Capillostrongyloides morae sp. n. (Nematoda: Capillariidae) from deep-sea fish (Teleostei, Moridae) in the western Mediterranean Sea

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Abstract: A new capillariid nematode, *Capillostrongyloides morae* sp. n., is described from specimens collected from the stomach and intestine of the common mora, *Mora moro* (Risso), and the Mediterranean codling, *Lepidion lepidion* (Risso) (both Gadiformes, Moridae), off the Mediterranean coasts of Spain. The new species shows similar morphological features as other congeneric species occurring in freshwater and marine fishes, but it differs in the length of the body and spicules, the size of the caudal bursa, and the presence of an elevated anterior vulvar lip. *Capillostrongyloides morae* sp. n. is the second species within the genus for which the presence of a stylet is reported, and the first one in which this structure along with the distribution of cephalic papillae and oral structures (e.g. lips and lobes) are clearly shown by using scanning electron microscopy.

Keywords: Gadiformes, *Lepidion lepidion*, *Mora moro*, Spain

The common mora, *Mora moro* (Risso), and the Mediterranean codling, *Lepidion lepidion* (Risso) (both Gadiformes: Moridae), are marine, bathypelagic fishes that occur in the Mediterranean Sea. Even though the depth range of both species varies from 450 to 2 500 m (Froese and Pauly 2013), the optimum range for *M. moro* in the western Mediterranean is narrower than that for *L. lepidion* (depths between 1 000–1 500 m vs 1 000–2 250 m, respectively – Stefanescu et al. 1992).

The diet of *M. moro* is based on benthic preys, chiefly epibenthic and suprabenthic crustaceans, fish and cephalopods (Carrassón et al. 1997); although living on the middle-upper slope (700–800 m), there is a clear tendency of this fish species to have a more general diet (benthic and pelagic preys – Relini Orsi 1976).

Lepidion lepidion, an endemic species to the Mediterranean, is the most abundant morid fish and the dominant species in the north-western Mediterranean at depths of 1 000–1 500 m (Stefanescu et al. 1992). This fish is a euryphagic predator that includes a broad range of benthic and benthopelagic preys in its diet (decapods, calanoid copepods, mysids and accidentally amphipods – Carrassón et al. 1997).

The parasite fauna of both species is poorly known. The parasitological information for *L. lepidion* is virtually

inexistent, whereas only four helminth species have been recorded so far in *M. moro*: larvae of the trypanorhynch cestode *Hepatoxylon trichuri* (Holtén, 1802), the nematode '*Ascaris morae*' Fourment, 1886 (nomen dubium), and the lepecreadiids *Neolepidapedon smithi* Bray et Gibson, 1989 and *Lepidapedon desclersae* Bray et Gibson, 1995 (see Bray and Gibson 1989, 1995, 1997, Waterman and Sin 1991, Bruce et al. 1994, Bray et al. 1999, 2009).

Recent helminthological examinations of specimens of *M. moro* and *L. lepidion* collected off the Mediterranean coast of Spain (between Catalonia coast and the Balearic Islands) revealed the presence of a new species of capillariid nematode, which is described herein.

MATERIALS AND METHODS

A total of 78 specimens of *Mora moro* and 174 *Lepidion lepidion* (total body length: 12.0–42.2 and 16.5–24.0 cm, respectively) was examined. Fish were caught using bottom trawling nets at depths of 1 000–1 900 m at three localities (off Barcelona, Ibiza and Mallorca) between the Balearic Islands and the Catalan coast of Spain (western Mediterranean). The collected nematodes were isolated from fresh or freshly frozen fish, washed in physiological saline, fixed in nearly boiling saline and preserved in 70% ethanol. For light microscopy, they were cleared in a mixture of glycerine and water in different concentrations. For scanning electron microscopy (SEM), specimens

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were postfixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical-point-dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 4 kV (GB low mode). Drawings were done with the aid of a camera lucida. All measurements are given in micrometres, unless otherwise indicated.

RESULTS

Family Capillariidae Railliet, 1915

Capillostrongyloides morae sp. n.

Figs. 1, 2

Description: Small, filiform, whitish nematodes. Anterior end of body slender, posterior end broader. Cuticle finely transversely striated, with two distinct lateral bacillary bands, extending along almost whole body length (Figs. 1D, 2D). Cephalic end rounded, separated from remaining body by a transverse incision (Fig. 2A). Mouth aperture slit-like, dorsoventrally oriented, surrounded by two lip-like lateral elevations and two lobes (Figs. 1B, 2A–C). Stylet conspicuous, finger-shaped, with broader basal part, distal tip pointed, protruding out from middle portion of oral opening. Cephalic papillae buried, arranged in two circles of six papillae each (four submedian and two lateral), and one lateral amphid on each lobe (Figs. 1B, 2A–C). Muscular oesophagus relatively short (Fig. 1A). Stichosome formed by a single row of about 29–38 stichocytes subdivided into 9–10 transverse annuli each; nuclei of stichocytes large (Fig. 1G). Nerve-ring surrounding muscular oesophagus between its first and second thirds (Fig. 1A). Excretory pore and deirids not visible. Two wing-like cells present at oesophago-intestinal junction (Fig. 1E).

Male (based on 11 specimens; measurements of holotype in parentheses): Body length 4.50–7.45 (6.40) mm, maximum width 54–69 (55). Width of bacillary bands 10–18 (18). Entire oesophagus 2.24–3.33 (2.97) mm long, representing 41–61% (47%) of whole body length. Muscular oesophagus 154–323 (248) long. Stichosome 2.10–3.12 (2.72) mm long, consisting of 29–33 (30) stichocytes. Nerve-ring 93–134 (93) from anterior end of body. Spicule well sclerotized, 325–373 (346) long, which represents 5–8% of body length; its anterior end expanded 9–16 (11) wide, posterior end rounded 5–10 (9) wide. Spicular sheath nonspinous (Figs. 1H, 2E,F). Posterior end of body rounded, provided with well-developed membranous bursa, supported by two digital projections not reaching posterior margin of bursa (Figs. 1C,H, 2E,F). One pair of poorly visible subventral papillae present at base of lateral lobes, plus one pair of lateral, flat, precloacal papillae (Figs. 1H, 2E). Length of tail including membranous bursa 12–21 (18), caudal membrane 6–11 (7) long and 18–27 (24) wide.

Female (based on 10 gravid specimens, measurements of allotype in parentheses): Body length 7.10–14.75

(14.75) mm, maximum width 62–112 (103). Width of lateral bacillary bands 11–20 (14). Entire oesophagus 2.76–5.10 (5.10) mm long, representing 33–40% (35%) of whole body length. Muscular oesophagus 265–404 (404) long. Stichosome 2.44–4.70 (4.70) mm long, consisting of 29–38 (34) stichocytes. Nerve-ring 101–180 (113) from anterior end of body. Vulva pre-equatorial, 4.30–9.70 (9.70) mm from posterior end of body and 36–161 (161) from posterior end of oesophagus. Anterior vulvar lip forming an appendix directed posteriorly, 22–40 (31) long and 11–48 (–) wide (Figs. 1E, 2H). Vagina muscular, directed posteriorly. Eggs arranged in a row in anterior part of uterus and in two in most distant region. Eggs oval, thin-walled, 50–66 × 22–34 (57 × 30), with smooth surface and slightly protruding polar plugs 4–5 × 7–9 (5 × 9) (Fig. 1F). Ovary extending posteriorly to level of rectum; rectum 22–82 (78) long. Caudal end of body rounded; anus subterminal; tail 5–9 (9) long (Figs. 1I, 2G).

One nongravid female: Body length 6.50 mm, maximum width 63. Lateral bacillary bands indistinct. Entire oesophagus 3.42 mm, representing 53% of whole body length. Muscular oesophagus length 294. Stichosome 3.10 mm long, consisting of 31 stichocytes. Nerve-ring 123 from anterior end of body. Vulva postequatorial, 3.00 mm from posterior end of body and 76 from posterior end of oesophagus. Anterior vulvar lip elevated, 24 long and 17 wide. Vagina muscular, directed posteriorly. Length of rectum 86. Tail 6 long.

Type host: Common mora, *Mora moro* (Risso) (Gadiformes: Moridae) (body length 12.0–42.2 cm).

Other host: Mediterranean codling, *Lepidion lepidion* (Risso) (Gadiformes: Moridae) (body length: 16.5–24.0 cm).

Site of infection: Stomach and intestine.

Type locality: Off Barcelona (40°44'30"N, 01°38'33"E; 1000–1400 m) western Mediterranean Sea, Spain (date of collection 15/10/2011).

Other localities: Off Barcelona (1000–1900 m for *L. lepidion*; 10/07/2010, 10/08/2010, 23/06/2011, 15/10/2011); off Ibiza (39°25'20"N, 01°19'13"E; 1000–1400 m for *M. moro*; 1000–1900 m for *L. lepidion*; 18/10/2011); off Mallorca (1000–1400 m for *M. moro*; 20/07/2010), all in the western Mediterranean Sea, Spain.

Prevalence and intensity: *Mora moro*: 15% (12 infected/78 examined), 2.3 nematodes per infected fish. *Lepidion lepidion*: 25% (43/174), 1.6.

Deposit of specimens: Holotype, allotype and one paratype ex *M. moro* at the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice (Cat. No. N – 1007); paratypes in the Natural History Museum (NHMUK), London (2 females and 1 male ex *M. moro*; No. 2013.5.20.1–3; 4 females and 3 males ex *L. lepidion* 2013.5.20.4–9).

Etymology: The specific name of this nematode relates that of the fish type host.

Fig. 1. *Capillostrongyloides morae* sp. n. **A** – anterior end of female, lateral view; **B** – cephalic end, apical view; **C** – posterior end of male, ventral view; **D** – lateral bacillary band; **E** – region of vulva, lateral view; **F** – egg; **G** – stichosome; **H** – posterior end of male, lateral view; **I** – posterior end of female, ventral view.

Fig. 2. *Capillostrongyloides morae* sp. n. SEM micrographs. **A, B** – cephalic end, apical and lateral views (arrows and arrowhead indicate stylet and lip-like lateral elevations, respectively); **C** – detail of mouth, apical view (arrowhead, arrow and asterisk indicate lip, stylet and amphid, respectively); **D** – lateral bacillary band; **E, F** – posterior end of male, lateral and subapical views (arrow indicates lateral papilla); **G** – posterior end of female, subventral view; **H** – region of vulva, sublateral view (arrow indicates elevated anterior vulvar lip). *Abbreviations:* a – submedian papilla of internal circle; b – submedian papilla of external circle; c – lateral papilla of external circle; L – lateral lobe; m – missing papilla; s – spicular sheath.

Table 1. Measurements of specimens of *Capillostrongyloides morae* sp. n. from two different hosts collected off the Mediterranean coast of Spain.

Host	<i>Mora moro</i>		<i>Lepidion lepidion</i>	
	Females (n = 5)	Males (n = 5)	Females (n = 5)	Males (n = 6)
Total body length*	11.20–14.75	4.68–7.45	7.10–9.70	4.50–5.76
Maximum body width	82–112	55–66	62–88	54–69
Entire oesophagus length*	3.66–5.10	2.33–3.33	2.76–3.63	2.24–2.75
(% of body length)	(33–39%)	(41–61%)	(35–40%)	(46–51%)
Muscular oesophagus length	265–404	207–323	316–385	154–307
Stichosome length*	3.30–4.70	2.10–3.12	2.44–3.28	2.10–2.50
No. of stichocytes	31–38	29–33	29–32	30–33
Nerve-ring to anterior body end	101–128	93–134	110–180	96–107
Width of bacillary band	14–20	10–18	11–14	10–13
Tail length	5–9	13–21	6–8	12–18
Vulva to posterior end of body*	7.00–9.70	-	4.30–6.00	-
Anterior vulvar lip length	27–40	-	22–30	-
Anterior vulvar lip width	11–48	-	14–17	-
Vulva to posterior end of oesophagus	41–161	-	36–81	-
Egg length**	50–61	-	54–66	-
Egg width**	22–30	-	24–34	-
Egg wall width	2	-	2	-
Rectum length	74–100	-	23–46	-
Caudal bursa length	-	7–8	-	6–11
Caudal bursa width	-	18–26	-	20–27
Spicule length	-	333–373	-	325–371

*in mm; ** more than 10 eggs measured in each gravid female.

DISCUSSION

By having a well-developed caudal bursa supported by two lateral, short and broad lobes, a non-spinous spicular sheath and the absence of caudal alae in the male, this new species can be placed in *Capillostrongyloides* Freitas et Lent, 1935. According to Moravec (2001), *Capillostrongyloides* is one of the allegedly valid genera within the family Capillariidae. This genus comprises nine species: *Capillostrongyloides ancistri* Moravec, Gelnar et Řehulka, 1987; *C. arapaimae* Portes Santos, Moravec et Venturieri, 2008; *C. congiopodi* Cantatore, Rossin, Lanfranchi et Timi, 2009; *C. fritschi* (Travassos, 1914); *C. norvegica* Moravec et Karlsbakk, 2000; *C. physiculi* (Johnston et Mawson, 1945); *C. sentinosa* (Travassos, 1927); *C. tandani* (Johnston et Mawson, 1940); and *C. tasmanica* (Johnston et Mawson, 1945). Two of these species, *C. physiculi* and *C. tandani*, were provisionally assigned to this genus because of their inadequate original descriptions. All species have been recorded from the digestive tract of fishes from different regions (Egypt, Norway, Tasman Sea, Brazil, South Australia and Mexico) (Moravec 2001, Portes Santos et al. 2008).

Most species of *Capillostrongyloides* exhibit a rather uniform morphology and are mainly differentiated by the length of spicules, the shape and length of caudal lobes and bursa, body length and presence/absence of elevated vulvar lips. The new species can be distinguished from *C. ancistri*, *C. fritschi*, *C. physiculi*, *C. sentinosa* and *C. tasmanica* by having larger spicules (longer than 325 µm), and from *C. arapaimae* and *C. congiopodi* by its shorter spicules (325–373 mm vs 0.78–1.80 mm and 1.05–1.23 mm, respectively).

The remaining species (*C. tandani*) was described only from females, the length of which falls within the lower range of that of the new species (7.1–8.6 mm vs 7.1–14.7 mm), but the anterior vulvar lip is not elevated, its host belongs to a different fish order (Siluriformes vs Gadiformes) and inhabits a very different habitat (fresh-water vs marine) and geographical region (South Australia vs Mediterranean coast of Spain) (Johnston and Mawson 1940).

The first species described from morid fishes, *C. physiculi*, was poorly described and provisionally listed within the genus (Moravec 1987), but differs in having smaller body sizes (female 5.8 mm, males 4.0–4.3 mm) and spicule length (270–320 µm), the absence of elevated vulvar lips, and a very different geographical distribution (Tasman Sea vs Mediterranean coast of Spain). The only congeneric species possessing similar biometrical features is *C. norvegica*, a nematode collected from a marine fish, *Enchelyopus cimbrius* (Linnaeus), from coastal waters of Norway (Moravec and Karlsbakk 2000).

Both nematode species have the anterior lip of vulva elevated, show similar body sizes (females 7.1–14.7 mm vs 7.8–12.3 mm, males 4.5–7.4 mm vs 4.0–5.7 mm) and spicule length (325–373 µm vs 273–366 µm), and were collected from gadiform fishes. However, *C. norvegica* is characterized by a highly developed membrane forming the male caudal bursa, somewhat longer eggs (63–75 µm vs 50–66 µm), smaller polar plugs (3 × 6 µm vs 4–6 × 7–9 µm), and the alleged presence of only small, posteriorly directed papillae on the median edge of caudal lobes (vs one subventral and one lateral caudal pairs) (Moravec and Karlsbakk 2000).

In fact, *C. morae* sp. n. is the first species of the genus with two pairs of caudal papillae, which could be a common feature for *Capillostrongyloides* spp., but until SEM studies are applied to other species, it remains uncertain. Additionally, it parasitizes a fish of the family Lotidae, which is limited in its distribution to the North Atlantic (British Islands and Barents Sea) (Froese and Pauly 2013). Perhaps *C. morae* and *C. norvegica* could be conspecific or closely related forms, but until molecular studies are carried out we consider them as different species.

It should be mentioned that the specimens of *C. morae* collected from *M. moro* were slightly larger than those found in *L. lepidion* (see Table 1). This difference can be

considered within the intraspecific variability produced by the local ecological conditions and physiological traits of hosts. A similar pattern was found by González-Solís and Moravec (2004) in nematodes of the genus *Orientatractis* Petter, 1966 parasitizing cichlid fishes in southern Mexico and Nicaragua.

Moravec (1999) pointed out that capillariids are one of the most difficult groups of helminths to study because their bodies are very fine and slender and researchers often overlooked many of their morphological details. During the present study, SEM observations helped to clarify and reveal the presence of structures poorly visible in other species of *Capillostrongyloides*. The oral opening harboured a conspicuous finger-like stylet and was surrounded by two lips and lobes in lateral position. Moreover, a hexaradiate pattern of 12 buried papillae surrounding mouth, occurring in trichinellosis (see Moravec 2001), was present. This is the second species within the genus for which a stylet is reported in the oral opening, because it was previously observed under light microscopy in *C. ancistri* by Moravec et al. (1987). Moreover, this is the first study in which SEM micrographs allowed to clearly ob-

serve the stylet, and distribution and number of oral and caudal papillae.

Nothing is known about the host specificity of *Capillostrongyloides* spp., but apparently they have some preferences for fishes of the orders Siluriformes and Gadiformes, since seven of the ten species reported so far (including the new species) have been found in those fish orders. This is the first species of *Capillostrongyloides* reported from deep-sea fishes in the Mediterranean, the second from the family Moridae and the fourth from gadiform fishes.

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REFERENCES

- BRAY R.A., GIBSON D.I. 1989: The Lepocreadiidae (Digenea) of fishes from the north-east Atlantic: review of the genus *Neolepidapedon* Manter, 1954, with a description of *N. smithi* n. sp. Syst. Parasitol. 13: 11–23.
- BRAY R.A., GIBSON D.I. 1995: The Lepocreadiidae (Digenea) of fishes of the north-east Atlantic: a review of the genus *Lepidapedon* Stafford, 1904. Syst. Parasitol. 31: 81–132.
- BRAY R.A., GIBSON D.I. 1997: The Lepocreadiidae Odhner, 1905 (Digenea) of fishes from the north-east Atlantic: summary paper, with keys and checklists. Syst. Parasitol. 36: 223–228.
- 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: S125–S144.
- BRAY R.A., WAESCHENBACH A., CRIBB T.H., WEEDALL G.D., DYAL P., LITTLEWOOD D.T.J. 2009: The phylogeny of the Lepocreadioidea (Platyhelminthes, Digenea) inferred from nuclear and mitochondrial genes: implications for their systematics and evolution. Acta Parasitol. 54: 310–329.
- BRUCE N.L., ADLARD R.D., CANNON L.R.G. 1994: Synoptic checklist of ascaridoid parasites (Nematoda) from fish hosts. Invertebr. Taxon. 8: 583–674.
- CARRASSÓN M., MATALLANAS J., CASADEVALL M. 1997: Feeding strategies of deep-water morids on the western Mediterranean slope. Deep-Sea Res. 44: 1685–1699.
- FROESE R., PAULY D. (Eds.) 2013: FishBase. World Wide Web electronic publication, www.fishbase.org, 06/2013.
- GONZÁLEZ-SOLÍS D., MORAVEC F. 2004: Two new nematode species, *Orientatractis campechensis* n. sp. and *Orientatractis chiapasensis* n. sp. (Nematoda: Atractidae) from cichlid fishes in southern Mexico and Nicaragua. J. Parasitol. 90: 1443–1449.
- MORAVEC F. 1987: Revision of Capillariid Nematodes (Subfamily Capillariinae) Parasitic in Fishes. Studie ČSAV No. 3, Academia, Praha, 141 pp.
- MORAVEC F. 1999: Trichinelloid nematodes parasitic in fishes. Helminthologia 36: 175–178.
- MORAVEC F. 2001: Trichinelloid Nematodes Parasitic in Cold-Blooded Vertebrates. Academia, Praha, Czech Republic, 429 pp.
- MORAVEC F., GELNAR M., ŘEHULKA J. 1987: *Capillostrongyloides ancistri* sp. n. (Nematoda: Capillariidae) a new pathogenic parasite of aquarium fishes in Europe. Folia Parasitol. 157–161.
- MORAVEC F., KARLSBAKK E. 2000: *Capillostrongyloides norvegica* sp. n. (Nematoda, Capillariidae) from the stomach of the marine fish, *Enchelyopus cimbrius*, from western Norway. Acta Parasitol. 45: 94–98.
- PORTES SANTOS C., MORAVEC F., VENTURIERI R. 2008: *Capillostrongyloides arapaimae* sp. n. (Nematoda: Capillariidae), a new intestinal parasite of the arapaima *Arapaima gigas* from the Brazilian Amazon. Mem. Inst. Oswaldo Cruz, Rio de Janeiro 103: 392–395.
- RELINI ORSI L. 1976: Note di biologia dell'alimentazione di *Moromor* (Risso 1810) (Osteichthyes, Moridae). Arch. Oceanogr. Limnol. 18: 375–387.
- STEFANESCU C., RUCABADO J., LLORIS D. 1992: Depth-size trends in western Mediterranean demersal deep-sea fishes. Mar. Ecol. Prog. Ser. 81: 205–213.
- WATERMAN P.B., SIN F.Y.T. 1991: Occurrence of the marine tapeworms, *Hepatoxylon trichiuri* and *Hepatoxylon megacephalum*, in fishes from Kaikoura, New Zealand. New Zeal. Nat. Sci. 18: 71–73.

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