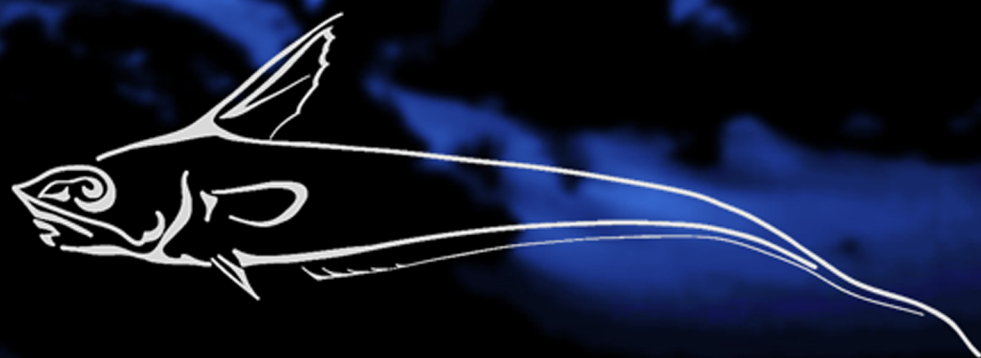


A Journey to the Depths of the Sea: Parasite
communities of the Alepocephalidae and the
Macrouridae in the Balearic Sea (NW Mediterranean)

David Pérez García
PhD Thesis 2017



Chapter V

A new species of *Hamaticolax* Ho & Lin, 2006
(Copepoda: Bomolochidae) from deep water
macrourids in the Mediterranean



A new species of *Hamaticolax* Ho & Lin, 2006 (Copepoda: Bomolochidae) from deep water macrourids in the Mediterranean

David Pérez-i-García · Maite Carrassón · Geoffrey Allan Boxshall

Received: 27 June 2016 / Accepted: 3 December 2016
© Springer Science+Business Media Dordrecht 2016

Abstract *Hamaticolax resupinus* n. sp. is described from specimens collected from the gill cavities of *Coelorinchus mediterraneus* Iwamoto & Ungaro and *Coryphaenoides mediterraneus* (Giglioli) (Gadiformes: Macrouridae) caught in the Western Mediterranean Sea at depths between 1,236 and 1,626 m. *Hamaticolax resupinus* n. sp. closely resembles *H. maleus* Oldewage, 1994, but differs from the latter by its smaller body size and in having a genital double-somite in the female that is markedly wider than the free abdominal somites and has strongly convex lateral margins. The new species is only the second bomolochid found on a macrourid host and is the first from depths in excess of 1,200 m. *Hamaticolax resupinus* n. sp. also represents the first parasitic copepod recorded from *Coe. mediterraneus* and only the third one from *Cor. mediterraneus* worldwide.

Introduction

The genus *Hamaticolax* Ho & Lin, 2006 (Bomolochidae Sumpf, 1871) is a small genus currently comprising ten species. Typically parasites of the gill cavity, these species have been reported from different families of fishes from both the Atlantic and Pacific Oceans: *Hamaticolax attenuatus* (Wilson, 1913) described from *Scorpaena plumieri* Bloch (Scorpaenidae) from off Jamaica; *Hamaticolax embiotocae* (Hanan, 1976) described from *Cymatogaster aggregata* Gibbons and other Embiotocidae from off California; *Hamaticolax galeichthyos* (Luque & Bruno, 1990) described from *Galeichthys peruvianus* Lütken (Ariidae) from off Perú; *Hamaticolax maleus* (Oldewage, 1994) described from *Malacocephalus laevis* (Lowe) (Macrouridae) from off South Africa; *Hamaticolax occultus* (Kabata, 1971) described from *Hippoglossoides elassodon* Jordan & Gilbert and *Lyopsetta exilis* (Jordan & Gilbert) (Pleuronectidae) from off British Columbia, Canada; *Hamaticolax paralabraxis* (Luque & Bruno, 1990) described from *Paralabrax humeralis* (Valenciennes) (Serranidae) from off Perú; *Hamaticolax prolixus* (Cressey, 1969) described from *Pleuronichthys coenosus* Girard (Pleuronectidae) and subsequently found on other Pleuronectidae plus some Batrachoididae, Cottidae, Cynoglossidae, Embiotocidae, Hexagrammidae, Paralichthyidae and Sciaenidae from off California; *Hamaticolax scutigerulus* (Wilson, 1935) described from *Pseudupeneus maculatus* (Bloch) (Mullidae)

D. Pérez-i-García · M. Carrassón (✉)
Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain
e-mail: Maite.Carrasson@uab.cat

G. A. Boxshall
Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

from off Belize; *Hamaticolax spinulus* (Cressey, 1969) described from *Scorpaena guttata* Girard (Scorpaenidae) and some Sebastidae and Hexagrammidae from off California; and *Hamaticolax unisagittatus* (Tavares & Luque, 2003) from *Centropomus undecimalis* (Bloch) (Centropomidae) from off Brazil (Wilson, 1913; Wilson, 1935; Cressey, 1969; Kabata, 1971; Hanan, 1976; Cressey, 1983; Luque & Bruno, 1990; Oldewage, 1994; Tavares & Luque, 2003).

During a parasitological survey of macrourid species from the deep-sea off Catalonia and the Balearic Islands (Western Mediterranean), a new bomolochid of the genus *Hamaticolax* was collected from the gills and inner surface of the opercula of *Coelorinchus mediterraneus* Iwamoto & Ungaro and *Coryphaenoides mediterraneus* (Giglioli). This is only the second *Hamaticolax* species reported from macrourids and is the first member of the Bomolochidae to be found in the deep Mediterranean Sea. This paper describes both sexes of the new *Hamaticolax* species from *Coe. mediterraneus* and *Cor. mediterraneus*, and provides a key to the species of the genus.

Materials and methods

Three individuals of *Coe. mediterraneus* (preanal length: 7.0–8.0 cm) and one of *Cor. mediterraneus* (preanal length: 3.2 cm) were captured during the oceanographic project ANTRMARE (Spanish Ministry of Science and Innovation) on the research vessel García del Cid with a semi-balloon otter-trawl (OTSB) fished at depths between 1,236 and 1,626 m off Barcelona and the Balearic Islands. Fish were measured, weighed, assigned unique codes and stored at -20°C on board until examination.

The copepods were removed from freshly thawed fish, washed in physiological saline and preserved in 70% ethanol. They were dissected and mounted in lactophenol as temporary slide preparations and examined on an Olympus microscope equipped with differential interference contrast optics. Drawings were made with the aid of a drawing tube. Measurements were made using an ocular micrometer and are presented as the range followed by the mean (in parentheses); all measurements are in millimetres. Morphological terminology follows Huys & Boxshall (1991). Host names were validated against FishBase

(Froese & Pauly, 2016). Type-material is deposited at the Natural History Museum, London (NHMUK).

Family Bomolochidae Sumpff, 1871 Genus *Hamaticolax* Ho & Lin, 2006

Hamaticolax resupinus n. sp.

Type-host: *Coelorinchus mediterraneus* Iwamoto & Ungaro (Gadiformes, Macrouridae).

Other hosts: *Coryphaenoides mediterraneus* (Giglioli) (Gadiformes, Macrouridae).

Type-locality: Off Barcelona, Western Mediterranean ($40^{\circ}41.96'N$, $01^{\circ}37.46'E$ – $40^{\circ}54.35'N$, $02^{\circ}06.06'E$); depth: 1,236–1,269 m; 10.vii.2010, 16.x.2011).

Other localities: Off Ibiza, Western Mediterranean ($39^{\circ}56.20'N$, $01^{\circ}37.91'E$; depth: 1,626 m; 19.xi.2011).

Type-material: Holotype female (NHMUK 2015.2974), allotype male (NHMUK 2015.2975), 9 female paratypes (2 damaged) and 2 male paratypes (1 damaged) (NHMUK 2015.2977–2986) ex *Coelorinchus mediterraneus*; 2 female paratypes and 2 male paratypes (1 damaged) (NHMUK 2015.2987–2990) ex *Coryphaenoides mediterraneus*.

Site on host: Gill filaments and inner surface of the opercula.

Etymology: The species name, *resupinus*, alludes to the reflexed form of the outer margin spines on the exopods of legs 3 and 4.

Description (Figs. 1–4)

Adult female. Body cycloform (Fig. 1A), 1.22 to 1.31 (1.26) long ($n = 10$); prosome length 0.79–0.92 (0.86), maximum width 0.65–0.79 (0.74). Prosome comprising broad cephalothorax and free second to fourth pedigerous somites; third somite not overlapping fourth in dorsal view (Fig. 1A). Cephalothorax bearing pair of acutely-pointed, tapering tines in rostral area (Fig. 1C). Urosome (Fig. 1B) 0.40–0.44 (0.43) long ($n = 9$), comprising fifth pedigerous somite, genital double-somite and 3 free abdominal somites. All urosomites wider than long; genital double-somite with strongly convex lateral margins, about 1.6 times wider than first free abdominal somite; anal somite weakly incised posteromedially. Surfaces of anterior urosomites smooth, lacking ornamentation; paired transverse rows of spinules present on ventral surface of anal somite and longitudinal row present

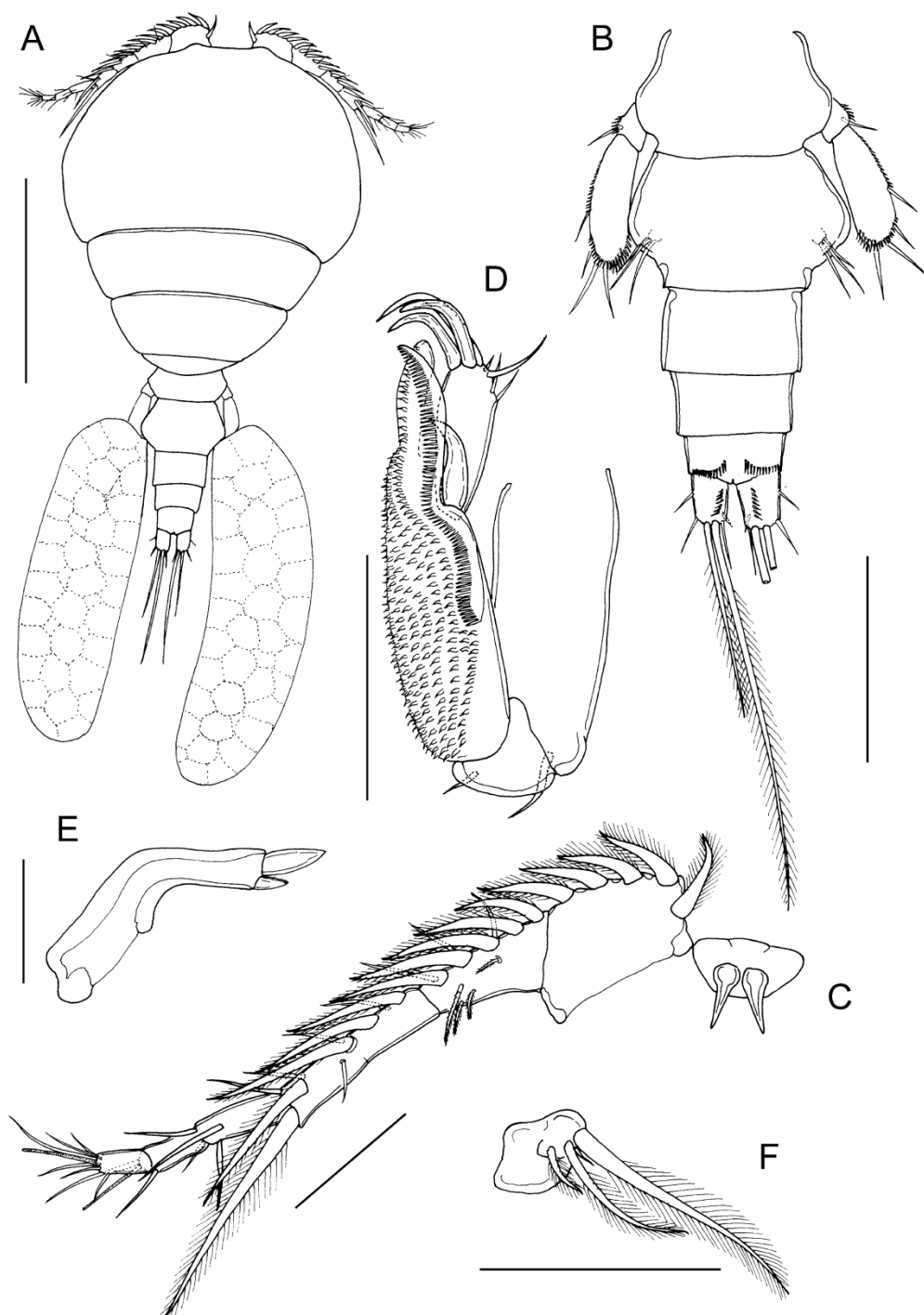


Fig. 1 *Hamaticolax resupinus* n. sp. Paratype female. A, Habitus, dorsal view; B, Urosome, ventral view; C, Antennule and rostrum; D, Antenna; E, Mandible; F, Maxillule. Scale-bars: A, 0.5 mm; B, 200 µm; C–D, F, 100 µm; E, 50 µm

ventrally on each caudal ramus (Fig. 1B). Caudal rami (Fig. 1B) about 1.3 times longer than wide, bearing single principal seta, plus 5 smaller setae.

Antennule (Fig. 1C) comprising broader proximal part and slender distal part; proximal part 3-segmented but third segment divided by partial suture; distal part slender, comprising 3 segments. First segment bearing 5 pilose setae, none modified; second segment bearing 5 pilose setae, 3 naked setae dorsally, and 4 short plumose setae on ventral surface; third segment bearing 5 pilose setae, distalmost long, extending beyond apex of antennule, 4 naked setae on dorsal surface plus 1 small naked seta on ventral surface; segments 4 to 6 with setal formula: 4, 2+1ae, 7+1ae.

Antenna (Fig. 1D) uniramous, 3-segmented; comprising long proximal segment (coxobasis) bearing single long seta, short middle (=first endopodal) segment armed with small naked seta and highly ornamented apical segment. Apical segment comprising partly fused second and third endopodal segments: proximal part (representing second endopodal segment) produced into blunt distal process ornamented with rows of spinules ventrally, continuous with multiple rows over ventral surface of segment, and armed with stout curved claw and pectinate process distally; distal part (third endopodal segment) bearing 3 curved claws and 4 unequal naked setae.

Labrum (Fig. 2A) wider than long, ornamented with 3 sensilla on ventral surface. Mandible (Fig. 1E) tipped with 2 unequal blades, each with single spinulate margin. Paragnath (Fig. 2A) bipartite; basal part ornamented with long setules, distal process blunt, covered with shorter setules. Maxillule (Fig. 1F) lobate, armed with 1 minute naked seta and 3 unequal pilose setae. Maxilla (Fig. 2B) 2-segmented; proximal segment (syncoxa) larger, unarmed; second segment (basis) narrowing distally, bearing 2 spinulate apical elements plus naked seta. Maxilliped (Fig. 2C) 3-segmented; comprising syncoxa, armed with seta in proximal half; basis armed with 1 pilose seta and vestigial seta located on medial margin; terminal (endopodal) segment forming sigmoid claw provided with short accessory process, and armed with spinulate seta proximally.

Legs 1 to 4 biramous, with armature as indicated in Table 1.

Leg 1 (Fig. 2D) modified, with flattened rami. Protopod with swollen, hirsute outer basal seta; inner coxal seta transformed into flattened broad, hirsute

element and inner basal element modified into short knob-like structure; basis ornamented with patches of fine surface spinules; interpodal sclerite with 2 rounded anterior lobes ornamented with short spinules around anterior margin. Exopod indistinctly 2-segmented, retaining almost complete suture between first and second segments but only partial suture between second and third; first segment with large spine at outer distal corner; compound distal segment bearing total of 4 outer spines and 6 plumose setae. Endopod 3-segmented: all endopodal segments ornamented with outer margin setules; second and third segments with inner margin setules; first segment with rows of fine surface spinules; third segment with minute spine located proximal to base of outermost seta.

Leg 2 (Fig. 2E) with 3-segmented rami; coxa with hirsute inner seta and basis with outer plumose seta and inner patch of spinules. All outer spines on exopodal segments unilaterally denticulate and provided with subterminal flagellum. Endopodal segments broad and flattened; outer margins of all segments ornamented with long setules, second and third segments with inner row of setules. Interpodal sclerite unornamented.

Leg 3 (Fig. 3A) with 3-segmented rami; coxa and basis armed with long inner and outer plumose setae, respectively; basis ornamented with inner patch of spinules. Exopodal segments with proximal outer spines reflexed over ramus. First exopodal segment with patch of spinules on outer distal margin. Endopodal segments broad, flattened and ornamented as in leg 2. Interpodal plate ornamented with paired patches of short spinules.

Leg 4 (Fig. 3B) with 3-segmented rami; coxa lacking inner seta; basis with outer plumose seta. All outer spines on exopodal segments finely denticulate, provided with subterminal flagellum and reflexed over ramus as in leg 3. Outer margins of all endopodal segments ornamented with long setules. Inner seta on first endopodal segment short, extending nearly to middle of second segment. Inner seta on second endopodal segment extending to about mid-length of third segment. Third segment with spinules present adjacent to bases of outer and inner apical spines; apical seta about as long as segment. Interpodal sclerite ornamented with paired patches of fine spinules as in leg 3.

Leg 5 (Fig. 2F) 2-segmented; protopodal segment small, ornamented with patch of spinules and armed

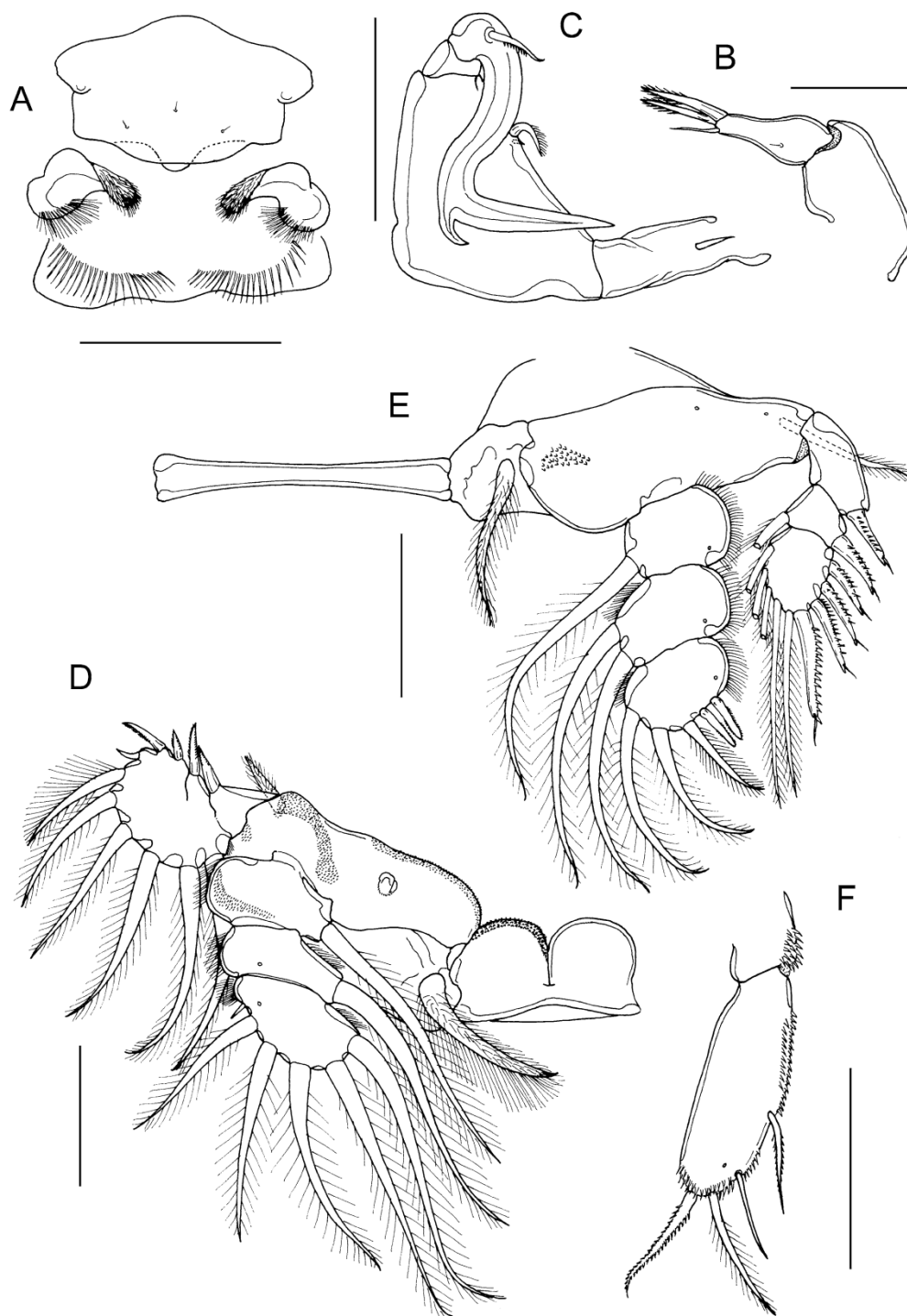


Fig. 2 *Hamaticolax resupinus* n. sp. Paratype female. A, Labrum and paragnaths *in situ*; B, Maxilla; C, Maxilliped; D, Leg 1; E, Leg 2; F, Leg 5. Scale-bars: A, C–F, 100 µm; B, 50 µm

Table 1 Armature of legs 1–4 of adult female *Hamaticolax resupinus* n. sp.

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-I	I-0; IV, 6	0-1; 0-1; I, 5
Leg 2	0-1	1-0	I-0; I-1; III, I, 5	0-1; 0-2; II, 3
Leg 3	0-1	1-0	I-0; I-1; II, I, 5	0-1; 0-2; II, 2
Leg 4	0-0	1-0	I-0; I-1; II, I, 5	0-1; 0-1; I, 1, I

with outer seta; free exopodal segment armed with spinulate spine at mid-length, outer naked subterminal spine, and inner spinulate and outer plumose terminal elements; inner distal spine longer than outer subterminal spine. Exopodal segment ornamented with spinules extending along outer margin, plus distal rows.

Leg 6 (Fig. 1B) represented by 3 short setae located in egg sac attachment area on genital double-somite.

Adult male. Body cycloform (Fig. 3C); 0.64–0.74 (0.69) long ($n = 2$). Prosome length 0.44–0.46 (0.45), maximum width 0.31–0.35 (0.33); comprising cephalothorax incorporating first pedigerous somite, and free second to fourth pedigerous somites. Rostral area without tines (Fig. 4A). Urosome (Fig. 3C) length 0.21–0.28 (0.24); comprising fifth pedigerous somite, elongate, pear-shaped genital somite, and two free abdominal somites. Ventral surface of first free abdominal somite naked (Fig. 3D). Anal somite weakly incised, ornamented with transverse row of spinules anteriorly and paired rows posteriorly (Fig. 3D). Caudal rami (Fig. 3D) ornamented with crescentic row of spinules ventrally; setation as in female.

Antennule (Fig. 4A) 5-segmented; proximal 2 segments only slightly more robust than distal 3 cylindrical segments. First segment with 5 robust pilose setae, none modified; compound second segment with 10 pilose setae, 5 naked setae dorsally, and 3 plumose and 2 naked setae ventrally; distalmost pilose element on second segment shorter than in female. Cylindrical distal segments with setal formula 4, 2+1ae and 7+1ae, respectively.

Antenna, mandible, maxillule and maxilla as in female.

Maxilliped (Fig. 3E) with broad syncoxa armed with plumose seta; basis robust, tapering distally, ornamented proximo-medially with multiple rows of short spinules and armed medially with 2 spinulate

setae; distal subchela incorporating unarmed endopodal segment, and bearing curved claw armed with long seta proximally; concave margin of claw ornamented with row of denticles, plus cluster at tip.

Legs 1 to 4 biramous with 3-segmented rami except 2-segmented endopod of leg 4; setal armature as indicated in Table 2.

Leg 1 (Fig. 4B) protopod and rami less flattened and less modified than in female. Coxa and basis distinct; coxa ornamented with row of spinules and armed with inner plumose seta (not swollen as in female); basis armed with swollen hirsute outer seta and ornamented with inner patch of spinules. Interpodal sclerite linear, ornamented with paired patches of small spinules. Outer spines on exopodal segments each finely spinulate along outer margin and provided with subterminal flagellum. All endopodal segments ornamented with patches of spinules on anterior surface near outer margins; second and third segments each with row of setules along inner margin.

Legs 2 and 3 (Fig. 4C–D) with outer spines on exopodal segments finely unilaterally spinulate, and bearing subterminal flagellum. First exopodal segment with distal patch of spinules near outer margin and short row of setules on inner margin. Long setules present on outer margins of endopodal segments. Interpodal sclerites linear, ornamented with paired patches of small spinules.

Leg 4 (Fig. 4E) with 3-segmented exopod and 2-segmented endopod; outer margin spines on exopod segments finely unilaterally spinulate and bearing subterminal flagellum. Outer distal patch of spinules and inner row of setules present on first exopodal segment. Inner plumose seta on proximal endopodal segment about twice as long as ramus, extending almost to tip of long seta on distal segment; distal endopodal segment with inner apical spine almost twice as long as outer spine; apical seta plumose, about twice as long as ramus. Long setules present on outer margins of both endopodal segments.

Leg 5 (Fig. 4F) 2-segmented; protopodal segment small, armed with outer seta; free exopodal segment ornamented distally with patches of spinules, and bearing 2 unequal terminal setae.

Remarks

The new species is similar to *Hamaticolax maleus*, the only other member of the genus reported from a

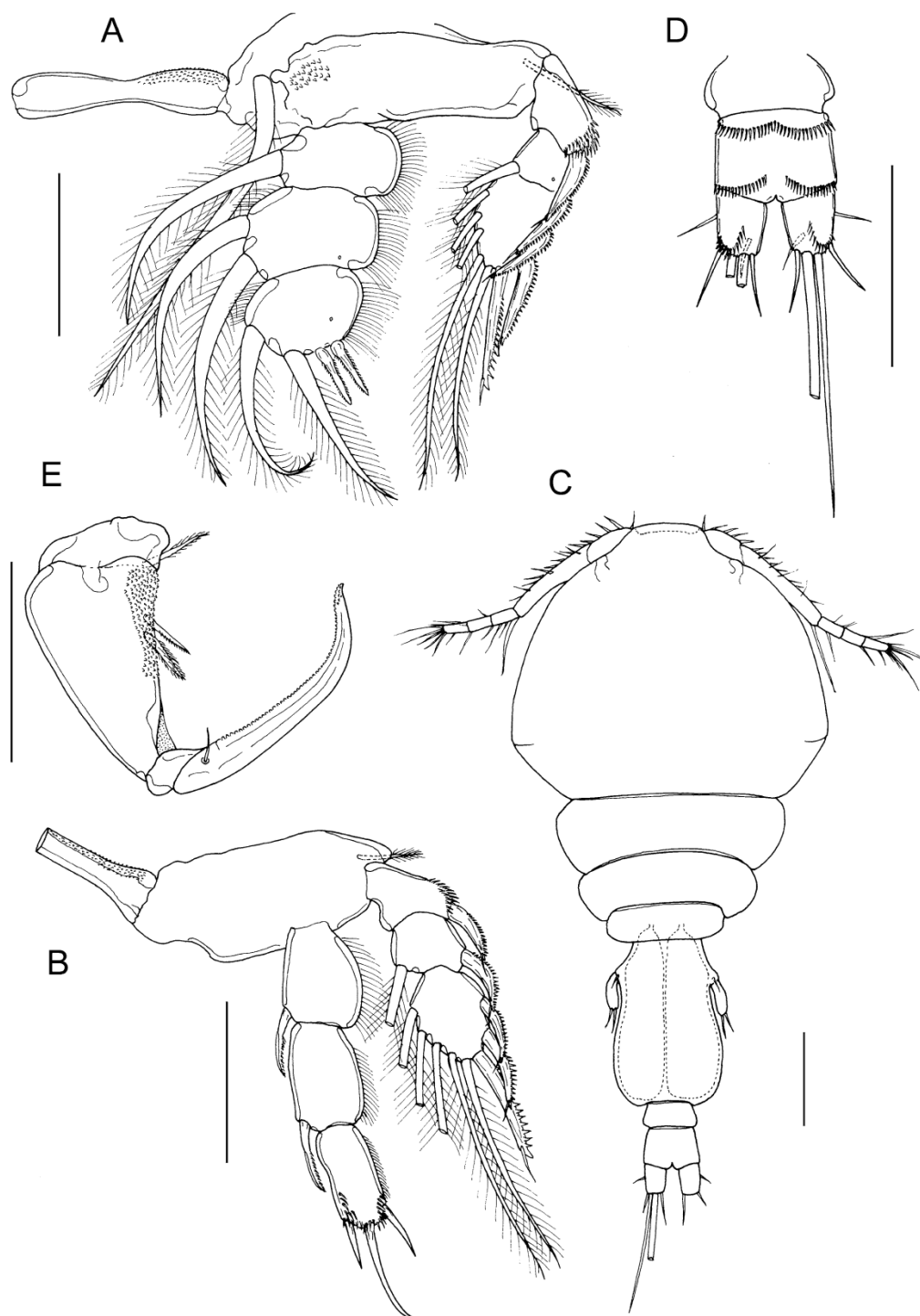


Fig. 3 *Hamaticolax resupinus* n. sp. Paratype female. A, Leg 3; B, Leg 4. C, Allotype male, habitus, dorsal; D, Male, postgenital somites and caudal rami, ventral; E, Male, maxilliped. Scale-bars: 100 μ m

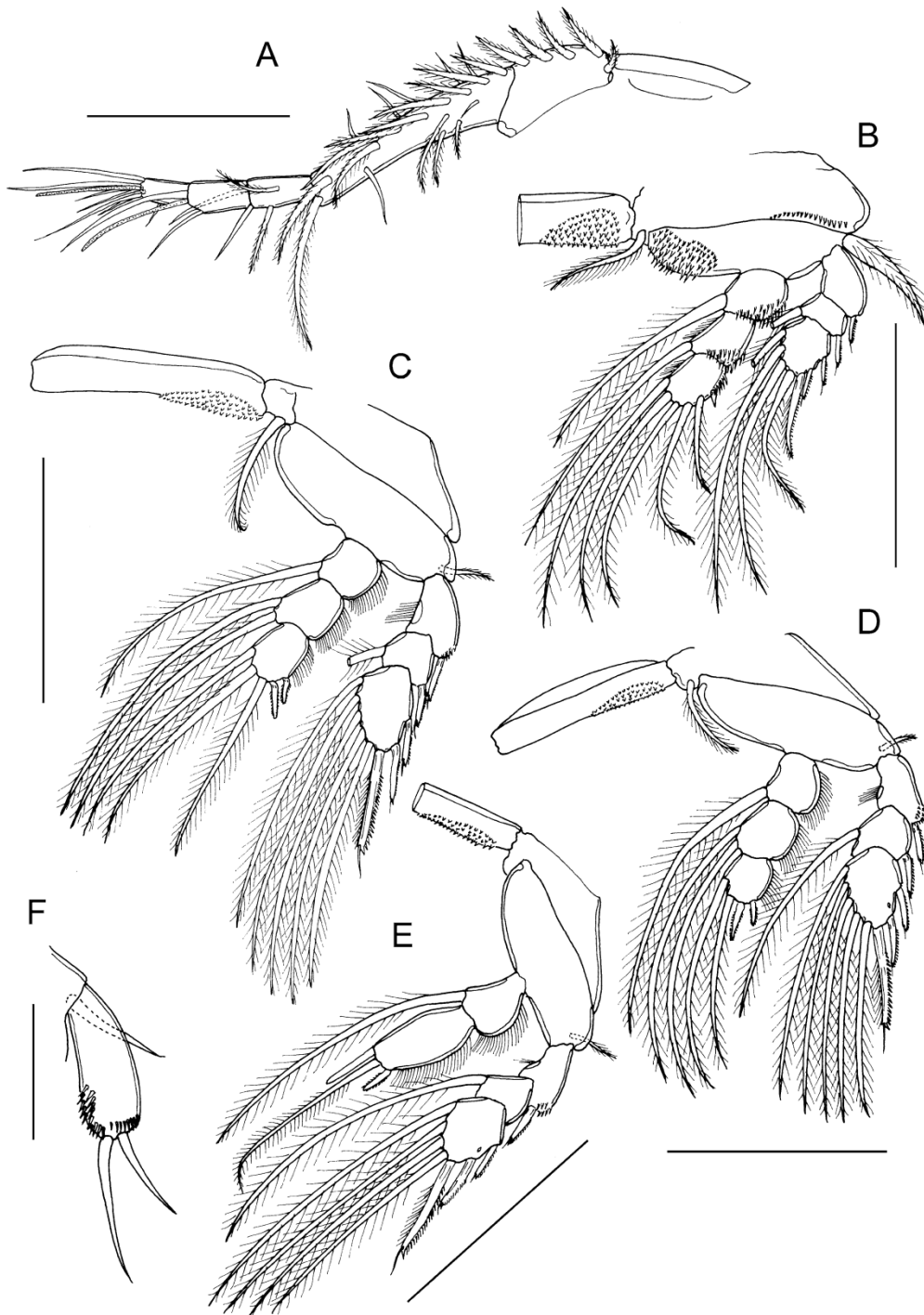


Fig. 4 *Hamaticolax resupinus* n. sp. Allotype male. A, Antennule and rostrum; B, Leg 1; C, Leg 2 (spinules on right side of interpodal sclerite omitted); D, Leg 3 (spinules on right side of interpodal sclerite omitted); E, Leg 4; F, Leg 5. Scale-bars: A–E, 100 µm; F, 50 µm

Table 2 Armature of legs 1–4 of adult male *Hamaticolax resupinus* n. sp.

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-0	I-0; I-1; II, 1, 4	0-1; 0-1; I, 5
Leg 2	0-1	1-0	I-0; I-1; II, I, 5	0-1; 0-2; II, 3
Leg 3	0-1	1-0	I-0; I-1; II, I, 5	0-1; 0-2; II, 2
Leg 4	0-0	1-0	I-0; 0-1; II, I, 4	0-1; I, 1, I

macrourid host. Detailed comparisons with *H. maleus* are difficult because of the number of observational errors and misinterpretations apparent in Oldewage's (1994) original description of *H. maleus*. Apparent differences, such as the lack of the small outer spine on the third endopodal segment of leg 1 in *H. maleus*, are almost certainly observational errors. Others errors are more difficult to interpret: the figures of the legs are incorrectly labelled in Oldewage (1994) and the illustrations do not match the information in the text. Using the exopodal setation formula as a reference we identify Oldewage's figure 2c as leg 2 (third exopodal segment with IV, 5) and figure 2d as leg 3 (third exopodal segment with III, 5). However the setation of the third endopodal segment is the same in both figures, whereas this segment of leg 3 typically has 2 setae and 2 spines in female bomolochids compared with 3 setae and 2 spines present on leg 2.

As a result of the inconsistencies in Oldewage (1994) we cannot rely on either the illustrations or the written descriptions of the limbs to provide reliable characters for species discrimination. The basic body shape is, however, more readily comparable. Oldewage (1994) shows that the genital double-somite is narrow and has more or less parallel lateral margins and he specifically states that "genital segment and three abdominal segments of equal diameter". In contrast, the genital double-somite of the new species has strongly convex lateral margins (Fig. 1B) and is about 1.6 times wider than the first free abdominal somite. The body length of the female was not given by Oldewage (1994) but from the scale provided with the figure, the body length can be calculated as 1.6 mm, distinctly longer than the maximum length (1.31 mm) of the new species. These differences in size and gross body morphology might be supported by characters based on the limbs, such as the different proportional lengths of the 4 setae on the maxillules,

but the uncertainty surrounding Oldewage's figures is so high that we are unable to rely on them. Clearly, *H. maleus* is in urgent need of redescription.

Hamaticolax maleus was found on the macrourid *Malacocephalus laevis* caught off the west coast of South Africa (Oldewage, 1994). The depth of capture was unknown, but this host fish is typically found between depths of 300 and 750 m (Geistdoerfer, 1990), although its overall depth range is 200 to 1,000 m according to Cohen et al. (1990). The hosts of the new species were collected in the western basin of the Mediterranean at depths of 1,236 to 1,626 m.

Discussion

Determining boundaries between some bomolochid genera has proven problematic (Vervoort, 1962, 1969; Cressey, 1984) and the genera *Acantholochus* Cressey, 1984 and *Hamaticolax* have shown a particularly high level of instability (Ho & Lin, 2006; Morales-Serna & Gómez, 2010). Morales-Serna & Gómez (2010) addressed this problem and provided new diagnoses of both *Acantholochus* and *Hamaticolax*. They transferred three species from *Acantholochus* to *Hamaticolax* and another three species in the opposite direction and then created keys to species of both genera. The key to *Acantholochus* was updated by Pashoal et al. (2013) who described a new species, *A. lamellatus* Pashoal, Cezar & Luque, 2013 from *Conodon nobilis* (Linnaeus) caught off Brazil. The new bomolochid described here is placed in *Hamaticolax* on the basis of the presence of a pair of rostral hooks in the female, the form of the proximal segments of the female antennule (i.e. the anterior margin of the first two segments is straight), and the presence of an accessory process on the claw of the maxilliped. However, we consider that generic boundaries are still rather labile and that the validity of bomolochid genera should be tested by a comprehensive phylogenetic analysis.

Attempting to identify our material from *Coelorchinus mediterraneus* and *Coryphaenoides mediterraneus* using the key of Morales-Serna & Gómez (2010) was problematic since the key contains a number of errors. The second couplet distinguishing between the type-species *Hamaticolax attenuatus* (Wilson, 1913) and *H. spinulus* (Cressey, 1969), uses two characters, the number of outer spines on the mid-

exopod segment of leg 3 and the total number of setal elements on the terminal exopod segment of leg 4. Morales-Serna & Gómez (2010) refer to two outer spines on the mid-exopod segment of leg 3, a character state not found in the entire order Cyclopoida. Indeed, in the whole of the Copepoda, two outer spines are present on the second exopodal segment of leg 3 only in the bizarre harpacticoid family Superornatiremidae (Huys, 1996). They also refer to the presence of only 5 elements on the terminal exopod segment of leg 4, a character state that would be extremely unusual for the Bomolochidae. Using Vervoort's (1969) redescription of *H. attenuatus*, which was based on re-examination of Wilson's (1913) type material, it is clear that the mid-exopod segment of leg 3 carries only one outer spine, as normal, and that the terminal exopod segment of leg 4 carries a total of 8 setal elements. So the characters provided for the key couplet do not serve to distinguish between the species. A new key is provided below, avoiding characters such as antennular segmentation which are often difficult to interpret as segments are often incompletely separated in bomolochids.

Key to the species of *Hamaticolax* (based on females)

- 1a Urosome as long as or longer than prosome 2
- 1b Urosome shorter than prosome 3
- 2a Hook-like spine on antenna extending beyond tip of pectinate process *H. attenuatus*
- 2b Hook-like spine on antenna not extending beyond tip of pectinate process *H. spinulus*
- 3a Second endopodal segment of legs 2 and 3 with 1 inner seta *H. unisagittatus*
- 3b Second endopodal segment of legs 2 and 3 with 2 inner setae 4
- 4a Apical segment of leg 4 endopod with 2 setal elements *H. galeichthys*
- 4b Apical segment of leg 4 endopod with 3 setal elements 5
- 5a Third exopodal segment of legs 3 and 4 each with 7 elements *H. embiotocae*
- 5b Third exopodal segment of legs 3 and 4 each with 8 elements 6

- 6a Endopod of leg 4 elongate, almost twice as long as exopod; third endopodal segment of leg 4 more than 4 times longer than wide *H. paralabracis*
- 6b Endopod of leg 4 typically about as long as exopod, at most 1.5 times longer; third endopodal segment of leg 4 at most 1.5 times longer than wide 7
- 7a Third exopodal segment of leg 2 with 8 elements (III, 5) 8
- 7b Third exopodal segment of leg 2 with 9 elements (IV, 5) 9
- 8a Leg 4 with long inner seta on first and second endopodal segments; seta on first segment reaching beyond distal margin of second and seta on second reaching beyond distal margin of third segment *H. occultus*
- 8b Leg 4 with short inner seta on first and second endopodal segments; seta on first segment not reaching beyond distal margin of second, and seta on second segment not reaching beyond distal margin of third *H. scutigeralus*
- 9a Leg 4 with long inner seta on first and second endopodal segments; seta on first segment reaching beyond distal margin of second and seta on second reaching beyond distal margin of third segment *H. prolixus*
- 9b Leg 4 with short inner seta on first and second endopodal segments; seta on first segment not reaching beyond distal margin of second, and seta on second segment not reaching beyond distal margin of third 10
- 10a Body length of adult female 1.6 mm; genital double-somite with linear lateral margins, about equal in width to free abdominal somites *H. maleus*
- 10b Body length of adult female 1.3 mm; genital double-somite with strongly convex lateral margins, markedly wider than free abdominal somites *H. resupinus* n. sp.

Hamaticolax resupinus n. sp. is the first species of the genus found below 1,300 m. Considering the bathymetric distribution of *Coe. mediterraneus* and *Cor. mediterraneus* in the studied area, this parasite could potentially be found up to 2,250 m depth, although it is likely to occur around 1,200 to 1,800 m, where its hosts are more abundant (Stefanescu et al., 1992, 1993). *Hamaticolax resupinus* n. sp. is the first

recorded parasitic copepod from *Coe. mediterraneus* and the third from *Cor. mediterraneus* worldwide, after *Chondacanthodes deflexus* Wilson, 1932 (see Kellermanns et al., 2009) and *Sarcotaces* sp. (see Bullock et al., 1986), and its first *Hamaticolax* species from the Mediterranean Sea. Bomolochids are uncommon parasites of Macrouridae. The most common copepod families reported from macrourids are the Chondracanthidae Milne Edwards, 1840, Lernaeopodidae Milne Edwards, 1840 and Sphyrriidae Wilson, 1919, especially the genera *Clavella* Oken, 1815 and *Lophoura* Kölliker, in Gegenbaur, Kölliker & Muller, 1853 (see Boxshall, 1998; Klimpel et al., 2009). However, the Macrouridae is a family with a worldwide distribution comprising over 400 species (Froese & Pauly, 2016), and only few have been targeted for extensive parasitological studies, such as *Cor. mediterraneus* (Kellermanns et al., 2009), *Coryphaenoides rupestris* Gunnerus (Campbell et al., 1980; Zubchenko, 1981) and *Macrourus berglax* Lacépède (Zubchenko, 1981; Palm & Klimpel, 2008). Therefore, as parasitological studies on members of the Macrouridae increase, the number of copepod parasites probably will do so, including the number of Bomolochidae.

Acknowledgements We thank all participants in the ANTROMARE 1 and 3 cruises and the staff of the Department of Animal Biology, Vegetal Biology and Ecology of the Universitat Autònoma de Barcelona (Spain) for their assistance during the oceanographic campaign.

Funding This study was supported by Spanish Science and Innovation Ministry project ANTROMARE (CTM2009-12214-C02-02). DPG benefited from a PIF PhD student grant of the Universitat Autònoma de Barcelona.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

References

- Boxshall, G. A. (1998). Host specificity in copepod parasites of deep-sea fishes. *Journal of Marine Systems*, *15*, 215–223.
- Bullock, A. M., Phillips, S. E., Gordon, J. D. M., & Roberts, R. J. (1986). *Sarcotaces* sp., a parasitic copepod infection in two deep-sea fishes, *Lepidion eques* and *Coelorhynchus occa*. *Journal of the Marine Biological Association U.K.*, *66*, 835–843.
- Campbell, R. C., Haedrich, R. L., & Munroe, T. A. (1980). Parasitism and ecological relationships among deep-sea benthic fishes. *Marine Biology*, *57*, 301–313.
- Cohen, D. M., Inada, T., Iwamoto, T., & Scialabba, N. (1990). *FAO species catalogue. Vol. 10. Gadiform fishes of the world (Order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date*. FAO Fish Synopses, 125(10), FAO, Rome, 442 pp.
- Cressey, R. (1969). Five new parasitic copepod from California inshore fish. *Proceedings of the Biological Society of Washington*, *82*, 409–427.
- Cressey, R. (1983). Parasitic copepods from the Gulf of Mexico and Caribbean Sea, II: Bomolochidae. *Smithsonian Contributions to Zoology*, *389*, 1–35.
- Cressey, R. (1984). A new genus of bomolochid copepod from eastern Pacific haemulid fishes. *Bulletin of Marine Sciences*, *35*, 182–186.
- Froese, R., & Pauly, D. (2016). Fishbase. World Wide Web electronic publication. www.fishbase.org, version (01/2016).
- Geistdoerfer, P. (1990). Macrouridae. In: Quero, J.C., Hureau, J.C., Karrer, C., Post, A. & Saldanha, L. (Eds). Check-list of the fishes of the eastern tropical Atlantic (CLOFETA). JNICT, Lisbon; SEI, Paris; and UNESCO, Paris. Vol. 2, 541–563 pp.
- Hanan, D. (1976). A new species of cyclopoid copepod, parasitic on shiner surfperch, *Cymatogaster aggregata* Gibbons, in Anaheim Bay and Huntington Harbor, California, with notes on *Bomolochus cuneatus* Fraser and *Ergasilus lizae* Krøyer. *Bulletin of the Southern California Academy of Sciences*, *75*, 22–28.
- Ho, J.-S., & Lin, C.-L. (2006). A new bomolochid copepod parasitic on marine fishes of Taiwan, with reassignment of species of *Holobomolochus* Vervoort, 1969. *Crustaceana*, *78*, 1369–1381.
- Huys, R. (1996). Superornatiremidae fam. nov. (Copepoda: Harpacticoida): An enigmatic family from North Atlantic anhihaline caves. *Scientia Marina*, *60*, 497–542.
- Huys, R., & Boxshall, G. A. (1991). *Copepod Evolution* (p. 468). London: The Ray Society.
- Kabata, Z. (1971). Four Bomolochidae (Copepoda) from fishes of British Columbia. *Journal Fisheries Research Board of Canada*, *28*, 1563–1572.
- Kellermanns, E., Klimpel, S., & Palm, H. W. (2009). Parasite fauna of the mediterranean grenadier *Coryphaenoides mediterraneus* (Giglioli, 1893) from the Mid-Atlantic Ridge (MAR). *Acta Parasitologica*, *54*, 158–164.
- Klimpel, S., Busch, M. W., Kellermanns, E., Kleinertz, S., & Palm, H. W. (2009). Metazoan Deep-Sea Fish Parasites. *Acta Biologica Benrodis, Supplementband II*. Heinrich-Heine University Düsseldorf, 384 pp.
- Luque, J. L., & Bruno, M. (1990). Two new species of *Acantholochus* Cressey, 1984 (Copepoda: Bomolochidae) parasitic on Peruvian marine fishes. *Journal of Natural History*, *24*, 241–249.
- Morales-Serna, F. N., & Gómez, S. (2010). A new bomolochid copepod parasitic on bullseye puffer *Sphoeroides*

- annulatus* (Jenyns) from Mexico, with reassignment of some species of *Acantholochus* Cressey and *Hamaticolax* Ho & Lin. *Zootaxa*, 2336, 36–50.
- Oldewage, W. H. (1994). A new species of *Holobomolochus* Vervoort, 1969 (Copepoda, Poecilostomatoida) from the west coast of South Africa. *Crustaceana*, 67, 324–328.
- Palm, H. W., & Klimpel, S. (2008). Metazoan fish parasites of *Macrourus berglax* Lacépède, 1801 and other macrourids in the north Atlantic: Invasion of the deep sea from the continental shelf. *Deep-Sea Research Part II*, 55, 235–242.
- Pashoal, F., Cezar, A. D., & Luque, J. L. (2013). A new species *Acantholochus* (Cyclopoida, Bomolochidae) parasitic on the barred grunt *Conodon nobilis* (Linnaeus, 1758) (Osteichthyes, Haemulidae) from Rio de Janeiro, Brazil. *Crustaceana*, 86, 212–220.
- Stefanescu, C., Lloris, D., & Rucabado, J. (1992). Deep-living demersal fishes in the Catalan Sea (western Mediterranean) below a depth of 1000 m. *Journal of Natural History*, 26, 197–213.
- Stefanescu, C., Lloris, D., & Rucabado, J. C. (1993). Deep-sea fish assemblages in western Mediterranean. *Deep-Sea Research Part I*, 40, 695–707.
- Tavares, L., & Luque, J. L. (2003). A new species of *Acantholochus* (Copepoda: Bomolochidae) parasitic on *Centropomus undecimalis* (Osteichthyes: Centropomidae) from the coastal zone of the State of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, 98, 241–245.
- Vervoort, W. (1962). A review of the genera and species of the Bomolochidae (Crustacea, Copepoda), including the description of some old and new species. *Zoologische Verhandelingen, Leiden*, 56, 1–111.
- Vervoort, W. (1969). Caribbean Bomolochidae (Copepoda: Cyclopoida). *Studies on the Fauna of Curaçao and other Caribbean Islands*, 28, 1–125.
- Wilson, C. B. (1913). Crustacean parasites of West Indian fishes and land crabs, with descriptions of new genera and species. *Proceedings of the United States National Museum*, 44, 189–277.
- Wilson, C. B. (1935). Parasitic copepods from the Dry Tortugas. *Papers from Tortugas Laboratory*, 29, 329–347.
- Zubchenko, A. V. (1981). Parasitic fauna of some Macrouridae in the northwest Atlantic. *Journal of the Northwest Atlantic Fishery Science*, 2, 67–72.

Chapter VI

Raphidascaris (Raphidascaris) macrouri n. sp. (Nematoda: Anisakidae)
from two deep-sea macrourid fishes in the Western Mediterranean:
Morphological and molecular characterisations



Contents lists available at ScienceDirect

Parasitology International

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

Raphidascaris (Raphidascaris) macrouri n. sp. (Nematoda: Anisakidae) from two deep-sea macrourid fishes in the Western Mediterranean: Morphological and molecular characterisations



David Pérez-i-García^{a,1}, María Constenla^{a,1}, Maite Carrassón^{a,*}, Francisco E. Montero^b, Anna Soler-Membrives^a, David González-Solís^{c,d}

^a Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain

^b Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Parc Científic, Universitat de València, PO Box 22085, 46071 Valencia, Spain

^c Laboratorio de Necton, El Colegio de la Frontera Sur (ECOSUR), Unidad Chetumal, Chetumal, Quintana Roo, Mexico

^d Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovska 31, 370 05 České Budějovice, Czech Republic

ARTICLE INFO

Article history:

Received 24 March 2015

Received in revised form 23 April 2015

Accepted 2 May 2015

Available online 12 May 2015

Keywords:

Raphidascaris (Raphidascaris) macrouri

Deep-water

Macrouridae

*Nezumia aequalis**Trachyrincus scabrus*

Spain

ABSTRACT

A new nematode species, *Raphidascaris (Raphidascaris) macrouri* n. sp. (Anisakidae), is described from male and female specimens found in the intestine, and occasionally in stomach and pyloric caeca, of two deep-water macrourid fishes (Gadiformes) off Barcelona, Mediterranean Sea: *Nezumia aequalis* (Günther) (type-host) and *Trachyrincus scabrus* (Rafinesque). Based on light and scanning electron microscopy examination, the new species shows similar morphological features as the other four valid species of the subgenus *Raphidascaris* Railliet & Henry, 1915, but it differs from *Raphidascaris (Raphidascaris) acus* (Bloch, 1779), *Raphidascaris (Raphidascaris) lutjani* Olsen, 1952 and *Raphidascaris (Raphidascaris) mediterraneus* Lèbre & Petter, 1983 in the high number of preloacal papillae (23–32) and from *Raphidascaris (Raphidascaris) gigi* Fujita, 1928 in the length of the spicules. Moreover, *Raphidascaris (Raphidascaris) macrouri* n. sp. exhibits a high variability on the number and distribution of caudal papillae, which was not recorded in the other four mentioned species. This is the first species of this subgenus reported from the family Macrouridae. Sequences of ITS1–5.8S–ITS2 region are analysed and compared with closely related nematode species. Molecular analysis confirmed the uniformity of the *R. (R.) macrouri* n. sp. between hosts.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Knowledge on the parasite fauna of deep-sea fishes in the Mediterranean has long been scarce. Most efforts have been focused on the identification and taxonomy of parasites in a range of deep-sea fish hosts in the Western Mediterranean [1–7]. Two common inhabitants of the western Mediterranean middle-slope [8,9] are *Nezumia aequalis* (Günther) and *Trachyrincus scabrus* (Rafinesque) (Gadiformes: Macrouridae), demersal deep-sea fishes distributed in the Atlantic Ocean and Mediterranean Sea [10].

During a parasitological study of these two fish species in the Western Mediterranean off Spain, an unknown anisakid nematode of the genus *Raphidascaris* Railliet & Henry, 1915 was found with a high prevalence, especially in *N. aequalis*. Although species of *Raphidascaris* are parasites of the digestive tract of a range of marine, brackish and freshwater fishes worldwide [11–14], adults of just a single species, *Raphidascaris (Raphidascaris) mediterraneus* Lèbre & Petter, 1983, have

been reported to parasitise *Chelidonichthys cuculus* (Linnaeus) and *Chelidonichthys obscurus* (Walbaum) in the Mediterranean [15,16], while larval forms of *Raphidascaris* sp. have been recorded in *Mora moro* [4].

This paper provides a description of a new species of the genus *Raphidascaris* from *N. aequalis* and *T. scabrus* based on detailed morphological (light and scanning electron microscopy) and molecular characterisation and phylogenetic analyses.

2. Materials and methods

2.1. Sample collection and morphological examination

Ten individuals of *N. aequalis* (preanal length: 2.8–5.8 cm) and four of *T. scabrus* (preanal length: 8.5–11.5 cm) were collected in 2007 and 2014 from the continental slope off Barcelona at depths between 558 and 1102 m. Sampling was carried out within the framework of the oceanographic projects BIOMARE and ANTRMARE (Spanish Ministry of Science and Innovation) on the research vessel García del Cid with a semi-balloon otter-trawl (OTSB) and a commercial fishing gear (BOU). Fishes were measured, weighed, assigned unique codes and stored at

* Corresponding author. Tel.: +34 935814637.

E-mail address: Maite.Carrasson@uab.cat (M. Carrassón).

¹ Equal contributors.

Table 1

GenBank accession numbers of sequences for the anisakid nematodes used in the analyses.

Species	Host	Country	GenBank no.	References
<i>Raphidascaris longispicula</i>	<i>Uroconger lepturus</i>	China	JN102362	[38]
<i>Raphidascaris lophii</i>		China	JF809816	[39]
<i>Raphidascaris lophii</i>	<i>Sebastes inermis</i>	Korea	JX974559	[40]
<i>Raphidascaris trichiuri</i>	<i>Muraenesox cinereus</i>	China	FJ009682	[41]
<i>Hysterothylacium fabri</i>	<i>Liparis tanakae</i>	China	KF736942	[42]
<i>Hysterothylacium fabri</i>	<i>Chelidonichthys spinosus</i>	Korea	JX974558	[40]
<i>Hysterothylacium fabri</i>		China	JQ520158	[38]
<i>Hysterothylacium aduncum</i>	<i>Zoarces viviparus</i>	Denmark	JX845137	unpublished
<i>Hysterothylacium zhoushanensis</i>	<i>Pseudorhombus oligodon</i>	China	JX028277	[38]
<i>Hysterothylacium deardorffstretorum</i>	<i>Paralichthys isosceles</i>	Brazil	JF730203	[43]
<i>Anisakis simplex</i>	<i>Balaenoptera acutorostrata</i>	Norway	JX535521	[44]
<i>Anisakis physeteris</i>	<i>Physeter macrocephalus</i>	Mediterranean Sea	JQ912693	[44]
<i>Anisakis ziphidarum</i>	<i>Mesoplodon grayi</i>	South Africa	JQ912691	[44]

– 20 °C. The nematodes were dissected out from fresh and freshly-frozen fish, washed in physiological saline and stored in 70% ethanol. For light microscopy examination, nematodes were cleared through a glycerine–water series. Drawings were made with the aid of an Olympus BX51 drawing tube. The type-material was deposited in the British Museum (Natural History) Collection at the Natural History Museum, London, UK (BMNH).

Nematode specimens (three males and two females) used for scanning electron microscopy were fixed in 10% buffered formaline, washed 4 times for 10 min each in 0.1 M phosphate buffer, postfixed in 1% (wt./vol.) osmium tetroxide with 0.7% ferrocyanide in phosphate buffer, washed in water, dehydrated in an ascending ethanol series (50, 70, 80, 90, and 95% for 10 min each and twice with 100% ethanol), and dried by critical point drying with CO₂. Samples were mounted on

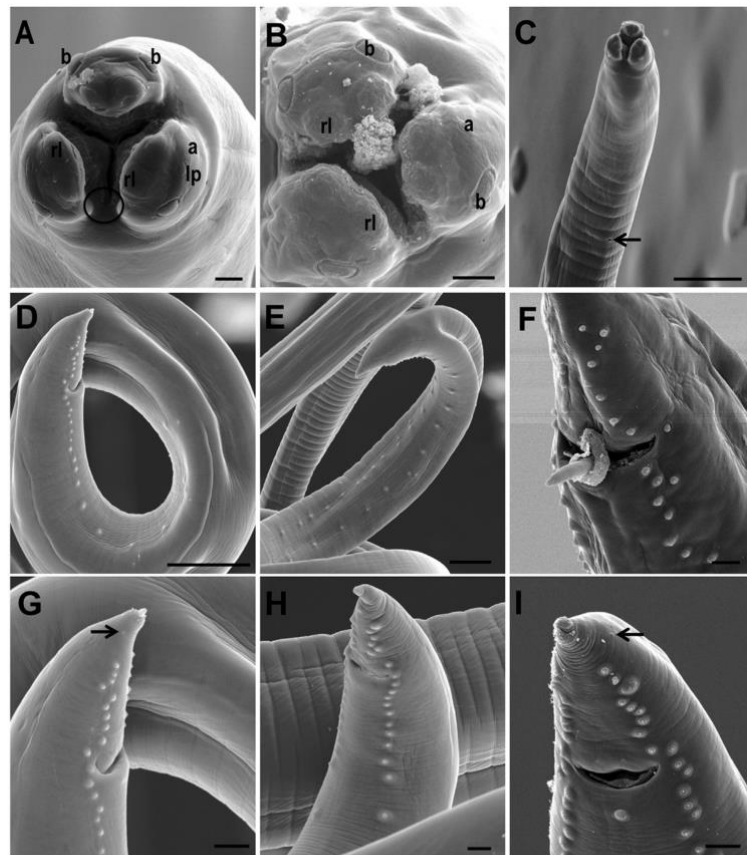


Fig. 1. *Raphidascaris (Raphidascaris) macrouri* n. sp., scanning electron micrographs. A, B – cephalic end of male, apical views (circle indicates group of small denticulations). C – anterior end of body, subventral view (arrow indicates excretory pore). D, E – posterior end of male, lateral and ventral views, respectively. F–I – region of cloaca, different specimens (arrows indicate phasmids). Abbreviations: a, amphid, b, submedian papilla, lp, lateral papilla, and rl, rounded lobe. Scale bars: A, B, F, G, H, and I, 10 µm; C, D, and E, 100 µm.

adhesive carbon films and coated with gold. Nematodes were observed with an S-570 scanning electron microscope (Hitachi Ltd., Japan) at an accelerating voltage of 10 and 15 kV. All measurements are in micrometres unless otherwise stated.

2.2. Molecular data

Four nematodes (one male and one female of each host species) were molecularly analysed. DNA from all samples was extracted with Qiagen TM (Valencia, California) DNeasy® Blood and Tissue Kit. The ITS1–5.8S–ITS2 region was amplified by polymerase chain reaction (PCR) using the primers A (forward: 5-GTC GAA TTC GTA GGT GAA CCT GCG GAA GGA TCA-3') and B (reverse: 5-GCC GGA TCC GAA TCC TGG TTA GTT TCT TTT CCT-3') [17] at 25 µM in 50 µL PCR reaction volume. PCR was performed under the following conditions: initial denaturation at 94 °C for 5 min, followed by 30 amplification cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min 10 s, and a final extension step at 72 °C for 7 min. PCR products were checked on GelRed Nucleid Acid Gel Stain in 1% agarose gels, purified and sequenced by Macrogen (Amsterdam, Netherlands). Sequences were aligned using Bioedit v. 7.2.5 [18] and variable sites were checked visually for accuracy.

Contiguous sequences were assembled using MEGA v.6 [19] and submitted to GenBank. Sequences from isolates were aligned using Muscle implemented in Mega v.6 with thirteen sequences for representatives of Anisakidae (species of *Raphidascaris*, *Hysterothylacium* and *Anisakis*, the latter used as outgroups) retrieved from GenBank (Table 1) and used in the phylogenetic analyses. The alignment was trimmed to the length of the shortest sequence and exclusion set (positions with ambiguous alignment) was defined using Gblocks [20] as implemented in SeaView v. 4 [21]. Maximum likelihood (ML) and

Bayesian inference (BI) analyses were conducted after analysis of the evolutionary substitution model with jModelTest 2.1.4 [22,23] using Akaike Information Criteria (AIC). The best-fitting model selected was the general time-reversible model with gamma distributed rate variation among sites (GTR + G). ML analysis was carried out in PhyML 3.0 [24], with a non-parametric bootstrap validation based on 1000 replicates. BI analysis was performed with MrBayes v.3.2 [25]. Log likelihoods were estimated over 10,000,000 generations using Markov Chain Monte Carlo (MCMC) searches on two simultaneous runs of four chains, samplings trees every 1000 generations. The first 2500 trees were discarded as “burn-in” and a consensus topology and nodal support estimated as posterior probability values [26] were calculated from the remaining trees. Pairwise genetic distances were calculated using the “uncorrected p-distance” model implemented in MEGA v.6.

3. Results

3.1. *R. (R.) macrouri* n. sp.

3.1.1. General

Medium-sized, yellowish nematodes with transversely striated cuticle. Lips nearly equal in size (dorsal lip slightly smaller than ventrolateral lips), wider than long, with lateral membranous flanges; pulp with two distinct anterior lobes. Dorsal lip with two double papillae; each ventrolateral lip bears one double subventral papilla, one small single papilla and lateral amphid (Fig. 1A–C). Interlabia and cervical alae absent. Oesophagus short; posterior half broader than anterior part (Fig. 2A–B). Ventriculus transversely oval; ventricular appendix relatively short, posteriorly directed. Excretory pore slightly posterior

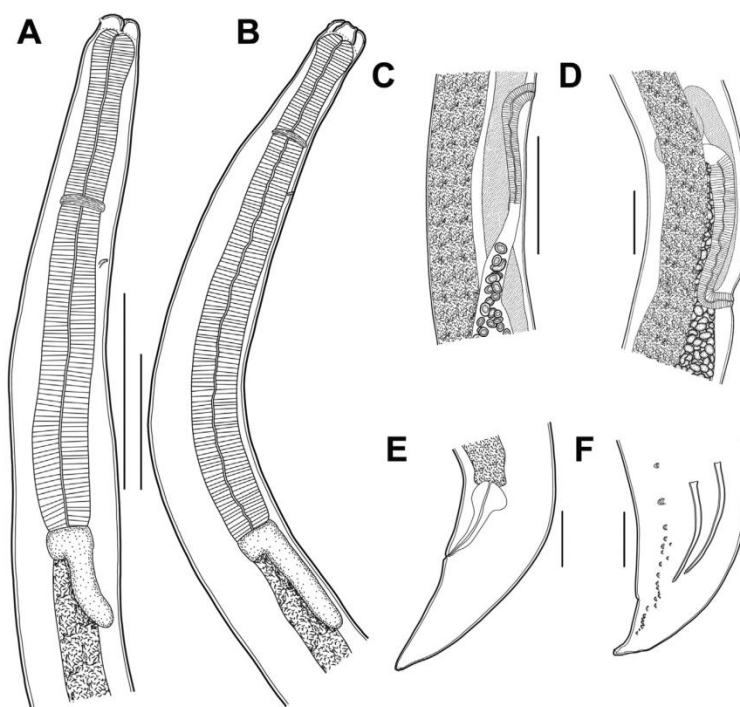


Fig. 2. *Raphidascaris (Raphidascaris) macrouri* n. sp. A, B – anterior end of male and female, respectively, lateral views. C – region of vulva with non-embryonated eggs, lateral view. D – region of vulva with embryonated eggs, lateral view. E, F – caudal end of female and male, respectively, lateral views. Scale bars: A, B, C, 500 µm; D, 300 µm; and E, F, 100 µm.

to level of nerve-ring. Tail of both sexes conical, ending in small process with nodular to digitiform protuberances (Figs. 2E; 3D–H).

3.1.2. Male (based on 11 specimens; four ex *N. aequalis* and seven ex *T. scabrus*; measurements of holotype in parentheses; Figs. 1–4; Table 2)

Length of body 8.77–18.01 (11.49) mm, maximum width 225–349 (256). Dorsal lip 30–39 (36) long; ventrolateral lips 39–46 (41) long. Length of oesophagus 0.86–1.46 (1.30) mm, representing 7–11 (11)% of body length, oesophagus maximum width 70–158 (104). Nerve-ring and excretory pore 293–408 (345) and 359–614 (548), respectively, from anterior extremity. Ventriculus 62–91 × 69–128 (91 × 102); ventricular appendix 221–425 (365) long, 35–81 (81) wide (Fig. 2A). Posterior end of body curved ventrally (Fig. 2F). Spicules subequal, alate, with proximal end expanded and distal end pointed; left spicule 180–240 (180) long, right spicule 191–294 (211) long; spicule length representing 1–2 (2)% of body length. Gubernaculum absent. A total of 31–42 (36) pairs of subventral papillae present, 23–32 (28) precloacal, 1–2 (2) adcloacal, the latter at level of cloaca (if single) or one somewhat anterior and the other somewhat posterior to cloaca (if two); and 5–9 (6) postcloacal (Figs. 1D–I, 4A–E). Most papillae small rounded (Fig. 4B) to acicular (Fig. 4E), subventral (Fig. 4D), although some papillae sublaterally displaced (Fig. 4C). Posteriormost precloacal, adcloacal and postcloacal papillae very small. Seventh pair of postcloacal papillae

sometimes triple (Fig. 3A). Single median papilla on the anterior cloacal lip of some individuals. Two small papilla-like structures on the posterior cloacal lip (Fig. 3C). Distribution of precloacal papillae variable, with three basic patterns: coupled papillae starting at the eleventh papilla from the cloaca (Fig. 4A); well-separated single papillae (Fig. 4B, D) or closely-located single papillae (Fig. 4E). Tail 123–175 (169) long, phasmids at last third (Fig. 11).

3.1.3. Gravid female (based on four specimens; two ex *N. aequalis* and two ex *T. scabrus*; measurements of allotype in parentheses; Figs. 1–3; Table 3)

Length of body 20.47–36.29 (20.47) mm, maximum width 441–602 (500). Dorsal lip 41–59 (59) long; ventrolateral lips 48–56 (55) long. Length of oesophagus 1.28–2.18 (1.82) mm, representing 5–9 (9)% of body length, maximum width 154–210 (175). Nerve-ring and excretory pore 338–571 (473) and 654–845 (671), respectively, from anterior extremity. Ventriculus 83–100 × 141–163 (100 × 141); ventricular appendix 339–484 (484) long, 66–96 (66) wide (Fig. 2B). Vulva situated in anterior region of body, at 5.28–6.59 (6.03) mm from anterior extremity, representing 16–29 (29)% of body length; vagina directed anteriorly from vulva in a specimen with embryonated eggs, and posteriorly in the remaining specimens. Uterus anterior (female with embryonated eggs) or posterior to vulva (remaining specimens) (Fig. 2C–D). Eggs numerous, suboval to almost rounded, thin-walled, smooth; size

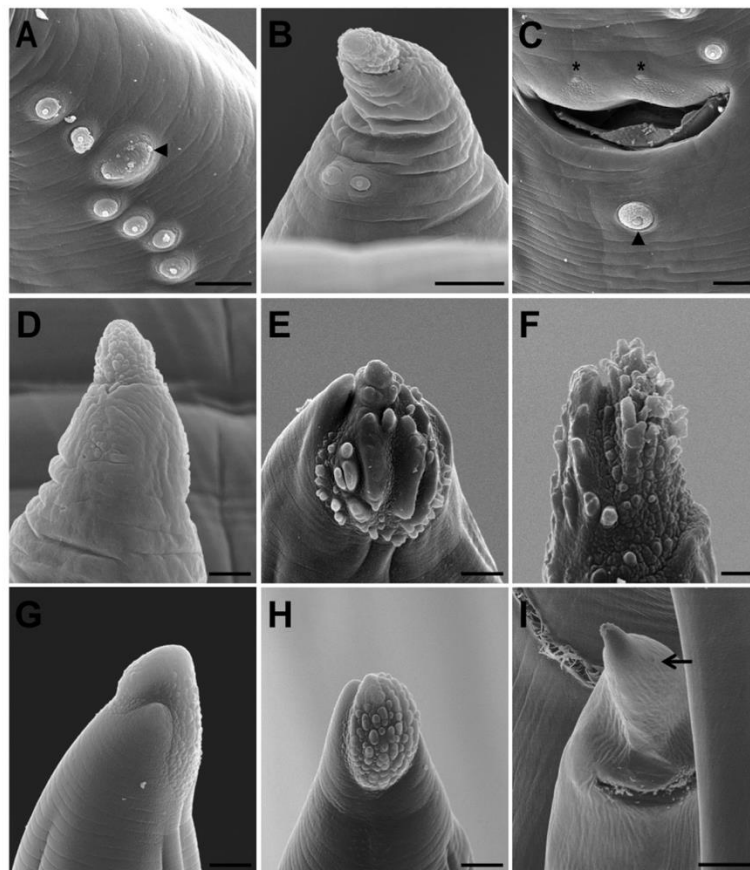


Fig. 3. *Raphidascaris (Raphidascaris) macrouri* n. sp., scanning electron micrographs. A – postcloacal papillae, subventral view (arrowhead indicates triple papilla). B – tail tip with a pair of ventral papillae. C – region of cloaca (arrowhead and asterisk indicate single anterior and posterior pair of papillae, respectively). D–H – tail tip, different specimens. I – tail of female, subventral view (arrow indicates phasmids). Scale bars: A, B, and C, 10 µm; D, E, G, and H, 5 µm; F, 2 µm; and I, 1 µm.

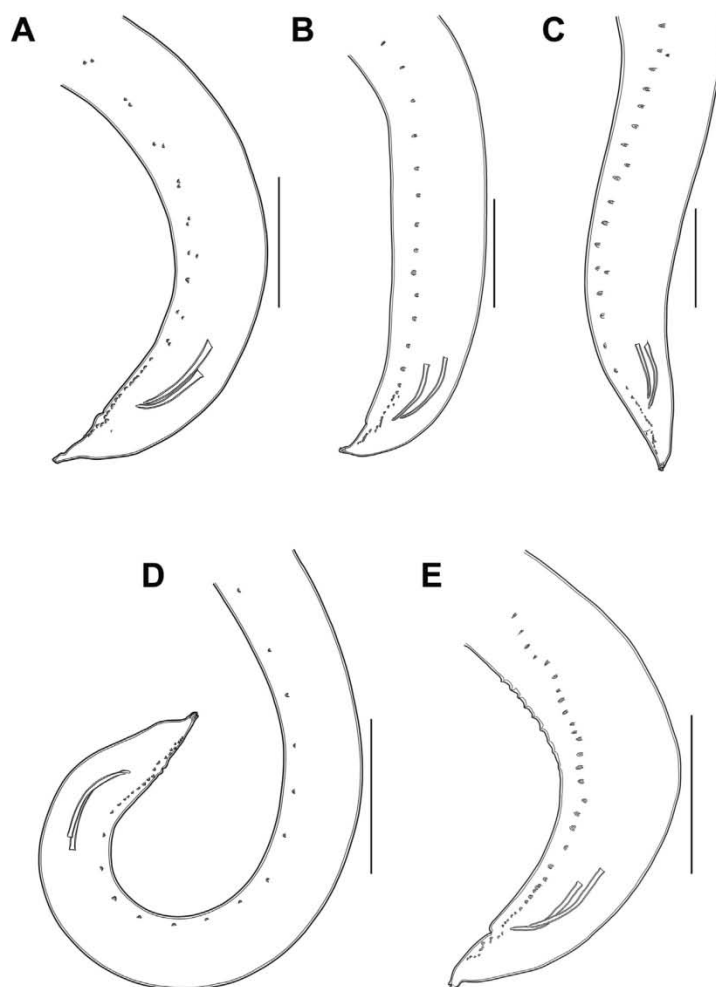


Fig. 4. *Raphidascaris (Raphidascaris) macrouri* n. sp. posterior end of male showing the variability in the distribution of caudal papillae. A, B, C – from *Nezumia aequalis*. D, E – from *Trachyrincus scabrus*. Scale bars: A, B, C, D, and E, 300 μ m.

46–59 \times 39–58 (56 \times 49). Tail 325–472 (325) long (Fig. 2E), phasmids at last third (Fig. 3I).

3.1.4. Non-gravid female (based on two specimens ex *N. aequalis*)

Length of body 11.70–14.30 mm, maximum width 292–377. Dorsal lip 40–54 long; ventrolateral lips 42–53 long. Length of oesophagus 1.03–1.15 mm, representing 8–9% of body length; maximum width 110–141. Nerve-ring at 346 from anterior extremity. Ventriculus 91 \times 127; ventricular appendix 281 long, with maximum width 60. Vulva at 3.02–3.97 mm from anterior extremity, representing 26–28% of body length; vagina directed posteriorly. Tail 252–303 long.

3.1.1. Taxonomic summary

Type-host: Common Atlantic grenadier *Nezumia aequalis* (Günther, 1878) (Gadiformes, Macrouridae) (preanal length 2.8–5.8 cm).

Other hosts: Roughsnout grenadier, *Trachyrincus scabrus* (Rafinesque, 1810) (Gadiformes, Macrouridae) (preanal length 8.5–11.5 cm).

Type-locality: Off Barcelona Spanish western Mediterranean. Ex *N. aequalis* (coordinates: 41° 05.25'N, 02° 05.14'E–41° 14.50'N, 02°

27.38'E; depth: 573–1102 m; dates: 28/04/2007, 26/06/2007, 05/07/2007, 19/07/2007, 28/12/2007, 06/05/2014, 19/05/2014); ex *T. scabrus* (coordinates: 41° 08.92'N, 02° 24.03'E–41° 14.52'N, 02° 27.36'E; depth: 558–783 m; dates: 06/03/2007, 11/05/2007, 19/07/2007).

Site of infection: Intestine, occasionally stomach and pyloric caeca.

Type-specimens: Holotype, allotype and paratypes at the BMNH: holotype and paratype ex *N. aequalis* (NHMUK.2015.5.19.1–2); paratypes: 3 males and 3 females ex *N. aequalis* (NHMUK.2015.5.19.3–8), 2 females and 7 males ex *T. scabrus* (NHMUK.2015.5.19.9–17).

Representative sequences: ITS1–5.8S–ITS2 region; GenBank accession numbers: KR232377 [R. (*R.*) *macrouri* ex *N. aequalis*]; KR232376 [R. (*R.*) *macrouri* ex *T. scabrus*].

Etymology: The specific name of this nematode relates to the genitive of the generic name of host family (i.e., Macrouridae).

3.2. Molecular characterisation

The four sequences of the ITS1–5.8S–ITS2 region of rDNA obtained herein based on material of *R. (*R.*) macrouri* n. sp. from both fish hosts

Table 2

Morphometric data for males of *Raphidascaris (Raphidascaris) macrouri* n. sp. from two hosts collected off the Mediterranean coast of Spain. All measurements are in micrometres unless otherwise stated.

Host	<i>Nezumia aequalis</i>	<i>Trachyrincus scabrus</i>
Locality	Off Barcelona	Off Barcelona
	n = 11	n = 7
Body length (mm)	11.40–13.04	8.77–18.01
Body maximum width	227–274	225–349
Dorsal lip length	31–37	30–39
Ventrolateral lip length	40–42	39–46
Oesophagus length (mm)	1.07–1.30	0.86–1.46
(% of body length)	(9–11)	(7–10)
Oesophagus width	104–119	70–158
Nerve-ring to anterior body end	345–380	293–408
Excretory pore to anterior body end	483–548	359–614
Ventriculus length	62–91	63–80
Ventriculus width	93–114	69–128
Ventricular appendix length	357–388	221–425
Ventricular appendix width	36–81	35–64
Left spicule length	180–199	180–240
Right spicule length	221–237	191–294
Spicule length as % of body length	2	1–2
Total no. of papillae	31–36	32–42
No. of precloacal papillae	24–28	23–32
No. of adcloacal papillae	2	1–2
No. of postcloacal papillae	5–7	7–9
Tail length	125–169	123–175

were 740 bp long and showed no intraspecific divergence. Therefore, only one sequence per host species was submitted to GenBank. The trees inferred from both BI and ML analyses had the same topology with similar nodal support (Fig. 5). Both analyses provided strong support for the affiliation of the new species to *Raphidascaris*, placing the new species as earlier divergent. Pairwise comparisons between the newly-obtained sequences for *R. (R.) macrouri* n. sp. with those for other species of *Raphidascaris* revealed divergence levels ranging from 13.2 to 13.6% (Table 4). It is worth noting that the interspecific divergence was much lower (0.3–4.3%) among the three species used in the comparisons, all belonging to the subgenus *Ichthyascaris*. Although representatives of *Anisakis* and *Hysterothylacium* formed strongly supported clades, the relationships of *Hysterothylacium zhoushanensis* and *Hysterothylacium deardoffverstreeterorum* were not resolved in our analyses.

Table 3

Morphometric data for gravid females of *Raphidascaris (Raphidascaris) macrouri* n. sp. from two hosts collected off the Mediterranean coast of Spain. All measurements are in micrometres unless otherwise stated.

Host	<i>Nezumia aequalis</i>	<i>Trachyrincus scabrus</i>
Locality	Off Barcelona	Off Barcelona
	n = 2	n = 2
Body length (mm)	20.47–23.67	32.41–36.29
Body maximum width	463–500	441–602
Dorsal lip length	41–59	48–50
Ventrolateral lip length	48–56	50–55
Oesophagus length (mm)	1.28–1.82	1.91–2.18
(% of body length)	5–9	6
Oesophagus width	154–175	210
Nerve-ring to anterior body end	338–473	447–571
Excretory pore to anterior body end	671 ^a	654–845
Ventriculus length	83–100	91 ^a
Ventriculus width	141–163	156 ^a
Ventricular appendix length	339–484	403–464
Ventricular appendix width	66–88	66–96
Vulva to anterior body end (mm)	5.28–6.03	5.67–6.59
(% of body length)	(22–29)	(16–20)
Egg-length	47–59	46–54
Egg-width	39–53	51–58
Tail length	325–355	399–472

^a n = 1.

4. Discussion

The nematode family Anisakidae Railliet & Henry, 1912 includes the subfamily Raphidascaridinae Hartwich, 1954 and tribe Raphidascaridinea Chabaud, 1965, where three genera are recognised, namely, *Raphidascaris* Railliet & Henry, 1915, *Raphidascaroides* Yamaguti, 1941 and *Thynnascaris* Dollfus, 1933 [27]. The genera *Ichthyascaris* Wu, 1949 and *Sprentascaris* Petter & Cassone, 1984 were erected to accommodate some species morphologically closely related to *Raphidascaris*; these were differentiated from the species in the latter genus by the presence of lateral alae and several well-developed postlabial cuticular elevations, respectively [28,29]. However, *Ichthyascaris* and *Sprentascaris* were recognised as subgenera of *Raphidascaris* in subsequent revisions [11,30]. Therefore, the genus *Raphidascaris* currently includes the subgenera *Ichthyascaris*, *Raphidascaris* and *Sprentascaris*.

The present material is assigned to *R. (Raphidascaris)* due to the presence of well-developed lips, excretory pore slightly posterior to the level of the nerve-ring, posteriorly directed ventricular appendix, and the lack of postlabial ornamentation, interlabia and intestinal caecum [27]. Four species are considered to belong to the nominotypical subgenus *Raphidascaris* [13,31]: *R. (R.) acus* (Bloch, 1779), which is found in different freshwater fishes (Anguilliformes, Gadiformes and Salmoniformes) in Europe, Asia and North America [27]; *R. (R.) gigi* Fujita, 1928 in freshwater fishes of the families Bagridae, Salmonidae and Plecoglossidae from Lake Biwa system, Japan [11]; *R. (R.) lutjani* Olsen, 1952, a parasite of the marine fish *Lutjanus analis* (Cuvier) (Lutjanidae) in the Gulf of Mexico [32]; and *R. (R.) mediterraneus* Lèbre & Petter, 1983 in *C. cuculus* and *C. obscurus* (both Triglidae) from the Mediterranean Sea [15,16].

R. macrouri n. sp. is the first species of the genus described from fishes of the family Macrouridae and is easily distinguishable from *R. lutjani* and *R. acus* in the high number of precloacal papillae (23–32 vs. 9–14 and 17–20, respectively) and length of spicules (180–294 vs. 1060–1090 and 440–1200 µm, respectively) [27,31]. *R. lutjani* can further be differentiated from *R. macrouri* n. sp. by the much larger size of body (males: 32.10–50.50 vs. 8.77–18.01 mm; females: 72.10 vs. 20.47–36.29 mm) and the more posterior position of vulva (33 vs. 16–29% of body length) [32]. *R. acus* exhibits longer oesophagus (1.60–5.15 vs. 0.86–2.18 mm), ventricular appendix (800–2660 vs. 221–484 µm), and eggs (70–100 × 66–78 vs. 46–58 × 41–57 µm) than the new species [27].

Raphidascaris gigi is very similar to *R. macrouri* n. sp. in the number of caudal papillae (37–42 vs. 31–42), but differs in the smaller body size (males: 5.90–8.46 vs. 8.77–18.01 mm; females: 14.25–18.90 vs. 20.47–36.29 mm), longer spicules (354–476 vs. 180–294 µm), longer ventricular appendix in females (612–639 vs. 339–484 µm) and a more anteriorly located vulva (6–7 vs. 16–29% of body length) [11].

R. macrouri n. sp. closely resembles *R. mediterraneus* in having similar length of oesophagus (0.86–2.18 vs. 1.47–1.90 mm), distance from excretory pore to anterior extremity of body (359–845 vs. 560–750 µm) and spicule length (180–294 vs. 200–230 µm), but differs in the smaller body size of males (8.77–18.01 vs. 15–30 mm) and exhibits a significantly high number of precloacal papillae (23–32 vs. 14–17) [15].

Morphometric variability was observed in the specimens of *R. macrouri* n. sp. collected from the two macrourid hosts. The specimens from *T. scabrus* were generally larger in comparison with those obtained from *N. aequalis* (Tables 2, 3). This difference may be related to the host size (*T. scabrus* is bigger than *N. aequalis*). The influence of the host size on the total length of parasites has already been reported in nematodes of freshwater and marine fishes [33–36]. Interestingly, the disposition of the vagina also varied among the gravid females of the new species. This structure was anteriorly directed from the vulva in specimens with embryonated eggs, whereas non-gravid females had a posteriorly directed vagina. Different vagina orientations during female development were also reported by Moravec and Nagasawa [11] in *R. gigi*. Probably, an advanced developmental stage and increase

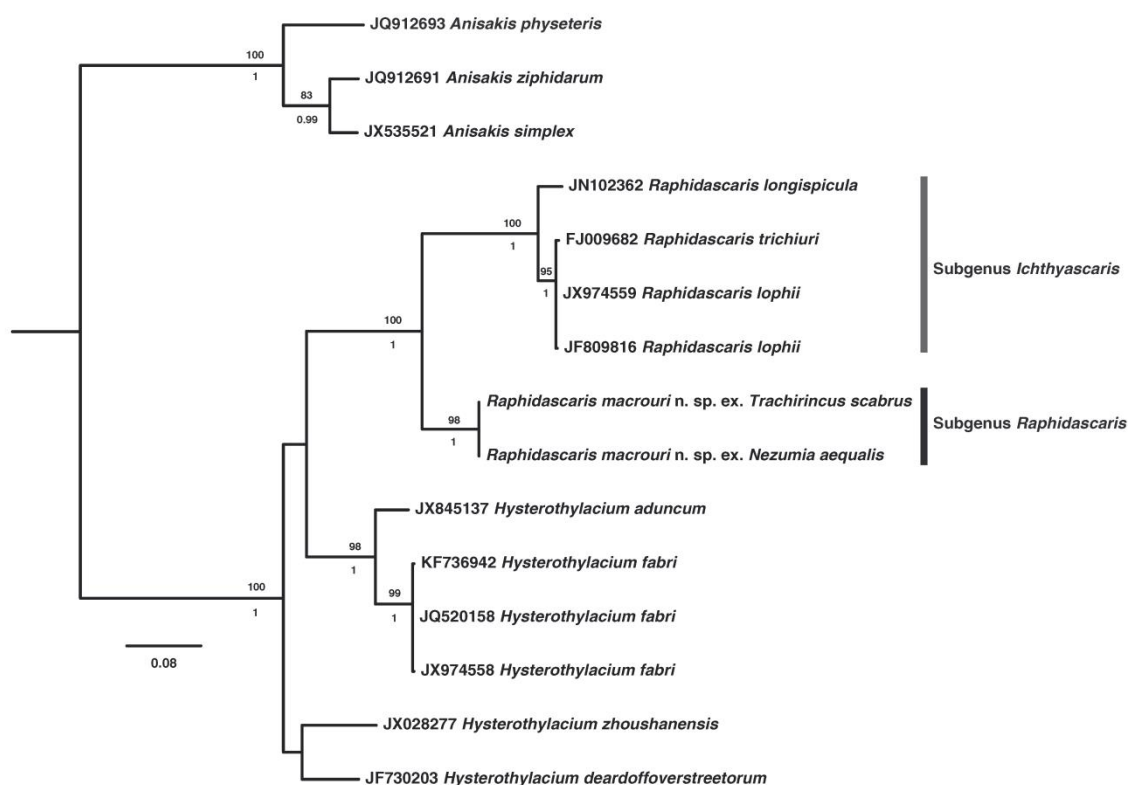


Fig. 5. Phylogenetic tree reconstructed using the newly-generated and retrieved from GenBank sequences for *Raphidascaris* species. Nodal support from Maximum Likelihood (ML) and Bayesian Inference (BI) analyses are indicated above and below the nodes. Outgroup: *Anisakis* species. The scale-bar indicates the expected number of substitutions per site.

in the number of eggs causes expansion of the uterus towards the anterior end and in consequence the vagina adopts anterior direction.

The number of caudal papillae in males of *R. macrouri* n. sp. (23–32 papillae) also showed a distinct variability in comparison to other species of *Raphidascaris*. Out of all the described species of this genus only two show a similar degree of variability in the number of preloacal papillae: *Raphidascaris* (*L.*) *vicentei* Santos, 1970 (syn.

Raphidascaris camura Deardorff & Overstreet, 1981) (31–40 papillae) and *Raphidascaris* (*S.*) *hypostomi* (Petter & Cassone, 1984) (17–26 papillae) [30,37].

In addition to the detailed morphological description, this study provides the first molecular characterisation of a Mediterranean nematode species of *Raphidascaris*. Both BI and ML analyses provided strong support for the generic affiliation of the new species. Even though there

Table 4

Pairwise p-distance values for the new species described and the other nematoda species included in this study for the molecular comparison, based on the sequenced ITS1–5.8S–ITS2 region of rDNA.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 <i>Raphidascaris macrouri</i> n. sp. ex. <i>Trachyrincus scabrus</i>															
2 <i>Raphidascaris macrouri</i> n. sp. ex. <i>Nezumia aequalis</i>	0.000														
3 JN102362 <i>Raphidascaris longispicula</i>	0.136	0.136													
4 JF809816 <i>Raphidascaris lophii</i>	0.132	0.132	0.042												
5 JX974559 <i>Raphidascaris lophii</i>	0.132	0.132	0.040	0.001											
6 FJ009682 <i>Raphidascaris trichiuri</i>	0.135	0.135	0.043	0.004	0.003										
7 KF736942 <i>Hysterothylacium fabri</i>	0.161	0.161	0.213	0.209	0.208	0.208									
8 JX974558 <i>Hysterothylacium fabri</i>	0.161	0.161	0.213	0.209	0.208	0.208	0.003								
9 JQ520158 <i>Hysterothylacium fabri</i>	0.162	0.162	0.213	0.209	0.208	0.208	0.001	0.001							
10 JX845137 <i>Hysterothylacium aduncum</i>	0.164	0.164	0.209	0.209	0.207	0.207	0.065	0.065	0.063						
11 JX028277 <i>Hysterothylacium zhoushanensis</i>	0.153	0.153	0.166	0.171	0.171	0.171	0.144	0.145	0.145	0.139					
12 JF730203 <i>Hysterothylacium deardorffoverstreetorum</i>	0.159	0.159	0.186	0.188	0.186	0.186	0.126	0.128	0.128	0.122	0.100				
13 JX535521 <i>Anisakis simplex</i>	0.297	0.297	0.299	0.293	0.293	0.295	0.288	0.288	0.286	0.275	0.266	0.244			
14 JQ912693 <i>Anisakis physeteris</i>	0.294	0.294	0.297	0.298	0.300	0.301	0.287	0.287	0.285	0.272	0.254	0.250	0.122		
15 JQ912691 <i>Anisakis ziphidarum</i>	0.283	0.283	0.294	0.290	0.290	0.291	0.284	0.284	0.283	0.279	0.263	0.249	0.052	0.119	

were differences in the number and distribution of caudal papillae in nematodes from both host species, the sequences proved that they were identical (Fig. 1). Unfortunately, the number of sequences of the ITS region for species of the Anisakidae, based on reliably identified material, is still very small. *R. macrourii* n. sp. represents the first species of the genus reported from macrourid fish and the second described from fish in the Mediterranean. Our study thus adds a new species to the mostly unknown parasite fauna of the deep-sea Mediterranean fishes.

Acknowledgements

We thank two anonymous reviewers for their comments and suggestions. This study was supported by the Spanish Science and Technology Ministry project BIOMARE (CTM2006-13508-C02-01MAR) and ANTRMARE (CTM2009-12214-C02-01/02); and partially supported by the Czech Science Foundation (Project No. P505/12/G112). We are grateful to Dr. A. Kostadinova for her valuable comments in the early version of this paper. David Pérez-i-García benefits from a PhD student grant (PIF2012-53279) of the Universitat Autònoma de Barcelona.

References

- Kensley B, Cartes JE. Records and distribution of *Sycesus infelix* in the deep Mediterranean (Crustacea: Isopoda: Aegidae). *J Mar Biol Assoc UK* 2003;83:775–7. <http://dx.doi.org/10.1017/S002531540300777Xh>.
- Constenla M, Carrassón M, Moyà CM, Fernàndez-Chacón A, Padrós F, Repullés-Albelda A, Montero FE. Parasitisation by *Bathycerca elongatum* (Digenea, Opecoelidae) in pyloric caeca of *Trachyrincus scabrus* (Teleostei, Macrouridae). *Dis Aquat Organ* 2011;96:239–47. <http://dx.doi.org/10.3354/dao202393>.
- Dallarés S, Georgieva S, Kostadinova A, Carrassón M, Gibson DI, Pérez-del-Olmo A. Morphometric and molecular characterisation of specimens of *Lepidapedon* Stafford, 1904 (Digenea: Lepidapedidae) from the deep-sea fish *Mora moro* (Risso) (Teleostei: Moridae) in the western Mediterranean. *Syst Parasitol* 2013;85:243–53. <http://dx.doi.org/10.1007/s11230-013-9426-7>.
- Dallarés S, Constenla M, Padrós F, Cartes JE, Solé M, Carrassón M. Parasites of the deep-sea fish *Mora moro* (Risso, 1810) from the NW Mediterranean Sea and relationship with fish diet and enzymatic biomarkers. *Deep Sea Res, Part I* 2014;92:115–26. <http://dx.doi.org/10.1016/j.dsr.2014.07.001>.
- González-Solis D, Carrassón M, Pérez-del-Olmo A. *Capillstrongyloides morae* sp. n. (Nematoda: Capillariidae) from deep-sea fish (Teleostei, Moridae) in the Western Mediterranean Sea. *Folia Parasitol* 2014;5683:63–8. <http://dx.doi.org/10.14411/fp.2014.003>.
- Mateu P, Montero FE, Carrassón M. Geographical variation in metazoan parasites of the deep-sea fish *Bathypteris mediterraneus* Bauchot, 1962 (Osteichthyes: Ipnopidae) from the Western Mediterranean. *Deep Sea Res, Part I* 2014;87:24–9. <http://dx.doi.org/10.1016/j.dsr.2014.01.008>.
- Pérez-del-Olmo A, Dallarés S, Carrassón M, Kostadinova A. A new species of *Bathycerca* (Digenea: Opecoelidae) from *Physicis blennoides* (Brünnich) (Gadiformes: Phycidae) in the Western Mediterranean. *Syst Parasitol* 2014;88:233–44. <http://dx.doi.org/10.1007/s11230-014-9491-6>.
- Stefanescu C, Lloris D, Rucabado J. Deep-sea fish assemblages in the Catalan Sea (western Mediterranean) below a depth of 1000 m. *Deep Sea Res I Oceanogr Res Pap* 1993;40:695–707. [http://dx.doi.org/10.1016/0967-0637\(93\)90066-C](http://dx.doi.org/10.1016/0967-0637(93)90066-C).
- Massuti E, Morales-Nin B, Stefanescu C. Distribution and biology of five grenadier fish (Pisces: Macrouridae) from the upper and middle slope of the northwestern Mediterranean. *Deep Sea Res Part I* 1995;42:307–30.
- Froese R, FishBase Pauly D. World Wide Web electronic publication. www.fishbase.org. 2014 version (08/2014).
- Moravec F, Nagasawa K. Redescription of *Raphidascaris gigi* Fujita, 1928 (Nematoda: Anisakidae), a parasite of freshwater fishes in Japan. *Syst Parasitol* 2002;52:193–8. <http://dx.doi.org/10.1023/A:1015785602488>.
- De Melo MD, dos Santos JN, Giese EG, dos Santos EGN, Santos CP. *Raphidascaris (Sprentascaris) lanfrediae* sp. nov. (Nematoda: Anisakidae) from the fish *Satanoperca jurupari* (Osteichthyes: Cichlidae). *Mem Inst Oswaldo Cruz* 2011;106:553–6.
- Moravec F, Justine J. *Raphidascaris (Ichthyascaris) etelidis* n. sp. (Nematoda, Anisakidae), a new ascaridoid nematode from lutjanid fishes off New Caledonia. *Zoosystema* 2012;34:113–21. <http://dx.doi.org/10.5252/z2012n1a4>.
- Jahantab M, Haseli M, Salehi Z. Morphological and genetic characteristics of the anisakid nematode *Raphidascaris acus* from the southwest Caspian Sea: evidence for the existence of sibling species within a species complex. *Parasitol Res* 2014;113:3419–25. <http://dx.doi.org/10.1007/s00436-014-4007-5>.
- Lèbre C, Petter AJ. Deux nouvelles espèces d'Ascarides (Nematoda) parasites de poissons téléostéens: *Raphidascaris mediterraneus* n. sp. complément de description de *Cucullanus micropapillatus* Törnquist, 1931 (Nematoda, Cucullanidae). *Bull Mus Nat Hist Nat Sect A Zool Biol Ecol Anim* 1983;2:491–505 (4e série, 5, section A).
- Petter AJ, Maillard C. Ascarides de poissons de Méditerranée occidentale. *Bull Mus Nat Hist Nat Sect A Zool Biol Ecol Anim* 1987;4:773–98 (4e série, 9, section A).
- D'Amelio S, Mathiopoulos KD, Santos CP, Pugachev ON, Webb SC, Picanco M, Paggi L. Genetic markers in ribosomal DNA for the identification of members of the genus *Anisakis* (Nematoda: Ascaridoidea) defined by polymerase-chain-reaction-based restriction fragment length polymorphism. *Int J Parasitol* 2000;30:223–6. [http://dx.doi.org/10.1016/S0020-7519\(99\)00178-2](http://dx.doi.org/10.1016/S0020-7519(99)00178-2).
- Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 1999;41:95–8.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013;30:2725–9. <http://dx.doi.org/10.1093/molbev/mst197>.
- Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000;22:540–52.
- Gouy M, Guindon S, Gascuel O. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 2010;27:221–4.
- Guindon S, Gascuel O. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Syst Biol* 2003;52:696–704.
- Darriba D, Taboada GL, Doallo R, Posada D. jModelTest2: more models, new heuristics and parallel computing. *Nat Method* 2012;9:772.
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhylML 3.0. *Syst Biol* 2010;59:307–21.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 2012;61:539–42.
- Huelsbeck JP, Ronquist F, Nielsen R, Bollback JP. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 2001;294:2310–4.
- Moravec F. Parasitic nematodes of freshwater fishes of Europe. Praha: Academia; 1994.
- Petter AJ, Cassone J. Nématodes de poissons du Paraguay; I. Ascaridoidea: *Sprentascaris*, n. gen. *Rev Suisse Zool* 1984;91:617–34.
- Bruce NL. *Hysterothylacium* Ward and Magath, 1917, and *Ichthyascaris* Wu, 1949, ascaridoid nematodes from Australian demersal fishes. *Mem Qld Mus* 1990;28:389–426.
- Moravec F, Kohn A, Fernandes BMM. First record of *Raphidascaris (Sprentascaris) hypostomi* (Petter et Cassone, 1984) comb. n. and *R. (S.) mahneri* (Petter et Cassone, 1984) comb. n. (Nematoda: Anisakidae) from Brazil with remarks on the taxonomic status of the genus *Sprentascaris* (Petter et Cassone, 1984). *Folia Parasitol* 1990;37:131–40.
- Smith JD. Taxonomy of *Raphidascaris* spp. (Nematoda, Anisakidae) of fishes, with a redescription of *R. acus* (Bloch, 1772). *Can J Zool* 1984;62:685–94.
- Olsen LS. Some nematodes parasitic in marine fishes. *Publ Inst Mar Sci* 1952;2:174–215.
- Morand S, Legendre P, Gardner SL, Hugot JP. Body size evolution of oxyurid (Nematoda) parasites: the role of hosts. *Oecol* 1996;107(2):274–82. <http://dx.doi.org/10.1007/BF00327912>.
- Sasal P, Jobet E, Fallex E, Morand S. Sexual competition in an acanthocephalan parasite of fish. *Parasitol* 2000;120:65–9. <http://dx.doi.org/10.1017/S0031182099005272>.
- González-Solis D, Moravec F. Two new nematode species, *Orientaltractis campechensis* n. sp. and *Orientaltractis chiapasensis* n. sp. (Nematoda: Atractidae) from cichlid fishes in Southern Mexico and Nicaragua. *J Parasitol* 2004;90(6):1443–9. <http://dx.doi.org/10.1645/GE-3366>.
- Isbert W, Montero FE, Carrassón M, González-Solis D. *Dichelyne (Cucullanellus) romani* n. sp. (Nematoda: Cucullanidae) in notacanthid fishes from the Northeast Atlantic and Western Mediterranean. *Syst Parasitol* 2015. <http://dx.doi.org/10.1007/s11230-015-9556-1>.
- Deardorff TL, Overstreet RM. *Raphidascaris camura* sp. n., *Hysterothylacium eurycheilum* (Olsen) comb. n., and comments on *Heterotryphum* Spaul (Nematoda: Ascaridoidea) in marine fishes. *J Parasitol* 1981;67:426–32. <http://dx.doi.org/10.2307/3280567>.
- Li L, Liu YY, Zhang LP. Morphological and molecular identification of *Hysterothylacium longilabrum* sp. nov. (Nematoda: Anisakidae) and larvae of different stages from marine fishes in the South China Sea. *Parasitol Res* 2012;111:767–77. <http://dx.doi.org/10.1007/s00436-012-2897-7>.
- Xu Z, Zhang LP, Liu BC, Li L. Morphological and molecular characterization of *Raphidascaris (Ichthyascaris) lophii* (Wu, 1949) (Nematoda, Anisakidae) from marine fishes from China, with a key to the species of the subgenus *Ichthyascaris*. *Acta Parasitol* 2012;57:316–22. <http://dx.doi.org/10.2478/s11686-012-0037-2>.
- Kim W-S, Jeon C-H, Kim J-H, Kim D-H, Oh M-J. Current status of anisakid nematode larvae infection in marine fishes caught from the coastal area of Korea between 2010 and 2012. *J Fish Pathol* 2012;25:189–97. <http://dx.doi.org/10.7847/jfp.2012.25.3.189>.
- Fang W, Xu S, Zhang S, Wang Y, Chen X, Luo D. Multiple primer PCR for the identification of anisakid nematodes from Taiwan Strait. *Exp Parasitol* 2010;124:197–201. <http://dx.doi.org/10.1016/j.exppara.2009.09.011>.
- Guo YN, Xu Z, Zhang LP, Hu YH, Li L. Occurrence of *Hysterothylacium* and *Anisakis* nematodes (Ascaridida: Ascaridoidea) in the tanaka's snailfish *Liparis tanaka* (Gilbert & Burke) (Scorpaeniformes: Liparidae). *Parasitol Res* 2014;113:1289–300. <http://dx.doi.org/10.1007/s00436-014-3767-2>.
- Knoff M, Felizardo NN, Iñiguez AM, Maldonado Jr A, Torres EJ, Pinto RM, Gomes DC. Genetic and morphological characterization of a new species of the genus *Hysterothylacium* (Nematoda) from *Paralichthys isosceles* Jordan, 1890 (Pisces: Teleostei) of the Neotropical Region, state of Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 2012;107:186–93.
- Mattiucci S, Cipriani P, Webb SC, Paoletti M, Marcer F, Bellisario B, Gibson DI, Nascetti G. Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). *J Parasitol* 2014;100:199–214. <http://dx.doi.org/10.1645/12-120.1>.

Conclusions

CONCLUSIONS

1) The parasite communities of *Alepocephalus rostratus* were formed by little number of species (seven metazoan species and one coccidian). Low richness together with the high proportion of larval forms are usually characteristic of meso- and bathypelagic fish species. This is probably due to the specialized diet of *A. rostratus* on planktonic organisms.

2) Tetrphyllidea fam. gen. sp. was the most prevalent parasite in *A. rostratus*. Being present in all samples, this might indicate a strong trophic link between the definitive host and *A. rostratus*. Molecular analyses related this parasite to the Phyllobothriinae, which are usually encountered as adults in sharks.

3) The nematodes Cucullaninae gen. sp. and *Hysterothylacium aduncum* and the digenean *Paraccacladium* sp. could be considered as potential tags of discrimination between populations of *A. rostratus* at different depths.

4) The great abundance and prevalence of Cucullaninae gen. sp. larvae (L3) at 1000 and 1400 m depth could be due to the greater consumption of calanoid copepods by the host at this depth. *Paraccacladium* sp., a digenean genus exclusively found in gelatinous planktonic feeders, increases its abundance at 1400–2000 m depth probably due to an increase on the predation of the Siphonophora *Chelophyes appendiculata*. The increasing abundance of *Hysterothylacium aduncum* with depth in *A. rostratus* might be related to an increase on consumption of different benthic crustaceans related to depth.

5) The abundance of *Paraccacladium* sp. was related to temperature probably because siphonophorans, intermediate host of this parasite, prefer warmer water masses; and Cucullaninae gen. sp. was associated with turbidity, presumably due to the latter promoted the abundance of calanoid copepods, the possible first intermediate host of Cucullaninae gen. sp.

6) The parasites Tetrphyllidea fam. gen. sp., *Anisakis physeteris* and *H. aduncum* were related to lower levels of activity for AChE in *A. rostratus*, probably due to a partial inhibition of the enzyme upon the burden of those parasites.

7) The LDH activity increased with the abundance of *Paracycloctyla cherbonnieri* and Tetrphyllidea fam. gen. sp., probably as consequence of an increase of the energy demand on the host due to the stress caused by parasitism.

8) Mediterranean *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus* parasite communities are fairly homogenous and poor in species compared to the ones of the few macrourid species studied in the Atlantic.

9) *Coelorinchus caelorhincus* had the most distinct parasite community. Being found at shallower depths, only three parasite species were encountered. Two of them, *Bathycreadium brayi* and Cucullanidae gen. sp. larvae, were exclusively found in this fish species.

10) *Coelorinchus mediterraneus* exhibited the highest number of parasite species (eight), *C. guentheri* and *Cor. mediterraneus* had less parasite richness. The digenean *Lepidapedon desclersae* and the nematode *Capillostrongyloides morae* were exclusively found in *Coe. Mediterraneus*, *Otodistomum* sp. in *C. guentheri* and *Gnathia* sp. in *Cor. mediterraneus*.

11) The parasite communities of *C. guentheri* and *Cor. mediterraneus* were dominated by juvenile and larval stages and especially homogenous probably due to the phylogenetic proximity of two hosts. Subtle differences found may be explained by the diet of *C. guentheri*, more specialized in benthic prey than that of *Cor. mediterraneus*.

12) Cucullanidae gen. sp. was the most abundant parasite of *C. caelorinchus*. The diet analysis related this nematode to the consumption of polychaetes, which are known to act as first intermediate hosts of some cucullanids.

13) *Lepidapedon desclersae* was the most abundant and prevalent parasite of *Coe. mediterraneus*. Its abundance was generally higher in the mainland slope, where the higher complexity of trophic webs respect the insular slope might promote the completion of complex life-cycles of digeneans.

14) *Hamaticolax resupinus* was reported from all four macrourids of the present thesis. Probably due to the high abundance of most of its hosts and their overlapping distributions in depth, this copepod can encounter enough hosts to complete its life cycle. This copepod was related to increasing levels of salinity in deep waters; an environmental factor which probably affects its naupliar stages.

15) *Hysterothylacium aduncum* was the most abundant and prevalent parasite in all macrourids, excluding *C. caelorhincus* which does not harbour it. As with *A. rostratus*, *H. aduncum* abundance tended to increase with depth and it is probably a key component of the parasite communities of the deep Mediterranean.

16) The activity of AChE decreased when the abundance of Cucullanidae gen. sp. increased, probably inhibited due to parasitism. However, the cucullanid larvae of *A. rostratus* originated the opposite response on the same enzyme and highlight the still poor knowledge about the relation between these variables.

17) *Coelorinchus mediterraneus*, *C. guentheri* and *Cor. mediterraneus* presented low prevalence of epitheliocystis, although in the middle slope it could reach high values. Temperature may be a limiting factor on the distribution of the chlamydiales that cause this pathology. Considering the high abundance of potential hosts in the lower slope (three different species), warm waters might limit the access of these bacteria.

18) No clear relationships between parasitism and most histological alterations could be found in the analysed macrourids. The low abundance of most of the encountered parasites could have restricted the burden on the host. The number and area occupied by macrophagic centres in liver and spleen could be related to Tetraphyllidea fam. gen. sp. in *Cor. mediterraneus* highlight a possible harmful effect.

19) *Hamaticolax resupinus* n. sp. is described for the first time from *Coe. mediterraneus* and *Cor. mediterraneus*. It is the second *Hamaticolax* species that parasitizes a macrourid species and it is the deepest bomolochid encountered worldwide. *Hamaticolax resupinus* is smaller than its closely related species *Hamaticolax maleus*, females have wider genital double-somite markedly wider than free abdominal somites and with convex lateral margins.

20) Characteristics that differentiates *Hamaticolax resupinus* n. sp. from the rest of the species of the genus are: an urosome shorter than the prosome; two inner setae in the second endopodal segment of legs 2 and 3; third exopodal segment of leg 2 with 9 elements (IV, 5); eight elements in the third exopodal segment of the legs 3 and 4; and short inner setae on the first and second endopodal segments of leg 4.

21) *Raphidascaaris (R.) macrouri* n. sp. is described for the first time from the deep Mediterranean macrourids *Nezumia aequalis* and *Trachyrincus scabrus*. This species differentiates from the rest of the subgenus by having high number of caudal papillae and short spicules.

22) High variability in the number and distribution of precloacal papillae is encountered in *R. macrouri* n. sp., which is not recorded in such extend in any other species of the genera. Molecular analyses on the ITS1–5.8S–ITS2 region confirm the species uniformity despite the variability in the papillae.

Bibliography

BIBLIOGRAPHY

- Anderson, R.M., May, R.M., 1979.** Population biology of infectious diseases: part I. *Nature* 280, 36–367.
- Angel, M.V., 1990.** Life in the Benthic Boundary Layer: connections to the mid-water and sea floor. *Philos. Trans. R. Soc. Lond.* 331, 15–28.
- Angel, M.V., 2003.** The pelagic environment of the open ocean. In: Tyler, P.A. (Ed.) *Ecosystems of the World, Vol. 28 Ecosystems of the Deep Ocean*. Elsevier, Amsterdam, pp. 39–80.
- Au, D., 2004.** The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. *Mar. Pollut. Bull.* 48(9–10), 817–834.
- Belló, A.R., Fortes, E., Belló-Klein, A., Belló, A.A., Llesuy, S., Robaldo, R., Bianchini, A., 2000.** Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen*. *Dis. Aquat. Organ.* 42(3), 233–236.
- Boudreau, B.P., Jørgensen, B.B., 2001.** *The Benthic Boundary Layer*. Oxford University Press, New York, USA, 405 pp.
- Boxshall, G., 1998.** Host specificity in copepod parasites of deep-sea fishes. *J. Mar. Syst.* 15, 215–223.
- Bray, R.A., 2004.** The bathymetric distribution of the digenean parasites of deep-sea fishes. *Folia Parasitol.* 51, 268–274.
- Bray, R.A., 2005.** Deep-sea parasites. In: Rohde, K. (Ed.) *Marine parasitology*. CSIRO PUBLISHING, Collingwood, Australia, pp. 366–369.
- Bray, R.A., Littlewood, D.T.J., Herniou, E.A., Williams, B., Henderson, R.E., 1999.** Digenean parasites of deep-sea teleosts: a review and case studies of intrageneric phylogenies. *Parasitology* 119(Supplement), S125S–144.
- Bucklin, A., Nishida, S., Schnack-Schiel, S., Wiebe, P.H., Lindsay, D., Machida, R.J., Copley, N.J., 2010.** Chapter 13: A Census of Zooplankton of the Global Ocean. In: McIntyre, A. (Ed.) *Life in the World's Oceans: Diversity, Distribution, and Abundance*. Wiley Blackwell, Oxford, pp. 247–265.
- Bush, A.O., 1990.** Helminth communities in avian hosts: determinants of pattern. In: Esch, G.W., Bush, A.O., Aho, J.M. (Eds.) *Parasite Communities: Patterns and Processes*. Chapman and Hall, London, pp. 197–232.

-
- Campbell, R.A., 1983.** Parasitism in the deep sea. In: Rowe, G.T. (Ed.) *The Sea. Vol. 8.* John Wiley & Sons, New York, pp. 473–552.
- Campbell, R.A., Haedrich, R.I., Munroe, T.A., 1980.** Parasitism and ecological relationships among deep-sea benthic fishes. *Mar. Biol.* 57, 301–313.
- Carrassón, M., Cartes, J.E., 2002.** Trophic relationships in a Mediterranean Deep-sea fish community: partition of resources, dietary overlap and connections within the benthic boundary layer. *Mar. Ecol. Prog. Ser.* 241, 41-55.
- Carrassón, M., Matallanas, J., 1998.** Feeding habits of *Alepocephalus rostratus* (Pisces: Alepocephalidae) in the Western Mediterranean Sea. *J. Mar. Biol. Assoc. U. K.* 78(4), 1295–1306.
- Carrassón, M., Matallanas, J., 2002.** Diets of the macrourids fishes in the western Mediterranean. *Mar. Ecol. Prog. Ser.* 234, 215–228.
- Cartes, J.E., 1994.** Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea. *Mar. Biol.* 120, 639–648.
- Cartes, J.E., 1998.** Feeding strategies and partition of food sources in deep-water decapod crustaceans (400-2300 m). *J. Mar. Biol. Assoc. U. K.* 78, 509–524.
- Cartes, J.E., Carrassón, M., 2004.** The influence of trophic variables in the depth-range distribution and zonation rates of deep-sea megafauna: the case of the Western Mediterranean assemblages. *Deep-Sea Res.* 51, 263–279.
- Cartes, J.E., Sardà, F., 1992.** Abundance and diversity of decapod crustaceans in the deep-Catalan Sea (Western Mediterranean). *J. Nat. Hist.* 26, 1305–1323.
- Cartes, J.E., Sardà, F., 1993.** Zonation of the deep-sea decapod fauna in the Catalan Sea (Western Mediterranean). *Mar. Ecol. Prog. Ser.* 94, 27–34.
- Cartes, J.E., Maynou, F., Sardà, F., Company, J.B., Lloris, D., Tudela, S., 2004.** The Mediterranean deep-sea ecosystems: an overview of their diversity, structure, functioning and anthropogenic impacts. In: *The Mediterranean deep-sea ecosystems: an overview of their diversity, structure, functioning and anthropogenic impacts, with a proposal for conservation.* IUCN, Málaga and WWF, Rome. pp. 9–38.
- Coad, B.W., Reist, J.D., 2004.** Annotated list of the arctic marine fishes of Canada. *Can. MS Rep. Fish Aquat. Sci.* 2674(iv).

- Cohen, D.M., Inada, T., Iwamoto, T., Scialabba, N., 1990.** Gadiform fishes of the world (order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes known to date. FAO Fish. Synop. 10, 1–442.
- Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., et al., 2010.** The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. PLOS ONE 5(8), e11842.
- Combes, C., 1996.** Parasites, biodiversity and ecosystem stability. Biodivers. Conserv. 5, 953–962.
- Cuttelod, A., García, N., Malak, D.A., Temple, H., Katariya, V., 2008.** The Mediterranean: A biodiversity hotspot under threat. In: Vié, J.-C., Hilton-Taylor, C., Stuart, S. (Eds.) *The 2008 Review of The IUCN Red List of Threatened Species*. IUCN Gland, Switzerland.
- Dallarés, S., Constenla, M., Padrós, F., Cartes, J., Solé, M., Carrassón, M., 2014.** Parasites of the deep-sea fish *Mora moro* (Risso, 1810) from the NW Mediterranean Sea and relationship with fish diet and enzymatic biomarkers. Deep-Sea Res. I 92, 115–126.
- Dallarés, S., Moyà-Alcover, C., Padrós, F., Cartes, J., Solé, M., Castañeda, C., Carrassón, M., 2016.** The parasite community of *Phycis blennoides* (Brünnich, 1768) from the Balearic Sea in relation to diet, biochemical markers, histopathology and environmental variables. Deep-Sea Res. I 118, 84–100.
- Danovaro R, Dinet A, Duineveld G, Tselepides A, 1999.** Benthic response to particulate fluxes in different trophic environments: A comparison between the Gulf of Lions-Catalan Sea (Western Mediterranean) and the Cretan Sea (Eastern Mediterranean). Progr. Oceanogr. 44(1–3), 287–312.
- Danovaro, R., Company, J.B., Corinaldesi, C., D’Onghia, G., Galil, B., Gambi, C., Gooday, A.J., Lampadariou, N., Luna, G.M., Morigi, C., Olu, K., Polymenakou, P., Ramírez-Llodra, E., Sabbatini, A., Sardà, F., Sibuet, M., Tselepides, A., 2010.** Deep-Sea Biodiversity in the Mediterranean Sea: The Known, the Unknown, and the Unknowable. PLoS ONE 5(8), 1–25.
- Dobson, A., Hudson, P.J., 1986.** Parasites, disease and the structure of ecological communities. Trends Ecol. Evol. 1, 11–15.

-
- D'Onghia, G., Maiorano, P., Sion, L., 2008.** A review on the reproduction of grenadiers in the Mediterranean with new data on the gonad maturity and fecundity. *Am. Fish Soc. Symp.* 63, 169–184.
- Eakins, B.W., Sharman, G.F., 2010.** Volumes of the World's Oceans from ETOPO1, NOAA National Geophysical Data Center, Boulder, CO.
- Emig, C.C., Geistdoerfer, P., 2004.** The Mediterranean deep-sea fauna: Historical evolution, bathymetric variations and geographical changes, *Carnets de Géologie/ Notebooks on Geology, Maintenon, Article 2004/01 (CG2004_A01_CCE-PG)*.
- Espinal-Carrión, T., López-López, E., 2010.** Helminths and lipid peroxidation in *Astyanax aeneus* (Pisces: Characidae) from a river in the humid subtropics of southeastern Mexico. *Dis. Aquat. Org.* 88(3), 215–224.
- Estrada, M., 1996.** Primary production in the northwestern Mediterranean. *Sci. Mar.* 60, 55–64.
- Feist, S.W., Longshaw, M., 2008.** Histopathology of fish parasite infections-importance for populations. *J. Fish Biol.* 73, 2143–2160.
- Fernández-Arcaya, U., Rotllant, G., Ramírez-Llodra, E., Recasens, L., Aguzzi, J., Flexas, M.M., Sánchez-Vidal, A., López-Fernández, P., García, J.A., Company, J.B., 2013.** Reproductive biology and recruitment of the deep-sea fish community from the NW Mediterranean continental margin. *Progr. Oceanogr.* 118, 222–234.
- Forbes, E., 1844.** Report on the Mollusca and Radiata of the Aegean Sea, and on their distribution, considered as bearing on geology. Report of the British Association for the Advancement of Science for 1843, 129–193.
- Froese, R., Pauly, D., 2016.** FishBase. World Wide Web electronic publication. www.fishbase.org, version (10/2016).
- Geistdoerfer, P., 1990.** Macrouridae. In: Quero, J.C., Hureau, J.C., Karrer, C., Post, A., Saldanha, L. (Eds.) *Check-list of the fishes of the eastern tropical Atlantic (CLOFETA) Vol. 2*. JNICT, Lisbon; SEI, Paris; and UNESCO, Paris, pp. 541–563.
- Golovan, G.A., Pakhorukov, N.P., 1980.** New data on the ecology and morphometry of *Alepocephalus rostratus* (Alepocephalidae). *J. Ichthyol.* 20, 77–84.
- Goren, M., Galil, B.S., 2015.** A checklist of the deep sea fishes of the Levant Sea, Mediterranean Sea. *Zootaxa* 3994(4), 507-530.

- Gupta, A.K., Agarwal, S.M., 1985.** Physiology of host-parasite relationships of *pallisentis nagpurensis* and *Senga visakhapatnamensis*, parasitizing liver of *Colisa lalia* and intestine of *Channa punctatus*. *Curr. Sci.* 54(17), 866–869.
- Hagen, N.T., 1996.** Sea urchin outbreaks and epizootic disease as regulating mechanisms in coastal ecosystems. In: Eleftheriou, A., Ansell, A.D., Smith, C.J. (Eds.) *Biology and Ecology of Shallow Coastal Waters*. Olsen & Olsen, Fredensborg, Denmark, pp. 303–308.
- Holmes, J.C., 1961.** Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala). I. General effects and comparison with crowding. *J. Parasitol.* 47, 209–216.
- Holmes, J.C., 1962.** Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala). II. Effects on growth. *J. Parasitol.* 48, 87–96.
- Hopkins, T.S., 1985.** Physics of the sea. In: R. Margalef (Ed.) *Key Environments: Western Mediterranean*. Pergamon Press, New York. pp. 100–125.
- Houston, K.A., Haedrich, R.L., 1986.** Food habits and intestinal parasites of deep demersal fishes from the upper continental slope east Newfoundland, northwest Atlantic Ocean. *Mar. Biol.* 92, 563–574.
- IHO [International Hydrographic Organization], 1953.** Limits of Oceans and Seas. Special Publication No. 23. 3d ed. Monte Carlo, Imp. Monegasque.
- Isajlović, I., Vrgoč, N., Zorica, B., Peharda, M., Krstulović Šifner, S., Piccinetti, C., 2009.** Age, growth and length–weight relationship of *Coelorinchus caelorhincus* (Risso, 1810) in the Adriatic Sea. *Acta Adriat.* 50(1), 23–30.
- Iwamoto, T., Ungaro, N., 2002.** A new grenadier (Gadiformes, Macrouridae) from the Mediterranean. *Cybium* 26(1), 27–32.
- Klimpel, S., Busch, M.W., Kellermanns, E., Kleinertz, S., Palm, H.W., 2009.** Metazoan deep-sea fish parasites. *Acta Biologica Benrodis Supplementband II*.
- Lafferty, K.D., 1997.** Environmental parasitology, what can parasites tell us about human impacts in the environment? *Parasitol. Today* 13, 251–255.

Lafferty, K.D., Thomas, F., Poulin, R., 2000. Evolution of host phenotype manipulation by parasites and its consequences. In: Poulin, R., Morand, S., Skorping, A. (Eds.) *Evolutionary Biology of Host-parasite Relationships: Theory Meets Reality*. Elsevier, Amsterdam, pp. 117–127.

Lafferty, K., Allesina, S., Arim, M., Briggs, C., De Leo, G., Dobson, A., Dunne, J., Johnson, P., Kuris, A., Marcogliese, D., Martínez, N., Memmott, J., Marquet, P., McLaughlin, J., Mordecai, E., Pascual, M., Poulin, R., Thielgtges, D., 2008. Parasites in food webs: the ultimate missing links. *Ecol. Lett.* 11(6), 533–546.

MacKenzie, K., 2002. Parasites as biological tags in population studies of marine organisms: an update. *Parasitology* 124, S153–S163.

MacKenzie, K., Abaunza, P., Campbell, N., 2005. The use of parasites as biological tags in multidisciplinary stock identification studies of small pelagic fish. *Parassitologia* 49(3), 119–123.

MacKenzie, K., Williams, H., Williams, B., McVicar, A.H., Siddall, R., 1995. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Adv. Parasitol.* 35, 85–144.

Macpherson, E., 1979. Ecological overlap between macrourids in the western Mediterranean Sea. *Mar. Biol.* 53, 149–159.

Madurell, T., Cartes, J.E., 2006. Trophic relationships and food consumption of slope dwelling macrourids from the bathyal Ionian Sea (eastern Mediterranean). *Mar. Biol.* 148, 1325–1338.

Manter, H.W., 1934. Some digenetic trematodes from deep-water fish of Tortugas, Florida. *Papers from the Tortugas Laboratory of Carnegie Institution of Washington* 28(16), 257–345.

Marcogliese, D.J., 1995. The role of zooplankton in the transmission of helminth parasites to fish. *Rev. Fish Biol. Fisher.* 5, 336–371.

Marcogliese, D.J., 2002. Food webs and the transmission of parasites to marine fish. *Parasitology* 124(07), S83–S99.

Marcogliese, D.J., 2003. Food webs and biodiversity: are parasites the missing link? *J. Parasitol.* 82(S), 389–399.

Marcogliese, D.J., 2004. Parasites: Small Players with Crucial Roles in the Ecological Theater. *EcoHealth* 1, 151–164.

- Marcogliese, D.J., 2005.** Parasites of the superorganism: Are they indicators of ecosystem health? *Int. J. Parasitol.* 35, 705–716.
- Marcogliese, D., Pietroock, M., 2011.** Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends Parasitol.* 27(3), 123–130.
- Mariotti, A., Struglia, M.V., Zeng, N., Lau, K-M., 2002.** The Hydrological Cycle in the Mediterranean Region and Implications for the Water Budget of the Mediterranean Sea. *J. Climate* 15, 1674–1690.
- Markle, D.F., Quéro, J.F., 1984.** Family Alepocephalidae. In: Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J., Tortonese, E. (Eds.) *Fishes of the North-eastern Atlantic and the Mediterranean*. UNESCO, Paris, pp. 228–253.
- Marshall, N.B., Merrett, N.R., 1977.** The existence of a benthopelagic fauna on the deep-sea. *Deep-Sea Res.* 24(supplement), 483–497.
- Massutí, E., Morales-Nin, B., Stefanescu C., 1995.** Distribution and biology of five grenadier fish (Pisces: Macrouridae) from the upper and middle slope of the northwestern Mediterranean. *Deep-Sea Res. I* 42(3), 307–330.
- Mauchline, J., Gordon, J.D.M., 1983.** Diets of clupeoid, stomiatoid and salmonoid fishes of the Rockall Trough, northeastern Atlantic Ocean. *Mar. Biol.* 77, 67–78.
- May, R.M., Anderson, R.M., 1979.** Population biology of infectious diseases: part II. *Nature* 280, 455–461.
- Maynou, F., Cartes, J.E., 2000.** Community structure of bathyal decapod crustaceans off south-west Balearic Islands (western Mediterranean): seasonality and regional patterns in zonation. *J. Mar. Biol. Assoc. U.K.* 80, 789–798.
- Miller, A.R., Tchernia, P., Channon, H., McGill, D.A., 1970.** Mediterranean sea atlas of Temperature, Salinity, Oxygen. Profiles and data from cruises of RV *Atlantis* and RV *Chain*. Vol. III. The Woods Hole Oceanographic Institution, Woods Hole, Mass.
- Minchella, D.J., Scott, M.E., 1991.** Parasitism: a cryptic determinant of animal community structure. *Trends Ecol. Evol.* 6, 250–254.
- Morales-Nin, B., Massutí, E., Stefanescu, C., 1996.** Distribution and biology of *Alepocephalus rostratus* from the Mediterranean Sea. *J. Fish Biol.* 48, 1097–1112.
- Moranta, J., Massutí, E., Palmer, M., Gordon, J.D.M., 2007.** Geographic and bathymetric trends in abundance, biomass and body size of four grenadier fishes along the Iberian coast in the western Mediterranean. *Prog. Oceanogr.* 72(1), 63–83.

-
- Moranta, J., Stefanescu, C., Massutí, E., Morales-Nin, B., Lloris, D., 1998.** Fish community structure and depth-related trends on the continental slope of the Balearic Islands (Algerian Basin, Western Mediterranean). *Mar. Ecol. Prog. Ser.* 171, 247–259.
- Morey, G., Moranta, J., Massutí, E., Grau, A., Linde, M., Riera, F., Morales-Nin, B., 2003.** Weight–length relationships of littoral to lower slope fishes from western Mediterranean. *Fish. Res.* 62(1), 89–96.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, A.B., Kent, J., 2000.** Biodiversity hotspots for conservation priorities. *Nature*, 403: 853–858.
- Noble, E.R., Orias, J.D., Rodella, T.D., 1972.** Parasitic fauna of the deepsea fish, *Macrourus ruspestris* (Gunnerus) from Korsfjorden, Norway. *Sarsia* 50, 47–50.
- Overstreet, R.M., 1997.** Parasitological data as monitors of environmental health. *Parassitologia* 39, 169–175.
- Palm, H., Klimpel, S., 2007.** Evolution of parasitic life in the ocean. *Trends Parasitol.* 23(1), 10–12.
- Poulin, R., Morand, S., 2000.** The diversity of parasites. *Q. Rev. Biol.* 75, 277–293.
- Price, P.W., Westoby, M., Rice, B., Atsatt, P.R., Fritz, R.S., Thompson, J.N., Mobley, K., 1986. Parasite mediation in ecological interactions. *Annu. Rev. Ecol. Evol. Syst.* 17, 487–505.
- Ramírez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C.R., Levin, L.A., Martínez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B.E., Smith, C.R., Tittensor, D.P., Tyler, P.A., Vanreusel, A., Vecchione, M., 2010.** Deep, diverse and definitely different: unique attributes of the world's largest ecosystem. *Biogeosciences* 7, 2851–2899.
- Sardà, F., Calafat, A., Flexas, M., Tselepidis, A., Canals, M., Espino, M., Tursi, A., 2004.** An introduction to Mediterranean deep sea biology. *Sci. Mar.* 68(S3), 7–38.
- Sures, B., 2005.** Effects of pollution on parasites, and use of parasites in pollution monitoring. In: Rohde, K. (Ed.) *Marine parasitology*. CSIRO Publishing, Collingwood, Australia, pp. 421–425.
- Sures, B. 2008.** Environmental Parasitology. Interactions between parasites and pollutants in the aquatic environment. *Parasite* 15(3), 434–438.

- Stefanescu, C., Lloris, D., Rucabado, J., 1992a.** Deep-living demersal fishes in the Catalan Sea (western Mediterranean) below a depth of 1000 m. *J. Nat. Hist.* 26, 197–213.
- Stefanescu, C., Rucabado, J., Lloris, D., 1992b.** Depth-size trends in western Mediterranean demersal deep-sea fishes. *Mar. Ecol. Prog. Ser.* 81, 205–213.
- Stefanescu, C., Lloris, D., Rucabado, J., 1993.** Deep-fish assemblages in the Catalan Sea (western Mediterranean) below a depth of 1000 m. *Deep-Sea Res.* 40(4): 695–707.
- Tecchio, S., Ramírez-Llodra, E., Sardà, F., Company, J., 2011.** Biodiversity of deep-sea demersal megafauna in western and central Mediterranean basins. *Sci. Mar.* 75(2), 341–350.
- Trubiroha, A., Kroupova, H., Wuertz, S., Frank, S.N., Sures, B., Kloas, W., 2010.** Naturally-induced endocrine disruption by the parasite *Ligula intestinalis* (Cestoda) in roach (*Rutilus rutilus*). *Gen. Comp. Endocrinol.* 166, 234–240.
- Zubchenko, A.V., 1981.** Parasitic fauna of some Macrouridae in the northwest Atlantic. *J. Northwest Atl. Fish. Sci.* 2, 67–72.

