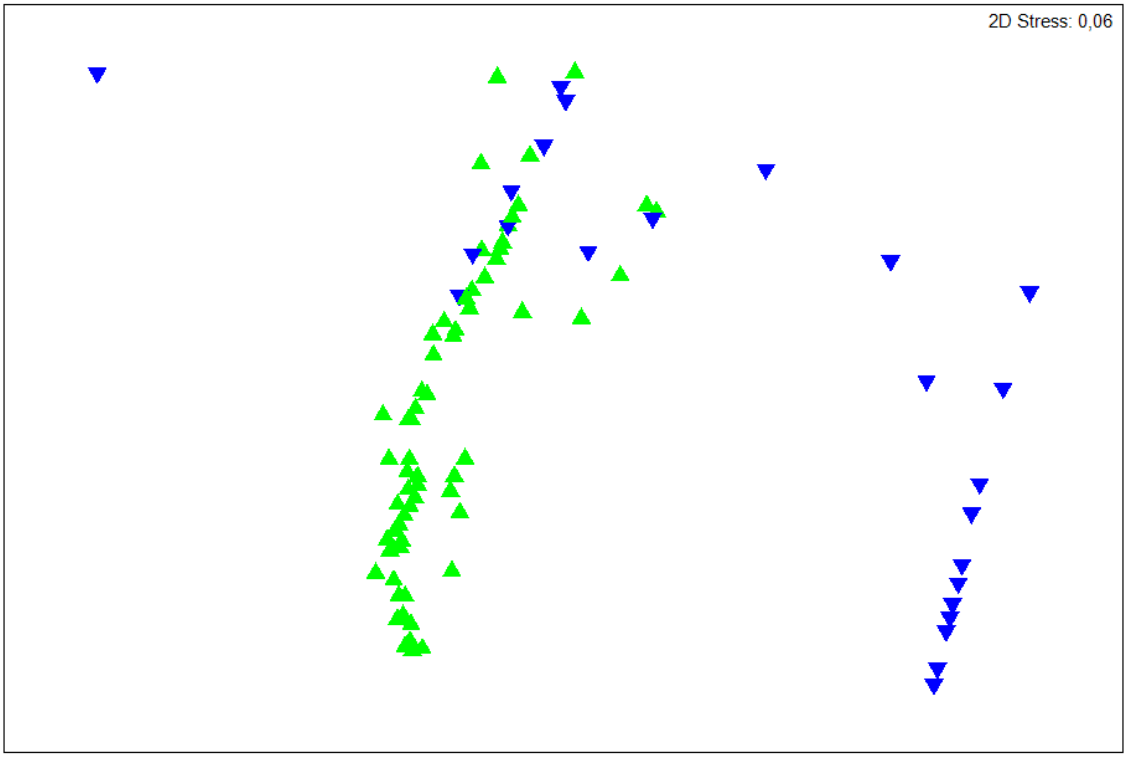

Figure 2. Multi-dimensional scaling (MDS) showing ordination of infracommunities of juvenile (▼) and adult (▲) specimens of *Galeus melastomus*.

Table 3. Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in juvenile specimens of *Galeus melastomus,* and means and standard deviations of fish parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (MD) and mean dominance (B-P)) in the different categorical groups of juvenile specimens of *G. melastomus*. N: sample size of *G. melastomus*; SR: species richness. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Mt, metacercaria; Pd, plerocercoid; Ps, plerocercus. Abbreviations for locations within host: G, gills; I, intestine; M, muscle; S, stomach; SW, stomach wall (encysted). Different superscript letters and numbers show significant differences among categorical groups in abundance and prevalence, respectively. Dashes indicate absence of the parasite.

	Stage	Location	Mainland slope								Insular slope							
			Barcelona summer				Barcelona autumn				Mallorca summer				Ibiza autumn			
			400-1,000 m		1,000-1,400 m		400-1,000 m		1,000-1,400 m		400-1,000 m		1,000-1,400 m		400-1,000 m		1,000-1,400 m	
N			**5**		**3**		**10**		**6**		**14**		**9**		**2**		**1**	
			P(%)	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**	**P(%)**	**MA±SD**
Digenea																		
Otodistomum sp.	Mt	S, SW	-	-	-	-	-	-	-	-	-	-	11	0.11 ± 0.33	50	0.50 ± 0.71	-	-
Cestoda																		
Ditrachybothridium macrocephalum	Ps, J, A	S, I	40^{1}	0.40 ± 0.55^{A}	67	1.00 ± 1.00	60^{1}	1.2 ± 1.55^{A}	50^{1}	1.17 ± 1.94^{A}	71^{1}	6.00 ± 5.68^{B}	33^{1}	1.89 ± 3.48^{A}	50	0.50 ± 0.71	100	1.00
Grillotia adenoplusia	Ps	M	-	-	-	-	20^{1}	1.00 ± 2.31^{A}	17^{1}	0.17 ± 0.41^{A}	-	-	78^{2}	7.67 ± 6.38^{B}	50	7.50 ± 10.61	-	-
Tetraphyllidea fam. gen. sp.	Pd	I	-	-	-	-	-	-	-	-	-	-	11	0.22 ± 0.67	-	-	-	-
Nematoda																		
Hysterothylacium aduncum	L3	S	-	-	-	-	10	0.10 ± 0.32	-	-	-	-	-	-	-	-	-	-
Hysterothylacium sp.	L3/L4	S	20	0.20 ± 0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proleptus obtusus	L3/L4/A	S, I	-	-	-	-	10	0.40 ± 1.26	-	-	-	-	-	-	-	-	-	-
Collarinema collaris	L3/L4	S	-	-	-	-	-	-	-	-	-	-	-	-	50	0.50 ± 0.71	-	-
Piscicapillaria baylisi	A	I	-	-	-	-	-	-	-	-	-	-	11	0.11 ± 0.33	-	-	-	-
Copepoda																		
Eudactylina vilelai	A	G	-	-	67	0.67 ± 0.58	-	-	-	-	-	-	11	0.11 ± 0.33	50	0.50 ± 0.71	-	-
TMA			0.60 ± 0.55^{A}		1.67 ± 1.15		2.70 ± 3.43^{AB}		1.33 ± 1.97^{A}		6.00 ± 5.68^{BC}		10.11 ± 5.06^{C}		9.50 ± 12.02		1.00	
SR			1		3		6		2		1		3		4		1	
MSR			0.60 ± 0.55^{A}		1.67 ± 1.15		1.40 ± 1.90^{A}		0.67 ± 0.82^{A}		0.71 ± 0.47^{A}		1.56 ± 0.73^{A}		2.50 ± 2.12		1.00	
MD (Brillouin's index)			0.00 ± 0.00^{A}		0.12 ± 0.21		0.20 ± 0.31^{A}		0.12 ± 0.20^{A}		0.00 ± 0.00^{A}		0.13 ± 0.17^{A}		0.24 ± 0.33		0.00	
B-P (Berger-Parker's index)			1.00 ± 0.00^{A}		0.89 ± 0.19		0.88 ± 0.21^{A}		0.83 ± 0.29^{A}		1.00 ± 0.00^{A}		0.94 ± 0.09^{A}		0.92 ± 0.12		1.00	

Table 4. Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in adult specimens of *Galeus melastomus,* and means and standard deviations of fish parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (MD) and mean dominance (B-P)) in the different categorical groups of adult specimens of *G. melastomus*. N: sample size of *G. melastomus*; SR: species richness. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Mt, metacercaria; Pd, plerocercoid; Ps, plerocercus. Abbreviations for locations within host: Ca, Abdominal cavity; G, gills; I, intestine; M, muscle; S, stomach; SW, stomach wall (encysted). Different superscript letters and numbers show significant differences among categorical groups in abundance and prevalence, respectively. Dashes indicate absence of the parasite.

	Stage	Location	Mainland slope								Insular slope					
			Barcelona summer				Barcelona autumn				Mallorca summer		Ibiza autumn			
			400-1,000 m		1,000-1,400 m		400-1,000 m		1,000-1,400 m		1,000-1,400 m		400-1,000 m		1,000-1,400 m	
N			9		23		7		8		12		3		8	
			P(%)	MA±SD	P(%)	MA±SD	P(%)	MA±SD	P(%)	MA±SD	P(%)	MA±SD	P(%)	MA±SD	P(%)	MA±SD
Monogenea																
Monogenea indet.	A	G	-	-	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Digenea																
Otodistomum sp.	Mt	S, SW	-	-	-	-	-	-	50^{1}	1.75 ± 1.91^{A}	8^{1}	1.17 ± 4.04^{A}	-	-	25^{1}	2.13 ± 5.25^{A}
Cestoda																
Ditrachybothridium macrocephalum	Ps, J, A	S, I	-	-	26^{1}	0.35 ± 0.65^{A}	29^{1}	0.29 ± 0.49^{A}	13^{1}	0.25 ± 0.71^{A}	8^{1}	0.08 ± 0.29^{A}	-	-	-	-
Grillotia adenoplusia	Ps	M, S, Ca	100^{1}	84.67 ± 66.34^{A}	100^{1}	53.04 ± 55.93^{A}	100^{1}	35.29 ± 38.66^{A}	100^{1}	79.13 ± 61.30^{A}	100^{1}	70.67 ± 67.27^{A}	100	32.00 ± 29.87	100^{1}	78.88 ± 63.16^{A}
Sphyriocephalus viridis	Pd	S	11	0.11 ± 0.33	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Hepatoxylon trichiuri	Pd	I	-	-	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Tetraphyllidea fam. gen. sp.	Pd	I, S	11^{1}	0.11 ± 0.33^{A}	13^{1}	0.35 ± 1.27^{A}	-	-	-	-	25^{1}	0.42 ± 0.90^{A}	-	-	13^{1}	0.75 ± 2.12^{A}
Nematoda																
Anisakis Type ll	L3	S	-	-	4	0.04 ± 0.21	-	-	-	-	-	-	-	-	-	-
Hysterothylacium aduncum	L3	I	-	-	-	-	14	0.29 ± 0.76	-	-	-	-	-	-	-	-
Anisakidae gen. sp.	L3	I	-	-	-	-	-	-	13	0.13 ± 0.35	-	-	-	-	-	-
Proleptus obtusus	L3	S	11	0.11 ± 0.33	-	-	14	0.14 ± 0.38	-	-	-	-	-	-	-	-
Collarinema collaris	A	S	11	0.11 ± 0.33	-	-	-	-	-	-	-	-	-	-	-	-
Piscicapillaria baylisi	A	I	22	0.22 ± 0.44	4	0.04 ± 0.21	-	-	-	-	8	0.42 ± 1.44	-	-	-	-
Copepoda																
Eudactylina vilelai	A	G	100^{1}	2.56 ± 1.81^{A}	52^{1}	1.61 ± 2.31^{A}	57^{1}	1.57 ± 1.90^{A}	88^{1}	2.00 ± 2.14^{A}	42^{1}	0.92 ± 1.51^{A}	67	2.33 ± 3.21	100^{1}	2.88 ± 3.00^{A}
TMA			87.89 ± 68.27^{A}		55.57 ± 56.12^{A}		37.57 ± 37.39^{A}		83.25 ± 61.08^{A}		73.67 ± 67.74^{A}		34.33 ± 32.93		84.63 ± 69.01^{A}	
SR			5		4		4		7		5		2		4	
MSR			2.67 ± 1.12^{A}		2.17 ± 1.11^{A}		2.14 ± 1.21^{A}		3.38 ± 1.85^{A}		2.08 ± 1.51^{A}		1.67 ± 0.58		2.50 ± 0.76^{A}	
MD (Brillouin's index)			0.16 ± 0.11^{A}		0.21 ± 0.22^{A}		0.28 ± 0.32^{A}		0.24 ± 0.32^{A}		0.14 ± 0.21^{A}		0.16 ± 0.14		0.21 ± 0.17^{A}	
B-P (Berger-Parker's index)			0.95 ± 0.06^{A}		0.91 ± 0.12^{A}		0.85 ± 0.19^{A}		0.91 ± 0.16^{A}		0.95 ± 0.08^{A}		0.94 ± 0.05		0.94 ± 0.05^{A}	

Significant positive relationships with host size were detected for individual total parasite abundance (GZM, χ^2= 208.819, p < 0.001), abundance of *Otodistomum* sp. (GZM, χ^2= 15.757, p < 0.001), Tetraphyllidea fam. gen. sp. (GZM, χ^2= 8.304, p= 0.004), *G. adenoplusia* (GZM, χ^2= 233.982, p < 0.001) and *E. vilelai* (GZM, χ^2= 34.712, p< 0.001) and for individual parasite richness (GZM, χ^2=14.350, p < 0.001) and diversity (r_s= 0.32, p= 0.001). In contrast, abundance of *D. macrocephalum* and individual dominance were significantly higher in juvenile sharks (GZM, χ^2= 66.678, p < 0.001 and r_s= -0.278, p= 0.004, respectively). Significant positive associations with host K were found for individual total parasite abundance (GZM, χ^2= 10.360, p= 0.001), abundance of *E. vilelai* (GZM, χ^2= 8.759, p= 0.003) and *G. adenoplusia* (GZM, χ^2= 14.071, p < 0.001) and Brillouin's diversity index (r_s= 0.364, p < 0.001). In contrast, abundance of *D. macrocephalum* and dominance index were negatively correlated with K (GZM, χ^2= 13.939, p < 0.001 and r_s= -0.367, p < 0.001, respectively). Negative correlations were detected between HSI and individual total abundance (GZM, χ^2= 24.288, p < 0.001), abundance of *E. vilelai* (GZM, χ^2= 6.182, p= 0.013), *Otodistomum* sp. (GZM, χ^2= 20.686, p < 0.001), Tetraphyllidea fam. gen. sp. (GZM, χ^2= 6.910, p= 0.009) and *G. adenoplusia* (GZM, χ^2= 26.048, p < 0.001). Individual total abundance and abundance of *G. adenoplusia* were positively linked to male GSI (GZM, χ^2= 5.554, p= 0.018 and χ^2= 5.993, p= 0.014, respectively), and individual total abundance and abundance of *Otodistomum* sp. and *G. adenoplusia* showed a positive relationship with female GSI (GZM, χ^2= 5.103, p= 0.024; χ^2= 4.131, p= 0.042 and χ^2= 5.282, p= 0.022, respectively).

Based on the observed differences on the parasite composition of the communities infecting juvenile and adult sharks, the description of the parasite assemblages of the two host size-groups is performed separately.

Within juvenile sharks, 26% of the fish were free of parasites. The parasites recovered from the infected hosts were grouped in a total of ten different parasite taxa (Table 3). Parasites most frequently found and classified as "common" (total P ≥ 8%) were *D. macrocephalum*, *G. adenoplusia* and *E. vilelai.* The most abundant parasites were the cestodes *D. macrocephalum* (TMA= 2.54) and *G. adenoplusia* (TMA= 1.90).

All adult specimens were infected by at least one parasite, and a total of 14 parasite taxa were identified (Table 4). Common parasites (total P ≥ 8%) were *Otodistomum* sp., *D. macrocephalum*, Tetraphyllidea fam. gen. sp., *G. adenoplusia* and *E. vilelai*. The most

abundant parasite was, by far, the cestode *G. adenoplusia* (TMA= 63.39) followed by the copepod *E. vilelai* (TMA= 1.83).

A significant effect of the factor locality-season-depth was found for TMA in juvenile sharks (GZM, χ^2= 20.873, $p < 0.001$). No differences among categorical groups were found for MSR, MD or B-P in the case of juvenile sharks or for TMA, MSR, MD and B-P in the case of adult sharks (GZM/Kruskal-Wallis, $p > 0.05$).

Figure 3 shows a plot of the first factorial plane of co-inertia analysis covering 99.996% of the total variance, mainly on the first axis (99.86% of the total inertia) of the FCA performed using component population data of the common parasites in juvenile sharks. Component populations of the three parasites strongly correlated with the first FCA axis (Cosine^2= 0.873–1.000). None of them was strongly correlated with the second FCA axis. From FCA and cluster analyses, three distinct assemblages of juvenile sharks could be distinguished depending on their parasite load:

Group A: Comprises samples from off Barcelona and Mallorca in summer at depth 1 and from off Barcelona in autumn at depth 2. These were characterized by the cestode *D. macrocephalum*, which was significantly more abundant in samples from off Mallorca in summer at depth 1 (GZM, χ^2= 19.271, p= 0.001). No significant differences among groups were detected for prevalence (GZM, $p > 0.05$).

Group B: Comprises samples from off Barcelona in autumn at depth 1, which were not characterized by any particular parasite.

Group C: Comprises samples from off Mallorca in summer at depth 2, which were characterized by the cestode *G. adenoplusia* and the copepod *E. vilelai*. The former parasite showed a significantly higher abundance and prevalence in this group (GZM, χ^2= 20.186, $p < 0.001$ and GZM, χ^2= 7.025, p= 0.03, respectively). *Eudactylina vilelai* was absent from all other groups.

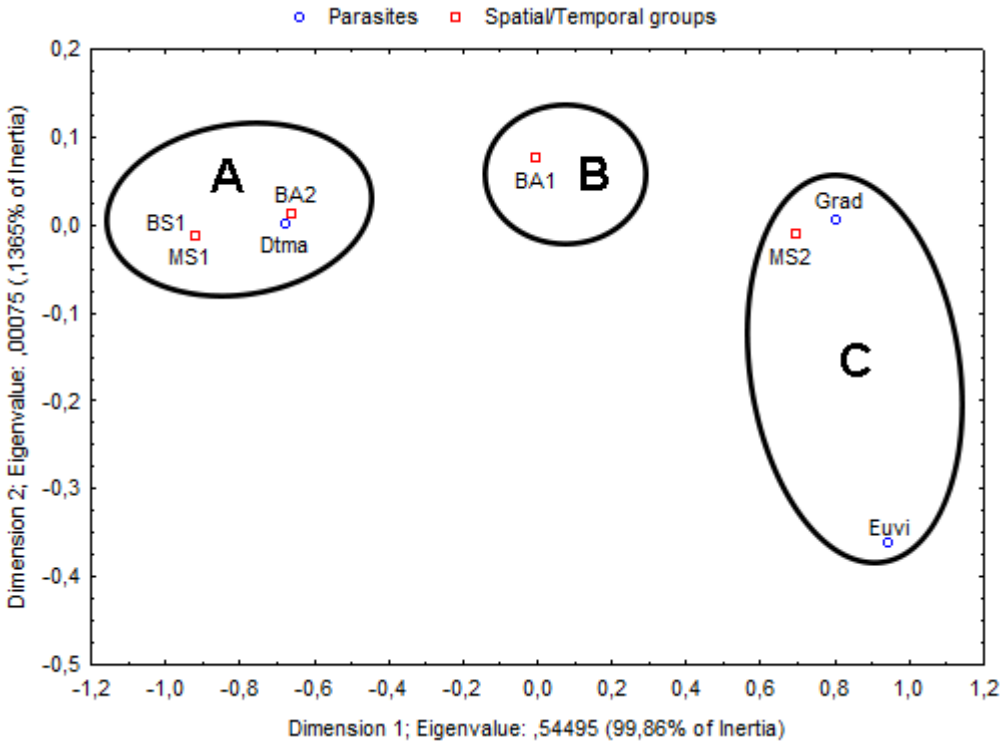

Figure 3. Plot of the first factorial plane of co-inertia of the factorial correspondence analysis (FCA) performed using component population data of the three common parasites (P > 10%) in juvenile specimens of *Galeus melastomus*. A/B/C refers to the groups established in the description of the parasite fauna. Abbreviations for locality-season groups (BA1, etc.) are defined in Fig. 1. Abbreviations for parasite names: Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*.

Figure 4 shows a plot of the first factorial plane of co-inertia analysis covering 82.81% of the total variance, mainly on the first axis (66.88% of the total inertia) of the FCA performed using component population data of the common parasites in adult sharks. Component populations of two parasites strongly correlated with the first FCA axis: *Otodistomum* sp. and *G. adenoplusia* (Cosine^2= 0.505–0.970). Only Tetraphyllidea fam. gen. sp. was significantly correlated with the second FCA axis: (Cosine^2= 0.509). From FCA and cluster analyses, two distinct assemblages of adult sharks could be distinguished depending on their parasite load:

Group A: Comprises samples from off Mallorca in summer and from off Barcelona and Ibiza in autumn at depth 2, which were characterized by the digenean *Otodistomum* sp. and the cestodes Tetraphyllidea fam. gen. sp. Although both parasites reached

maximum abundances in samples from off Ibiza in autumn at depth 2, no significant effects were detected neither for abundance or prevalence (GZM, $p > 0.05$).

Group B: Comprises samples from off Barcelona in autumn at depth 1 and from off Barcelona in summer at both depths, which were characterized by the cestodes *D. macrocephalum* and *G. adenoplusia* and by the copepod *E. vilelai*. The two former parasites were most abundant in samples from off Barcelona in summer at depths 2 and 1, respectively. No significant effects were detected neither for abundance or prevalence (GZM, $p > 0.05$).

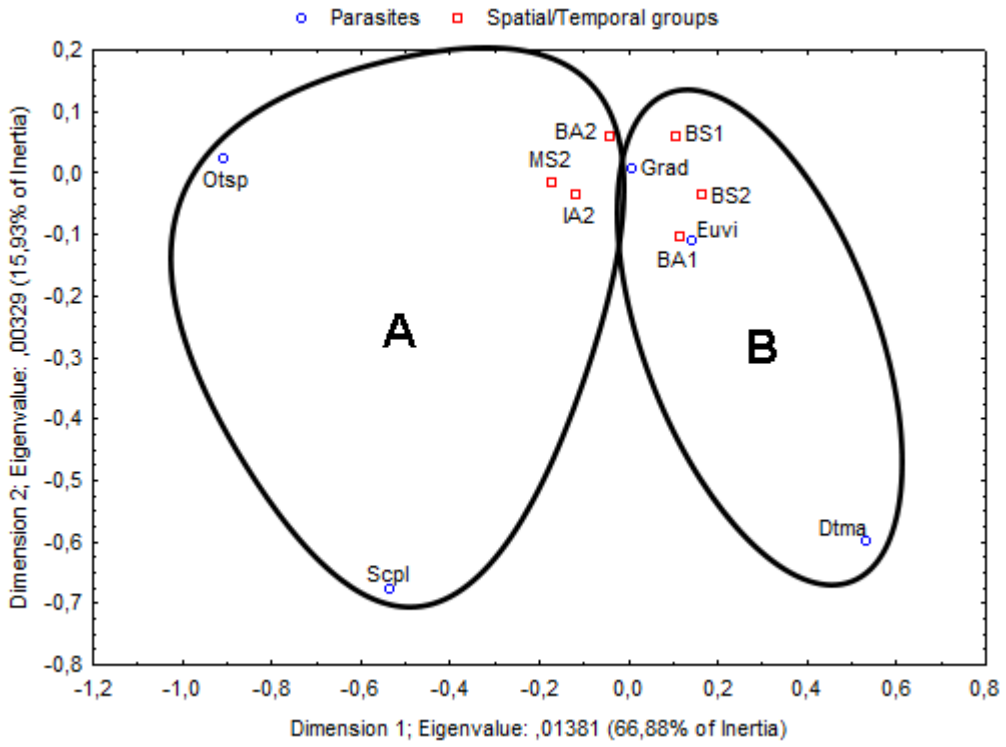

Figure 4. Plot of the first factorial plane of co-inertia of the factorial correspondence analysis (FCA) performed using component population data of the five common parasites (P > 10%) in adult specimens of *Galeus melastomus*. A/B refers to the groups established in the description of the parasite fauna. Abbreviations for locality-season groups (BA1, etc.) are defined in Fig. 1. Abbreviations for parasite names: Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*; Otsp, *Otodistomum* sp.; Scpl, *Scolex pleuronectis* (Tetraphyllidea fam. gen. sp.).

The CCA assessing the relationships between the abundance of main parasites and environmental variables explained 84.9% of the constrained variance in the first two axes (Fig. 5). At the right-lower part of the plot, the strongest associations were found between high turbidity levels and the abundance of the parasites *D. macrocephalum* and *P. baylisi*, linked to hauls from 500–700 m depth. High salinity levels were related to the nematodes *H. aduncum* and *P. obtusus*. At the right-upper part of the plot and more weakly, O_2 levels were linked to the cestodes Tetraphyllidea fam. gen sp., *S. viridis* and larval anisakid nematodes.

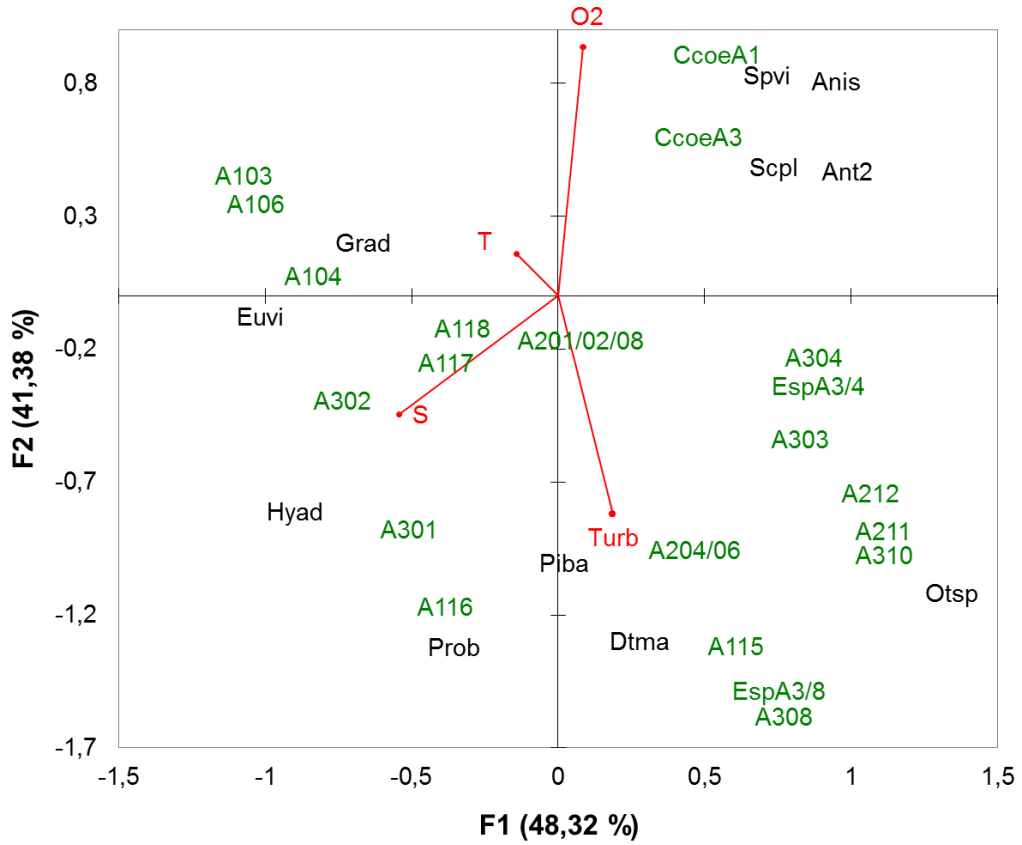

Figure 5. Canonical correspondence analysis (CCA) showing relationships between the abundance of parasites infecting *Galeus melastomus*, *Etmopterus spinax* and *Centroscymnus coelolepis* and environmental data. Abbreviations for parasites names: Anis, Anisakidae gen. sp; Ant2, *Anisakis* Type II; Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*; Hyad, *Hysterothylacium aduncum*; Otsp, *Otodistomum* sp.; Piba, *Piscicapillaria baylisi*; Prob, *Proleptus obtusus*; Scpl, *Scolex pleuronectis* (Tetraphyllidea fam. gen. sp.); Spvi, *Sphyriocephalus viridis*. Abbreviations for environmental variables: O2, oxygen concentration; S, salinity; T, temperature; Turb, turbidity.

3.2.2. Etmopterus spinax

The percentage of uninfected sharks was 36%. In infected specimens, a total of two parasite taxa were found (Table 5), of which only one of them (i.e. Tetraphyllidea fam. gen. sp.) was considered common.

Table 5. Developmental stage, location within host, prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites found in *Etmopterus spinax* and *Centroscymnus coelolepis*, and means and standard deviations of fish parasitological descriptors (total mean abundance (TMA), mean species richness (MSR), mean diversity (MD) and mean dominance (B-P)), fish total length (TL), condition factor (K), hepatosomatic index (HSI), adults gonadosomatic index (GSI) and acetylcholinesterase (AChE), lactate dehydrogenase (LDH) and citrate synthase (CS) activities. N: sample size of *C. coelolepis* and *E. spinax*, (*): number of females. Abbreviations for developmental stages: A, adult; J, juvenile; L, larvae; Pd, plerocercoid; Ps, plerocercus. Abbreviations for locations within host: Ca, Abdominal cavity; I, intestine; K, kidney; L, liver; M, muscle; S, stomach; SW, stomach wall (encysted). Dashes indicate absence of the parasite.

	Etmopterus spinax				***Centroscymnus coelolepis***			
	Stage	**Location**	**Total**		**Stage**	**Location**	**Total**	
N(*)			**11(6)**				**10(7)**	
			P(%)	**MA±SD**			**P(%)**	**MA±SD**
Cestoda								
Aporhynchus norvegicus	A	I	9	0.09 ± 0.30	–	–	–	–
Grillotia adenoplusia	–	–	–	–	Ps	M, SW, Ca, K	30	29.70 ± 93.22
Sphyriocephalus viridis	–	–	–	–	Pd	S	50	2.20 ± 3.08
Clistobothrium sp.	–	–	–	–	J	I	10	0.10 ± 0.32
Monorygma sp.	–	–	–	–	A	I	20	0.20 ± 0.42
Tetraphyllidea fam. gen. sp.	Pd	I, L	64	10.36 ± 28.58	Pd	I	100	896.00 ± 2,665.50
Cestoda indet.	–	–	–	–	A	I	10	0.10 ± 0.32
Nematoda								
Anisakidae gen. sp.	–	–	–	–	L3	S	10	0.10 ± 0.32
Anisakis Type II	–	–	–	–	L3	M, SW	20	0.30 ± 0.67
TMA			10.45 ± 28.55				928 ± 2,658	
SR			2				8	
MSR			0.73 ± 0.65				2.50 ± 1.18	
MD (Brillouin's index)			0.05 ± 0.13				0.25 ± 0.25	
B-P (Berger-Parker's index)			0.93 ± 0.19				0.85 ± 0.18	
TL			23.59 ± 9.69				38.58 ± 14.34	
K			0.43 ± 0.07				0.71 ± 0.20	
HSI			9.28 ± 3.75				10.68 ± 7.06	
GSI			5.90 ± 8.75				3.51 ± 7.02	
AChE			78.61 ± 37.52				19.15 ± 12.71	
LDH			5,170 ± 796				4,877 ± 1,043	
CS			13.98 ± 16.75				1.92 ± 1.87	

Abundance of Tetraphyllidea fam. gen. sp. showed positive correlations with fish TL (GZM, χ^2= 5.678, p= 0.017) and HSI (GZM, χ^2= 5.911, p= 0.015). No relationship between the abundance of Tetraphyllidea fam. gen. sp. and K was detected (GZM, p > 0.05).

No particular associations were detected between the parasites of *E. spinax* and any environmental variable (Fig. 5).

3.2.3. Centroscymnus coelolepis

All examined sharks were infected by at least one parasite. A total of eight different parasite taxa were recovered (Table 5), of which the following three were considered common: *G. adenoplusia*, *S. viridis* and Tetraphyllidea fam. gen. sp. Furthermore, *G. adenoplusia*, Anidakidae gen sp. and *Anisakis* Type II constitute new host records.

Significant positive correlations with fish size and HSI were detected for total individual parasite abundance (GZM, χ^2= 4.801, p= 0.028 and GZM, χ^2= 4.712, p= 0.03, respectively), abundance of Tetraphyllidea fam. gen. sp. (GZM, χ^2= 7.497, p= 0.006 and GZM, χ^2= 7.787, p= 0.005, respectively) and *S. viridis* (GZM, χ^2= 7.862, p= 0.005 and GZM, χ^2= 7.256, p= 0.007, respectively). No effects of total individual parasite abundance, abundance of Tetraphyllidea fam. gen. sp. or *S. viridis* were detected on fish K or GSI (GZM, p > 0.05). Abundance of *G. adenoplusia* and individual richness, diversity and dominance were not related to fish TL or condition factors (GZM/r_s, p > 0.05).

The CCA assessing the relationships between the abundance of main parasites and environmental variables evidenced an association between the abundance of Tetraphyllidea fam. gen. sp., *S. viridis* and anisakid larval nematodes and near-bottom O_2 levels (Fig. 5, right-upper part of the plot).

3.3. Dietary composition of sharks and its relationship with the parasite fauna

Diet of *G. melastomus*, which will not be described in detail herein, was highly diversified (69 different prey items were identified, most of them to genus/species level). For the CCA on parasite-prey relationships (Fig. 6) only 16 prey-groups were considered. These groups represented 87.9% of the 499 prey identified (excluding items like scales and foraminiferans) in terms of number, and a higher proportion in terms of wet weight.

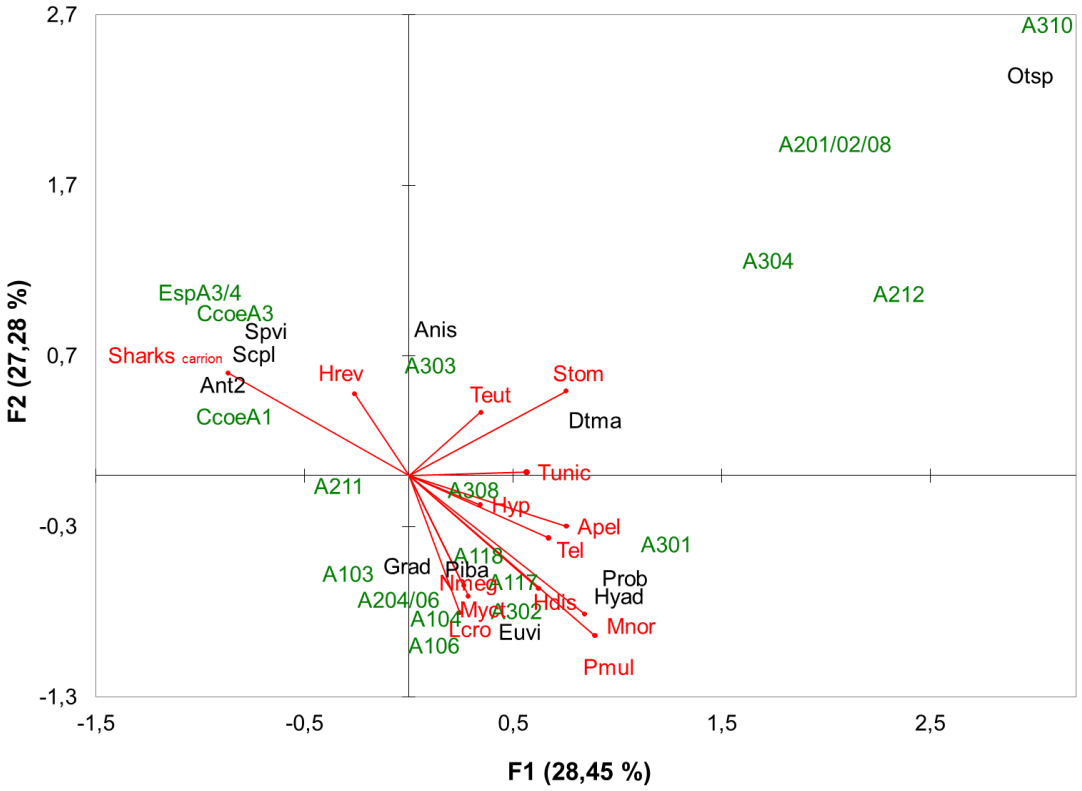

Figure 6. Canonical correspondence analysis (CCA) showing relationships between the abundance of main parasites and main prey-items found in *Galeus melastomus*, *Etmopterus spinax* and *Centroscymnus coelolepis*. Abbreviations for parasites names: Anis, Anisakidae gen. sp; Ant2, *Anisakis* Type II; Dtma, *Ditrachybothridium macrocephalum*; Euvi, *Eudactylina vilelai*; Grad, *Grillotia adenoplusia*; Hyad, *Hysterothylacium aduncum*; Otsp, *Otodistomum* sp.; Piba, *Piscicapillaria baylisi*; Prob, *Proleptus obtusus*; Scpl, *Scolex pleuronectis* (Tetraphyllidea fam. gen. sp.); Spvi, *Sphyriocephalus viridis*. Abbreviations for prey names: Apel, *Acantephyra pelagica*; Hdis, *Heteroteuthis dispar*; Hrev, *Histioteuthis reversa*; Hyp, Hyperiidea; Lcro, *Lampanyctus croccodilus*; Mnor, *Meganyctiphanes norvegica*; Myct, Myctophidae; Nmeg, *Nematoscelis megalops*; Pmult, *Pasiphaea multidentata*; Stom, Stomiidae; Tel, Teleostei; Teut, Teutoidea; Tunic, Tunicata.

The diet of this shark was mainly based on benthopelagic-mesopelagic prey, including also some benthic prey. In terms of number, decapods (*Pasiphaea multidentata* and *Acanthephyra pelagica*), euphausiids (mainly *Meganyctiphanes norvegica*) and myctophid fish (*Lampanyctus croccodilus*) were dominant among pelagic prey, but tunicates were also included among the groups considered in the CCA. The main benthopelagic prey were squids (*Histioteuthis* sp., *Heteroteuthis dispar*) and fish (among those identified, *Lepidion lepidion* and some macrourids). Among benthic (not swimming) prey, crabs/lobsters (e.g. *Monodaeus couchi*, *Calocaris macandreae*,

Munida tenuimana) were dominant. In terms of weight, squids, fish and decapods were the most important prey.

In *G. melastomus* diet changed as a function of fish size, although the rather low number of small specimens, mainly juveniles, available (n=23) did not allow to include size as factor in the CCA. Diet of small specimens was mainly based on *M. norvegica* (44.1% of prey), with *P. multidentata* and squids (both 8.9% of prey) as secondary prey. Meso-bathypelagic shrimps (*P. multidentata*, *A. pelagica*: 29.0% of prey) and squids (*H. dispar*, unidentified: 16.3% of prey) were comparatively more important in the diet of large (adult) specimens, in which *M. norvegica* only represented 14.9% of the total prey.

Etmopterus spinax preyed on mesopelagic decapods (*Sergestes* spp., *Pasiphaea* spp.), euphausiids and myctophids at < 1,000 m, while the diet at > 1,000 m was almost exclusively based on cephalopods (*Histioteuthis* spp.).

Centroscymnus coelolepis is the deepest distributed species (see Table 1), and its diet was essentially based on squids and carrion.

The CCA relating the abundance of individual parasites and prey explained 55.7% of the constrained variance in the first two axes (Fig. 6). Most of the parasites linked to diet items recovered from *G. melastomus* grouped at the right part of the plot. Hauls were not ordered as a function of depth, but as a function of season and of prey swimming capacity. At the right-upper part of the plot, the digenean *Otodistomum* sp., the cestode *D. macrocephalum* and unidentified larval anisakid nematodes were related to squids (Teuthoidea) and Stomiid fish associated to samples collected in October (A3 hauls). At the right-lower part of the plot, the cestode *G. adenoplusia*, the nematode *P. baylisi* and the copepod *E. vilelai* were most related to the euphausiid *Nematoscelis megalops* and to myctophid fish. The nematodes *H. aduncum* and *P. obtusus* were associated to mesopelagic (migratory) prey such as decapods (*P. multidentata*) and euphausiids (*M. norvegica*).

The larval cestodes Tetraphyllidea fam. gen. sp. were the only parasites found in the specimens of *E. spinax* included in the CCA (all from hauls at > 1,000 m depth), and appeared linked to the squids *Histioteuthis* spp., practically the only prey recovered from *E. spinax* guts (Fig. 6).

Parasites from *C. coelolepis* (the cestodes Tetraphyllidea fam. gen. sp. and *S. viridis*, and the nematode *Anisakis* Type 2) were associated to carrion (shark food falls) at the left-upper part of the plot (Fig. 6).

3.4. Relationship of enzymatic markers with parasite infection and fish biological parameters

3.4.1. Galeus melastomus

Mean activity levels (in nmol/min/mg prot) ranged across groups from 10.72 to 32.55 for AChE, from 1,330 to 2,925 for LDH and from 18.70 to 64.68 for CS (Table 2). For AChE, V_{max} was 14.1 nmol/min/mg prot. and K_m was 0.112 mM, with an average catalytic efficiency of 125.

A significant effect of the factor locality-season-depth was found for AChE (GLM, $F_{(5, 78)}= 5.697$, $p < 0.001$), LDH ($F_{(5, 74)}= 7.568$, $p < 0.001$) and CS ($F_{(5, 75)}= 6.787$, $p < 0.001$) activity levels. Highest activity levels of the three enzymes assessed were recorded in samples from off Barcelona in autumn at depth 2 (Table 2).

Significant negative relationships were detected between AChE activity and fish TL (GLM, $F_{(1, 88)}= 46.192$, $p < 0.001$) and between LDH activity and fish K (GLM, $F_{(1, 83)}= 7.551$, $p= 0.007$). Acetylcholinesterase activity levels were negatively related to total parasite abundance (GZM, $\chi^2= 23.588$, $p < 0.001$), abundance of *E. vilelai* ($\chi^2= 8.032$, $p= 0.005$), *Otodistomum* sp. ($\chi^2= 4.112$, $p= 0.043$) and *G. adenoplusia* ($\chi^2= 22.366$, $p < 0.001$), as also were LDH and CS activity levels to the abundance of *Otodistomum* sp. (GZM, $\chi^2= 6.922$, $p= 0.009$ and $\chi^2= 13.959$, $p < 0.001$, respectively).

3.4.2. Etmopterus spinax

Acetylcholinesterase, LDH and CS mean activity levels were 78.61, 5,170 and 13.98 nmol/min/mg prot, respectively (Table 5). For AChE, V_{max} was 54.1 nmol/min/mg prot and K_m was 0.119 mM, with an average catalytic efficiency of 454.6.

Acetylcholinesterase activity levels were negatively correlated with HSI (GLM, $F_{(1, 7)}= 5.693$, $p= 0.048$) and with the abundance of Tetraphyllidea fam. gen. sp. (GZM, $\chi^2= 4.850$, $p= 0.028$). No other significant associations were found between the parameters assessed for this species.

3.4.3. Centroscymnus coelolepis

Acetylcholinesterase, LDH and CS mean activity levels were 19.15, 4,877 and 1.92 nmol/min/mg prot, repectively (Table 5). For AChE, V_{max} was 43.3 nmol/min/mg prot and K_m was 0.043 mM, with an average catalytic efficiency of 1,007.

Acetylcholinesterase activity was negatively associated with fish TL (GLM, $F_{(1, 8)}$= 18.544, p= 0.003) and HSI (GLM, $F_{(1, 8)}$= 13.053, p= 0.007), while CS showed a positive relationship with the same two biological parameters (GLM, $F_{(1, 8)}$= 75.503, p= 0.0001 and $F_{(1, 8)}$= 29.250, p= 0.001, respectively). Total individual parasite abundance, abundance of Tetraphyllidea fam. gen. sp. and *S. viridis* correlated negatively with AChE (GZM, χ^2= 9.279, p= 0.002; χ^2= 14.386, p= 0.0001 and χ^2= 7.100, p= 0.008, respectively). Abundance of Tetraphyllidea fam. gen. sp. and *S. viridis* showed a negative association with LDH as well (GZM, χ^2= 6.225, p= 0.013 and GZM, χ^2= 5.938, p= 0.015, respectively). No other significant associations were found between the parameters assessed for this species.

3.5. Relationships of histological observations with parasite infection, enzymatic activities and fish biological parameters

3.5.1. Galeus melastomus

Frequent presence of coccidian oocysts was detected in histological sections of the spiral valve (Fig. 7A). Oocysts with four sporocysts were heterogeneously distributed and preferentially located basally in the epithelium of the intestinal mucosa, reaching in some cases the underlying lamina propria. Oocysts wall was colourless, while sporocysts showed a refringent surface and contained two eosinophilic sporozoites, each with a conspicuous granule of basophilic material. Oocysts were round or oval in shape, measuring from 11.2 to 15.9 μm in diameter (mean diameter= 13.5, n= 21). Although the methodology used in the present study made accurate identification of these microparasites not possible, the presence of four sporocysts, each containing two sporozoites, within each oocyst readily allows placing them within the family Eimeridae. Overall prevalence of oocysts was 55%. In infected specimens, OC/mm^2 ranged between 3 and 200 (mean number= 32). No significant differences in OC/mm_2 were detected across locality-season-depth groups (Kruskal-Wallis, $p > 0.05$). No associations were found between OC/mm^2 and fish TL, condition indices, enzymatic activity levels or MM/mm^2 (r_s, $p > 0.05$ in all cases).

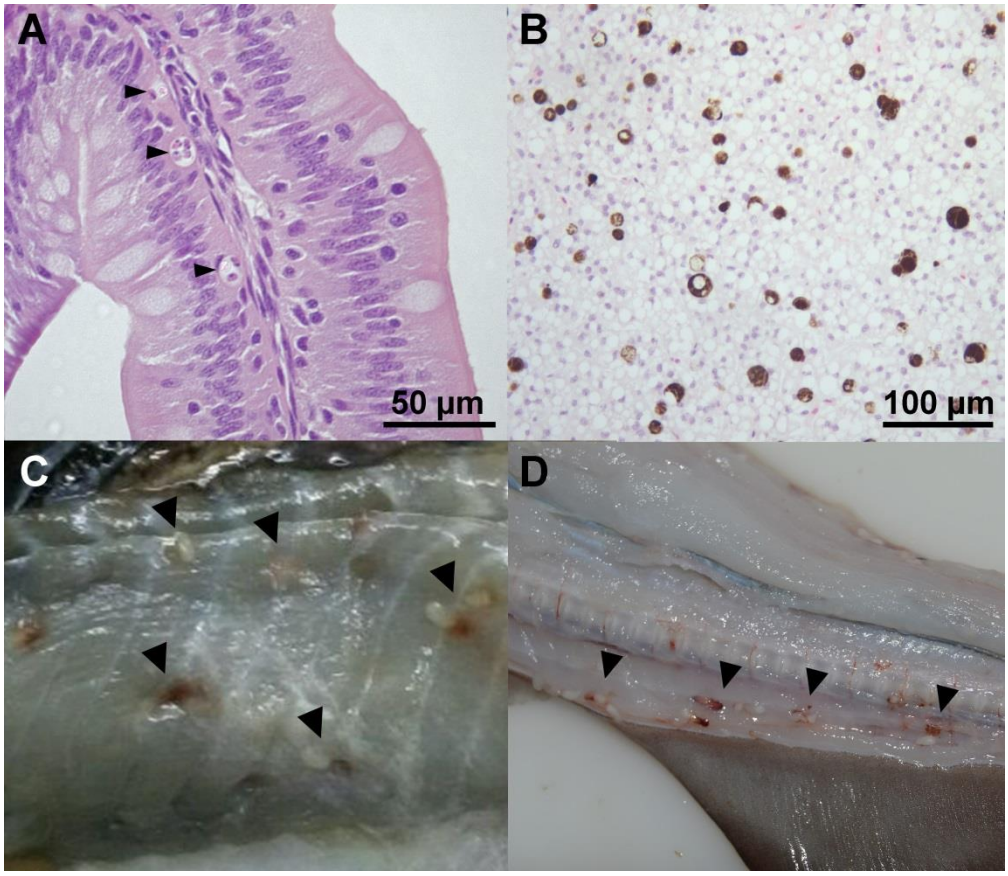

Figure 7. Photomicrographs of histological pathologies and alterations found in histological sections of different organs of *Galeus melastomus*. A, Coccidian oocysts in the intestinal mucosa (arrowheads); B, Melano-macrophages in hepatic tissue; C, Larval stages of the cestode *Grillotia adenoplusia* encapsulated in the abdominal musculature (arrowheads); D, Larval stages of the cestode *G. adenoplusia* encapsulated in the tail musculature (arrowheads).

Melano-macrophages were homogenously distributed across all hepatic and some splenic sections (Fig. 7B). Their mean densities across groups ranged from 34.11 to 383.18 MM/mm^2 (Table 2). Density of melano-macrophages significantly increased with fish size and female GSI (GLM, $F_{(1, 77)}$= 88.941, $p < 0.001$ and $F_{(1, 46)}$= 4.723, p= 0.035, respectively), but was inversely associated to HSI (GLM, $F_{(1, 77)}$= 43.061, $p < 0.001$). Positive relationships between MM/mm^2 and total parasite abundance (GZM, χ^2= 33.362, $p < 0.001$), abundance of *E. vilelai* (GZM, χ^2= 12.346, $p < 0.001$) *Otodistomum* sp. (GZM, χ^2=27.481, $p < 0.001$) and *G. adenoplusia* (GZM, χ^2= 36.971, $p < 0.001$) and the dominance index (r_s= -0.312, p= 0.007) were detected. In contrast, MM/mm^2 correlated negatively with the abundance of *D. macrocephalum* (GZM, χ^2= 17.163, $p < 0.001$) and Tetraphyllidea fam. gen. sp. (GZM, χ^2= 10.403, p= 0.001) and with the diversity index (r_s= 0.350, p= 0.002). An interaction with fish TL was detected

in the analyses assessing the association between MM/mm^2 and the abundances of Tetraphyllidea fam. gen. sp. and *G. adenoplusia*. Finally, significant positive relationships were detected between MM/mm^2 and LDH (GLM, $F_{(1, 59)}$= 13.582, p < 0.001) and CS ($F_{(1, 58)}$= 10.589, p= 0.002) activities.

Macroscopical lesions associated to encapsulated larval stages of the parasite *G. adenoplusia* were detected in the muscular tissues of all adult specimens of *G. melastomus* (Fig 7C, D). These parasites were sometimes recovered from the stomach walls, but were mainly found in the musculature of the abdominal and tail regions. In the latter area they concentrated in large numbers. Damage of the caudal musculature was observed in highly parasitized sharks. In these regions, the affected tissue was much softer than normal.

No other relevant histological alterations were detected in the mentioned organs or in gill or gonad sections.

3.5.2. Etmopterus spinax and Centroscymnus coelolepis

No relevant alterations were observed in histological sections of the examined organs of *E. spinax* and *C. coelolepis*. No quantitative analysis of MM was carried out for these species since, in contrast with *G. melastomus*, only a very slight pigmentation was detected in some liver sections.

4. Discussion

4.1. General features of the parasite communities of G. melastomus, E. spinax and C. coelolepis in the northwestern Mediterranean Sea

Shark species have been barely addressed as regards their parasite communities, and *G. melastomus* from the Balearic Sea shows the highest total richness values reported to date (15 vs. 2–11 different parasite taxa, see Henderson and Dunne, 1998; Moore, 2001; Palm and Schröeder, 2001; Henderson et al., 2002; Chambers, 2008; Isbert et al., 2015; Dallarés et al., in press). As regards small-sized demersal sharks examined for parasites, they show moderate to high richness values compared to the rest of sharks addressed (Henderson and Dunne, 1998; Moore, 2001; Henderson et al., 2002; Dallarés et al., in press). This trend is likely related to their benthopelagic feeding habits: the higher availability of potential intermediate hosts close to the seafloor results in more complex

and diverse fish parasite communities in this environment (Campbell et al., 1980; Marcogliese, 2002).

The parasite community of *G. melastomus* in the Balearic Sea, which is herein described in full for the first time, is thus characterized by high abundance, richness and diversity values when compared to other sharks. Furthermore, high dominance values are attained by most infracommunities as a result of the high preponderance of a single parasite species (i.e. *D. macrocephalum* in small and *G. adenoplusia* in large fish).

A recent study (Dallarés et al., in press) has also addressed the parasite community of this species in the mainland slope of the Balearic Sea; however, despite having been conducted in the same area and at similar depth ranges, the structure and composition of parasite infracommunities described in the cited and present studies show marked differences. Overall mean parasite abundance of present specimens is extremely higher (40.83 vs. 1.04), the main reason being that in the first description of the parasite community of *G. melastomus* the most important parasite in terms of abundance in adult and large-sized juvenile sharks (i.e. the larval trypanorhynch cestode *G. adenoplusia*) was not collected. As described by Dallarés et al. (in press), all organs were thoroughly examined with the exception of the musculature, where, as evidenced by present results, *G. adenoplusia* is almost exclusively located. Mean infracommunity richness and diversity are somewhat higher in present specimens (1.85 vs. 0.6 and 0.16 vs. 0.09, respectively). Since no differences exist between both samples concerning size-ranges of the sharks examined or localities, seasons and depths sampled, these differences might be due either to stochastic factors or to long-term changes in benthic and/or suprabenthic faunal assemblages (as those reported by Cartes et al., 2009) in turn affecting parasite availability and transmission.

This represents the first attempt to describe the parasite community infecting *E. spinax* in the Mediterranean Sea. With a total of only two different parasites recovered, parasite infracommunities of *E. spinax* from the present study are characterized by extremely low richness and diversity values and a very high dominance index, which may be related with the pelagic feeding habits of this species (Carrassón et al., 1992, present results). As a rule, pelagic fish possess impoverished parasite communities compared to those living close to the seafloor, as has been for instance observed in mediterranean myctophid teleosts (Mateu et al., 2015), due to the generalized scarcity of potential intermediate hosts compared to the benthic environment (Campbell et al., 1980; Marcogliese, 2002).

Present parasitological results for *E. spinax* contrast with the outcomes by Klimpel et al. (2003), who recovered seven different parasite taxa from 37 juvenile specimens of *E. spinax* in the North Sea. Apparently, these dissimilarities could not be attributed to dietary differences between Atlantic and Mediterranean sharks (i.e. a more diverse diet of the former that would presumably favour the acquisition of more parasite taxa), since juvenile specimens of *E. spinax* from the Atlantic essentially consumed one euphausiid crustacean and one teleost species (Klimpel et al., 2003), while those from the Mediterranean feed onto a wider array of prey (present results). The absence of ectoparasites, which do not rely on host feeding patterns for transmission, in Mediterranean specimens further suggests that other factors than diet may be responsible for the lower parasite richness and diversity observed in present sharks. Some authors have reported a generalized decrease in parasite richness and abundance in Mediterranean with respect to Atlantic teleosts (Pérez-del-Olmo et al. 2009b; Mattiucci et al. 2014; Constenla et al., 2015), and factors such as lower fish size, feeding intensity, dietary diversity and general faunal biomass and abundance in the Mediterranean Sea have been suggested as responsible for this pattern (Constenla et al., 2015). However, comparative data for condrichthyans are at present scarce (parasite communities of only two shark species have been described in the Mediterranean to date, see Dallarés et al., in press), and the low number of specimens of *E. spinax* available in the present study does not allow any generalization.

The parasite community infecting *C. coelolepis* is addressed herein for the first time, and seems characterized by moderate richness and diversity, and overall lower dominance values than those reported for the rest of sharks studied in the Mediterranean (Dallarés et al., in press; present results). The total parasite richness observed in *C. coelolepis* from the Mediterranean Sea resembles that reported for other bathydemersal sharks of similar size in the Atlantic: Palm and Schröder (2001) reported six, seven and nine different parasite taxa from *Heptranchias perlo*, *Deania profundorum* and *Deania histricosa*, respectively, in eastern Atlantic waters. However, parasitological data for *C. coelolepis* presented herein should be considered as representative of mainly juvenile hosts, as these constituted the majority of specimens sampled, and those reported by Palm and Schröder (2001) are from adult specimens (H.W. Palm, personal comm.). The positive correlation between total parasite abundance and body length, coupled with the fact that the largest shark examined harboured the parasite infracommunity with highest richness, indicates that parasite richness and abundance in adult C. *coelolepis* will

probably reach higher values than those presented herein. As outlined for *E. spinax*, the number of specimens available for *C. coelolepis* was low and the outcomes of the present study should thus be considered preliminary and wait to be confirmed by future additional studies.

4.2. The parasite community of G. melastomus in relation to host ontogeny and environmental gradients

A marked differentiation in the parasite communities between ontogenic stages of *G. melastomus* can be clearly appreciated, as highlighted by the MDS and PERMANOVA results. As pointed out by Campbell et al. (1980), changes in parasite fauna during the life history of the host can be directly attributed to changes in its feeding habits. Accordingly, an important ontogenic diet shift has been reported for *G. melastomus* in the study area (Carrassón et al., 1992; present results), with juveniles preying on smaller prey, mainly euphausiids, and adults preferring larger ones including decapod crustaceans, cephalopods and fish. Overall, parasite infracommunities of adult specimens of *G. melastomus* are more abundant, richer and more diverse than those of juvenile hosts. On the one hand, the much higher parasite abundance observed in adult specimens of *G. melastomus* in the present study is mainly explained by the accumulation of histozoic parasites (mainly *G. adenoplusia* in the muscular tissue, see section 4.3.) throughout the lifespan of the fish. On the other hand, the increased parasite richness and diversity may be related to a diversification of the diet in adult specimens, as reported by Carrassón et al. (1992), which implies the consumption of a greater variety of potential intermediate hosts and, therefore, the exposure to a larger diversity of parasites. The preferential consumption of larger prey belonging to higher trophic levels that may harbour more diverse parasite assemblages than the small crustaceans consumed by juvenile sharks is also a plausible explanation.

The generalized homogeneity of parasite communities in juvenile and adult specimens of *G. melastomus* across environmental gradients (i.e. seasons, localities and depth ranges) is altogether surprising. However, although general infracommunity structure does not show environmental-related variations, composition of infracommunities does differ across environmental gradients as a consequences of differences in prevalence and abundance of individual parasite species (see section 4.3.), which is further highlighted by the outcomes of the FCAs.

4.3. Patterns on individual parasites in relation to fish size, diet and environmental gradients and variables

The diphyllidean cestode *D. macrocephalum* was the most important parasite in terms of abundance in juvenile specimens of *G. melastomus*, and steadily decreased in number in adult hosts. This pattern can be explained by the ontogenic diet shift undergone by *G. melastomus* (Carrassón et al., 1992, present results). For diphyllideans, a filter-feeding crustacean is believed to act as first and a shrimp or crab as second intermediate host before the parasite infects the elasmobranch final host (Tyler, 2006). Indeed, larval stages of diphyllideans have been recovered from euphausiid and decapod crustaceans, among others (see Bray and Olson (2004) and references therein), and Dallarés et al. (in press) suggested that the decapod *C. macandreae*, more abundant in the diet of juvenile sharks according to Carrassón et al. (1992), could be the main transmitter of this parasite to *G. melastomus*. Present diet results suggest that the euphausiid *M. norvegica*, the most abundant prey in juvenile hosts, is the most probable source of larval *D. macrocephalum* infecting *G. melastomus* in the Balearic Sea, although *C. macandreae* was also identified among shark's prey and should not be discarded as potential intermediate host.

Ditrachybothrdium macrocephalum was strongly associated to high turbidity levels in the CCA relating parasites and environmental variables. This association was already found by Dallarés et al. (in press) in samples of *G. melastomus* from the same area and 549–809 m depth. Increased water turbidity generally involves more food availability for zooplankton and an increased abundance of these organisms enhance the aggregation of benthopelagic fish (Cartes et al., 2013), which could favour the transmission of this parasite.

The trypanorhynch cestode *G. adenoplusia* was the most abundant and prevalent parasite in adult specimens of *G. melastomus* (note that prevalence reached 100% in all groups), and was also recovered from the largest *C. coelolepis*.

Trypanorhynchs use copepods as first and other invertebrates or small fish as second intermediate hosts (Palm, 2004), and those belonging to the genus *Grillotia* are commonly found in elasmobranchs (Beveridge and Campbell, 2007). *Grillotia adenoplusia* from *G. melastomus* was linked to myctophid fish, which are known to prey on copepods in the sampled area (Bernal et al., 2015). This result is consistent with the outcomes of Dallarés et al. (2014), where the taxon Trypanorhyncha fam. gen. sp. (which grouped specimens of three different species, of which more than 90% belonged

to the genus *Grillotia* (unpublished results)) was linked to myctophids as well. Dallarés et al. (2016) reported an association between *Grillotia* cf. *erinaceus* and the teleost *Gaidropsarus biscayense*. These coincidences suggest that the infection of benthopelagic predatory fish with plerocerci of *Grillotia* is likely to occur, in many cases, through the consumption of fish prey in turn feeding on copepods.

Grillotia adenoplusia was essentially recovered from the musculature, where it was specially located in the caudal region. The progressive acquisition of this parasite throughout the lifespan of the host results in the accumulation of large numbers of plerocerci in the muscular tissue. The preferential location of this parasite in the caudal fin may be explained by the hunting strategy followed by its final host, which would likely be a large shark such as *Dalatias licha* or *Hexanchus griseus*, larger species inhabiting the same depths as *G. melastomus* in the Mediterranean and that feed on other chondrychtians, especially the former (Matallanas, 1982; Stefanescu et al., 1993; Ebert, 1994). Furthermore, *G. adenoplusia* has been recorvered from *H. griseus* in the Atlantic (Beveridge and Campbell, 2013). Indeed, Seamone et al. (2014) demonstrated that the optimal attack strategy for large sharks preying onto smaller relatives is a tail-on approach, i.e. attacking from behind, which would presumably enhance the transmission of parasites located in the tail region. The same pattern of distribution of plerocerci of *Grillotia* has been observed in *C. coelolepis* from the present study and in *E. spinax* from the Atlantic (W. Isbert, personal comm.), which suggests that the tail-on approach is a common hunting strategy among shark predators.

An additional trypanorhynch, *S. viridis*, was recovered from *G. melastomus* and *C. coelolepis* and was found linked to high near-bottom O_2 levels in the latter, which are known to enhance biomass of copepod crustaceans (Cartes et al. 2013) and would presumably enhance the transmission of this parasite. Moreover, *S. viridis* was also found associated to carrion (of shark origin), which could point to an acquisition of this parasite through consumption of infected shark remains. As regards their final host, adult specimens of *S. viridis* have been mainly recovered from *D. licha* (Dallarés et al., 2017), which preys on small sharks in the study area (Matallanas, 1982). The high presence of larval stages of *S. viridis* recovered from *C. coelolepis* suggests that this shark could be an usual prey of *D. licha* in the lower slope of the Balearic Sea.

Similarly to *S. viridis*, Tetraphyllidean larval stages collectively known as *Scolex pleuronectis* are believed to use copepods as first intermediate hosts (Klimpel et al., 2010) and were linked to O_2 levels. These cestodes use different large invertebrates and

fish as paratenic and second intermediate hosts before maturing in a chondrichthyan final host (Euzet, 1994; Klimpel et al., 2010). As suggested by present results, *E. spinax* becomes infected through consumption of cephalopods, while *C. coelolepis* may acquire Tetraphyllidea fam. gen. sp. through feeding on infected carrion (at least in part). The recovery of larger numbers of *S. viridis* and Tetraphyllidea fam. gen. sp. in the biggest specimens of *C. coelolepis* could point to a higher consumption of carrion by larger sharks.

Digenean metacercariae of the genus *Otodistomum* were mostly found encysted in the stomach wall of *G. melastomus* and were more abundant in larger hosts due to accumulation through time (in the same way as described for *G. adenoplusia*), as is characteristic of histozoic parasites (Dallarés et al., 2014). Knowledge on the life cycle of *Otodistomum* is scarce. These digeneans are known to use fish as second intermediate hosts and to mature in elasmobranchs (Rocka, 2006). Although first intermediate hosts are not known for *Otodistomum*, molluscs act as such for digeneans (Gibson, 2002) and, in this sense, *Otodistomum* was associated to squids in the CCA with prey abundance data, which could shed some light on the infection pathway followed by this parasite.

Despite the low prevalence and abundance levels of the nematodes *H. aduncum* and *P. obtusus* in *G. melastomus*, it is worth to note the associations detected between their abundance and high salinity levels. *Hysterothylacium aduncum* has been also linked to salinity levels in the teleost *Phycis blennoides* and in the shark *Scyliorhinus canicula* in the Balearic Sea, in the same way as *P. obtusus* in *G. melastomus* from the same area (Dallarés et al., 2016; Dallarés et al., in press). According to Cartes et al. (2013), salinity levels decrease linearly from 400 m to 1,000–1,200 m depth in the mainland and insular slopes of the Balearic Sea, which is consistent with the association of high salinity levels with hauls of 600–700 m depth (i.e. A301, A302) in the CCA plot relating parasite abundance with environmental variables (actually, *H. aduncum* and *P. obtusus* were only recovered from specimens sampled on the upper slope). Relationships with higher salinity levels could be an indirect effect of higher salinity at shallower depths (associated to the Levantine Intermediate Waters, LIW) in our sampling. In addition, near-bottom salinity levels in the upper slope of the Balearic Islands have been found to correlate with suprabenthos biomass (Cartes et al., 2008). To these depths have been found associated euphausiids and hyperiid amphipods in the mainland slopes (Cartes et al., 2013), as well as the greatest decapod biomass and

diversity of all the bathymetric range encompassed by the continental slope (Fanelli et al., 2013). *Proleptus obtusus* is believed to use decapod crustaceans as intermediate hosts (Moravec, 2007 and references therein; Dallarés et al., in press), while *H. aduncum* can use copepods, isopods, amphipods and mysids as first and larger invertebrates as second intermediate hosts (Køie, 1993; Klimpel and Rückert, 2005). Therefore, environmental conditions in the upper slope seem to favour the abundance of the crustaceans needed by *H. aduncum* and *P. obtusus* to complete their life cycles, and can explain the higher abundance of these nematodes.

The nematode *P. baylisi,* recovered from *G. melastomus,* is known to parasitize scyliorhinid sharks in the adult form (Moravec, 2001; Dallarés et al., in press, present results) but its biology and life cycle are essentially unknown. Nothing is known about its intermediate hosts (if any), but this nematode has been linked to the euphausiid *N. megalops* and myctophid fish in the CCA relating parasite abundance to host prey. Its association to high water turbidity levels could further suggest that its intermediate hosts are favoured by increased levels of organic matter.

In contrast to heteroxenous parasites (i.e. those with indirect life cycles), which are trophically transmitted, prevalence and abundance patterns of monoxenous parasites (i.e. those using a single host to complete their life cycle), such as copepods, are not explained by the feeding habits of their hosts. Other factors, such as abiotic parameters or host population dynamics, determine instead the infection levels of these parasites (Skinner, 1982; Bagge et al., 2004). In this sense, *E. vilelai* was to some extent linked to higher salinity levels, which could be related to the above-explained about depth-related trends at LIW levels. It is possible that the associated increased suprabenthos biomass favours the aggregation of specimens of *G. melastomus* due to higher prey availability. As demonstrated by Bagge et al. (2004), infection levels by directly transmitted parasites scale as a function of fish population size (i.e. overall availability of hosts). Such increase in the quantity of hosts could enhance transmission of *E. vilelai*, which would be translated into higher abundances of this parasite. In the same way as suggested for *G. melastomus*, myctophid fish could also aggregate as a result of increased biomass of benthopelagic fauna, and these coinciding favourable environmental conditions may explain the correlation observed between the abundance of *E. vilelai* and of myctophid fish. As regards the higher copepod abundance in larger hosts, it might indicate that these parasites accumulate in host gills with time, in a similar way as histozoic parasites in tissues.

Concerning the only microparasite detected (i.e. a coccidian of the family Eimeridae, in the intestinal mucosa of *G. melastomus*), it probably belongs to the species *Eimeria palavensis*, described from *G. melastomus* in the NW Mediterranean (Marquès and Capapé, 2001). The observed shape and size of oocysts are consistent with the description provided by the mentioned authors (11.2 to 15.9 μm in diameter in present specimens vs. 12.0 to 14.4 μm). However, some morphological features were impossible to observe due to the methodology used in sample processing and additional observations should be carried out in order to confirm the identity of this coccidian.

4.4. Enzymatic activity levels (AChE, LDH, CS) in relation to fish biological parameters and parasite abundance

Acetylcholinesterase is a key enzyme involved in neurotransmission (Solé et al., 2008, 2010), LDH is a glycolytic enzyme involved in anaerobic metabolism and burst swimming capacity and CS is a key regulating enzyme of the Krebs cycle, and its activity reflects oxygen consumption in aerobic metabolism (Bernal et al., 2003; Drazen and Seibel, 2007, Drazen et al., 2015).

To the best of our knowledge, no data for the muscular enzymatic activities measured in the present study is available in the literature for the three sharks addressed, except for AChE in *G. melastomus*.

A recent study with the blue shark *Prionace glauca* (Alves et al., 2015) reported a catalytic efficiency for muscular AChE similar to that of *E. spinax* and within the range observed considering the three sharks studied herein.

Parasites can be considered as natural stressors to their hosts and, as such, are expected to alter stress and/or metabolic biomarkers. To date, few studies have addressed the effects of parasite infections on enzymatic activity levels in fish (Gupta and Agarwal, 1985; Dautremepuits et al., 2003; Podolska and Napierska, 2006; Pérez-i-García et al., 2015; Dallarés et al., 2014, 2016) and general patterns in relation to this aspect are yet to be described. The negative associations consistently observed between AChE activity levels and parasite abundance (either total or of individual parasite species) in the three sharks addressed may suggest a certain compromise on this enzyme activity caused by parasite infection and its associated stress. Pérez-i-García et al. (2015) and Dallarés et al. (2016) reported similar trends in the teleosts *Alepocephalus rostratus* and *P. blennoides* off the same area, which supports this hypothesis. However, different contrasting trends have been reported by some authors in other fish species (Gupta and

Agarwal, 1985; Podolska and Napierska, 2006). The negative correlations also observed between LDH activity levels and abundance of some parasites have not been reported elsewhere and actually contradict the outcomes by Pérez-i-García et al. (2015), where an opposite trend was observed. These inconsistencies warn against a too simplistic interpretation of these results, especially considering that enzymes respond to many different factors (Solé et al., 2010) that might further interact among them yielding complex activity patterns.

A negative association between AChE activity levels and body size, as observed for *G. melastomus* and *C. coelolepis*, is a generalized trend commonly observed in many fish (Koenig and Solé, 2014; Dallarés et al., 2016) and it is considered to respond to a proportionality between AChE activity and muscular cell surface, which decreases with increasing body size (Lundin, 1962).

Although the comparison of enzymatic activities among the sharks addressed is not one of the main purposes of the present study, it is worth to note that mean CS activity values are much lower in *C. coelolepis* than in *G. melastomus* or *E. spinax* and that AChE activity values reach much higher values in *E. spinax* than in the other two species. Metabolic rates linked to oxygen consumption, in turn related to CS activity, are usually lower in deep-dwelling fishes than in those with a shallower distribution (Treberg et al., 2003; Drazen et al., 2005; Drazen et al., 2015). These trends have been explained on the basis of the visual-interactions hypothesis, which predicts declines in metabolism with depth for visual animals due to a reduction of the distances over which predators and prey interact coupled with lower need for rapid locomotory capacity to chase prey (Drazen and Seibel, 2007). The deeper distribution of *C. coelolepis* could thus account for the observed difference in CS activity. The overall higher AChE activity levels of *E. spinax* could be also explained on the basis of the different ecological habits of these species. Solé et al. (2008) and Solé et al. (2010) reported lower AChE activities in fish with lower mobility and linked to the benthos than in those with high swimming capacity and, therefore, more pelagic habits. These differences were attributed to the need for more mobile species of higher metabolic activities to hunt prey or escape predators (Solé et al., 2010). Of the three sharks addressed, *E. spinax* shows strongly pelagic habits while *G. melastomus* and *C. coelolepis* are demersal species feeding on benthic and benthopelagic prey (Carrassón et al., 1992), which can explain the higher AChE activity levels observed in the former species.

4.5. Fish condition indices in relation to parasites

Parasites showing positive relationships with the gonadosomatic index and the condition factor were more abundant in larger sharks and vice versa. Since these somatic indices also scaled with fish body length, the detected associations are most probably the consequence of these tendencies rather than of a true impact of parasite loads on fish condition. However, the repeated negative correlations detected between the hepatosomatic index and parasite loads, either total or of individual parasites, could be attributed to a detrimental effect of parasites on fish hepatic resources, as suggested by Dallarés et al. (2016) for the teleost *P. blennoides*. This pattern was not detected for *E. spinax* or *C. coelolepis*, and we hypothesize that only in cases in which parasites inflict severe damage to their host (as, for example, *G. adenoplusia* in the muscular tissues of *G. melastomus*, see sections 3.5.1. and 4.6. for details), the re-allocation of liver resources to face infection damage is clearly reflected in the hepatosomatic index.

4.6. Histological assessment in relation to parasites and fish condition indices

Melano-macrophages (MM) are pigmented specialized macrophagic cells occurring mainly in the haemopoyetic tissues of different organs and that contain heterogeneous materials, such as different pigments including melanin, cell debris or lipid droplets (Agius and Roberts, 2003). These structures accumulate degraded materials and metabolic wastes, and respond to the presence of chemicals, pollutants or pathogens (Fournie et al., 2001; Anderson et al., 2003; Carrassón et al., 2008) and to biological factors (e.g. age, size, reproduction or nutritional condition) (Agius, 1985; Montero et al., 1999; Jordanova et al., 2008). The characteristic features of MM in sharks have been described to date in a very low number of species (Pulsford et al., 1982; Agius and Agbede, 1984; Borucinska et al., 2009). In contrast to teleosts, in which the spleen is preferred, the liver is the most adequate organ to quantify these structures in the case of sharks (Borucinska et al., 2009). The observation that MM in *G. melastomus* do not aggregate forming the classical ‘centres’ described in teleosts (Carrassón et al., 2008; Dallarés et al., 2014) is in accordance with previous observations performed in shark species (Borucinska et al., 2009).

Since these structures accumulate throughout the lifespan of the fish (Agius and Roberts, 2003), their number in examined tissues usually scales with body size (Brown and George, 1985; Dallarés et al., 2014, 2016), which is further confirmed by present

results from *G. melastomus*. The almost complete absence of MM in hepatic sections of *E. spinax* and *C. coelolepis* could be attributed to the fact that all examined sections of these species were obtained from juvenile individuals. Increases of hepatic melano-macrophages with reproductive maturity have been reported in teleosts (Elston et al., 1997; Jordanova et al., 2008) and explained on the basis of a liver remodelling phase after the spawning period (see Jordanova et al. (2008) and references therein). A similar pattern might occur in sharks, as suggested by the correlation (weak though) detected between density of MM and female gonadosomatic index in *G. melastomus*.

The correlations detected between density of MM and the activities of the two enzymatic indicators of metabolic activity assessed in the present study (i.e. LDH and CS) are meaningful, since increased metabolism should enhance the production of metabolic waste and, consequently, the formation of MM. The same trend was reported by Dallarés et al. (2014) in the teleost *M. moro* for LDH activity.

As highlighted by Dallarés et al. (2016), caution should be taken when attributing an increased density of MM to parasite infections. Despite the significant associations detected between the former and the abundance of some parasites in the present study, interactions with fish length occurred in some analyses. In the rest of cases, although an interaction was not detected, parasites displaying more abundance in larger sharks showed positive relationships with MM density and vice versa. Therefore, the results obtained suggest that fish size is far more determinant than parasite abundance in explaining MM quantitative variations and that these structures will probably respond to parasites only when these inflict appreciable damage to host tissues (as reported by Dezfuli et al., 2007).

We do not know to which extent muscular tissues infested with larval stages of *G. adenoplusia* lost their functionality. It is plausible that this occurred in the heavily affected tail musculature of adult sharks, taking into account the altered structure of the musculature affected. If this was the case, it could be hypothesized that this parasite would decrease the chance of escaping predators of *G. melastomus*, which would thus enhance the probability of parasite transmission.

Unfortunately, no muscular tissues were freshly fixed in formaline and an accurate histopathological analysis of the tissular alterations induced by *G. adenoplusia* was not possible in the present study. Montero et al. (2015) performed a preliminary histological assessment of these lesions, also in *G. melastomus* from the Mediterranean Sea, and concluded that the parasites were located within dilated muscular fibers and

compressing the adjacent cells. However, no observations regarding the destruction of the muscular tissue were made, probably because the studied sharks were not large adults (as deduced from the prevalence of infection provided, 38.5%, when adult sharks show a prevalence of 100%) and the infestation levels by the parasite were still low.

5. Conclusions

In conclusion, the parasite community of *G. melastomus* is characterized by high abundance, richness and diversity. The cestodes *Ditrachybothridium macrocephalum* and *Grillotia adenoplusia* dominate the infracommunities of juvenile and adult specimens, respectively, in this host. A differentiation of parasite communities, linked to a diet shift, has been observed between ontogenic stages of this species. *Etmopterus spinax* displays a highly depauperate parasite community, which contrasts with results from the Atlantic Ocean. The parasite community of *C. coelolepis*, addressed herein for the first time, shows moderate richness and diversity.

Detailed parasite-prey relationships have been discussed and possible transmission pathways suggested for parasites of the three hosts. Parasites were mostly related to high water turbidity and O_2 levels, which enhance zooplankton proliferation and likely thus enhance parasite transmission. The nematodes *H. aduncum* and *P. obtusus* were in turn linked to high salinity levels, as already reported by previous studies, associated to high biomass and diversity of benthic and benthopelagic crustaceans, which may enhance nematode transmission. A decrease of acetylcholinesterase activity and lower hepatosomatic index, possibly linked to infection-related stress, have been observed. However, caution is recommended when using general fish condition indices and enzymatic activities to assess the impact of parasite infections on fish health. Lesions associated to encapsulated larvae of *G. adenoplusia* have been observed in the muscle of *G. melastomus*, especially in the tail region, which can be indicative of the hunting strategy of its final host and may compromise the escape response of *G. melatomus* thus facilitating parasite transmission.

Conflict of interest

The authors of the present study declare that they have no conflict of interest.

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CHAPTER 7 - MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF *DITRACHYBOTHRIDIUM MACROCEPHALUM* REES, 1959 (CESTODA: DIPHYLLIDEA) FROM *GALEUS MELASTOMUS* RAFINESQUE IN THE WESTERN MEDITERRANEAN

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Morphological and molecular characterisation of *Ditrachybothridium macrocephalum* Rees, 1959 (Cestoda: Diphyllidea) from *Galeus melastomus* Rafinesque in the Western Mediterranean

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Abstract New morphological, molecular and ecological data for *Ditrachybothridium macrocephalum* Rees, 1959 (Cestoda: Diphyllidea) are presented and discussed based on specimens recovered from the blackmouth catshark *Galeus melastomus* Rafinesque (Scyliorhinidae) in the Western Mediterranean. A redescription of the plerocercus of this parasite is provided and new data on immature and mature worms including the first description of the eggs are reported, based on light and scanning electron microscopy observations. Analysis of 28S rDNA (domains D1–D3) sequences from plerocerci, immature and adult specimens revealed that they are conspecific with specimens from the North East Atlantic. Although previous authors considered that museum specimens identified as *D. macrocephalum* may represent more than one species, examination of type- and voucher material revealed no relevant morphological differences between museum specimens and the present material. Information on infection levels of *D. macrocephalum* is provided from a large number of host specimens (n = 170). This species was more abundant in juvenile than in adult hosts and on the middle slope than on the upper slope; this may be related to ontogenetic and bathymetric diet shifts of *G. melastomus*.

Sara Dallarés and Ana Pérez-del-Olmo contributed equally towards this study.

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Introduction

Members of the cestode order Diphyllidea van Beneden in Carus, 1863 are currently assigned to six genera infecting the spiral valves of different elasmobranchs, mainly batoids (Caira et al., 2013). The genus *Ditrachybothridium* Rees, 1959 was erected to accommodate *D. macrocephalum* Rees, 1959, based on ten immature and two adult but not yet gravid specimens from *Leucoraja fullonica* (L.) and *Leucoraja circularis* (Couch) and the small-spotted catshark *Scyliorhinus canicula* (L.) from off the west coast of Scotland, North East (NE) Atlantic (Rees, 1959). The second and only other species of the genus, *Ditrachybothridium piliformis* Faliex, Tyler & Euzet, 2000, was described from *Galeus priapus* Séret & Last from off Vanuatu (South Pacific) (Faliex et al., 2000).

Available data on the distribution and host specificity of the parasites of this genus are scarce and exclusively based on specimens of *D. macrocephalum* collected in the NE Atlantic. Williams (1960) recorded this species from *S. canicula* and *L. fullonica* (as *Raja fullonica*) and Bray & Olson (2004) recovered plerocerci from the scyliorhynid *Apristurus laurussonii* (Saemundsson) and the rajid *Rajella* cf. *bigelowi*. Additionally, the latter authors provided a brief description of this larval stage and characterised individuals collected from both hosts molecularly. Tyler (2006) carried out a revision of the genus in which he included a description of the only mature (gravid) specimen found to date (accession no. BMNH 1973.6.11.11–13) from the blackmouth catshark *Galeus melastomus* Rafinesque off North West Utsira (North Sea), as well as the first ultrastructural description based on scanning electron micrographs of the scolex. However, no detailed data exist on the morphology of the scolex and eggs of *D. macrocephalum*. Furthermore, as a result of re-examination of the museum material, Caira et al. (2013) suggested that the specimens currently assigned to *D. macrocephalum* may actually comprise more than one species and highlighted the need of a detailed morphological and molecular characterisation of this species.

The present study represents the first record of *D. macrocephalum* from the deep-sea scyliorhinid *G. melastomus* in the Mediterranean. Molecular and morphological data are provided for the plerocerci, immature and adult worms as well as information on the infection levels of this parasite in its definitive host of different size classes and from different depths and seasons.

Materials and methods

Collection and processing of the material

A total of 170 specimens of *G. melastomus* was collected using a semi-balloon otter trawl at depths between 550 m and 1,236 m in 2007 (40°34.45′N, 01°26.44′E – 41°7.85′N, 02°13.34′E) and 2010, 2011 and 2014 (41°04.28′N, 02°10.81′E – 41°14.49′N, 02°29.34′E) on the continental slope of the Balearic Sea (off Barcelona, Spain). Immediately upon capture, elasmobranchs were measured (total length, TL). Cestodes collected in 2014 were recovered from the spiral valves, killed in almost boiling saline solution and fixed in 10% buffered formalin. Elasmobranchs from earlier collections (i.e. 2007, 2010 and 2011) were frozen at −20°C in individual plastic bags directly on board for further examination. In the laboratory, fish were dissected and thoroughly examined under stereomicroscope for the presence of parasites according to a standardised protocol. Specimens of *D. macrocephalum* were preserved in 70% ethanol. Segments of plerocerci, immature and mature specimens were preserved in 100% molecular grade ethanol for molecular analyses.

Whole mounts were stained with Mayer's hydrochloric carmine or iron acetocarmine, dehydrated through an alcohol series, cleared in dimethyl phthalate and mounted in Canada balsam. Cross sections of the strobila (15 μm thick) were stained with hematoxylin-eosin, using standard histological methodology (Scholz & Hanzelová, 1998). Several specimens of all developmental stages were prepared for scanning electron microscopy (SEM) following the procedure outlined by Kuchta & Caira (2010). Terminology of microtriches follows Chervy (2009). All measurements are in micrometres unless otherwise stated and are presented as the range followed by the mean, the standard deviation (when number of measurements ≥25) and the number of measurements taken (n) in parentheses.

Voucher specimens are deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences (IPCAS C–693). The following type- and voucher specimens were examined from the Natural History Museum, London, UK (BMNH): three paratypes (BMNH 1959.8.4.193–196) and six voucher specimens (BMNH 1976.4.13.39–41; BMNH 2004.1.6.1–5) of *D. macrocephalum* and one paratype of *D. piliformis* (BMNH No.1999.10.6.1–2).

Molecular analyses

Genomic DNA was isolated from the terminal proglottid of five plerocerci, one immature and one mature cestode and used to amplify the D1–D3 regions of the nuclear large subunit ribosomal DNA (28S rDNA). Extraction was performed using a Qiagen™ (Valencia, California, USA) DNeasy® Tissue Kit following manufacturer's instructions. Polymerase chain reaction (PCR) amplifications were performed as described in Constenla et al. (2014) using the

primers and conditions described in Fyler et al. (2009). PCR amplicons were purified using a Qiagen™ MinElute® PCR Purification Kit and sequenced directly for both strands using the PCR primers, LSU5 and 1200F. Sequences were assembled and edited using Mega v6 (Tamura et al., 2013) and submitted to GenBank under accession numbers KR653219–KR653221. Sequences were aligned using Clustal W as implemented in MEGA v6 together with published sequences by Bray & Olson (2004) and Caira et al. (2013) for *D. macrocephalum* (GenBank AY584865), *Andocadoncum meganae* Abbott & Caira, 2014 (GenBank KC860137; annotated as Echinobothriidae gen. n., sp. n. 1 in GenBank), *Ahamulina catarina* Marques, Jensen & Caira, 2012 (GenBank KC860132) and *Coronocestus* n. sp. 1 *sensu* Caira et al. (2013) (GenBank KC860134); the latter taxon was used as an outgroup.

Maximum likelihood (ML) and Bayesian inference (BI) algorithms were used for phylogenetic tree reconstruction. Prior to analyses the best-fit model of nucleotide substitution was selected with jModelTest 2.1.1 (Guindon & Gascuel, 2003; Darriba et al., 2012) using the Bayesian Information Criterion (BIC); this was the Hasegawa-Kishino-Yano model including estimates of invariant sites (HKY + I). ML analyses were performed in PhyML 3.0 (Guindon et al., 2010) with a non-parametric bootstrap validation based on 100 replicates. BI analyses were carried out in MrBayes 3.2 (Ronquist et al., 2012) using Markov Chain Monte Carlo (MCMC) searches on two simultaneous runs of four chains during 10^7 generations, sampling trees every 10^3 generations. The first 25% of the sampled trees were discarded as 'burn-in' and the consensus tree topology and nodal support were estimated from the remaining samples as posterior probability values (Huelsenbeck et al., 2001). The calculation of the distance matrix (p-distance model) was performed with Mega v6.

Assessment of the infection levels

Descriptors of the parasite infection of fish follow Bush et al. (1997). Prevalence (P in %) and mean abundance (MA) were calculated for plerocerci and immature/adult worms, except for the samples collected in 2007, in which the developmental stage of the parasites was not determined.

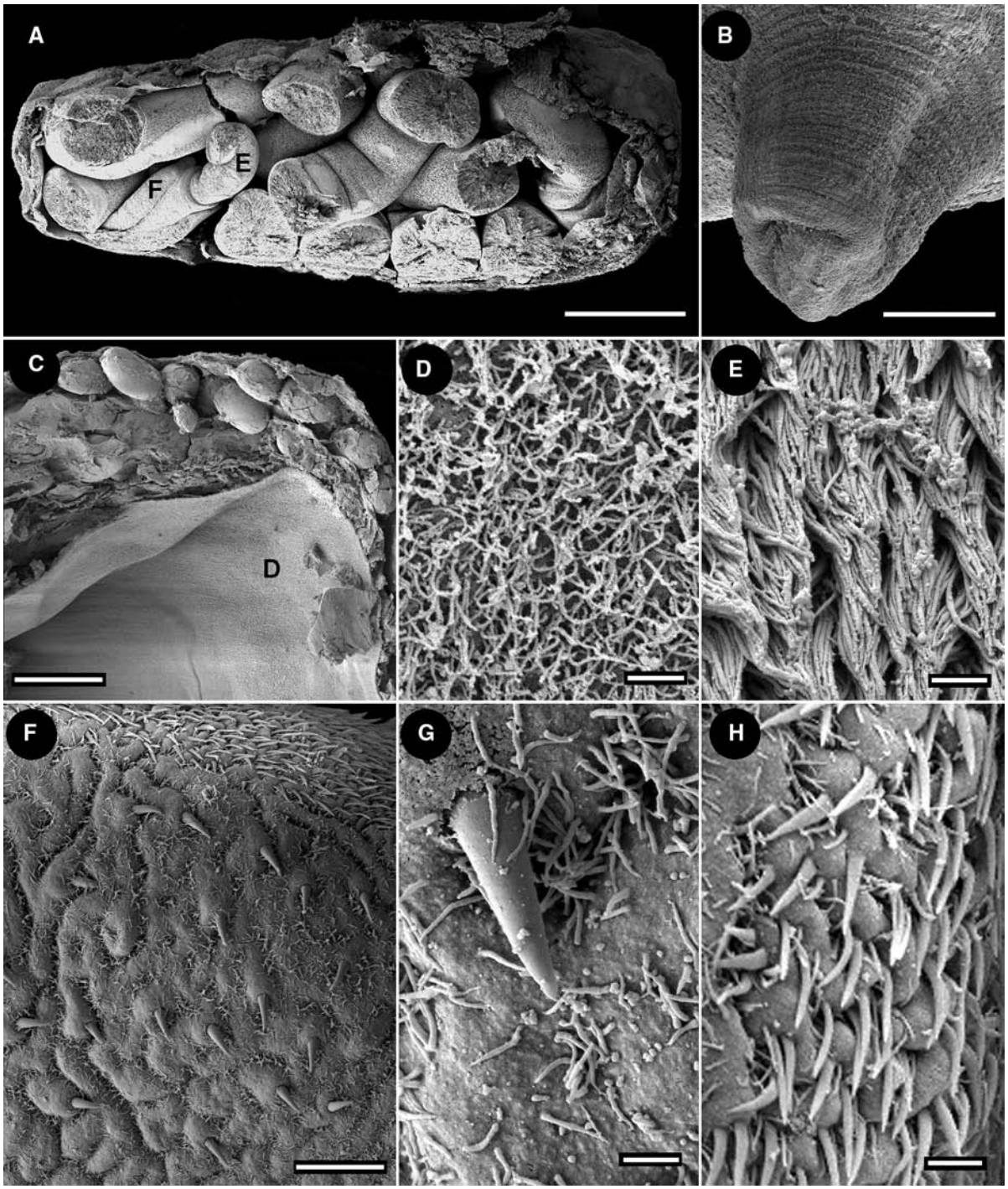
Sharks were grouped in relation to (i) size: two size-classes, size-class 1 (TL $<$ 40 cm) and size-class 2 (TL $\geq$ 40 cm), corresponding to juveniles and adults, respectively (MacPherson, 1980); (ii) depth: two depth categories, depth 1 (500–999 m) and depth 2 (1,000–1,400 m), corresponding to the upper and middle slopes, respectively (D'Onghia et al., 2004); and (iii) season (spring, summer and autumn). Since no data from different depths were available for the samples collected in spring, data from these specimens were excluded from the analyses related to depth.

Using individual fish as replicate samples, differences in abundance and prevalence of the sum of plerocerci and adult stages of *D. macrocephalum* were tested by means of generalized linear models (GZM) for the factors season, depth and size-class (applying negative binomial model for abundance and logistic model for prevalence data).

Morphological characterisation of specimens of *D. macrocephalum*

Material examined: (i) ten plerocerci (one live plerocercus, two manually-excysted cestodes in whole mounts, two plerocerci in longitudinal sections, one manually-excysted cestode in cross sections, and two plerocerci and two manually-excysted cestodes for SEM); (ii) one immature cestode (whole mount); (iii) five fully-mature (gravid) cestodes (two complete strobilae in whole mounts, three scolices for SEM); and (iv) eggs from frozen material for light microscopy and SEM.

Live plerocerci were brownish in colour, with almost transparent double wall, oval or elongate in shape, and displayed a contraction-extension behaviour resulting in a crawling movement with directional displacement. Whole-mounted plerocerci measured 2,395–7,692 (4,466; n = 4) in length and 1,022–1,815 (1,418; n = 4) in width (Fig. 1A). In the anterior part of the plerocercus, the inner wall formed a narrow channel connecting the cavity with the strobila and the exterior (Fig. 2A). The latter was associated with muscular tissue forming a sphincter-like structure. The opposite extremity showed an invagination on the external surface associated internally with strong musculature (Figs. 1B, 2B). The external surface was smooth, with weak transverse striations on both extremities (Fig. 1B). The inner wall was covered by capilliform filitriches *c.*2 long on its inner surface (Fig. 1D). Several transparent thin-



walled oval inclusions (*c*.80 × 50) were observed between both layers (Figs. 1A, C, 2C); these were more abundant on the extremes of the plerocercus, especially at the posterior end, and histological sections revealed the presence of unidentified, poorly-stained material within them (Fig. 2C). Almost the entire cavity of the plerocercus was occupied by the coiled immature cestode (Fig. 1A), fully strobilated

◀**Fig. 1** Scanning electron micrographs of plerocercus of *Ditrachybothridium macrocephalum* ex *Galeus melastomus* from the Western Mediterranean. A, Fraction of plerocercus; B, Detail of posterior opening of the plerocercus; C, Detail of inner and outer layers of the plerocercus with inclusion bodies; D, Detail of inner wall of the plerocercus covered with capilliform filitriches; E, Detail of cephalic peduncle covered with long capilliform filitriches; F, Proximal bothrial surface with large thorn-shaped spines and capilliform filitriches at boundary of distal bothrial surface covered with trifurcate spinitriches; G, Detail of large thorn-shaped spines interspersed with capilliform filitriches; H, Detail of gladiate spinitriches interspersed with capilliform filitriches. *Note*: small letters correspond to figures showing higher magnification images of these surfaces. *Scale-bars*: A, 500 μm; B, C, 100 μm; D, E, G, H, 1 μm; F, 10 μm

with visible genital primordia and differentiated scolex. The scolex had no specific position, whereas the end of the strobila was located on the posterior part and attached to the inner wall (Fig. 1A). The scolex consisted of the scolex proper and a short cephalic peduncle. The scolex proper consisting of paired bothria measured 764–954 × 374–448 (n = 2). The distal bothrial surface was covered with gladiate spinitriches (*c.*1 long) and interspersed with capilliform filitriches (Fig. 1F, H). The proximal surface was armed with large thorn-shaped spines *c.*10 long and interspersed with capilliform filitriches (Fig. 1F, G). The spines may also be covered by tegument (Fig. 1F). The strobilae liberated from plerocerci were *c.*8–16 mm long, comprised 36–42 proglottides and were covered with long capilliform filitriches (Fig. 1E).

Total length, number of proglottides and sizes of scolex and bothria of non-encysted immature cestodes were similar to those in encysted individuals.

Mature cestodes measured *c.*45–60 mm in length and comprised 49–52 proglottides: 24–31 immature proglottides, 36–1,000 (378 ± 284; n = 54) long and 415–795 (530 ± 90; n = 55) wide (first immature proglottides 36–268 long; late immature proglottides 292–1,000 long); 5 mature proglottides, 1,048–1,518 (1,241; n = 9) long and 805–1,133 (881; n = 9) wide; and 13–23 gravid proglottides, 1,398–4,024 (2,286 ± 632; n = 30) long and 878–2,289

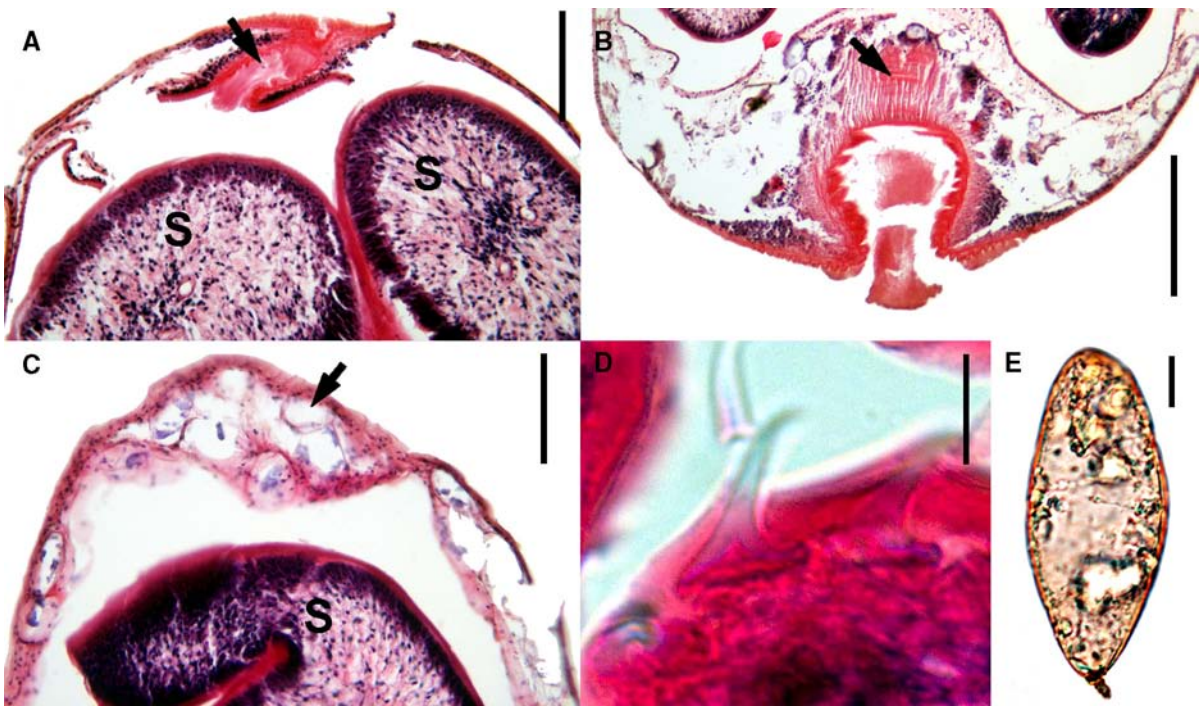


Fig. 2 Photomicrographs of histological sections of plerocercus of *Ditrachybothridium macrocephalum* ex *Galeus melastomus* from the Western Mediterranean. A, Anterior part of plerocercus (*arrow* indicates the narrow channel connecting the cavity with the strobila and the exterior); B, Posterior part of plerocercus (*arrow* indicates thick musculature); C, Detail of inner and outer layers of plerocercus (*arrow* indicates inclusion body); D, Detail of spines; E, Egg. *Abbreviation*: S, strobila. *Scale-bars*: A–C, 100 μm; D, E, 10 μm

(1,420 ± 416; n = 32) wide. Bothria measured 1,244–1,434 in length and 512–578 in width (n = 2) (Fig. 3A, B). Distal bothrial surfaces were covered with gladiate, coniform or trifurcate spinitriches, interspersed with capilliform filitriches (Fig. 3D–F, I). Proximal bothrial surfaces were armed with large thorn-shaped spines, 11–23 (16 ± 2.1; n = 28) long, and interspersed with capilliform filitriches (Figs. 2D, 3C, G, H); spines may be covered by tegument (Fig. 3C). The cephalic peduncle and strobila were covered with long capilliform filitriches *c.*5 long (not shown).

Eggs were spindle-shaped, thin-walled, with smooth shells without ornamentation and with mucron at one pole, 50–62 (56 ± 3; n = 25) long and 20–26 (24 ± 1; n = 25) wide (Fig. 2E).

Molecular characterisation of specimens of *D. macrocephalum*

A total of seven partial 28S rDNA (domains D1–D3, 1,182 nt) sequences was generated from five plerocerci, one immature and one mature specimen. All newly-generated sequences were identical and differed by 0.5% (4 nt) from the sequences of *D. macrocephalum* generated by Bray & Olson (2004) from two plerocerci ex *R.* cf. *bigelowi* and *A. laurussonii* collected in the NE Atlantic.

Both model-based algorithms (ML and BI) produced identical trees with *A. catarina*, *A. meganae* and *D. macrocephalum* forming a monophyletic clade. Sequences for *D. macrocephalum* obtained from *G. melastomus* collected in the Mediterranean and NE Atlantic formed a strongly supported lineage (Fig. 4).

Assessment of the infection levels

A total of 53 worms was found. The overall prevalence and mean abundance of *D. macrocephalum* were 21% and 0.31 ± 0.75 worms per fish, respectively (Table 1). The overall percentage of plerocerci with respect to the total number of worms was 28% (62%, 38% and 7% in spring, summer and autumn samples, respectively). Values for prevalence and mean abundance of pooled developmental stages, plerocerci and immature/adult worms found in host groups stratified by size-class and depth by seasons are given in Table 1.

Fig. 3 Scanning electron micrographs of mature *Ditrachybothridium macrocephalum* ex *Galeus melastomus* from the Western Mediterranean. A, Scolex, dorsoventral view; B, Scolex, lateral view; C, Detail of distal (*right*) and proximal (*left*) bothrial surface, showing protuberances containing spines; D–F, I, Details of distal bothrial surface showing gladiate, coniform or trifurcate spinitriches; G, H, Details of thorn-shaped spines. *Note*: small letters correspond to figures showing higher magnification images of these surfaces. *Scale-bars*: A, B, 100 μm; C, 10 μm; D–F, I, 1 μm; G, H, 2 μm

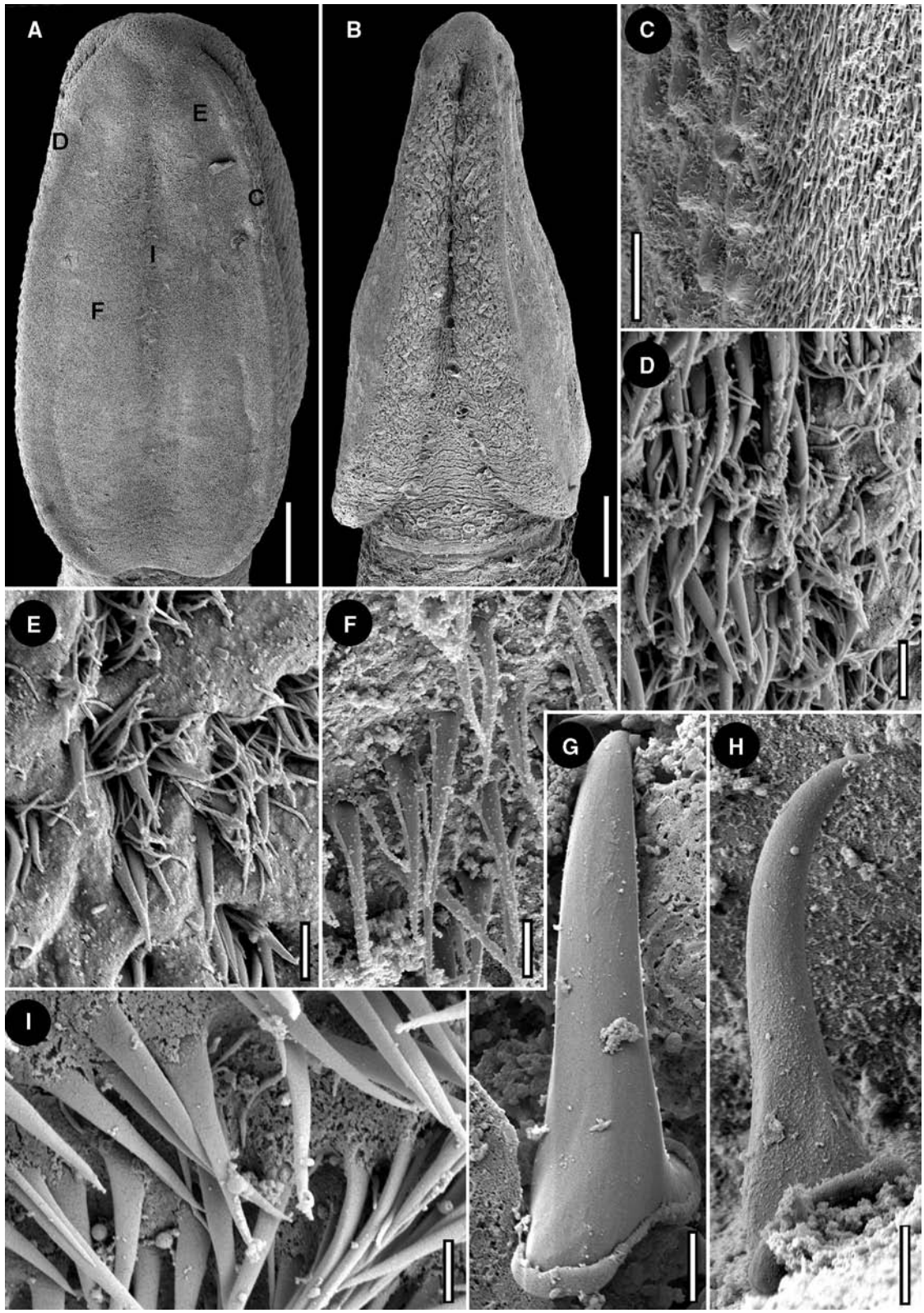
No interaction was found among the factors depth, season and host size-class for the total sample of *D. macrocephalum* (GZM, $p > 0.05$). Since an interaction between the factors season and depth was found for cestode prevalence and mean abundance (GZM, $\chi^2 = 6.544$, $p = 0.011$ and $\chi^2 = 6.901$, $p = 0.009$, respectively), the effect of the factor depth was tested separately within each season. A significant effect of the factor depth was detected in the summer sample, with higher parasite prevalence and mean abundance in sharks from greater depths (i.e. 1,000–1,400 m) (GZM, $\chi^2 = 9.544$, $p = 0.002$ and $\chi^2 = 10.454$, $p = 0.001$, respectively). No differences between depth strata for parasite prevalence or abundance were detected in autumn (GZM, $p > 0.05$). A host size-effect was also found, with significantly higher mean parasite abundance in smaller/juvenile sharks (GZM, $\chi^2 = 4.511$, $p = 0.034$). No effect of the size-class was found for parasite prevalence (GZM, $p > 0.05$).

Discussion

This study provides morphological, molecular and ecological data on different developmental stages of *D. macrocephalum* from its definitive host in the Mediterranean for the first time.

Molecular analyses confirmed that the plerocerci, inmature and adult specimens recovered from *G. melastomus* during the present study and the specimens studied by Bray & Olson (2004) are conspecific.

The plerocercus of *D. macrocephalum* was first described by Bray & Olson (2004) from *A. laurussonii* (Scyliorhinidae) and *R.* cf. *bigelowi* (Rajidae) in the NE Atlantic. When compared to those from the Mediterranean, an almost identical morphology was observed, with some slight differences that we attribute to differences in the state of development of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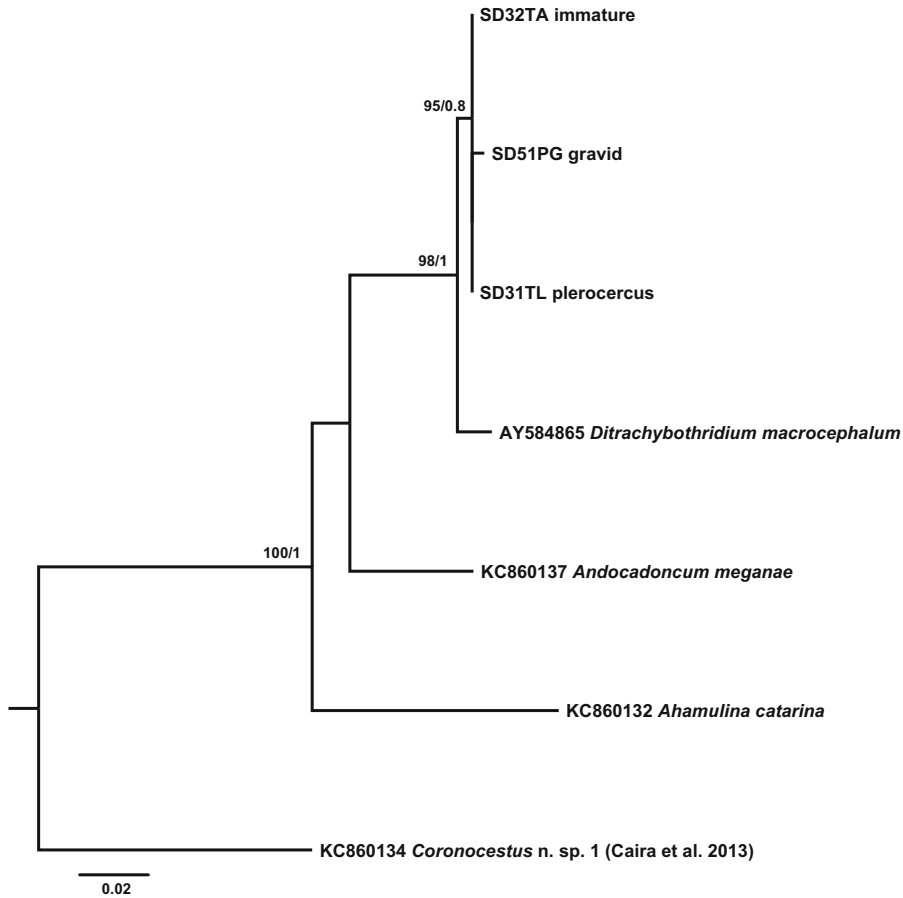

Fig. 4 Maximum likelihood (ML) phylogram reconstructed using the newly-generated and retrieved from the GenBank 28S rDNA sequences for *Ditrachybothridium macrocephalum*, *Andocadoncum meganae* and *Ahamulina catarina* with nodal support from ML and Bayesian inference (BI) analyses indicated as ML/BI. Outgroup: *Coronocestus* n. sp. 1 (Caira et al. 2013). The scale-bar indicates the expected number of substitutions per site

Table 1 Number (n) of specimens of *Galeus melastomus* examined and prevalence (P) and mean abundance (MA ± standard deviation, SD) of *Ditrachybothridium macrocephalum* within size-groups of sharks, depth strata, seasons and in the total sample

		n[a]	Pooled developmental stages[a]		n[b]	Plerocerci[b]		Immature/ adult worms[b]	
			P (%)	MA ± SD		P (%)	MA ± SD	P (%)	MA ± SD
Size-class 1 (TL < 40 cm)		83	25	0.40 ± 0.91	45	9	0.09 ± 0.29	42	0.64 ± 1.11
Size-class 2 (TL ≥ 40 cm)		87	17	0.23 ± 0.54	58	17	0.19 ± 0.44	12	0.14 ± 0.40
Depth 1 (< 1,000 m)	All seasons	125	18	0.26 ± 0.66	63	13	0.14 ± 0.40	27	0.37 ± 0.79
	Spring	30	37	0.43 ± 0.63	30	23	0.27 ± 0.52	17	0.17 ± 0.38
	Summer	70	3	0.03 ± 0.17	14	–	–	14	0.14 ± 0.36
	Autumn	25	40	0.68 ± 1.14	19	5	0.05 ± 0.23	5	0.84 ± 1.21
Depth 2 (≥ 1,000 m)	All seasons	45	29	0.47 ± 0.94	40	15	0.15 ± 0.36	23	0.35 ± 0.89
	Summer	29	28	0.38 ± 0.68	26	19	0.19 ± 0.40	19	0.23 ± 0.51
	Autumn	16	31	0.63 ± 1.31	14	7	0.07 ± 0.27	29	0.57 ± 1.34
Total		170	21	0.31 ± 0.75	103	14	0.15 ± 0.38	25	0.36 ± 0.83

[a] Based on samples of 2007, 2010, 2011 and 2014; [b] Based on samples of 2010, 2011 and 2014

the worms. The observed variations consisted in a better developed musculature of the posterior part of the plerocercus, a smaller number of inclusion bodies and a longer strobila in the present material.

Bray & Olson (2004) used the term "vacuoles" to define the inclusion bodies observed between the two walls of the plerocercus. However, this terminology should not be considered since vacuoles are intracellular organelles surrounded by a membrane that may contain a variety of substances (e.g. water, secretions or enzymes) (Venes, 2001) whereas the structures present in the plerocercus of *D. macrocephalum* are much larger than and external to surrounding cells, and are delimited by a shell-like wall. The inclusion bodies contained some poorly stained material, which could not be identified; histochemical techniques would be required in order to confirm the nature of their content.

Caira et al. (2013) observed differences in bothrial length and width and a diversity of "spines" when examining museum material identified as *D. macrocephalum* (paratypes: accession nos. BMNH 1973.6.11.11–13; 2004.1.6.6–11; 2004.1.6.1–5; 2001.19.5) and suggested that they represent several distinct species. We observed no substantial differences in the size and shape of the scolex and bothria between the newly-collected specimens and the museum material examined in the present study. However, there were differences in the length of the spines on the proximal surfaces of the scolex. The spines in the present material were 16 μm long on average, similar to those in the material of Williams (1960) (18 μm; BMNH 1976.4.13.39–40 and 1976.4.13.41), but distinctly shorter than those in the specimens described by Bray & Olson (2004) (33 μm; BMNH 2004.1.6.1–5) and in the type-material collected by Rees (1959) (23 μm; BMNH 1959.8.4.193–196). These structures were considered as big coniform spinitriches by Caira et al. (2013). However, microtriches are described as cellular surface structures (Chervy, 2009; Poddubnaya & Mackiewicz, 2009), while in the histological sections from the present study they appeared embedded in the syncytial tegument, even reaching the muscular layers beneath it (Fig. 2E). The fact that they were still internalised (covered by tegument) and only appearing as protuberances on the surface of the scolex of adult and larval specimens also indicates that they are not surface structures of the tegument.

The presence of trifurcate spinitriches on the distal bothrial surface is typical for diphyllideans, including *D. macrocephalum* (see Caira et al., 2013; Abbot & Caira, 2014). In the present study, the distal bothrial surface of some mature worms was covered with trifurcate spinitriches (Fig. 3F), but most studied specimens were covered with gladiate or coniform spinitriches instead (Figs. 1H, 3D, E, I). Since their bases were not clearly visible, in some cases they may actually represent trifurcate spinitriches.

This study provides the first data on the eggs of *D. macrocephalum*. Their morphological characteristics are similar to those described for *D. piliformis* by Faliex et al. (2000); however, in *D. macrocephalum* they are smaller in size (56 × 24 *vs* 75 × 29 in *D. piliformis*) and tend to be slightly less elongated.

The higher abundance of *D. macrocephalum* in juvenile than in adult sharks, and from the middle slope with respect to the upper slope, is probably related to ontogenetic and bathymetric diet shifts of the definitive hosts, as it has been reported by Carrassón et al. (1992) for *G. melastomus* from the same locality.

Despite the predominance of strict host-specificity in diphyllidean cestodes (oioxeny; Caira et al., 2013; Abbot & Caira, 2014), *D. macrocephalum* seems to exhibit an extremely wide host spectrum (euryxeny) being reported from three scyliorhinids (*S. canicula*, *A. laurussonii* and *G. melastomus*), one squalid [*Etmopterus spinax* (L.)] and four species of rajids (*L. fullonica*, *L. circularis*, *R.* cf. *bigelowi* and *Rajella fyllae* Lütken) (Rees, 1959; Williams, 1960; Bray & Olson, 2004; Isbert et al., 2015; present study). However, mature specimens have only been found in *G. melastomus*, suggesting that the other hosts may not represent definitive, but rather accidental or unspecific hosts in which the life-cycle is not completed.

Our results expand the knowledge on the geographical range of *D. macrocephalum* from the NE Atlantic into the Mediterranean Sea. In contrast, the distribution of *D. piliformis* seems to be restricted to the Pacific Ocean (Tyler, 2006). Given the fact that until now, only a few scyliorhinid species have been examined for parasites (Tyler, 2006), a much greater diversity of *Ditrachybothridium* spp. is likely to be discovered and the geographical distribution of the species within the genus might expand in future studies.

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CHAPTER 8 - REVISION OF THE FAMILY SPHYRIOCEPHALIDAE PINTNER, 1913 (CESTODA: TRYPANORHYNCHA), WITH THE DESCRIPTION OF *HETEROSPHYRIOCEPHALUS ENCARNAE* N. SP. AND REDESCRIPTIONS OF TWO SPECIES OF *SPHYRIOCEPHALUS*

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Revision of the family Sphyriocephalidae Pintner, 1913 (Cestoda: Trypanorhyncha), with the description of *Heterosphyriocephalus encarnae* n. sp. and redescriptions of two species of *Sphyriocephalus*

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ABSTRACT

The family Sphyriocephalidae Pintner, 1913, which comprises the genera *Hepatoxylon* Bosc, 1811, *Sphyriocephalus* Pintner, 1913 and *Heterosphyriocephalus* Palm, 2004, is revised from newly-collected and museum material. *Heterosphyriocephalus encarnae* n. sp. is described from the pelagic thresher, *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae) collected from the Pacific Ocean off Boca del Alamo, Mexico. This species can be readily distinguished from the rest of sphyriocephalids by its small size, low number of proglottids and long velum with a characteristically irregular and folded border, among other features. The tentacles show a distinctive basal armature, and a heteroacanthous typical metabasal armature with heteromorphous hooks. Redescriptions are provided for *Sphyriocephalus tergestinus* Pintner, 1913 and *S. viridis* (Wagener, 1854) Pintner 1913 based on novel morphological data. A phylogenetic analysis including the available sequences of sphyriocephalid species plus new generated sequences of *S. tergestinus* has been performed, from which *S. tergestinus* is allocated into *Heterosphyriocephalus* as *H. tergestinus* n. comb. New dichotomous keys for the determination of genera of Sphyriocephalidae are provided, as well as new generic diagnoses for *Sphyriocephalus* and *Heterosphyriocephalus* and keys for the determination of species within both genera. Although the morphology of the genus *Hepatoxylon* is not addressed in the present study, the available sequence of the type-species has been incorporated in the phylogenetic analysis and its relationship to the other two genera of the family is discussed.

1. Introduction

The family Sphyriocephalidae Pintner, 1913 was erected to accommodate two species, *Sphyriocephalus viridis* (Wagener, 1854) Pintner 1913, collected as adults from the kitefin shark *Dalatias licha* (Bonnaterre) (Squaliformes: Dalatiidae) and the gulper shark *Centrophorus granulosus* (Bloch and Schneider) (Squaliformes: Centrophoridae), and *Sphyriocephalus tergestinus* Pintner, 1913, collected as adults from the thresher shark *Alopias vulpinus* (Bonnaterre) (Lamniformes: Alopiidae) and as plerocercoids from the teleosts *Lepidopus caudatus* (Euphrasen) (Perciformes: Trichiuridae) and *Brama brama* (Bonnaterre) (Perciformes: Bramidae). Both trypanorhynch species were originally described from the Mediterranean Sea off Trieste, Italy. Following the re-description by Dollfus [1], this family included trypanorhynch cestodes that were characterized by (i) a large and muscular scolex, (ii) two circular bothria with fleshy rims and fused margins, (iii) tentacles emerging from the bothrial cavity, (iv) a basal swelling and characteristic basal armature, (v) transversely or obliquely oriented bulbs, (vi) a retractor muscle attached at the anterior part of the bulb, (vi) absence of a cirrus-sac (i.e. the cirrus is invaginated in the distal part of the ejaculatory duct), (vii) eggs with two prolongations on opposite ends and (viii) plerocercoid larval stages lacking a blastocyst.

Guiart [2] described a third species, *Sphyriocephalus alberti* Guiart, 1935, on the basis of plerocercoid stages from the Portuguese dogfish, *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello (Squaliformes: Somniosidae) collected from the Mediterranean Sea off Calvi, France. This species was considered a synonym of *S. viridis* by Dollfus [1]. The synonymization was later confirmed by Bussieras [3] based on a detailed re-examinations of the type material. The latter author provided a very accurate description of the tentacular armature for *S. viridis*, which had initially been described as homeoacanthous, and noted that the disposition of hooks in the metabasal tentacular armature of this species clearly followed a heteroacanthous pattern. His suggestion that this feature could also be present in closely related species was later supported by Beveridge and Campbell [4], who examined two plerocercoids of *S. tergestinus* from the blue hake *Macruronus novaezelandiae* (Hector) (Gadiformes: Merluccidae), collected off

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Tasmania, Australia, and confirmed that a heteroacanthous pattern is present in the metabasal armature.

In contrast, *Sphyriocephalus pelorosoma* Heinz and Dailey, 1974, described from a single adult specimen found in the bigeye thresher, *Alopias superciliosus* (Lowe) (Lamniformes: Alopiidae) from the Pacific Ocean off Bolsa Chica State Beach, California [5], and *Sphyriocephalus dollfusi* Bussieras and Aldrin, 1968, described from a single plerocercoid from the stomach of the bigeye tuna *Thunnus obesus* (Lowe) (Perciformes: Scombridae) from the Atlantic Ocean off the western African coast [6], are considered to possess homeoacanthous armature patterns [7].

More recently, Palm [7] erected the new genus *Heterosphyriocephalus* Palm, 2004 to accommodate *H. oheulumiae* Palm, 2004 based on plerocercoids found in the sickle pomfret, *Taractichthys steindachneri* (Döderlein) (Perciformes: Bramidae) from the Indian Ocean off Pelabuhan Ratu, Indonesia and in the yellowfin tuna *Thunnus albacares* (Bonnaterre) (Perciformes: Scombridae) from an unknown locality. Species of this genus are characterized and differ from species of *Sphyriocephalus* in the possession of a typical heteroacanthous, heteromorphous armature and absence of a distinct basal swelling and basal armature.

Pintner [8] considered that the genera *Dibothriorhynchus* Blainville, 1828 (now a synonym of *Hepatoxylon* Bosc, 1811) and *Sphyriocephalus* form the subfamily Sphyriocephalinae. However, the former genus was later placed within the family Hepatoxylidae by Dollfus [9], who only recognized *Sphyriocephalus* in the Sphyriocephalidae and thus refuted the synonymy of both families. Later [10] synonymized the Hepatoxylidae with the Sphyriocephalidae based on their morphological affinities regarding the tentacular armature, oncotaxy, attachment site of the retractor muscle in the bulb and features of the surface ultrastructure [7].

At present, the family Sphyriocephalidae comprises the genera *Hepatoxylon*, *Heterosphyriocephalus* and *Sphyriocephalus*. The genus *Sphyriocephalus* currently includes four valid species, namely *S. dollfusi*, *S. pelorosoma*, *S. tergestinus* and *S. viridis*. However, the necessity of a re-examination of the type material of *S. tergestinus* and *S. viridis* (to confirm their armature patterns) and of adequate re-descriptions based on museum and newly collected material (in order to provide a complete description for both species) has been stressed by several authors [3,4,7].

In the present study, the family Sphyriocephalidae is revised and a reconfiguration of genera is performed based on molecular and morphological data. A new species of *Heterosphyriocephalus* from the pelagic thresher, *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae) collected from the Pacific Ocean off Boca del Alamo, Mexico, is described. Furthermore, redescriptions are provided for *Sphyriocephalus tergestinus* and *S. viridis* based on museum and newly collected material. New generic diagnoses are provided for *Sphyriocephalus* and *Heterosphyriocephalus*, as well as keys for the determination of both genera and species.

2. Materials and methods

2.1. Collection of specimens

Adult and juvenile worms and plerocercoids of all species addressed in the present study were recovered from specimens of *A. pelagicus* from the Gulf of California off Boca del Alamo (23°53′N, 109°48′W) (Mexico); *A. vulpinus* from off Bouharoun (36°40′N, 04°40′E) (Algeria); off Cap de Creus (42°20′N, 03°15′E), Sant Pol de Mar (41°35′N, 02°38′E) and Tarragona (41°02′N, 01°18′E) (Spain) and *D. licha* from off Dellys (36°55′N, 03°53′E) (Algeria) and off Nouméa (23°00′S, 167°11′E) (New Caledonia). Cestodes were placed in warm saline solution and either fixed in 10% buffered formalin and then transferred to 70% ethanol or preserved in 70% ethanol directly. In the case of the worms collected in Spain, the terminal proglottid of the strobila was preserved in pure ethanol prior to fixation. Additional plerocercoids of *S. viridis* were obtained from frozen specimens of *Galeus melastomus* Rafinesque from off Tarragona (40°35′N, 01°27′E) (Spain), *Mora moro* (Risso) from off Barcelona (40°59′N, 02°01′E) (Spain) and *Ce. coelolepis* from off Barcelona (41°04′N, 03°17′E) and Majorca (40°39′N, 03°07′E) (Spain).

2.2. Morphological study

For morphological observations, worms were stained either in celestine blue or iron acetocarmine, dehydrated in a graded series of ethanols, cleared in methyl salicylate or in clove oil and studied as permanent mounts in Canada balsam. Some of the specimens were skinned with a scalpel blade before mounting in order to reveal internal structures. Some tentacles were detached from the scolex and studied as semi-permanent mounts in pure glycerin or as permanent mounts in Canada balsam. Pieces of strobila of each species were embedded in paraffin, sectioned at 4–5 μm and stained with hematoxylin and eosin for histology. Several samples of scolices, proglottids and eggs from all three species addressed were prepared as described by Kuchta and Caira [11] for scanning electron microscopy (SEM).

Type and voucher material has been deposited in the South Australian Museum (SAM), Adelaide (Australia); the Instituto de Biología of the Universidad Autónoma de México (IBUNAM), Mexico City (Mexico); the Lawrence R. Penner Parasitology Collection (LRP) at the University of Connecticut, Storrs, Connecticut (USA); the Muséum National d'Histoire Naturelle (MNHN), Paris (France); the National Museum of Natural History (Smithsonian Institution, NMNH), Suitland (USA); and the Helminthological Collection of the Universitat Autònoma de Barcelona (UABhc), Barcelona (Spain).

Drawings were made with the aid of a drawing tube attached to an Olympus BH light microscope with Nomarski interference contrast. Measurements were obtained with a stage micrometer. Scolex measurements of worms preserved in ethanol were obtained from digital images taken with a Jenoptik ProgRes C3 camera attached to a Leica DM light microscope. All measurements are presented in micrometers, unless otherwise stated, as the range followed, in parentheses, by the mean ± standard deviation and the number of measurements taken (n).

The following comparative museum material was made available for examination through the courtesy of Dr. Helmut Sattmann (Naturhistorisches Museum, Vienna, Austria): five voucher specimens (VNHM 2068, 2069, 2073, 2569 and 2570) of *S. viridis* and two syntypes (VNHM 2071 and 2076), nine paratypes (VNHM 2062–2067, 2072, 2074 and 2075) and two voucher specimens (VNHM 2567 and 2568) of *S. tergestinus* (see Table 1 for details).

2.3. Molecular and phylogenetic analyses

Genomic DNA was isolated from the terminal proglottid of three adult specimens of *S. tergestinus* from *A. vulpinus* from off Cap de Creus (Spain) and used to amplify the D1–D3 regions of the nuclear large subunit ribosomal DNA (28S rDNA). Extraction was performed using Qiagen™ (Valencia, California, USA) DNeasy® Tissue Kit following manufacturer's instructions. Polymerase chain reaction (PCR) amplifications were performed following Constenla et al. [12] using the primers and conditions described in Fyler et al. [13]. PCR amplicons were purified using Qiagen™ MinElute® PCR Purification Kit and sequenced directly for both strands using the PCR primers, LSU5 and 1200F.

Sequences were assembled and edited using Mega v6 [14] and submitted to GenBank under accession numbers KX570645–KX570647. Sequences were aligned using Muscle as implemented in MEGA v6 together with published sequences by Palm et al. [15] and Olson et al. [16,17] for *H. oheulumiae* (GenBank FJ572941), *Hepatoxylon trichiuri* (Holten, 1802) (GenBank FJ572943), *S. viridis* (GenBank FJ572940), *Sphyriocephalus* sp. (GenBank AF286974) and *Paroncomegas araya*

Table 1
Summary data for the type-material of *Sphyriocephalus tergestinus* and *S. viridis* from the Naturhistorisches Museum Wien (VNHM) collected by Pintner and re-examined in the present study.

Accession no.	Species label	Host species	Locality	Material	Medium	Amended identification
VNHM2062	*S. viridis*[a]	*L. caudatus*	Neapel, 1890	Cross-sections of plerocercoid	Permanent mount	*H. tergestinus*
VNHM2063	*S. viridis*[a]	*L. caudatus*	Neapel, 1890	Cross-sections of plerocercoid	Permanent mount	*H. tergestinus*
VNHM2064	*S. viridis*[a]	*L. caudatus*	Neapel, 1890	Longitudinal sections of plerocercoid	Permanent mount	*H. tergestinus*
VNHM2065	*S. viridis*	*L. caudatus*	Neapel, 1890	Tentacle fragments	Permanent mount	*H. tergestinus*
VNHM2066	*S. viridis*	*L. caudatus*	Neapel, 1890	Cross-sections of plerocercoid	Permanent mount	*H. tergestinus*
VNHM2067	*S. viridis*	*A. vulpinus*[b]	–	Sections of gravid segments	Permanent mount	*H. tergestinus*
VNHM2068	*S. viridis*	*D. licha*[c]	Neapel, 1890	Tentacle fragments	Permanent mount	
VNHM2069	*S. viridis*	*C. granulosus*	Neapel, 1890	Single tentacle	Permanent mount	
VNHM2071	*S. tergestinus*	*A. vulpinus*[b]	Trieste, 1898	Tentacles	Permanent mount	
VNHM2072	*S. viridis*	*A. vulpinus*[b]	Trieste, 1898	Longitudinal sections of scolex & cross-sections of gravid segment	Permanent mount	*H. tergestinus*
VNHM2073	*S. viridis*	*D. licha*[c]	Neapel, 1890	Longitudinal sections of scolex	Permanent mount	
VNHM2074	*S. viridis*	*A. vulpinus*[b]	Trieste	Tentacles	Permanent mount	*H. tergestinus*
VNHM2075	*S. viridis*	*A. vulpinus*[b]	Trieste	Strobila fragments	Permanent mount	*H. tergestinus*
VNHM2076	*S. tergestinus*	*A. vulpinus*[b]	Trieste, 1923	Strobila fragments	Permanent mount	
VNHM2567	*S. tergestinus*	*A. vulpinus*[b]	Trieste, 1898	Entire specimen, fragmented	Ethanol	
VNHM2568	*S. tergestinus*	*A. vulpinus*[b]	Trieste, 1898	Specimen fragments	Ethanol	
VNHM2569	*S. viridis*	*C. granulosus*	Neapel, 1890	Entire specimen	Ethanol	
VNHM2570	*S. viridis*	*C. granulosus*	Neapel, 1890	Specimen fragments	Ethanol	

[a] Labelled as *Tertrarhynchus viridis*.
[b] Labelled as *Alopecias vulpes*.
[c] Labelled as *Scymnorhinus lichia*.

(Woodland, 1934) (GenBank DQ642801); the latter taxon was used as an outgroup.

SeaView v4 interface [18] was used to select blocks of evolutionarily conserved sites. Maximum likelihood (ML) and Bayesian inference (BI) algorithms were used for phylogenetic tree reconstruction after determination of the best-fit model of nucleotide substitution with jModelTest v2.1.1 [19] using the Akaike Information Criterion (AIC). The best-fitting model selected was the general time-reversible model with gamma distributed rate variation among sites (GTR + G). ML analysis was performed in PhyML v3.0 [20] with a non-parametric bootstrap of 100 replicates. BI analysis was carried out with MrBayes v3.2 [21]. Log likelihoods were estimated over 10^7 generations using Markov Chain Monte Carlo (MCMC) searches on two simultaneous runs of four chains, samplings trees every 10^3 generations. The first 25% of the sampled trees were discarded as "burn-in" and a consensus topology and nodal support estimated as posterior probability values [22] were calculated from the remaining trees. Pairwise genetic distances were calculated using the "uncorrected p-distance" model implemented in MEGA v6.

3. Results

3.1. Description of a new species and redescriptions of S. tergestinus and S. viridis

The following diagnoses are based on newly collected material and observations of the type material of *S. viridis* and *S. tergestinus* deposited in VNHM. Re-examination of the latter material revealed that many microscope slides had been labeled incorrectly. The amended identification of slides is presented in Table 1, alongside additional information.

A new species of *Heterosphyriocephalus* is described, and redescriptions are provided for *S. viridis* and *S. tergestinus* (which is herein transferred to *Heterosphyriocephalus* based on morphological and molecular results).

3.1.1. Heterosphyriocephalus encarnae n. sp.

Type-host: *Alopias pelagicus* Nakamura (Lamniformes: Alopiidae).

Type-locality: Gulf of California off Boca del Alamo, Mexico (23°53′N, 109°48′W).

Site of infection: Stomach.

Type material: Holotype and paratype in IBUNAM (Nos. CNHE 10182 and 10183). Paratypes in NMNH (No. USNM 1421484), SAM (Nos. AHC 36260–36264) and LRP (Nos. LRP 8918–8924).

Etymology: This species is dedicated to both the mother and grandmother, Encarnación Villar and Encarnación Sánchez (respectively) of the first author (SD) [= Encarna (abbreviated form of Encarnación), *encarnae*].

Description (Figs. 1–4).

[Based on 13 whole mounts of fully mature worms, 1 immature worm and 1 plerocercoid; 4 worms preserved in ethanol; 2 specimens used for SEM; 6 tentacles detached from scolices and placed in glycerine or mounted in Canada balsam; 6 serial-sectioned proglottids.]

Cestodes anapolytic, 9.5–34.0 (18.3 ± 7.0; $n = 12$) mm long (Fig. 1A), with 34–57 (47 ± 8; $n = 10$) proglottids in fully mature strobilae (Fig. 1A); maximum width at level of terminal gravid proglottids (Fig. 1A), strobila with 15–39 (26 ± 7; $n = 11$) immature proglottids; 3–9 (6 ± 2; $n = 12$) mature proglottids and 6–24 (14 ± 6; $n = 12$) gravid proglottids.

Scolex craspedote (Figs. 1A, C, 2A, G, E), compact, 1507–1938 (1721 ± 149; $n = 11$) long by 1723–2322 (1991 ± 157; $n = 11$) wide; maximum width at level of velum (in dorso-ventral view); pars bothrialis 1200–1815 (1389 ± 186; $n = 11$) long by 1714–2139 (1928 ± 189; $n = 4$) wide (Figs. 1A, C, 2A, G); bothria two in number, oval, 1200–1815 (1389 ± 186; $n = 11$) long by 1246–1523 (1407 ± 90; $n = 8$) wide, with fused posterior margins, with thick, fleshy rims, mildly delimited (Figs. 1A, C, 2A, G); bothrial pits absent; distal bothrial surface with medial groove, covered with acicular filitriches; proximal bothrial surface covered with small cylindrical projections; projections 15 long by 4 wide, covered with acicular filitriches (Fig. 2J), decreasing in size towards scolex peduncle (Fig. 2I), disappearing towards apex of scolex and posterior margin of velum (Fig. 2E). Apex of scolex, peduncle and velum covered with acicular filitriches (Fig. 2I); pars vaginalis shorter than pars bothrialis (Fig. 1C), 692–1046 (882 ± 100; $n = 11$) long by 1553–1999 (1769 ± 133; $n = 12$) wide; tentacle sheaths sinuous (Fig. 1C); pars bulbosa 346–584 (451 ± 69; $n = 13$) long by 1692–2230 (1956 ± 151; $n = 12$) wide (Fig. 1C); retractor muscles attach to anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent (Fig. 1B); bulbs compact, oval (Fig. 1B, C), 631–923 (778 ± 95; $n = 13$) long by 246–354 (296 ± 31; $n = 13$) wide, in transverse orientation; bulb width/length ratio 1.0:2.3–3.0 (2.6 ± 0.2; $n = 13$); pars post-bulbosa absent (Fig. 1C); velum present, with irregular and folded

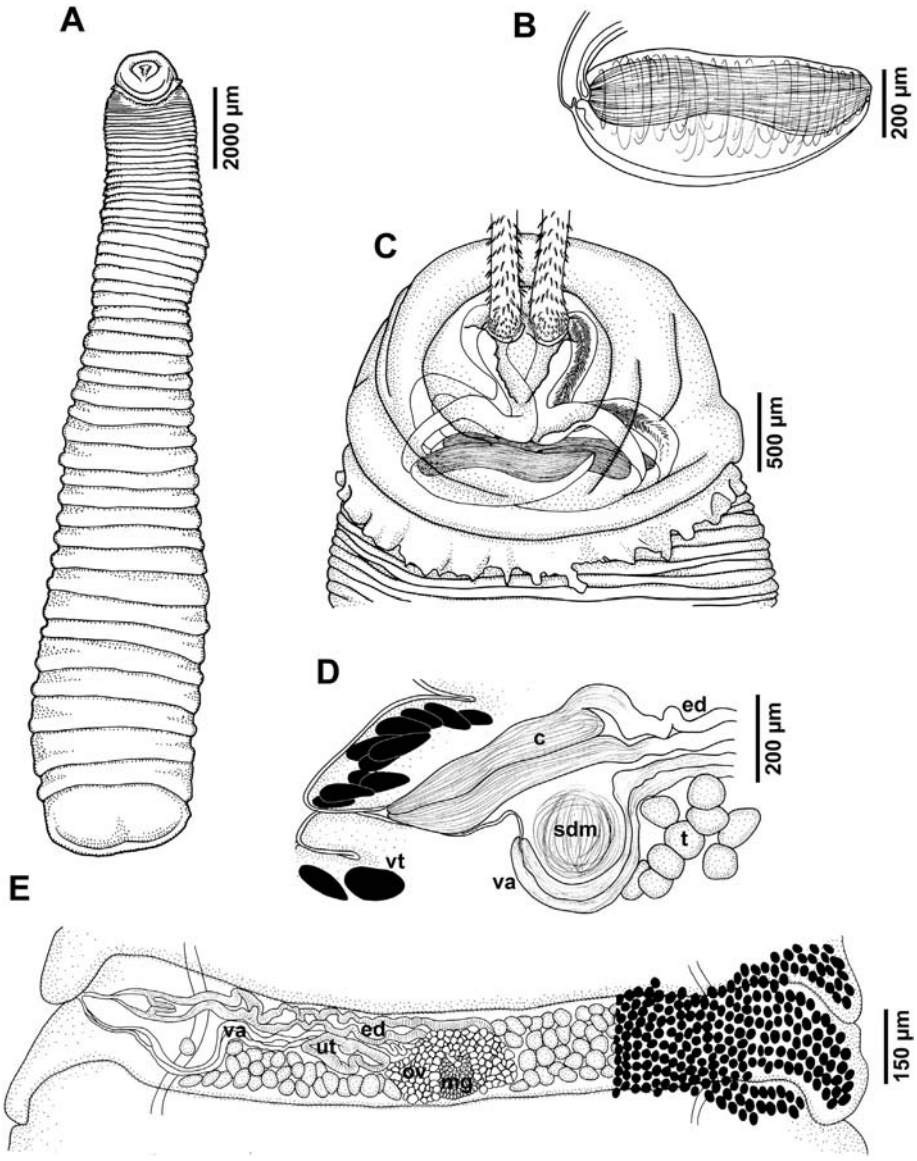

Fig. 1. Line drawings of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, outline of entire specimen, dorsoventral view; B, bulb; C, scolex, dorsoventral view; D, terminal genitalia; E, mature segment. *Abbreviations*: c, cirrus; ed, ejaculatory duct; mg, Mehlis' gland; ov, ovary; sdm, spherical dense mass; t, testis; ut, uterus; va, vagina; vt, vitelline follicles.

border (Figs. 1C, 2A, G, E), 231–615 (387 ± 92; $n = 13$) long, scolex width at level of velum in dorso-ventral view 1753–2322 (2017 ± 172; $n = 12$); scolex ratio (pars bothrialis:pars vaginalis:pars bulbosa) 1.0:0.5–0.9:0.3–0.4 (1.0:0.6 ± 0.1:0.3 ± 0.1; $n = 11$).

Fully everted tentacle 1504–1773 (1610 ± 108; $n = 5$) long, tentacle diameter without hooks 112–190 (146 ± 23; $n = 10$) at base (Fig. 3D–F), 158–216 (182 ± 23; $n = 10$) at basal swelling (Fig. 3D–F), 119–197 (144 ± 23; $n = 10$) in metabasal region (Fig. 3A–C), 107–137 (123 ± 12; $n = 10$) near tip (Fig. 3A–C). Metabasal armature typical heteroacanthous, heteromorphous (Figs. 2B–D, 3A–F); hooks hollow, in ascending half spiral rows. Hook files begin on antibothrial surface (Fig. 3A, D) and terminate on bothrial surface of tentacle (Figs. 2B, C, 3C, F); 8 hooks per principal row; hooks 1 and 1' abut (Fig. 3A, D). Hooks 1(1') falcate in early metabasal armature (Figs. 3A, B, 4B) 77–92 (87 ± 5; $n = 13$) long, base 45–54 (51 ± 3; $n = 13$) long, with transition to uncinate shape after third metabasal row (Figs. 3A, B, 4B), 62–83 (74 ± 8; $n = 9$) long, base 44–51 (48 ± 3; $n = 9$) long; hooks 2(2') falcate in early metabasal armature (Figs. 3A, B, 4B), 82–95 (90 ± 4; $n = 14$) long, base 45–59 (52 ± 5; $n = 14$) long, with transition to uncinate shape after third metabasal row (Figs. 3A, B, 4B), 62–80 (73 ± 7; $n = 8$) long, base 44–57 (50 ± 5; $n = 8$) long; hooks 3(3') falcate (Figs. 3A–C, 4B), 79–98 (90 ± 6; $n = 15$) long, base 48–57 (53 ± 3; $n = 15$) long; hooks 4(4') falcate (Figs. 3A–C, 4B), 77–109 (90 ± 10; $n = 15$) long, base 48–59 (54 ± 4; $n = 15$) long; hooks 5(5') falcate (Figs. 3B, C, 4B), 77–103 (88 ± 7; $n = 15$) long, base slightly narrower, 39–56 (49 ± 6; $n = 15$) long; hooks 6(6') falcate (Figs. 3B, C, 4B), 72–94 (84 ± 7; $n = 15$) long, base 41–54 (47 ± 4; $n = 15$) long; hooks 7(7') falcate (Figs. 3B, C, 4B), slightly smaller, 68–88 (77 ± 5; $n = 15$) long, base narrower, 36–50 (42 ± 4; $n = 15$) long; hooks 8(8') falcate (Figs. 3B, C, 4B), smaller, 57–83 (70 ± 8; $n = 15$) long, base narrower, 30–45 (37 ± 5; $n = 15$) long. Anterior extension of base longer for all hooks near base of tentacle (Fig. 3A–C).

Distinctive basal armature present (Fig. 3D–F); row 1 of basal hooks uncinate (Figs. 3D–F, 4A), 27–42 (33 ± 5; $n = 15$) long, base extended anteriorly, 24–35 (28 ± 3; $n = 15$) long. Basal swelling present (Fig. 3D–F), projected towards antibothrial surface, with 7–8 rows of hooks; rows 2–4 of basal hooks on antibothrial and bothrial surfaces falcate (Figs. 3D–F, 4A), 29–38 (31 ± 3; $n = 15$) long, base 15–21 (18 ± 2;

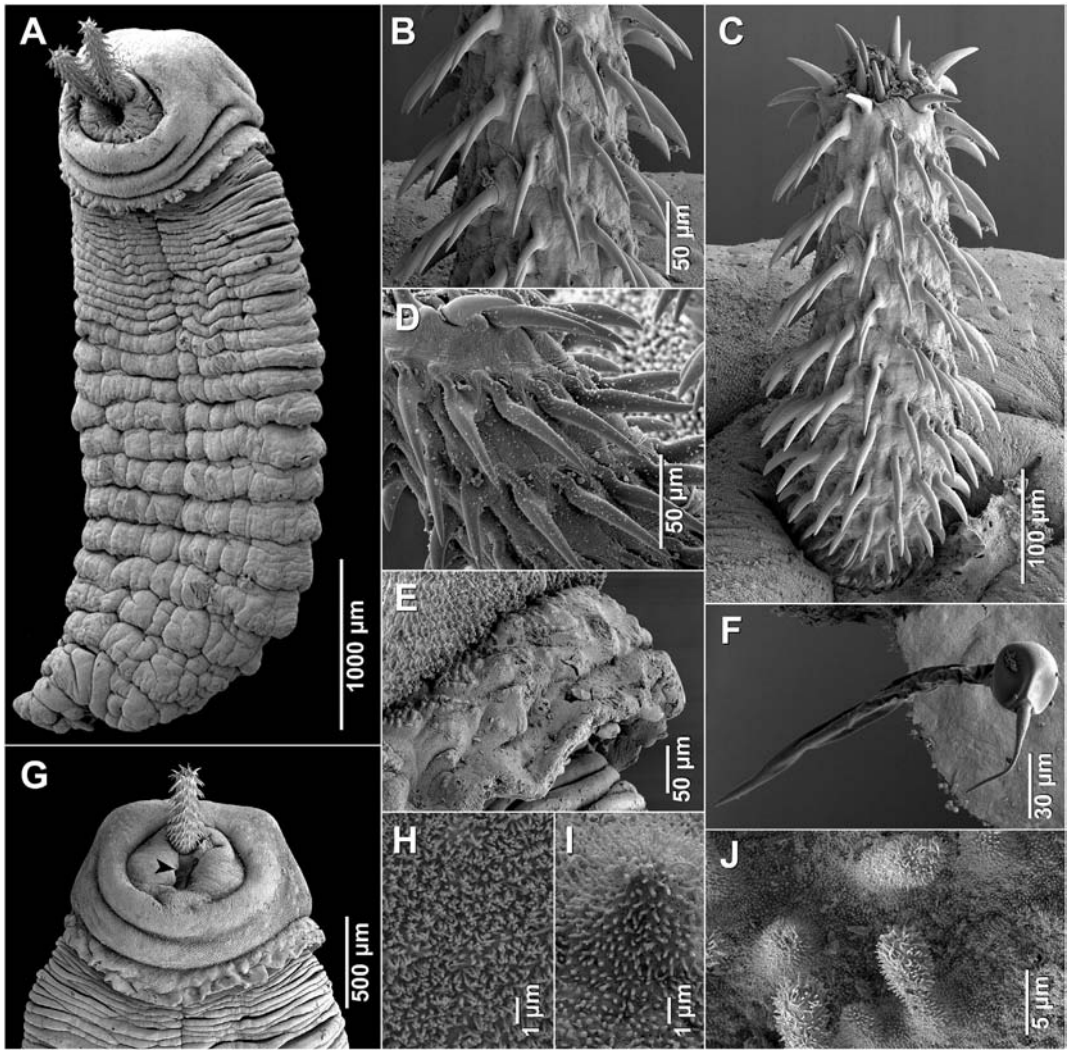

Fig. 2. Scanning electron micrographs of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, complete specimen, dorsoventral view; B, metabasal tentacular armature, bothrial surface; C, basal and metabasal tentacular armature, bothrial surface; D, metabasal tentacular armature, internal surface; E, velum, detailed view; F, egg; G, scolex, dorsoventral view, arrowhead shows medial groove in distal bothrial surface; H, acicular filitriches covering strobila; I, small projection with acicular filitriches covering scolex peduncle; J, small cylindrical projections with acicular filitriches covering proximal bothrial surface.

$n = 15$) long; rows 5–7 of basal hooks on antibothrial and bothrial surfaces uncinate (Figs. 3D–F, 4A), 17–27 (21 ± 3; $n = 15$) long, base 13–18 (15 ± 2; $n = 15$) long; rows 2 and 3 of basal hooks on internal and external surfaces falcate (Figs. 3D–F, 4A), 23–35 (30 ± 4; $n = 12$) long, with elongate base, 12–20 (17 ± 2; $n = 12$) long; rows 4–9 of basal hooks on internal and external surfaces uncinate (Figs. 3D–F, 4A), densely arranged in mosaic pattern, increasing in size towards metabasal armature, 14–26 (21 ± 4; $n = 15$) long, with characteristically broad base 14–23 (18 ± 3; $n = 15$) long. First principal rows begin at level of hook row 11 (Fig. 3D–F).

Proglottids craspedote (Figs. 1A, E, 2A), covered with acicular filitriches (Fig. 2H); first immature proglottids 31–62 (42 ± 12; $n = 12$) long by 1622–2653 (1998 ± 282; $n = 12$) wide; first mature proglottids 292–461 (359 ± 62; $n = 11$) long by 1922–3737 (2782 ± 573; $n = 11$) wide; first gravid proglottids 369–784 (479 ± 110; $n = 12$) long by 1992–3868 (2963 ± 613; $n = 12$) wide; terminal gravid proglottid 385–877 (522 ± 136; $n = 12$) long by 2046–3907 (3065 ± 621; $n = 12$) wide. Genital pores pre-equatorial (Fig. 1E), covered by velum of adjacent anterior proglottid; pore inconspicuous; cirrus-sac absent (Fig. 1D, E); cirrus unarmed, invaginated in the ejaculatory duct in immature and early mature proglottids (Fig. 1E); ejaculatory duct runs parallel and close to anterior margin of proglottid until reaching to distal ovarian lobe on aporal side (Fig. 1E), tortuous in proximal and mid-regions, dilated in final portion; seminal vesicles absent (Fig. 1D, E). Testes occupy complete intervascular space (Fig. 1E), in 2–3 layers, exclusively preovarian (Fig. 1E); testes 59–98 (85 ± 12; $n = 15$) long by 35–50 (45 ± 4; $n = 15$) wide; total number of testes 264–273 (268 ± 5; $n = 3$); testes distribution 87–99 (92 ± 6; $n = 3$) postporal; 165–186 (176 ± 11; $n = 3$) aporal; preporal testes absent.

Vagina enters genital atrium posterior to ejaculatory duct (Fig. 1D, E), turns encircling a characteristic spherical dense mass at distal part (Fig. 1D), runs posterior and parallel to ejaculatory duct and anterior to uterus, turns towards and runs posterior to ovarian isthmus at level of Mehlis' gland; seminal receptacle absent (Fig. 1E). Ovary at posterior margin of proglottid (Fig. 1E), bilobed in dorsoventral view; ovarian lobes 98–183 (160 ± 42; $n = 4$) long by 110–189 (143 ± 37; $n = 4$) wide in early mature proglottids, 183–305 (242 ± 50; $n = 4$) long by 110–281 (191 ± 73; $n = 4$) wide in late mature proglottids. Mehlis' gland large (Fig. 1E), between ovarian lobes, 79–134 (117 ± 25; $n = 4$) long by 92–122 (101 ± 15; $n = 4$) wide in early mature proglottids, 104–195 (157 ± 39; $n = 4$) long by 153–253 (190 ± 44; $n = 4$) wide in late mature proglottids. Vitelline follicles circummedullar (Fig. 1E),

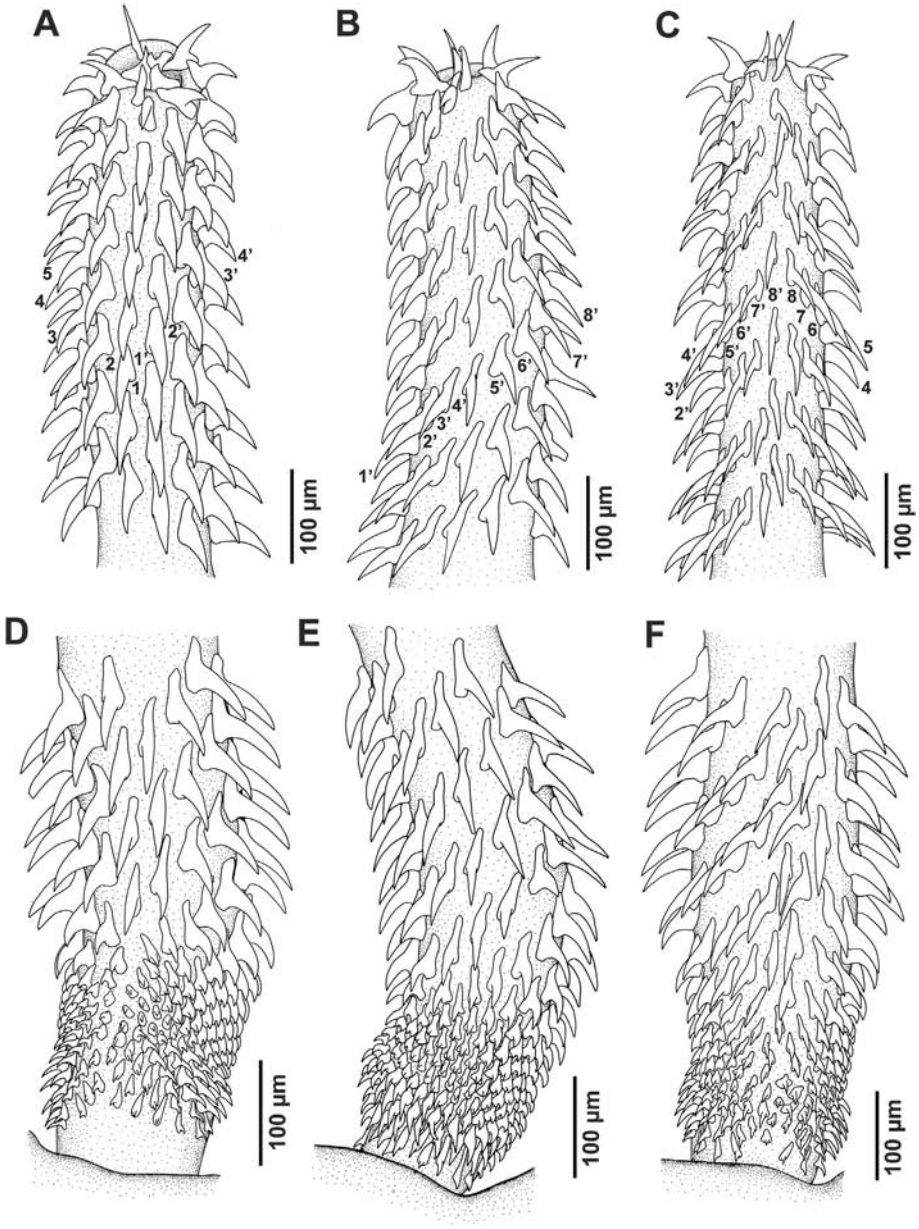

Fig. 3. Line drawings of tentacular armature of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, metabasal tentacular armature, antibothrial surface; B, metabasal tentacular armature, internal surface; C, metabasal tentacular armature, bothrial surface; D, basal tentacular armature, antibothrial surface; E, basal tentacular armature, external surface; F, basal tentacular armature, bothrial surface.

absent in ovarian region, 15–24 (20 ± 3; $n = 15$) in diameter in early mature proglottids, 21–40 (33 ± 6; $n = 15$) in late mature proglottids. Uterus preformed (Fig. 1E), fusiform to plicate, parallel and posterior to vagina, displaced porally. Uterine pore present, displaced porally.

Eggs thin-walled, 29–37 (33 ± 2; $n = 20$) in diameter, with two opposite prolongations (Fig. 2F), first prolongation short and filiform, 49–77 (61 ± 7; $n = 20$) long, second prolongation larger and wider, 261–288 (273 ± 8; $n = 20$) long; egg surface rough.

Remarks

Heterosphyriocephalus encarnae n. sp. can be readily distinguished from other sphyriocephalid species by its small size, small number of proglottids, a long velum with a characteristically irregular and folded margin, presence of small cylindrical projections covering the proximal bothrial surface, scolex peduncle and anterior part of the velum and the absence of a pars post-bulbosa. The metabasal tentacular armature is typical heteroacanthous, heteromorphous with eight hooks per principal row. This species is further characterized by the low number of testes in an exclusively pre-ovarian distribution and the absence of seminal vesicles.

Heterosphyriocephalus encarnae n. sp. resembles the type-species, *H. oheulumiae*, in similar proportions of the bothria and bulbs and a typical heteroacanthous, heteromorphous metabasal armature. However, it differs from the type-species in a number of features, such as (i) a much smaller scolex (1507–1938 vs. 6500–9625, respectively), (ii) the possession of a velum (vs. absence of a velum, respectively), (iii) the pars bothrialis extending beyond the pars bulbosa (vs. pars bothrialis and pars bulbosa slightly or not overlapping, respectively), (iv) tentacles with a basal swelling and characteristic basal armature (vs. absence of both features, respectively), (v) fewer principal hooks in the metabasal armature (8 vs. 11–13, respectively), (vi) larger lengths and

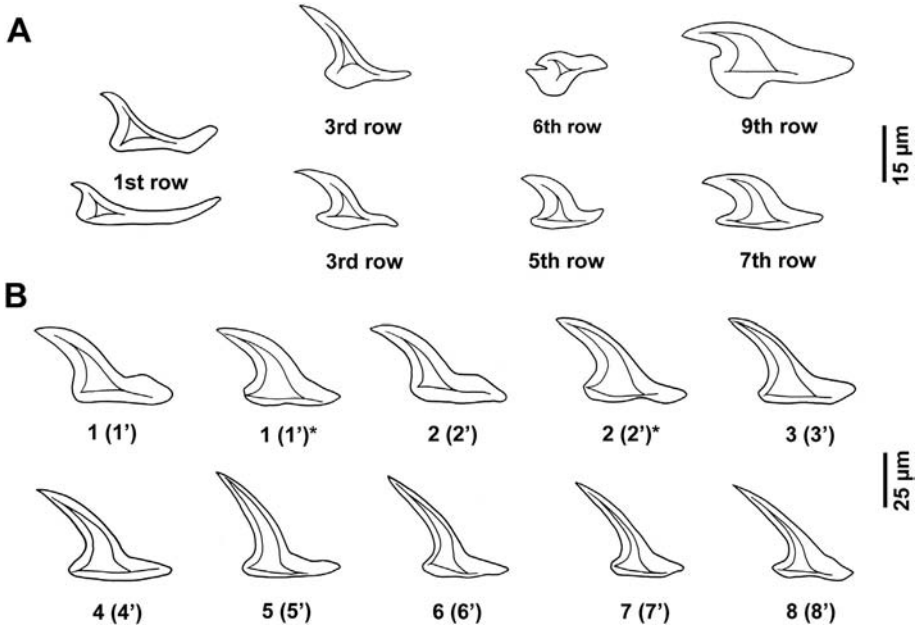

Fig. 4. Line drawings of individual tentacular hooks of *Heterosphyriocephalus encarnae* n. sp. from *Alopias pelagicus* Nakamura from the Pacific Ocean off Boca del Álamo, Mexico. A, basal individual hooks. Upper figures show hooks on internal and external surfaces, while lower figures show hooks on antibothrial and bothrial surfaces; B, individual hooks on metabasal tentacular armature. *Change to uncinate shape as progressing further into metabase.

base lengths of basal hooks (14–38 × 12–21 vs. 10 × 5 long, respectively) and (vii) larger lengths and base lengths of metabasal hooks (57–108 × 30–59 vs. 18–60 × 18–43 long, respectively).

The examination of adult specimens and characterization of proglottids of *H. oheulumiae*, with special focus on the morphology of the eggs and the reproductive structures (e.g., the presence/absence of seminal vesicles, the seminal receptacle, the characteristic spherical dense mass at the distal part of the vagina observed in *H. encarnae* n. sp. and *S. tergestinus*) is necessary before an accurate diagnosis of the genus and comparison among species can be made. New observations might reveal additional similarities between *H. oheulumiae* and its congeners.

The morphological resemblance between the new species and *S. tergestinus,* as well as the results of the molecular analyses presented below, resulted in the allocation of *S. tergestinus* into *Heterosphyriocephalus* (see below).

3.1.2. Heterosphyriocephalus tergestinus (Pintner, 1913) n. comb.

Synonyms: *Sphyriocephalus tergestinus* Pintner, 1913 (new synonym).

Type-host: *Alopias vulpinus* (Bonnaterre) [*Alopecias vulpes* (Gmelin) sensu Pintner (1913); *Vulpecula marina* Garman sensu Dollfus (1942)] (Lamniformes: Alopiidae). *Additional hosts*: Plerocerci: *Aphanopus carbo* Lowe (Perciformes: Trichiuridae), *Brama brama* (Bonnaterre) (*B. raji* Bloch and Schneider sensu [23]) (Perciformes: Bramidae), *B. dussumieri* Cuvier (Perciformes: Bramidae), *Conger conger* (L.) (Anguilliformes: Congridae), *Lepidopus caudatus* (Euphrasen) (*Le. argenteus* Bonnaterre sensu [24]) (Perciformes: Trichiuridae), *Macruronus novaezelandiae* (Hector) (Gadiformes: Merlucciidae), *Taractichthys steindachneri* (Döderlein) (Perciformes: Bramidae), *Trachurus picturatus* (Bowdich) (Perciformes: Carangidae).

Adults: *Euprotomicrus bispinatus* (Quoy and Gaimard) (Squaliformes: Dalatiidae), *Isurus oxyrinchus* Rafinesque (*Isuropsis glaucus* (Müller and Henle) sensu [31]; *I. glaucus* (Müller and Henle) sensu [1]) (Lamniformes: Lamnidae).

Type-locality: Mediterranean Sea: off Trieste, Italy.

Additional localities:

Mediterranean Sea: Italy: off Naples [23], Palermo [23] and Messina [23]; Spain: off Cap de Creus (present study), Sant Pol de Mar (present study) and Tarragona (present study); Algeria: off Bouharoun (present study); Atlantic Ocean: Spain: from the Gulf of Cádiz [25]; Portugal: off Sesimbra [26] and Madeira [26–28]; North Atlantic Ocean: from the Great Meteor Seamount (GMS) [29]; Pacific Ocean: USA: from the Johnston Atoll and Hawaii (locality unknown) [30], from the eastern coastline of Japan (locality unknown) [31,32], from the western coastline of Mexico (locality unknown) [30], from the north-central Pacific Ocean (locality unknown, see [30]); Indian Ocean: Indonesia: off Pelabuhan Ratu [7]; Tasman Sea: Australia: off Tasmania (locality unknown, see [4]).

Site of infection: Stomach and spiral valve (plerocerci and adult worms); body cavity and gills (plerocerci).

Deposited new material: NMNH (Nos. USNM 1421485–1421487), SAM (Nos. AHC 36249–36253 and AHC 47775), UABpc (Nos. C1–13 and C19).

Prevalence: 100% (3 sharks infected in Spain).

Intensity: 5–7 (in sharks from Spain).

Re-description (Figs. 5–8)

[Based on 5 whole mounts of fully mature worms; 13 fully mature worms preserved in ethanol; 3 specimens used for SEM; 4 tentacles detached from scolices and placed in glycerine or mounted in Canada balsam; 2 serial-sectioned proglottids.]

Cestodes anapolytic, 44–480 (214 ± 152; $n = 18$) mm long (Fig. 5A), with 126–260 (182 ± 43; $n = 15$) proglottids in fully mature strobilae; maximum width at level of terminal gravid proglottids; strobila with 73–130 (100 ± 16; $n = 14$) immature proglottids, 6–29 (14 ± 7; $n = 15$) mature proglottids, 8–151 (73 ± 47; $n = 16$) gravid proglottids.

Scolex craspedote (Figs. 5A, B, 6A), compact, 1393–2600 (2034 ± 294; $n = 13$) long by 1541–2254 (1974 ± 225; $n = 12$) wide; maximum width at level of bothria (in lateral view); pars bothrialis 1232–1969 (1487 ± 208; $n = 11$) long by 1714–3146 (2647 ± 378; $n = 13$) wide (Fig. 6A); bothria two in number, oval, 889–1435 (1185 ± 136; $n = 15$) long by 1143–1523 (1331 ± 114; $n = 16$) wide, with fused posterior margins, with very thick, fleshy rims, well delimited

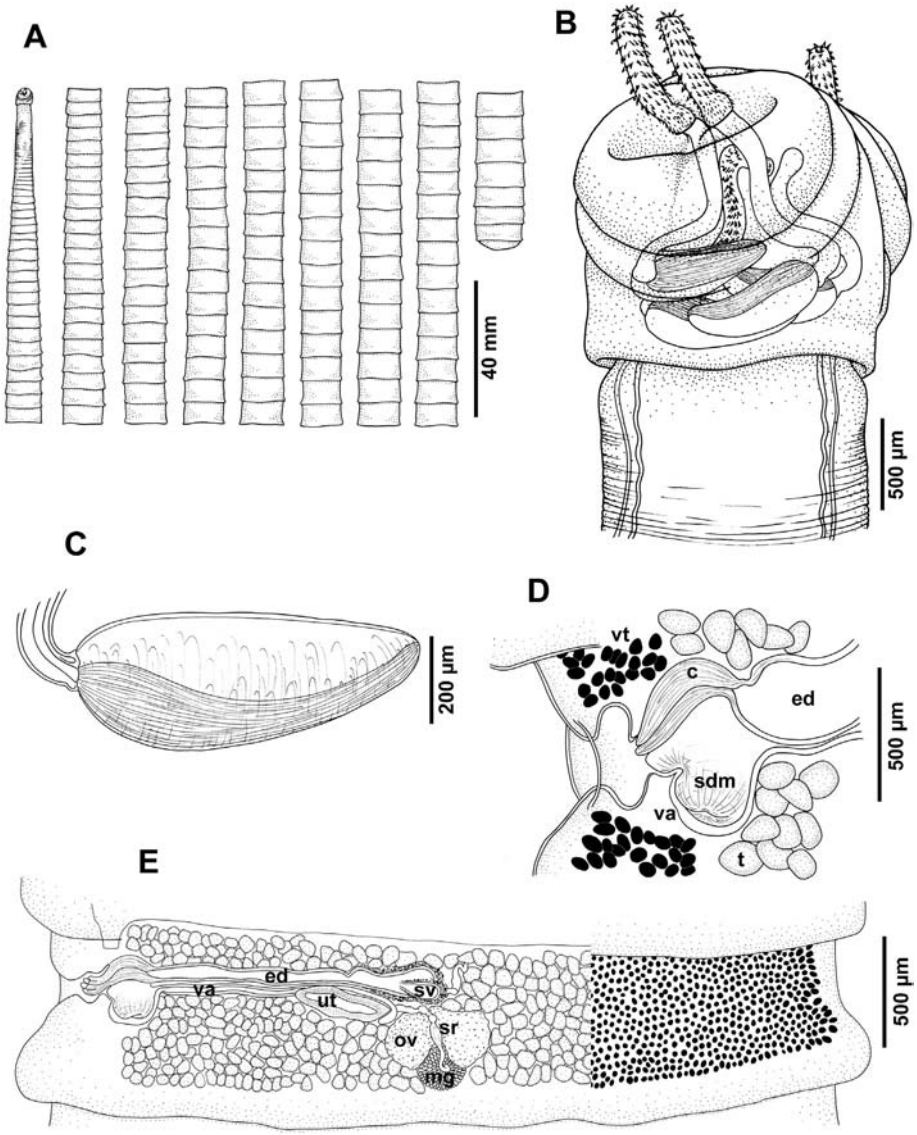

Fig. 5. Line drawings of *Heterosphyriocephalus tergestinus* n. comb. from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain (A) and off Bou Haroun, Algeria (B–E). A, outline of entire cestode, dorsoventral view; B, scolex, dorsoventral view; C, bulb; D, terminal genitalia; E, mature segment. *Abbreviations*: c, cirrus; ed, ejaculatory duct; mg, Mehlis' gland; ov, ovary; sdm, spherical dense mass; sr, seminal receptacle; sv, seminal vesicle; t, testis; ut, uterus; va, vagina; vt, vitelline follicles.

(Figs. 5A, B, 6A, F); distal bothrial surface with medial ridge (Figs. 5B, 6F); bothrial pits absent; pars vaginalis shorter than pars bothrialis, 1061 ($n = 1$) long by 1676–1861 ($n = 2$) wide; tentacle sheaths sinuous (Fig. 5B); pars bulbosa 569–692 (633 ± 62; $n = 3$) long by 1676–1846 ($n = 2$) wide; retractor muscles attach to anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent (Fig. 5B, C); bulbs compact, oval (Fig. 5B, C), 689–896 (800 ± 71; $n = 10$) long by 238–396 (314 ± 46; $n = 10$) wide, in transverse orientation; bulb width/length ratio 1.0:2.0–3.0 (2.6 ± 0.3; $n = 10$); pars post-bulbosa 108–400 (246 ± 147; $n = 3$) long (Fig. 5B); velum present (Figs. 5A, B, 6A), 92–185 (144 ± 47; $n = 3$) long, covered with small projections (Fig. 6J); projections c. 5 long by c. 5 wide (Fig. 6K), decreasing in size towards scolex peduncle, disappearing towards apex of scolex; scolex width at level of velum in dorso-ventral view 1584–1940 (1797 ± 151; $n = 4$); scolex ratio (pars bothrialis:pars vaginalis:pars bulbosa) 1.0:1.9:2.8 ($n = 1$).

Fully everted tentacle 1311–1540 (1439 ± 98; $n = 5$) long, tentacle diameter without hooks 122–207 (138 ± 28; $n = 9$) at base (Fig. 7A–C), 168–220 (183 ± 16; $n = 9$) at basal swelling (Fig. 7B), 128–159 (140 ± 10; $n = 9$) in metabasal region (Figs. 6B–D, 7A–C), 118–151 (136 ± 10; $n = 9$) near tip (Figs. 6B–D, 7A–C). Metabasal armature typical heteroacanthous, heteromorphous (Figs. 6B–D, 7A–C); hooks hollow, in ascending half spiral rows. Hook files begin on antibothrial surface (Figs. 6C, 7A) and terminate on bothrial surface of tentacle (Figs. 6D, 7C); 8–9 hooks per principal row, following an irregular pattern; hooks 1 and 1' abut (Figs. 6C, 7A). Hooks 1(1') falcate in early metabasal armature (Figs. 7A, B, 8B), 76–83 (79 ± 3; $n = 8$) long, base 44–50 (47 ± 2; $n = 8$) long, with transition to uncinate shape after fifth metabasal row (Figs. 7A, B, 8B), 67–82 (75 ± 4; $n = 10$) long, base 47–58 (55 ± 3; $n = 10$) long; hooks 2(2') falcate in early metabasal armature (Figs. 7A, B, 8B), 79–85 (84 ± 2; $n = 8$) long, base 45–54 (51 ± 3; $n = 8$) long, with transition to uncinate shape after fifth metabasal row (Figs. 7A, B, 8B), 68–83 (75 ± 4; $n = 10$) long, base 50–59 (55 ± 3; $n = 10$) long; hooks 3(3') falcate (Figs. 7A, B, 8B), 70–86 (75 ± 6; $n = 9$) long, base slightly narrower, 50–55 (51 ± 2; $n = 9$) long; hooks 4(4') falcate (Figs. 7A, B, 8B), larger, 77–95 (84 ± 7; $n = 9$) long, base slightly wider, 51–58 (55 ± 2; $n = 9$) long; hooks 5(5') falcate (Figs. 7A–C, 8B), slightly larger, 77–100 (88 ± 8; $n = 13$) long,

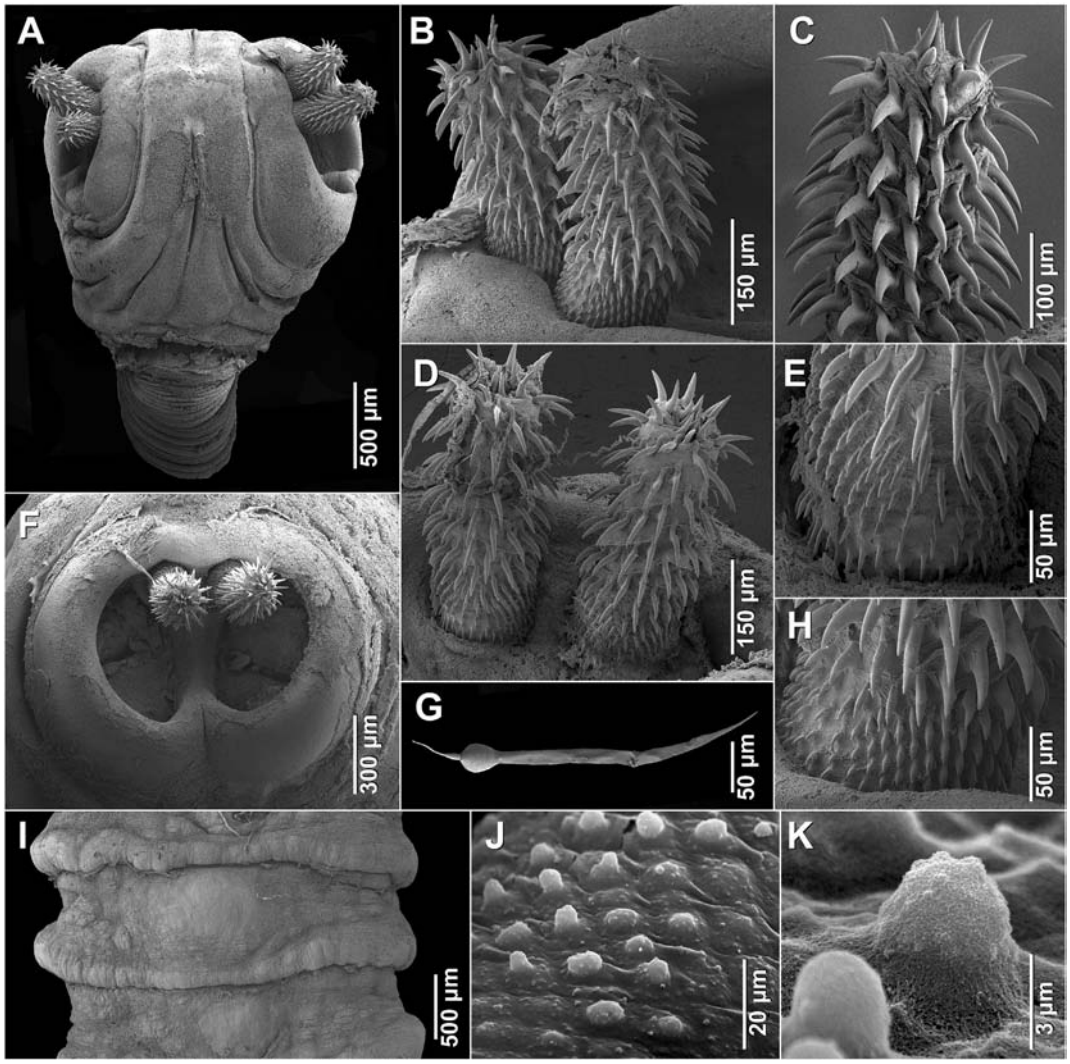

Fig. 6. Scanning electron micrographs of *Heterosphyriocephalus tergestinus* n. comb. from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain. A, scolex, lateral view; B, full tentacles, external/internal to antibothrial surface; C, metabasal tentacular armature, antibothrial surface; D, entire tentacle, bothrial surface; E, basal tentacular armature, bothrial surface; F, bothria, detailed view; G, egg; H, basal tentacular armature, external to antibothrial surface; I, segments of mature strobila; J, small projections covering the velum; K, detail view on small projection on velum.

base 50–57 (53 ± 2; $n = 13$) long; hooks 6(6') falcate (Figs. 7B, C, 8B), larger, 68–98 (79 ± 10; $n = 15$) long, base slightly narrower, 41–56 (50 ± 5; $n = 15$) long; hooks 7(7') falcate (Figs. 7B, C, 8B), slightly smaller, 61–92 (75 ± 10; $n = 15$) long, base slightly narrower, 37–54 (47 ± 5; $n = 15$) long; hooks 8(8') falcate (Figs. 7B, C, 8B), slightly smaller, 56–80 (70 ± 6; $n = 12$) long, base narrower, 32–45 (41 ± 4; $n = 12$) long; hooks 9(9') falcate (Figs. 7C, 8B), smaller, 53–63 (58 ± 5; $n = 4$) long, base slightly narrower, 32–36 (34 ± 2; $n = 4$) long. All hooks decrease slightly in size towards metabasal region (Figs. 7A–C).

Distinctive basal armature present (Figs. 6B, D, E, H, 7A–C); row 1 of basal hooks uncinate (Figs. 7A–C, 8A), 32–46 (39 ± 3; $n = 12$) long, base extended anteriorly, 24–35 (31 ± 3; $n = 12$) long. Basal swelling present (Figs. 6B, D, E, H, 7A–C), projected towards antibothrial surface, with 9–10 rows of hooks; rows 2–4 of basal hooks on antibothrial and bothrial surfaces falcate (Figs. 7A–C, 8A), 23–33 (28 ± 4; $n = 11$) long, base 9–23 (17 ± 4; $n = 11$) long; rows 5–7 of basal hooks on antibothrial and bothrial surfaces uncinate (Figs. 7A–C, 8A), 14–27 (20 ± 4; $n = 11$) long, base 9–18 (14 ± 2; $n = 11$) long; rows 2 and 3 of basal hooks on internal and external surfaces falcate (Figs. 7A–C, 8A), 38–44 (40 ± 2; $n = 10$) long, with elongate base, 21–27 (24 ± 2; $n = 10$) long; rows 4–9 of basal hooks on internal and external surfaces uncinate (Figs. 7A–C, 8A), densely arranged in mosaic pattern, increasing in size towards metabasal armature, 18–38 (25 ± 6; $n = 10$) long, with characteristically broad base, 15–24 (19 ± 3; $n = 10$) long. First principal rows begin at level of hook row 11 (Fig. 7A–C).

Proglottids craspedote (Figs. 5A, E, 6I); first immature proglottids 8–12 (10 ± 2; $n = 4$) long by 1492–1830 (1687 ± 175; $n = 3$) wide; first mature proglottids 369–584 (492 ± 109; $n = 4$) long by 2999–3860 (3364 ± 398; $n = 4$) wide; first gravid proglottids 1061–1338 (1188 ± 140; $n = 4$) long by 4245–4783 (4587 ± 241; $n = 4$) wide; terminal gravid proglottid 1323–2169 (1712 ± 427; $n = 4$) long by 2476–3799 (3004 ± 700; $n = 4$) wide. Genital pores equatorial (Fig. 5E); pore conspicuous; cirrus-sac absent (Fig. 5D, E); cirrus unarmed, with unarmed sheath, invaginated in the ejaculatory duct in immature and early mature proglottids; ejaculatory duct runs parallel and close to anterior margin of proglottid until reaching to Mehlis' gland in early mature proglottids and overpassing distal ovarian lobe on aboral side in late mature proglottids (Fig. 5E), dilated in final portion, where it connects with large pyriform seminal vesicle (Fig. 5E). Testes occupy complete intervascular space (Fig. 5E), in 2–3 layers, mainly preovarian, some posterior to ovary and Mehlis' gland; testes 90–127 (107 ± 12;

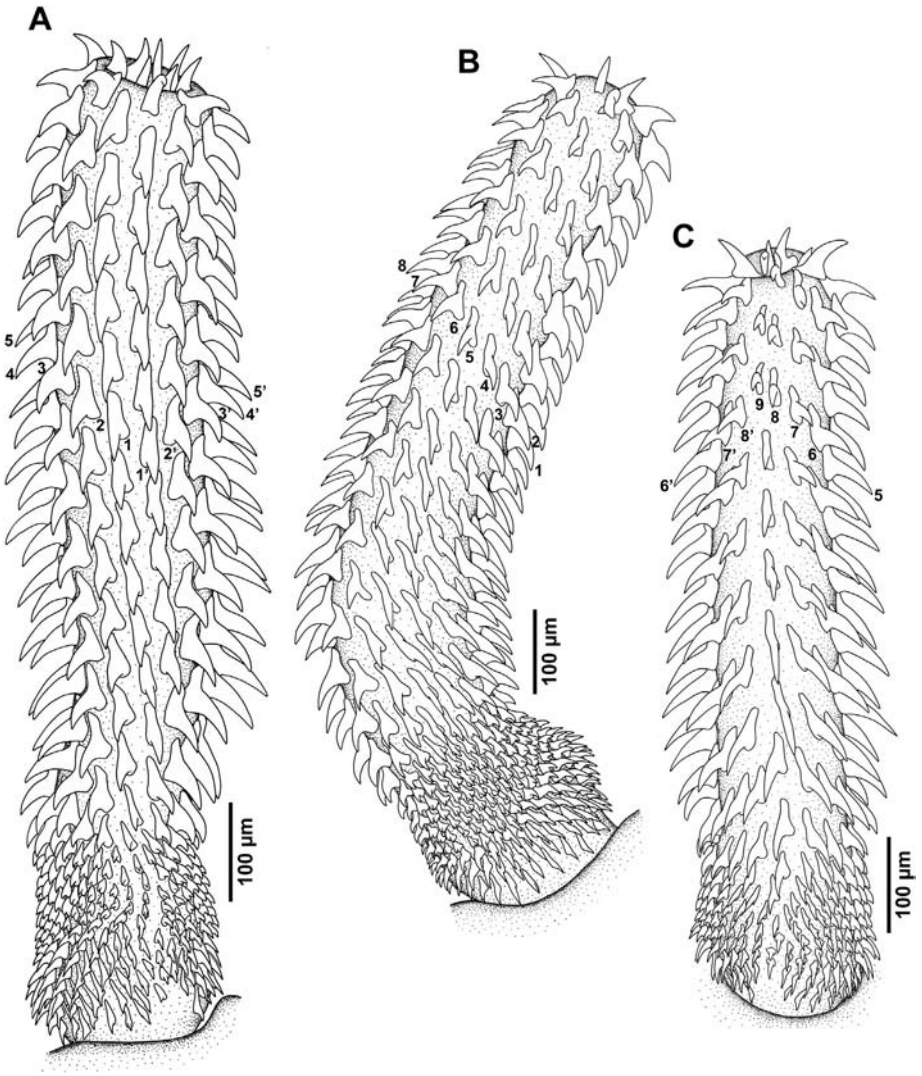

Fig. 7. Line drawings of tentacular armature of *Heterosphyriocephalus tergestinus* n. comb. from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain. A, tentacular armature, antibothrial surface; B, tentacular armature, internal surface; C, tentacular armature, bothrial surface.

$n = 15$) long by 55–72 (63 ± 6; $n = 15$) wide; total number of testes 570–715 (658 ± 62; $n = 4$); testes distribution 70–105 (93 ± 17; $n = 4$) preporal, 185–268 (233 ± 36; $n = 4$) postporal, 313–358 (333 ± 22; $n = 4$) aporal.

Vagina enters genital atrium posterior to ejaculatory duct (Fig. 5D, E), turns encircling a characteristic spherical dense mass at distal part (Fig. 5D, E), runs posterior and parallel to ejaculatory duct and anterior to uterus, turns towards and runs posterior to ovarian isthmus at level of Mehlis' gland; small and elongate seminal receptacle present (Fig. 5E), becoming larger with maturity. Ovary in posterior region of proglottid (Fig. 5E), not reaching to posterior margin, bilobed in dorsoventral view; ovarian lobes 162–229 (198 ± 28; $n = 4$) long by 122–223 (188 ± 46; $n = 4$) wide in early mature proglottids, 274–451 (367 ± 73; $n = 4$) long by 226–335 (288 ± 53; $n = 4$) wide in late mature proglottids. Mehlis' gland between ovarian lobes (Fig. 5E), reaching to posterior margin of proglottid, 113–162 (145 ± 22; $n = 4$) long by 107–204 (171 ± 44; $n = 4$) wide in early mature proglottids, 220–274 (241 ± 27; $n = 4$) long by 232–311 (281 ± 34; $n = 4$) wide in late mature proglottids. Vitelline follicles circummedullar (Fig. 5E), absent in ovarian region, 11–18 (15 ± 3; $n = 15$) in diameter in early mature proglottids, 34–61 (45 ± 9; $n = 15$) in late mature proglottids. Uterus preformed (Fig. 5E), fusiform, parallel and posterior to vagina, displaced porally, sometimes inducing a large round swelling at side of late gravid proglottids (Fig. 5A). Uterine pore present, displaced porally.

Eggs thin-walled, 34–40 (37 ± 1; $n = 20$) in diameter, with two opposite prolongations (Fig. 6G), first prolongation short and filiform, 57–76 (67 ± 6; $n = 20$) long, second prolongation larger and wider, 303–376 (335 ± 21; $n = 20$) long; egg surface rough.

Remarks

This species is characterized by a typical heteroacanthous, heteromorphous metabasal armature with eight to nine hooks per principal row following an irregular pattern. Other features characteristic for this species are the presence of small projections covering the velum and scolex peduncle, genital pores in an equatorial position, the presence of a large pyriform seminal vesicle entering the proximal part of the ejaculatory duct and the presence of a small seminal receptacle.

Phylogenetic analysis based on 28S rDNA (Fig. 9) has shown that this species is a congener to *H. oheulumiae*. Consequently, *S. tergestinus* is hereby transferred to the genus *Heterosphyriocephalus* as *Heterosphyriocephalus tergestinus* n. comb. Furthermore, the relocation of this species is supported by the presence of shared morphological features with *H. encarnae* (the only species of the genus for which complete morphological data exist), which differ from morphological characteristics of members of the genus *Sphyriocephalus*.

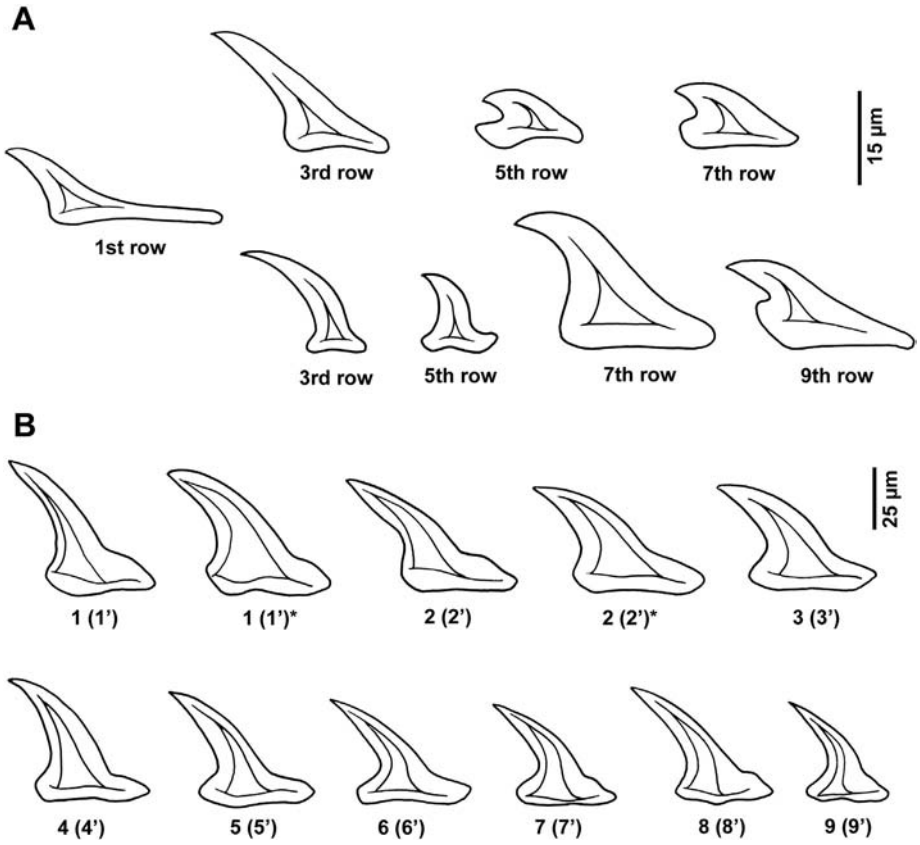

Fig. 8. Line drawings of individual tentacular hooks of *Heterosphyriocephalus tergestinus* n. comb. from *Alopias vulpinus* (Bonnaterre) from the Mediterranean Sea off Cap de Creus, Spain. A, basal individual hooks. Upper figures show hooks on internal and external surfaces, while lower figures show hooks on antibothrial and bothrial surfaces; B, individual hooks on metabasal tentacular armature. *Change to uncinate shape as progressing further into metabase.

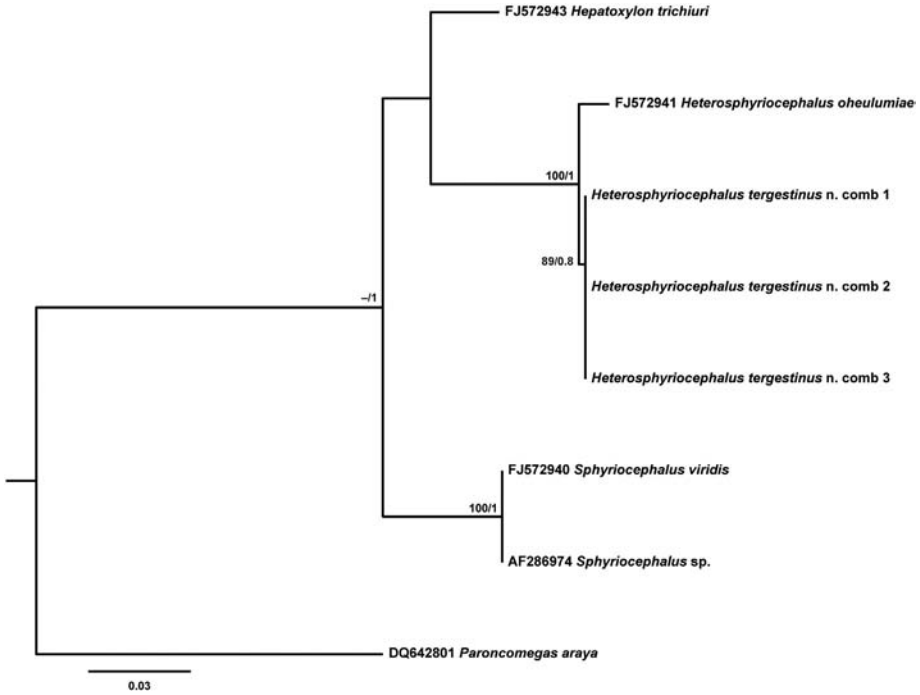

Fig. 9. Maximum likelihood (ML) phylogram reconstructed using the newly generated and retrieved from GenBank 28S rDNA sequences for *Heterosphyriocephalus tergestinus* n. comb., *Heterosphyriocephalus oheulumiae*, *Hepatoxylon trichiuri*, *Sphyriocephalus viridis* and *Sphyriocephalus* sp. with nodal support from ML and Bayesian inference (BI) analyses indicated as ML/BI. Outgroup: *Paroncomegas araya*. The scale-bar indicates the expected number of substitutions per site.

Heterosphyriocephalus tergestinus n. comb. resembles the type-species of the genus, *H. oheulumiae*, in similar bothria and bulb proportions and in the presence of a typical heteroacanthous, heteromorphous armature pattern. However, it differs from *H. oheulumiae* in (i) a smaller plerocercoid larval stage (3–4 vs. 6 mm, respectively), (ii) a much smaller scolex (1393–2600 vs. 6500–9625, respectively), (iii) the possession of a velum (vs. absence of a velum, respectively), (iv) a pars bothrialis reaching posteriorly beyond the pars bulbosa (vs. pars bothrialis not reaching to beyond the pars bulbosa, respectively), (v) a larger tentacle diameter (128–159 vs. 95–135, respectively), (vi) the presence of a basal swelling and characteristic basal armature (vs. absence of both features, respectively), (vii) a metabasal armature with fewer hooks per principal row (8–9 vs. 11–13, respectively), (viii) larger lengths and base lengths of basal hooks (13–44 × 9–27 vs. 10 × 5 long, respectively) and (ix) larger lengths and base lengths of metabasal hooks (53–100 × 32–59 vs. 18–60 × 18–43 long, respectively).

Heterosphyriocephalus tergestinus n. comb. is morphologically very similar to *H. encarnae*. Both species share many morphological features, such as the presence of a velum, similar scolex proportions, a pars bothrialis extending beyond the pars bulbosa and the presence of small projections covering different parts of the scolex. The tentacle diameters of both species are also very similar, as are the basal and metabasal tentacular armature patterns and the shape and sizes of basal and metabasal hooks. Both species possess a characteristic, spherical, dense mass at the distal part of the vagina, which seems to be an exclusive feature of the genus. The eggs are almost identical in shape and size but show a slightly longer prolongation in *H. tergestinus* n. comb.

However, *H. tergestinus* n. comb. can be readily differentiated from *H. encarnae* in (i) different body sizes (200–300 mm vs. 9.5–34 mm, respectively), (ii) the number of proglottids (>150 vs. 34–57, respectively), (iii) a shorter velum (92–185 vs. 231–615, respectively), (iv) the absence of small cylindrical projections covering the proximal bothrial surface (vs. the presence of such projections in this area, respectively), (v) the presence of a pars post-bulbosa (vs. absence of a pars post-bulbosa, respectively), (vi) an irregular pattern of 8–9 hooks per principal row in the metabasal tentacular armature (vs. consistently 8 hooks per principal row, respectively), (vii) the number of testes (570–715 vs. 264–273, respectively), (viii) the presence of a large, pyriform seminal vesicle in the proximal part of the ejaculatory duct (vs. absence of this structure, respectively), and (ix) the presence of a small seminal receptacle (vs. absence of a seminal receptacle, respectively).

The spherical dense mass observed at the proximal part of the vagina was already noted and described by Pintner [8] and Yamaguti [31]. The latter author interpreted this structure as a distal vaginal sphincter, which was rejected later by Dollfus [1]. Following Pintner [8], Dollfus [1] described it as a muscular mass bearing elongate spines with rounded bases in a regular pattern. Although we also consider this structure as a large and nearly spherical muscular mass as observed in histological sections and whole mounts, we have not been able to observe the spines described by former authors. We believe that Pintner [8] misinterpreted either the muscular fibers forming this mass, which are thick and abundant and very easily observed in sections and whole mounts, or the cellular nuclei that are interspersed with such fibers and that in histological sections often appear elongate and triangular in shape as elongate spines. As mentioned by Dollfus [1], this structure seems to be unique within the Eucestoda.

The detailed morphological description of two species of this genus (i.e. *H. encarnae* and *H. tergestinus*), provided in the present study, as well as the confirmation of their phylogenetic relationships based on molecular results (presented in Section 3.3), enables us to provide a more accurate generic diagnosis. However, as it was stated above, an examination of adult specimens of the type-species becomes necessary in order to identify some unknown morphological characteristics and to complete the generic diagnosis.

3.1.3. Sphyriocephalus viridis (Wagener, 1854) Pintner, 1913

Synonyms: *Tetrarhynchus viridis* Wagener, 1854; *Rhynchobothrium viride* Wagener, 1854 (sensu Parona, 1894); *Sphyriocephala viridis* (Wagener, 1854) Pintner, 1913 [sic]; *Sphyriocephala richardi* Guiart, 1935 [sic]; *Sphyriocephalus richardi* Guiart, 1935; *Sphyriocephala alberti* Guiart, 1935 [sic]; *Sphyriocephalus alberti* Guiart, 1935.

Type-host: *Dalatias licha* (Bonnaterre) (Squaliformes: Dalatiidae).

Additional hosts:

Plerocerci: *Alepocephalus rostratus* Risso (Osmeriformes: Alepocephalidae), *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello (Squaliformes: Somniosidae), *Dalatias licha* (Bonnaterre) (Squaliformes: Dalatiidae), *Galeus melastomus* Rafinesque (*Pristiurus melanostomus* (Rafinesque) sensu [8]) (Carcharhiniformes: Pentachidae), *Mora moro* (Risso) (Gadiformes: Moridae) (new host record), *Pseudotriakis microdon* de Brito Capello (*Pseudotriacis microdon* Capello sensu [2]) (Carcharhiniformes: Pseudotriakidae), *Squalus acanthias* L. (Squaliformes: Squalidae), *Synaphobranchus brevidorsalis* Lloyd (*Sy. pinnatus* (Gronovius) sensu [2]) (Anguilliformes: Synaphobranchidae), *Trachyrincus scabrus* (Rafinesque) (*Lepidoleprus trachyrhynchus* Risso sensu [8]) (Gadiformes: Macrouridae), *Xiphias gladius* L. (Perciformes: Xiphiidae).

Adults: *Alopias superciliosus* Lowe (Lamniformes: Alopiidae), *Centrophorus granulosus* (Bloch and Schneider) (Squaliformes: Centrophoridae).

Type-locality: Mediterranean Sea off Nice, France.

Additional localities: Mediterranean Sea: France: off Calvi [2]; Italy: off Naples [23] and Trieste [23]; Algeria: off Bou Ismaïl (Castiglione sensu [3]) and Dellys (present study); Spain: off Barcelona (present study) and Majorca (present study); Atlantic Ocean: Cape Verde (locality unknown, see [1,2]), Portugal: Azores (locality unknown, see [1,2]), from the Gulf of Guinea [33]; north-eastern Atlantic Ocean: locality unknown [34]; tropical, equatorial and central South Atlantic Ocean: localities unknown [35]; Pacific Ocean: USA: off Bolsa Chica State Beach, California [5], New Caledonia: off Nouméa (present study).

Site of infection: stomach (plerocerci and adult worms), body cavity (plerocerci).

Deposited new material: NMNH (No. USNM 1421488), SAM (Nos. AHC 36254–36259 and AHC 47776), MNHN (No. JNC395), UABpc (Nos. C14–18).

Re-description (Figs. 9–12)

[Based on 6 whole mounts of fully mature worms and 3 scolices; 8 specimens used for SEM; 6 detached tentacles in glycerin or Canada balsam; 2 serial-sectioned proglottids; several plerocerci and fragments of adult worms preserved in ethanol.]

Cestodes anapolytic, with >200 proglottids in fully mature strobilae (Fig. 10A); maximum length >20 cm (Fig. 10A); maximum width at level of terminal gravid proglottids (Fig. 10A); strobila with 141 (n = 1) immature proglottids, 27–99 (n = 2) mature proglottids, >99 (n = 1) gravid proglottids.

Scolex acraspedote (Figs. 10A, B, 11A), compact, 4952–6183 (5632 ± 405; n = 6) long by 3614 (n = 1) wide, bearing a small apical protuberance (Figs. 10A, 11B); maximum width at level of bothria (in lateral view); pars bothrialis 2415–3737 (3111 ± 405; n = 7) long by 3876–4322 (4086 ± 224; n = 3) wide (Figs. 10A, 11A); bothria two in number, oval, well delimited, 1779–3076 (2546 ± 412; n = 12) long by 2207–2907 (2599 ± 288; n = 7) wide, with fused posterior margins, with very thick, fleshy rims (Figs. 10A, B, 11A, B); distal bothrial surface markedly concave, with medial ridge (Fig. 11B); bothrial pits absent; pars vaginalis longer than pars bothrialis, 2830–3783 (3402 ± 451; n = 5) long by 3399 (n = 1) wide (Fig. 10B); tentacle sheaths sinuous (Fig. 10B); pars bulbosa 1230–1846 (1630 ± 277; n = 4) long by 2907–3614 (n = 2) wide (Fig. 10B); retractor muscles attach at anterior part of bulbs (Fig. 10C); prebulbar organs and gland cells inside bulbs absent (Fig. 10B, C); bulbs compact, oval (Fig. 10B, C), 1200–1707 (1454 ± 197; n = 10) long by 615–723 (672 ± 34; n = 10) wide, in transverse orientation; bulb width/length ratio

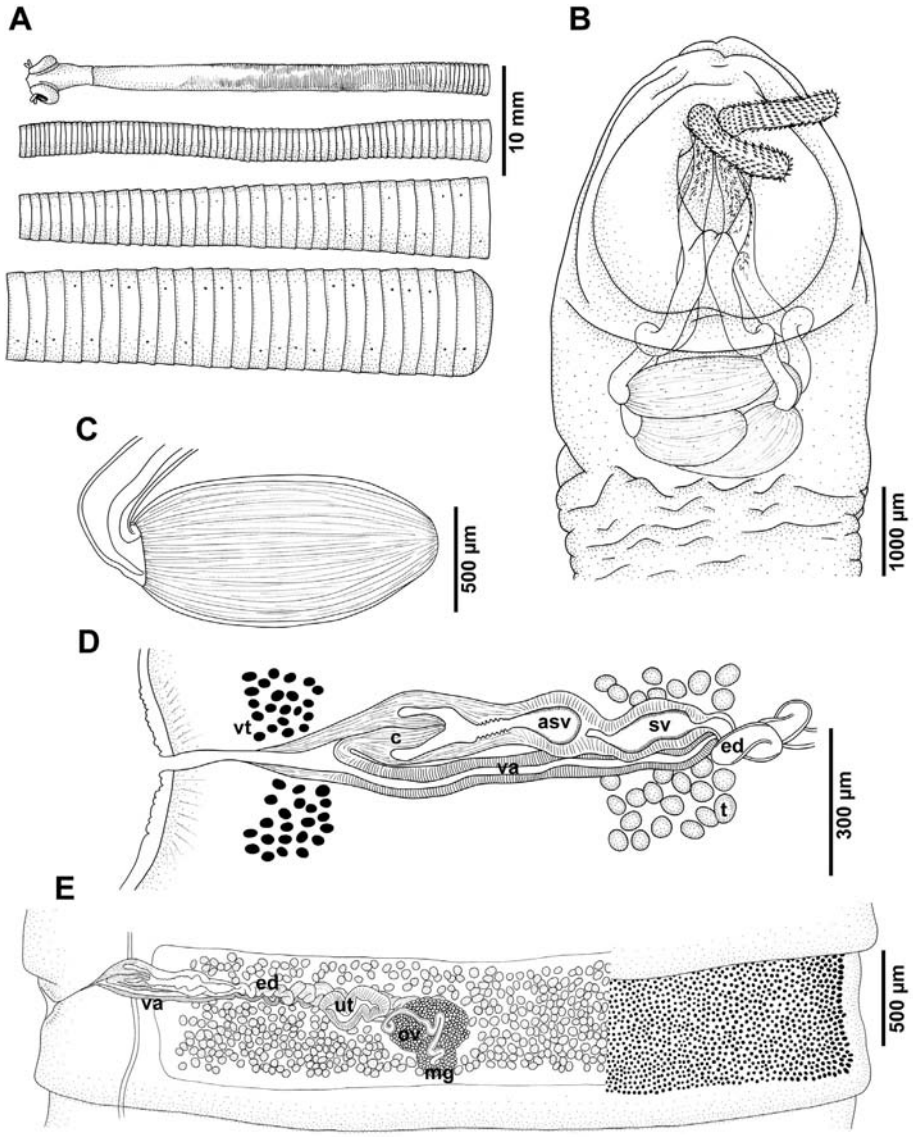

Fig. 10. Line drawings of *Sphyriocephalus viridis* from *Dalatias licha* (Bonnaterre) from the Mediterranean Sea off Dellys, Algeria (A, C–E) and the Pacific Ocean off Nouméa, New Caledonia (B). A, outline of entire specimen, scolex in lateral view, strobila in dorsoventral view; B, scolex, dorsoventral view; C, bulb; D, terminal genitalia; E, mature segment. *Abbreviations*: asv, accessory seminal vesicle; c, cirrus; ed, ejaculatory duct; mg, Mehlis' gland; ov, ovary; sv, seminal vesicle; t, testis; ut, uterus; va, vagina; vt, vitelline follicles.

1.0:1.8–2.6 (2.2 ± 0.3; $n = 10$); pars post-bulbosa prominent, 477–923 (665 ± 187; $n = 34$) long (Fig. 10B); scolex ratio (pars bothrialis:pars vaginalis:pars bulbosa) 1.0:1.0–1.2:0.4–0.8 (1.0:1.1 ± 0.1:0.5 ± 0.2; $n = 5$). Scolex peduncle covered with acicular filitriches. Scolex delimited from strobila by a narrow constriction (Fig. 10A, B).

Fully everted tentacle 2876–4353 (3511 ± 760; $n = 3$) long; tentacle diameter without hooks 223–246 (234 ± 12; $n = 3$) at base (Figs. 11G, 12A–C), 246–288 (265 ± 21; $n = 3$) at basal swelling (Figs. 11C, G, 12A–C), 238–261 (251 ± 11; $n = 3$) in transition area towards metabasal region (Figs. 11C, F, G, 12A–C), 215–269 (243 ± 25; $n = 4$) in metabasal region (Figs. 11F, G, 12A–C), 223–261 (243 ± 19; $n = 4$) near tip (Figs. 11F, G, 12A–C). Metabasal armature typical heteroacanthous, heteromorphous (Figs. 11C, F, G, 12A–C); hooks hollow, in ascending half spiral rows. Hook files begin on antibothrial surface (Figs. 11G, 12A) and terminate on bothrial surface of tentacle (Fig. 12C); 10–13 hooks per principal row, decreasing in number towards metabasal region; hooks 1 and 1' abut (Figs. 11G, 12A). Metabasal hooks of identical shape, differ in size on different tentacular surfaces (Figs. 11F, G, 12A–C, 13B). Hooks on antibothrial surface of transition area towards metabasal region uncinate (Figs. 12A, 13B), 86–92 (90 ± 2; $n = 8$) long, base 60–70 (65 ± 4; $n = 8$) long; hooks on antibothrial surface of metabasal region uncinate (Figs. 12B, 13B), slightly larger, 83–107 (95 ± 9; $n = 10$) long, base 52–69 (62 ± 5; $n = 10$) long; hooks on external and internal surfaces of transition area towards metabasal region uncinate (Figs. 11C, 12A, C, 13B), 80–96 (89 ± 6; $n = 10$) long, base 52–70 (64 ± 5; $n = 10$) long; hooks on external and internal surfaces of metabasal region uncinate (Figs. 12A, C, 13B), larger, 92–107 (101 ± 6; $n = 10$) long, base wider, 64–75 (71 ± 4; $n = 10$) long; hooks on bothrial surface of transition area towards metabasal region uncinate (Figs. 12B, 13B), 73–95 (90 ± 7; $n = 10$) long, base 52–70 (62 ± 6; $n = 10$) long; hooks on bothrial surface of metabasal region uncinate (Figs. 12B, 13B), larger, 98–119 (110 ± 8; $n = 10$) long, base wider, 67–86 (78 ± 8; $n = 10$) long.

Distinctive basal armature and basal swelling present (Figs. 11C, F, G, 12A–C); basal swelling with 8–9 rows of hooks on antibothrial surface; rows 1–5 of basal hooks on antibothrial and bothrial surfaces falcate (Figs. 12B, 13A), 41–56 (50 ± 5; $n = 10$) long, base 26–32 (29 ± 2; $n = 10$) long; rows 6–13 of basal hooks on antibothrial and bothrial

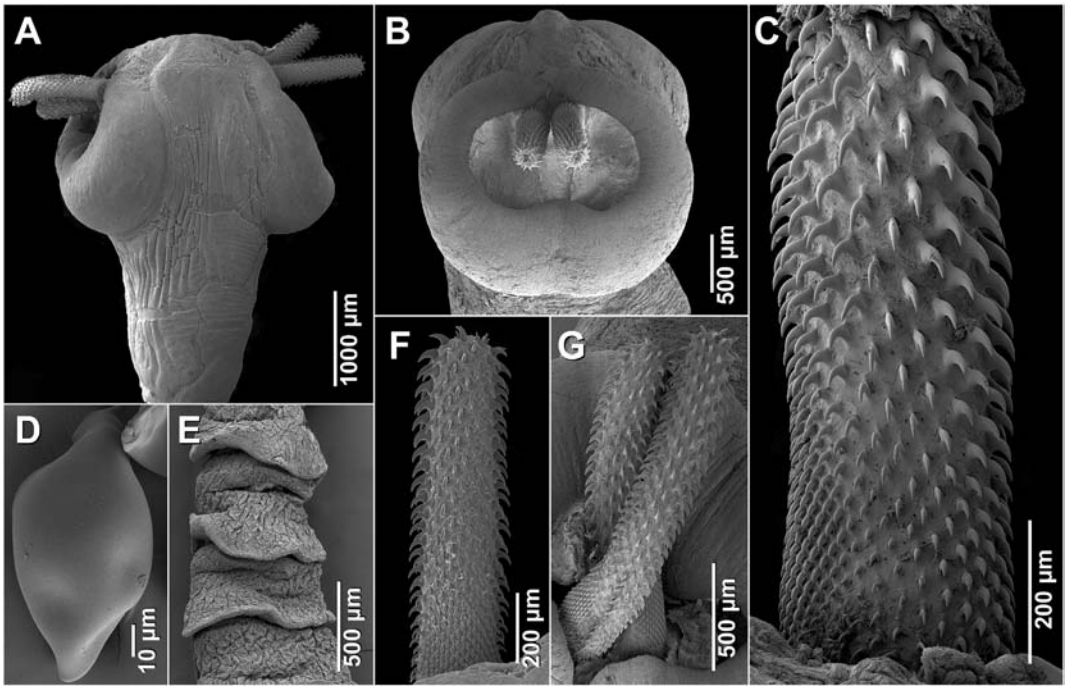

Fig. 11. Scanning electron micrographs of *Sphyriocephalus viridis* from *Dalatias licha* (Bonnaterre) from the Mediterranean Sea off Dellys, Algeria. A, scolex, lateral view; B, pars bothrialis, detailed view; C, basal to metabasal tentacular armature, antibothrial surface; D, egg; E, segments of mature strobila; F, proximal to distal metabasal tentacular armature, external surface; G, basal and metabasal tentacular armature, antibothrial surface.

surfaces uncinate (Figs. 12B, 13A), 37–53 (44 ± 6; $n = 10$) long, base 26–39 (33 ± 5; $n = 10$) long. Rows 1–3 of basal hooks on internal and external surfaces falcate (Figs. 12A, C, 12A), 42–62 (53 ± 5; $n = 10$) long, base 21–32 (26 ± 3; $n = 10$) long; rows 4–13 of basal hooks on internal surface uncinate (Figs. 11C, 12A, C, 13A), 29–44 (36 ± 5; $n = 10$) long, base 27–33 (30 ± 2; $n = 10$) long; rows 4–13 of basal hooks on external surface uncinate (Figs. 11C, 12A, C, 13A), larger, 44–58 (51 ± 4; $n = 10$) long, base longer, 36–47 (42 ± 4; $n = 10$) long. All basal hooks increase in size as progressing further into transition area towards metabasal region (Figs. 11C, 12A–C). First principal rows begin at level of hook row 13 (Figs. 12A–C). Boundary between transition area and metabasal armature around hook row 19 (Figs. 12A–C).

Proglottids craspedote (Figs. 10A, 11E), covered by acicular filitriches; first immature proglottids 15–21 ($n = 2$) long by 1578–3322 ($n = 2$) wide; first mature proglottids 523–761 (625 ± 121; $n = 4$) long by 2476–7000 (4348 ± 1952; $n = 4$) wide; first gravid proglottids 646–1261 (1038 ± 270; $n = 4$) long by 3676–6500 (4901 ± 1196; $n = 4$) wide; terminal gravid proglottid 1546 ($n = 1$) long by 3783 ($n = 1$) wide. Genital pores pre-equatorial (Fig. 10E); pore inconspicuous; cirrus-sac absent (Fig. 10D, E); cirrus unarmed, invaginated in ejaculatory duct in immature and early mature proglottids (Fig. 10D, E); accessory seminal vesicle round (Fig. 10D, E), 150–162 (156 ± 5; $n = 5$) in diameter, followed by seminal vesicle, oval to pyriform (Fig. 10D, E), 217–272 (241 ± 20; $n = 5$) long by 110–150 (126 ± 15; $n = 5$) wide; accessory seminal vesicle and seminal vesicle located posterior to cirrus (Fig. 10D, E); ejaculatory duct strongly coiled, runs parallel and close to anterior margin of proglottid until reaching to ovarian isthmus (Fig. 10E). Testes occupy complete intervascular space (Fig. 10E), in 2–3 layers, mainly preovarian, some posterior to ovary and Mehlis' gland (Fig. 10E); testes oval (Fig. 10D, E), 81–101 (91 ± 5; $n = 15$) long by 55–73 (61 ± 5; $n = 15$) wide; total number of testes 965–1100 (1038 ± 68; $n = 3$); testes distribution 100–135 (119 ± 18; $n = 3$) preporal, 280–370 (335 ± 48; $n = 3$) postporal, 563–595 (583 ± 18; $n = 3$) aporal.

Vagina joins posteriorly the ejaculatory duct to form an hermaphroditic duct (Fig. 10D, E), runs posterior and parallel to ejaculatory duct, turns towards and runs posterior to ovarian isthmus at level of Mehlis' gland (Fig. 10E); seminal receptacle absent. Ovary in posterior region of proglottid (Fig. 10E), not reaching to posterior margin, bilobed in dorsoventral view; ovarian lobes 239–349 (305 ± 54; $n = 4$) long by 166–331 (245 ± 77; $n = 4$) wide in early mature proglottids, 349–509 (425 ± 68; $n = 5$) long by 184–319 (255 ± 48; $n = 5$) wide in late mature proglottids. Mehlis' gland large (Fig. 10E), round, positioned between ovarian lobes, almost reaching to posterior margin of proglottid, 162–288 (234 ± 54; $n = 4$) long by 172–264 (221 ± 42; $n = 4$) wide in early mature proglottids, 202–368 (314 ± 68; $n = 5$) long by 239–380 (311 ± 63; $n = 5$) wide in late mature proglottids. Vitelline follicles circummedullar (Fig. 10E), absent in ovarian region, 18–31 (24 ± 3; $n = 15$) in diameter in early mature proglottids, 34–46 (40 ± 3; $n = 15$) in late mature proglottids. Uterus preformed (Fig. 10E), fusiform, highly folded, parallel and overlapping vagina and ejaculatory duct, displaced porally. Uterine pore present (Fig. 10A), displaced porally.

Eggs thin-walled, variable in shape, oval to pyriform (Fig. 11D), normally pointed on both sides and with mucron at one pole, side opposite to mucron occasionally completely blunt; with no prolongations present; 82–121 (95 ± 10; $n = 20$) long by 55–73 (61 ± 5; $n = 20$) wide; egg surface smooth.

Remarks

This species was originally described by Wagener [36] as *Tetrarhynchus viridis*. Pintner [23] redescribed the species, allocated it within the genus *Sphyriocephalus* and erected the family Sphyriocephalidae. Although later authors reviewed the work of Wagener [36] and Pintner [23] and addressed different features of the morphology of this species [1,3,7], the description remained incomplete and several morphological characteristics were still unknown. The present work provides the most advanced redescription of this species and accurately addresses the features that until now have remained unknown or dubious.

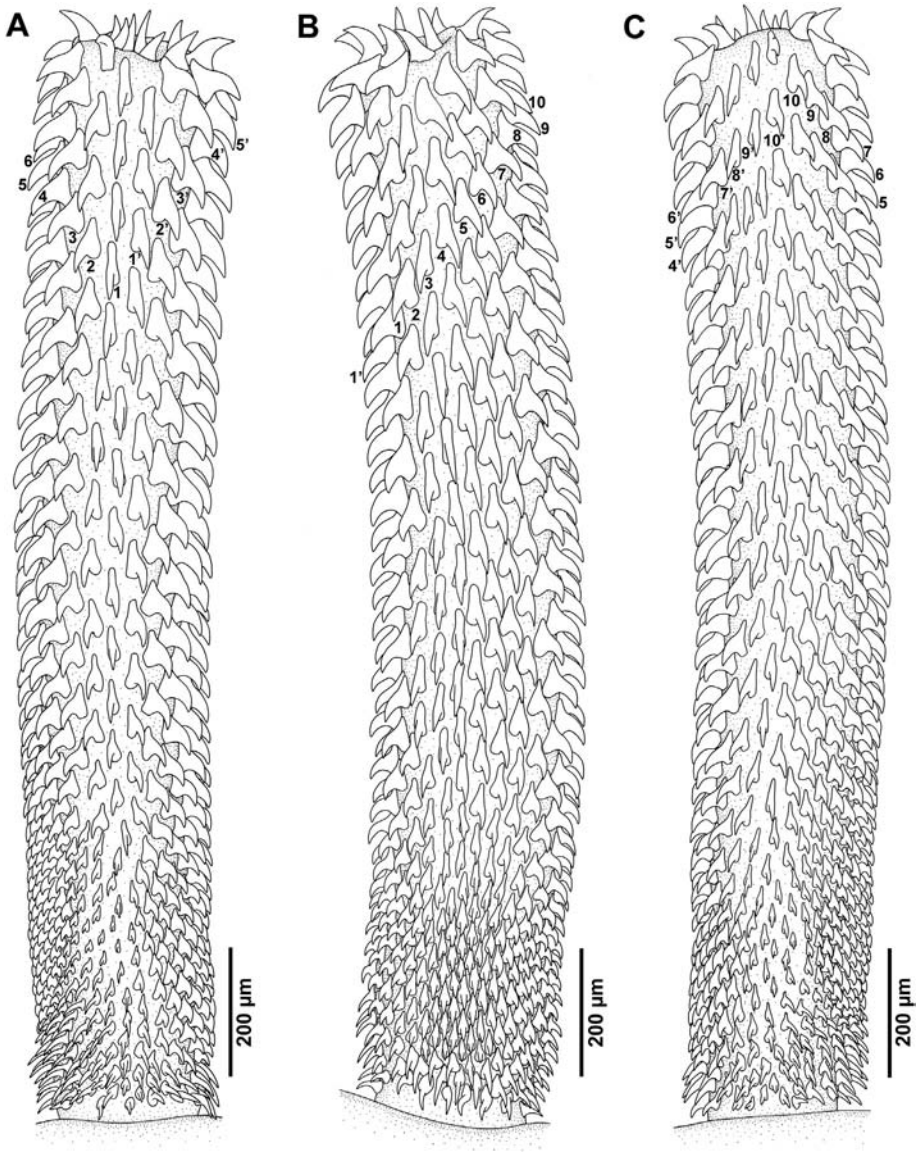

Fig. 12. Line drawings of tentacular armature of *Sphyriocephalus viridis* from *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello from the Mediterranean Sea off Majorca, Spain. A, tentacular armature, antibothrial surface; B, tentacular armature, internal surface; C, tentacular armature, bothrial surface.

Sphyriocephalus viridis can be characterized by (i) the presence of a small protuberance on the apical part of the scolex, (ii) a different tentacle diameter (i.e. smaller than in *Sphyriocephalus pelorosoma* and *Sphyriocephalus dollfusi* but larger than in members of the genus *Heterosphyriocephalus*), (iii) a different size of the uncinate hooks arranged in mosaic pattern on internal (i.e. smaller hooks) and external (i.e. larger hooks) surfaces of the basal tentacular armature, (iv) the presence of two seminal vesicles in the distal part of the ejaculatory duct, (v) the possession of a large number of testes (i.e. around 1000) and (vi) a different size and shape of eggs (i.e. oval to pyriform, without any prolongations and with mucron on one pole).

Sphyriocephalus viridis resembles its congeners *S. pelorosoma* and *S. dollfusi* in similar proportions of the scolex and bothria (Table 2) and in a similar number of principal hooks in the metabasal armature (i.e. 20–23 hooks). Furthermore, it also resembles *S. dollfusi* in its possession of an acraspedote scolex.

However, *S. viridis* can be differentiated from both *S. pelorosoma* and *S. dollfusi* in a number of features, such as (i) a larger body size (i.e. total length up to 300 mm vs. up to 118 and 71 mm, respectively), (ii) a different orientation of bulbs (i.e. transversely vs. longitudinally oriented, respectively), (iii) differently shaped bulbs (i.e. compact vs. elongate in the other two species, respectively), (iv) longer tentacles (i.e. 2876–4353 vs. 2000–2125 and 2020–2530 in length, respectively), (v) a smaller tentacle diameter (i.e. basal armature: 223–246 vs. 475–500 and 460–600, respectively; metabasal armature: 215–269 vs. 375–450 and 375–475, respectively), (vi) the presence of a basal swelling and a characteristic basal armature (vs. absence of both features, respectively), (vii) a typical heteroacanthous, heteromorphous metabasal armature (vs. a homeoacanthous, homeomorphous metabasal armature, respectively) and (viii) smaller metabasal hooks with narrower base (95–110 × 62–78 vs. 150–160 × 135–140 and 126–145 × 110–126, respectively). *Sphyriocephalus viridis* can be further distinguished from *S. pelorosoma* by (i) the absence of a velum (vs. presence of a velum, respectively) and (ii) a different shape of eggs (i.e. absence vs. presence of a long prolongation, respectively).

As it has been stated above for the type species of *Heterosphyriocephalus*, detailed observations on the morphology of *S. pelorosoma* and *S. dollfusi* with special focus on characteristics of the reproductive system are necessary in order to differentiate between *S. viridis* and its congeners.

As Dollfus [1] noted, the apical protuberance of the scolex was described and clearly illustrated by Wagener [36] but ignored by Pintner

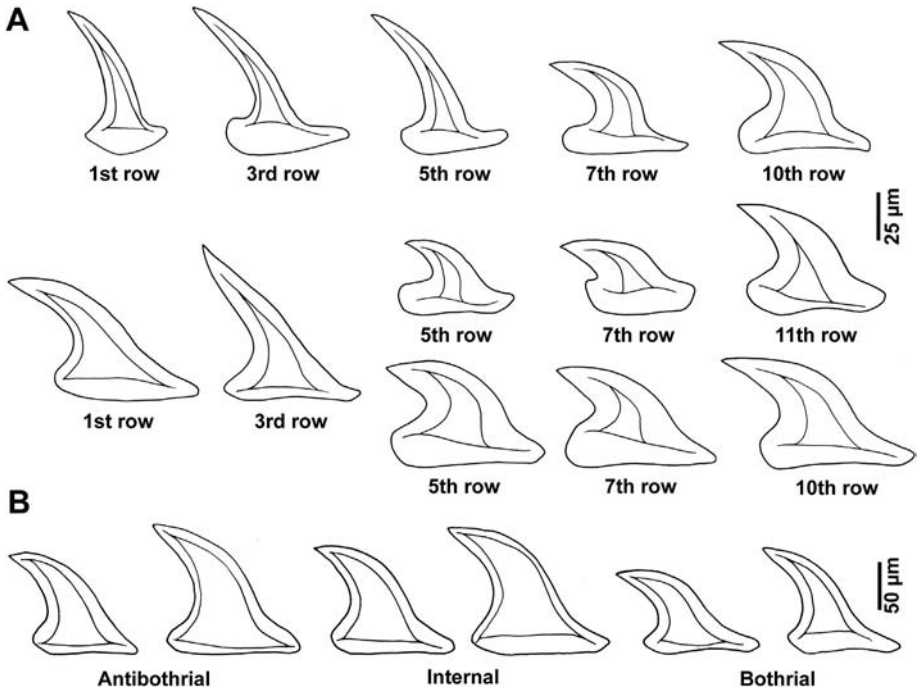

Fig. 13. Line drawings of individual tentacular hooks of *Sphyriocephalus viridis* from *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello from the Mediterranean Sea off Majorca and Barcelona, Spain. A, basal individual hooks. Upper figures show hooks on antibothrial and bothrial surfaces, while lower figures show hooks on internal and external surfaces (hooks of internal surface are shown on upper level; hooks of external surface are shown on lower level); B, individual hooks on metabasal tentacular armature. Left figures on each tentacular surface show hooks on transition area from basal to metabasal region, while right figures on each tentacular surface show hooks on metabasal region.

[23]. Histological sections revealed a small concavity in the centre, with radially departing muscular fibers.

Palm [7] described the scolex of *S. viridis* as slightly craspedote with the presence of a short velum. However, Dollfus [1] stated that the posterior limitation of the scolex is marked by an irregular constriction instead of a velum. According to this author, this line corresponds to the posterior margin of a velum, which is not free, and he considered the scolex as "cryptocraspedote" (i.e. apparently acraspedote due to the reduction and fusion with the strobila of the real velum). A velum has not been observed in any of the examined specimens (i.e. museum specimens and new material). Therefore, the scolex of *S. viridis* is considered as acraspedote.

Although this species was originally thought to have an homeoacanthous armature pattern, Bussieras [3] re-examined the type specimens of *S. alberti* (now *S. viridis*) deposited in the Oceanographic Museum in Monaco and noted that the metabasal armature follows a typical heteroacanthous pattern. According to this author, this feature could only be detected in the distal part of the tentacle, since the half-spiral rows were not distinguishable in the early metabasal armature. Herein we confirm this pattern observed by Bussieras [3] and

Table 2
Metrical data of species of *Sphyriocephalus* and *Heterosphyriocephalus*. *Abbreviations*: TL, total length; NS, number of segments; SL, scolex length; SW, scolex width; PboL, pars bothrialis length; PboW, pars bothrialis width; BoL, bothrium length; BoW, bothrium width; PvaL, pars vaginalis length; PvaW, pars vaginalis width; PbuL, pars bulbosa length; PbuW, pars bulbosa width; BL, bulb length; BW, bulb width; PpbuL, pars postbulbosa length.

Species / measurements	*S. viridis*	*S. dollfusi*	*S. pelorosoma*	*H. oheulumiae*	*H. tergestinus* n. comb.	*H. encarnae* n. sp.
TL (mm)	>200	71	69–118	–	44–480	9.5–34.0
NS	>200	–	>300	–	126–260	34–57
SL	4952–6183	5000–5375	6000–6500	6,5–9625	1393–2600	1507–1938
SW	3614	3000–5800	6550–6675	2475–4000	1541–2254	1723–2322
PboL	2415–3737	2020–3000	2625–2750	1950–2000	1232–1969	1200–1815
PboW	3876–4322	3829[a]	–	2545[b]	1714–3146	1714–2139
BoL	1779–3076	2020	–	1450–1750	889–1435	1200–1815
BoW	2207–2907	2140–2500	–	1225–1350	1143–1523	1246–1523
PvaL	2830–3783	2020–3375	2940–4000	1800–2800	1061	692–1046
PvaW	3399	3286[a]	–	–	1676–1861	1553–1999
PbuL	1230–1846	2000–2500	2390–2750	925	569–692	346–584
PbuW	2907–3614	3371[a]	–	–	1676–1846	1692–2230
BL	1200–1707	2025–2330	2200–2390	750–925	689–896	631–923
BW	615–723	620–1000	750–1000	275–425	238–396	246–354
PpbuL	477–923	–	9640	–	108–400	0
Ratios						
BL:BW	1.8–2.6	2.3–3.5	2.3–3.0	1.9–3.3	2.0–3.0	2.3–3.0
PvaL:PboL	1.0–1.2	1.2–1.4	1.0–1.2	2.1–2.2	1,9	0.5–0.9
PbuL:PboL	0.4–0.8	1.1–1.7	1.2–1.6	2.0–3.0	2,8	0.3–0.4

[a] Inferred from line-drawing of Bussieras & Aldrin (1968, Fig. 1).
[b] Inferred from line-drawing of Palm (2004, Fig. 69a).

determine that *S. viridis* possesses a typical heteroacanthous armature, with half-spiral rows starting on the antibothrial surface and terminating on the bothrial surface. This characteristic is only visible in vastly extended tentacles, since in the early metabasal armature hooks are fairly small and close to one another which makes it hard to observe this pattern. This fact has also been stated by Bussieras [3]. However, Bussieras [3] misidentified the tentacular surfaces on which the patches of small, uncinate hooks forming a mosaic pattern on the basal armature are located. He stated that these hooks are located on bothrial and antibothrial surfaces, whereas these structures are in fact located on the internal and external tentacular surfaces.

Former authors noted the presence of a "globular-pyriform seminal vesicle" [1] in the distal part of the ejaculatory duct. Although this suggests only a single structure, the present study revealed the presence of two distinct seminal vesicles in a tandem position. Both vesicles are clearly separated, connected only by a short and narrow duct. At present, this feature is unique within the family Sphyriocephalidae and none of the species in this group possesses seminal vesicles in the distal part of the ejaculatory duct. However, examinations of adult specimens of *S. pelorosoma* and *S. dollfusi* are necessary in order to determine whether this characteristic resembles a generic feature. The presence of one, two or even three seminal vesicles in the distal part of the ejaculatory duct is a common feature in several groups of trypanorhynch cestodes (e.g. all members of the order Gymnorhynchoidea, some members of Eutetrarhynchidae or Otobothriidae) and seems to be highly variable, even among genera of the same family [7].

The eggs of *S. viridis* were described by Wagener [36] and Pintner [23] as bearing a "spiniform process" on either side. However, examination of eggs from the new material reveals that, although most eggs are pointed at both sides, only one side bears a true, spiniform process or mucron. In fact, egg shapes can vary significantly from semi-circular or oval to even pyriform, with one mucronated pole and a completely blunt opposite pole. Eggs described in the original description by Wagener [36] were also smaller than the ones observed in the present study (i.e. 75 × 45 vs. 95 × 61, respectively). Based on these differences, we can only speculate that he could have examined eggs that belong to a different species or which had been observed in utero.

3.2. Keys and generic diagnoses

3.2.1. Key to the genera of Sphyriocephalidae

Features of the segment morphology presented in the key only apply for species for which adult specimens are well-known (i.e. *S.viridis*, *H. encarnae* and *H. tergestinus*).

1 Tentacles short, bulbous or conical in shape, emerging from anterior bothrial margins; bothria elongate; two sets of genitalia per proglottid..... ***Hepatoxylon* Bosc, 1811**

- Tentacles long and cylindrical in shape, emerging from inside the bothria; bothria round or oval; one set of genitalia per proglottid..... 2

2 Bulbs >1200 in length and 600 in width; two seminal vesicles present in distal part of ejaculatory duct; absence of spherical dense mass at the distal part of the vagina; tentacles >2000 in length; absence of small projections covering different parts of the scolex..... ***Sphyriocephalus* Pintner, 1913**

- Bulbs <1000 in length and 500 in width; seminal vesicles absent or only a single seminal vesicle present in proximal part of ejaculatory duct; presence of spherical dense mass located at the distal part of the vagina; tentacles <1800 in length; presence of small projections covering different parts of the scolex..... ***Heterosphyriocephalus* Palm, 2004**

3.2.2. Genus Sphyriocephalus Pintner, 1913

Diagnosis: Scolex large, compact, craspedote or acraspedote. Bothria two in number, oval, well delimited, with fused posterior margins, with thick, fleshy rims; bothrial pits absent; tentacle sheaths sinuous or straight; retractor muscles attach at anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent; bulbs compact, oval, in transverse or longitudinal orientation. Four elongate tentacles; metabasal armature homeoacanthous, homeomorphous or typical heteroacanthous, heteromorphous; hooks hollow. Distinctive basal armature and basal swelling present or absent. Proglottids (unknown for *S. pelorosoma* and *S. dollfusi*) craspedote. Genital pores marginal, irregularly alternating; cirrus-sac absent; cirrus unarmed, invaginated in ejaculatory duct; two seminal vesicles present in distal part of ejaculatory duct, posterior to cirrus. Testes oval, occupy complete intervascular space, in 2–3 layers, mainly preovarian. Vagina joins posteriorly the ejaculatory duct to form an hermaphroditic duct; seminal receptacle absent. Ovary in posterior region of proglottid, bilobed in dorsoventral view; Mehlis' gland posterior, between ovarian lobes. Vitelline follicles circummedullar, absent in ovarian region. Uterus preformed, displaced porally. Uterine pore present, displaced porally. Eggs thin-walled, variable in shape.

Type-species: S. viridis (Wagener, 1854) Pintner, 1913.

Key to the species of *Sphyriocephalus*

1 Metabasal tentacular armature typical heteroacanthous, heteromorphous; basal swelling and characteristic basal armature present; tentacles >2800 in length and <300 in diameter; bulbs in transverse orientation..... ***S. viridis* (Wagener, 1854) Pintner, 1913**

- Metabasal tentacular armature homeoacanthous, homeomorphous; basal swelling and characteristic basal armature absent; tentacles <2600 in length and >350 in diameter; bulbs in longitudinal orientation..... **2**

2 Scolex craspedote, >6.0 mm in length; pars vaginalis of similar length or shorter than pars bothrialis; metabasal hooks >150 in length..... ***S. pelorosoma* Heinz & Dailey, 1974**

- Scolex acraspedote, <5.4 mm in length; pars vaginalis longer than pars bothrialis; metabasal hooks <145 in length..... ***S. dollfusi* Bussieras & Aldrin, 1968**

3.2.3. Genus Heterosphyriocephalus Palm, 2004

Diagnosis: Scolex large, compact, craspedote or acraspedote. Bothria two in number, oval, well delimited, with fused posterior margins, with thick, fleshy rims; bothrial pits absent; small projections covering different parts of the scolex (i.e. scolex peduncle, velum, proximal bothrial surface) (latter character unknown for *H. oheulumiae*); tentacle sheaths sinuous; retractor muscles attach at anterior part of bulbs; prebulbar organs and gland cells inside bulbs absent; bulbs compact, oval, in transverse orientation. Four elongate tentacles; metabasal armature typical heteroacanthous, heteromorphous or homeomorphous; hooks hollow. Distinctive basal armature and basal swelling present or absent. Proglottids (unknown for *H. oheulumiae*) craspedote. Genital pores marginal, irregularly alternating; cirrus-sac absent; cirrus unarmed, invaginated in ejaculatory duct; external seminal vesicle present or absent. Testes oval, occupy complete intervascular space, in 2–3 layers, mainly

preovarian. Vagina enters genital atrium posterior to ejaculatory duct; seminal receptacle present or absent. Ovary in posterior region of proglottid, bilobed in dorsoventral view; Mehlis' gland between ovarian lobes. Vitelline follicles circummedullar, absent in ovarian region. Uterus preformed, displaced porally. Uterine pore present, displaced porally. Eggs thin-walled, with two opposite prolongations; first prolongation short and filiform, second prolongation elongate and wide.

Type-species: H. oheulumiae Palm, 2004.

Key to the species of *Heterosphyriocephalus*

1 Scolex acraspedote, >6 mm in length; metabasal tentacular armature with 11–13 hooks per principal row; basal swelling and characteristic basal armature absent..... ***H. oheulumiae* Palm, 2004**

- Scolex craspedote, <5 mm in length; metabasal tentacular armature with <10 hooks per principal row; basal swelling and characteristic basal armature present..... **2**

2 Worms between 200 and 300 mm in length and with >150 proglottids; bothria well delimited; proximal bothrial surface lacking small projections; pars post-bulbosa present; scolex velum with regular border, not folded; large, pyriform seminal vesicle in proximal part of ejaculatory duct; seminal receptacle present..... ***H. tergestinus* n. comb. (Pintner, 1913)**

- Worms between 10 and 34 mm in length and with fewer than 100 proglottids; bothria less delimited; proximal bothrial surface covered with small projections; pars post-bulbosa absent; scolex velum with irregular and folded border; seminal vesicles absent; seminal receptacle absent..... ***H. encarnae* n. sp.**

3.3. Relationships between species of sphyriocephalids based on molecular data

Three identical partial 28S rDNA (domains D1–D3, 1250 nt) sequences were generated from three adult specimens of *H. tergestinus*.

Both ML and BI algorithms produced trees with identical topology, which are illustrated in Fig. 9. The molecular analysis has shown that the newly generated sequences of *H. tergestinus* grouped with that of *H. oheulumiae* ex. *T. steindachneri* collected in Indonesia [15] forming a strongly supported monophyletic clade. The sequences for both species differed by 1.0–1.1% (13–14 nt). *Hepatoxylon trichiuri* ex. *Taractes rubescens* (Jordan & Evermann) (Perciformes: Bramidae) from Indonesia [15] formed a clade with members of *Heterosphyriocephalus*, though not strongly supported, and differed by 5.5–6.0% (69–75 nt) from the newly generated sequences. The next clade combined the sequences of *S. viridis* ex *D. licha* from Indonesia [15] and *Sphyriocephalus* sp. ex. *D. licha* from northeastern Atlantic Ocean (off the southwestern part of Ireland) [16], thus confirming the identity of the sequence obtained by Olson et al. [16]. The sequences of the latter species were identical and differed by 7.9–8.2% (99–103 nt) from our newly generated sequences.

4. Discussion

4.1. Contributions to the knowledge of sphyriocephalid tapeworms

This is the first comprehensive study on the family Sphyriocephalidae including morphological and molecular data.

With the description of *H. encarnae* and the allocation of *S. tergestinus* in the genus *Heterosphyriocephalus* this genus is no longer monotypic and now includes three valid species (i.e. the two mentioned species and the type-species *H. oheulumiae*). Therefore, it now has an equal number of species as the genus *Sphyriocephalus*, but might become the most speciose genus within the family given that *S. pelorosoma* and *S. dollfusi* might be transferred to it, either as a single species or separately, as it has been suggested by Palm [7] (see below).

The genus *Sphyriocephalus* now consists of three valid species, out of which only the type-species, *S. viridis*, is adequately characterized. *Sphyriocephalus pelorosoma* was originally described on the basis of a single, adult specimen [5] and *S. dollfusi* only on the basis of a single plerocercoid [6]. Despite the recovery of adults for both species (several for *S. pelorosoma* and only one for *S. dollfusi*) in Indonesia off Pelabuhan Ratu by Palm [7,37], information and measurements regarding the genital organs and eggs are almost entirely absent. Although Palm [7] considered both species valid, he suggested that they might be conspecific, due to their high morphological similarity, with the exception of slight differences in some scolex measurements (see Table 2) and hook sizes. Furthermore, the eggs of *S. pelorosoma* possess a long filament [7], for which the length has not been provided. According to this description, such eggs clearly differ from those found in *S. viridis*. These eggs could resemble those of *H. encarnae* and *H. tergestinus*, which bear a short and filiform prolongation and a larger and wider one (present study, see Sections 3.1.1 and 3.1.2). This trait might indicate a possible allocation of *S. pelorosoma* and *S. dollfusi* to the genus *Heterosphyriocephalus*, for which the egg shape is highly characteristic. However, further studies completing the morphological description for both species and including molecular data will be required to confirm the synonymy of *S. pelorosoma* and *S. dollfusi*, which will furthermore clarify their phylogenetic position within the Sphyriocephalidae.

The genus *Hepatoxylon* remains unchanged. Although the morphology of its members has not been addressed in the present study, the available sequence of the type-species *He. trichiuri* has been incorporated in the phylogenetic analyses and its relation to other members of the family are discussed.

4.2. Phylogenetic relationships

As suggested by morphology (see remarks section for *H. tergestinus* n. comb. in Section 3.1.2) and confirmed by molecular data (Fig. 9), *H. tergestinus* n. comb. and *H. oheulumiae* are congeners and therefore have to be placed within the same genus.

One of the questions that arises from the phylogenetic analysis is the position of the genus *Hepatoxylon* within the Sphyriocephalidae and in relation to the remaining genera. According to present results, *He. trichiuri* groups with members of *Heterosphyriocephalus*, while species of *Sphyriocephalus* remain as a separate clade. This configuration, although weakly supported, agrees with the results published by Palm et al. [15] based on 18S rDNA and 28S rDNA. However, according to morphological data, *Sphyriocephalus* and *Heterosphyriocephalus* are the most similar genera within the family and one might expect that their members group together while representatives of *Hepatoxylon* form a separate clade. Palm [15] carried out a molecular study on the phylogeny of trypanorhynch cestodes, where the same topology as the one presented herein was observed: *H. oheulumiae* and *He. trichiuri* grouped together, while *S. viridis* appeared in a more derived position. As highlighted by Bazsalovicsová et al. [38], this conflict between morphology and genetic data is of high relevance in taxonomy and systematics. Scholz et al. [39] encountered the same situation with species of the genus *Khawia* Hsü, 1935 (Cestoda: Caryophyllidea), which was regarded as a possible consequence of morphological divergence (without the corresponding genetic differentiation) which might have occurred after the switch of one of two related parasite species to a different host group. Following this argument, *Hepatoxylon* is the only genus of the family which parasitizes carcharhiniform sharks, while the other two genera seem restricted to lamniform and squaliform selachians. Within these, the family Alopiidae seems to be of special importance, since all valid species within *Sphyriocephalus* and

Heterosphyriocephalus (with the exception of *H. oheulumiae*, for which the final host remains unknown) have been recovered from this host family (see below). This possible host-related phenotypic plasticity, despite frequently encountered in multiple other parasite groups [39–42], is not yet well understood, and furthermore highlights the importance of combining morphological with genetic data in systematic studies.

4.3. Host-specificity

Adult specimens of trypanorhynchs vary greatly in their host specificity, from species with a euryxenous to oioxenous host specificity [43,44]. Larval stages, on the other hand, are known to show a very low host specificity [45].

Overall, plerocerci of *S. viridis* and *H. tergestinus* have been reported from a large number of teleosts acting as intermediate hosts (see Sections 3.1.2 and 3.1.3). The same has been observed for members of *Hepatoxylon* [1]. When addressing the adult forms of sphyriocephalids, it can be stated that the most well-known species are able to parasitize a diverse array of mostly pelagic selachians as their final hosts. Adults of *S. viridis* and *H. tergestinus* have each been reported from three hosts belonging to two different orders and three different families [i.e. *S. viridis* from *A. superciliosus* (Lamniformes: Alopiidae), *D. licha* (Squaliformes: Dalatiidae) and *C. granulosus* (Squaliformes: Centrophoridae); *H. tergestinus* from *A. vulpinus* (Lamniformes: Alopiidae), *E. bispinatus* (Squaliformes: Dalatiidae) and *I. oxyrinchus* (Lamniformes: Lamnidae)] ([3,5,8,23,25,30,31,32,34,36], present study). Similarly, the adults of *He. trichiuri* have been recovered from four hosts belonging to three different orders and four different families, namely *Carcharias* sp. (Lamniformes: Odontaspididae), *Lamna nasus* (Bonnaterre) (Lamniformes: Lamnidae), *Prionace glauca* (L.) (Carcharhiniformes: Carcharhinidae) and *Somniosus microcephalus* (Bloch & Schneider) (Squaliformes: Somniosidae)) (citations listed in Dollfus [1]).

In contrast, adult specimens of *S. pelorosoma* and *S. dollfusi* are solely known from *A. superciliosus* (Lamniformes: Alopiidae) [7,37], *H. encarnae* from *A. pelagicus* (Lamniformes: Alopiidae) (present study), and *He. megacephalum* from *Carcharodon carcharias* (L.) (Carcarhiniformes, Carcharhinidae) [1]. Until now, *H. oheulumiae* has not been found in its adult form [7].

Taking into account the low host-specificity shown by adults of the most well-known sphyriocephalids, we hypothesize that more definitive hosts are likely to emerge and will add to the list of infected host species for the least-known or recently described species of this group.

4.4. Different patterns of host-infection among genera

Despite an existing overlap in host preference among the three genera in terms of orders and families of selachians serving as definitive hosts, some patterns can be revealed. The genera *Sphyriocephalus* and *Heterosphyriocephalus* seem restricted to the orders Lamniformes and Squaliformes. Within these two orders, the family Alopiidae seems to be of a special importance, since all the existing species within both genera (with the exception of *H. oheulumiae*, for which the definitive host has not yet been described) have been reported from alopiids. In contrast, *Hepatoxylon* represents the only genus that has been reported from sharks of the order Carcharhiniformes. Members of Lamniformes and Squaliformes also serve as definitive hosts, but in these cases the families differ as follows: Alopiidae and Dalatiidae represent typical definitive hosts for members of *Sphyriocephalus* and *Heterosphyriocephalus*, while members of *Hepatoxylon* have been recorded from Somniosidae and Odontaspididae. The family Lamnidae seems to be infected by members of both groups (namely by *H. tergestinus* and *He. trichiuri*).

4.5. Patterns in the geographical distribution

Sphyriocephalus viridis and *H. tergestinus* both possess an interoceanic distribution (molecularly confirmed for the former species by Palm [15]). Both species have been reported from the Atlantic (including the Mediterranean Sea) and Pacific Oceans and *H. tergestinus* also occurs in the Indian Ocean (see Sections 3.1.2 and 3.1.3 for a list of localities). This is not surprising given the cosmopolitan distribution patterns and migratory habits of the typical final hosts of both species. *Alopias vulpinus*, which acts as the final host for *H. tergestinus*, is a cosmopolitan species of tropical and temperate waters [46] with highly migratory habits (sensu [47]), while *D. licha*, typically hosting the adults of *S. viridis*, is found at the continental shelves and slopes of tropical and temperate latitudes worldwide [46].

In the case of *H. encarnae* we assume a presence in the Indian and Pacific Oceans since its final host, *A. pelagicus*, seems restricted to the tropical areas in the Indo-Pacific [46]. However, there is a partial lack of reliable records due to its confusion with *A. vulpinus* (sensu [47]).

Alopias superciliosus, same as *A. vulpinus*, occurs in tropical and temperate waters and displays a cosmopolitan distribution [46]. Therefore, *S. dollfusi* and *S. pelorosoma* are expected to follow the same distributional pattern. At present, *S. dollfusi* has been recorded from the Atlantic and Indian Oceans [6,7] and *S. pelorosoma* from the Indian and Pacific Oceans [5,7].

Although three species of *Alopias* are officially recognized, Eitner [48] suggested the presence of a fourth unrecognized species based on fixed allelic differences among individuals in a group of specimens identified as *A. superciliosus*. Since each species of *Alopias* seems to host its corresponding sphyriocephalid parasite (*S. dollfusi* and *S. pelorosoma* would make an exception if they are confirmed as congeners) we could speculate the possible existence of an additional sphyriocephalid species parasitizing this (at present) undescribed alopiid shark.

In the case of *Hepatoxylon*, the type species *He. trichiuri* also has a cosmopolitan distribution, as it was recorded from the Atlantic and Pacific Oceans as well as from the Mediterranean Sea (see Dollfus [1] for a list of references; [4]). *Hepatoxylon megacephalum* has only been recorded from the Pacific Ocean [4] and the Mediterranean Sea (see Dollfus [1] for references). Although only very few records exist for the latter species, its final host, Ca. *carcharias* is found worldwide mostly in temperate waters and undergoes interoceanic migrations (sensu [47]). Therefore, we expect further geographical records for *H. megacephalum* in the future.

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CONCLUSIONS

CONCLUSIONS

1. The parasite communities of the teleosts *Mora moro* and *Phycis blennoides* are described for the first time, and are characterized by high parasite abundance, richness and diversity. *Mora moro* hosts 18 different endoparasite taxa, of which 17 are new host records, and *P. blennoides* hosts 20 different parasite taxa, of which 11 constitute new host records.

2. The nematodes Anisakidae gen. sp., *Anisakis* Type II and larval forms of tetraphyllidean cestodes are suggested as tags for differentiating populations of *M. moro* in the Balearic Sea.

3. Parasite infracommunities of *P. blennoides* vary with depth as a result of an ontogenic diet shift coupled to a bathymetric migration of this host, in turn associated to a different composition of macroinvertebrate communities along the continental slope.

4. Parasite infracommunities of *M. moro* and *P. blennoides* are more abundant, rich and diverse in the mainland than in the insular slope due to the existence of more complex and abundant benthopelagic faunal assemblages in the former, in turn associated to more favourable environmental and hydrographic conditions (i.e. higher O_2 levels and increased organic matter linked to submarine canyons).

5. Parasites communities of shark species are described for the first time from the Mediterranean Sea. To date, no shark parasite communities had been described in this area.

6. The parasite community of the shark *Scyliorhinus canicula* is characterized by low richness and diversity, and high dominance of the nematode *Proleptus obtusus*, which reaches very high abundance levels. A total of five parasites have been recovered, of which one constitutes a new host record.

7. The parasite community of the shark *Galeus melastomus* is described for the first time. It is characterized by high abundance, moderate richness and diversity values and high dominance of the cestodes *Ditrachybothridium macrocephalum*, in the case of juveniles, and *Grillotia adenoplusia*, in the case of adult sharks. A total of 15 metazoan parasites, of which 13 constitute new host records, and one microparasite have been recovered.

8. Parasite infracommunities of *G. melastomus* show lower richness and abundance off Vilanova than off Besós sampling sites, which is possibly due to the vicinity of the latter locality to the Besós submarine canyon, associated to more complex invertebrate communities as a result of higher nutrient availability.

9. Parasite infracommunities of adult specimens of *G. melastomus* are more abundant, rich and diverse than those of juveniles. This pattern is mainly associated to an ontogenic diet shift undergone by this shark, according to which adult sharks consume larger prey including crustaceans, cephalopods and fish.

10. The parasite community of juvenile specimens of the shark *Etmopterus spinax* is described for the first time in the Mediterranean Sea. It is characterized by extremely low richness and diversity values, with only two parasite species recovered, which contrasts with the parasite assemblages of juvenile *E. spinax* described from the Atlantic Ocean. The number of specimens available of this species was low and parasitological results should thus be considered preliminary.

11. The parasite community of the shark *Centroscymnus coelolepis* is described for the first time, and it is characterized by moderate richness and diversity. A total of eight parasites have been recovered, of which three constitute new host records. The number of specimens available of this species was low and parasitological results should thus be considered preliminary.

12. The abundance patterns of coelozoic parasites are mainly determined by feeding rates or dietary shifts of the host, while histozoic parasites accumulate in different tissues throughout the lifespan of the host and show a positive correlation with its size.

13. Multivariate Canonical Correspondence Analyses can be a powerful and reliable tool for inferring the environmental drivers of parasite abundance patterns, as suggested by the numerous coincidences found among the parasite communities of the hosts addressed regarding relationships between the abundance of individual parasites and environmental variables.

14. High O_2 concentration of water masses, linked to the mainland slope, enhances the abundance of parasites with indirect life cycles as a result of zooplankton proliferation and the associated aggregation of potential intermediate hosts. In a similar way, increased water turbidity favours parasite transmission and increases parasite loads due

to an increase of zooplankton and suprabenthic invertebrate communities linked to higher nutrient availability.

15. High water salinity levels, associated to the upper slope, seem to be linked to higher abundance of the nematodes *Hysterothylacium aduncum* and *P. obtusus*, probably because salinity correlates with the abundance of decapods and mysids, among others, which are used by these nematodes as intermediate hosts. In turn, high water temperature levels increase the abundance of monogenean parasites, likely due to enhanced egg hatching success and reduced time to maturity, as previously reported for these parasites.

16. Multivariate Canonical Correspondence Analyses can be a useful tool for elucidating the transmission pathways of heteroxenous parasites, as suggested by the numerous coincidences found between the parasite-prey relationships determined in the different studies and what is known about the life cycles of the parasites addressed. Such detailed parasite-prey relationships provide a better understanding of the life cycles of the parasites addressed. Already known transmission patterns have been confirmed in many cases, and new ways of infection have been suggested in others.

17. A possible inhibition of acetylcholinesterase activity due to parasite infection-related stress has been detected in *P. blennoides*, *G. melastomus*, *E. spinax* and *C. coelolepis*. Furthermore, increased lipid peroxidation levels with higher abundance of the acanthocephalan *Echinorhynchus* sp. and the nematode *H. aduncum* have been detected in *P. blennoides*, possibly pointing to oxidative stress induced by parasitism. However, enzymes respond to many different factors that can interact among them yielding complex activity patterns, and inconsistencies observed for other enzymatic biomarkers or parasites, and in the literature, warn against a too simplistic interpretation of these results.

18. Lower citrate synthase activity has been observed in *C. coelolepis* compared to *G. melastomus* and *E. spinax*, possibly due to a deeper distribution of this shark. Higher acetylcholinesterase activity has been observed in *E. spinax* compared to *G. melastomus* and *C. coelolepis*, possibly explained by the more pelagic habits of this shark.

19. In general, parasite burden does not seem to have a significant effect on fish general condition indices, which points to a negligible impact of parasite loads on general fish

condition when infection levels are not abnormally harmful or high. Caution should be taken when attributing variations of such indices, which respond to many different factors, to parasite infection.

20. Parasite burden does not seem to have a significant impact on the number and/or surface of splenic melano-macrophage or macrophage centres in the case of teleosts, or on the number of hepatic melano-macrophages in the case of *G. melastomus*. Since these structures respond to multiple factors, caution must be taken when attributing their variations to parasite infection.

21. The presence of cysts of unknown etiology is reported from *M. moro* and *P. blennoides*. In *P. blennoides*, these structures further reach the highest prevalences recorded to date in any fish from the study area.

22. Lesions associated to larval stages of the cestode *G. adenoplusia* have been observed in the musculature of all adult specimens of *G. melastomus*. These parasites accumulate on the tail region, possibly as a strategy to enhance their transmission onto the final host. Furthermore, the large accumulation of these larvae and associated destruction of the muscular tissues could compromise escape response of the host and favour its capture by the parasite final host.

23. Detailed morphological, molecular and ecological data of different developmental stages (plerocerci and adults) of the cestode *D. macrocephalum* are provided for the first time from its definitive host, the blackmouth catshark *G. melastomus*, in the Mediterranean Sea. Furthermore, morphological data on the eggs of *D. macrocephalum* are provided for the first time.

24. Mediterranean specimens of *D. macrocephalum* are conspecific with the material for the northeast Atlantic Ocean, as revealed by molecular results and morphological examination of type and voucher material. Morphological differences between specimens from the two localities are attributed to different developmental conditions and intraspecific phenotypic plasticity.

25. The higher abundance of *D. macrocephalum* in juvenile than in adult *G. melastomus*, and in the middle than in the upper slope, is in all likelihood related to ontogenic and bathymetric diet shifts undergone by its host.

26. The new species *Heterosphyriocephalus encarnae* n. sp. is described, and two already existing species, *Sphyriocephalus viridis* (the type species of the family Sphyriocephalidae) and S*phyriocephalus tergestinus* are further redescribed based on morphological and molecular data of newly-collected and museum material. *Heterosphyriocephalus encarnae* n. sp. can be readily distinguished from the rest of sphyriocephalid species by its small size, low number of proglottids, long velum with characteristically folded border, presence of cylindrical projections covering specific parts of the scolex, a typical heteroacanthous, heteromorphous, metabasal armature with eight hook per principal row, low number of testes in an exclusively pre-ovarian distribution and the absence of seminal vesicles.

27. The small cylindrical projections described in *H. encarnae*, only detectable by scanning electron microscopy, have also been observed in specific parts of the scolex of *S. tergestinus* and are reported for the first time in cestodes.

28. *Sphyriocephalus tergestinus* is allocated into *Heterosphyriocephalus* as *Heterosphyriocephalus tergestinus* n. comb. based on the results of a new phylogenetic analyses performed with available molecular data for sphyriocephalids and with new sequences generated from specimens from the Mediterranean Sea.

29. New generic dichotomous keys are provided for the family Sphyriocephalidae, as well as new generic diagnoses and keys for the determination of species for the genera *Sphyriocephalus* and *Heterosphyriocephalus* based on updated morphological descriptions and the results of the phylogenetic analysis.

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