



**Echinocephalus inserratus sp. n. (Nematoda: Gnathostomatidae) from the stingray *Pastinachus ater* (Dasyatidae) and new records of congeneric and some other nematode larvae from teleost fishes off New Caledonia**

František Moravec, Jean-Lou Justine

► **To cite this version:**

František Moravec, Jean-Lou Justine. *Echinocephalus inserratus* sp. n. (Nematoda: Gnathostomatidae) from the stingray *Pastinachus ater* (Dasyatidae) and new records of congeneric and some other nematode larvae from teleost fishes off New Caledonia. *Folia Parasitologica*, 2021, 68, 10.14411/fp.2021.014 . hal-03268554

**HAL Id: hal-03268554**

**<https://hal.sorbonne-universite.fr/hal-03268554>**

Submitted on 23 Jun 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Research Article

OPEN ACCESS

# ***Echinocephalus inserratus* sp. n. (Nematoda: Gnathostomatidae) from the stingray *Pastinachus ater* (Dasyatidae) and new records of congeneric and some other nematode larvae from teleost fishes off New Caledonia**

František Moravec<sup>1</sup>  and Jean-Lou Justine<sup>2</sup> 

<sup>1</sup>Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic;

<sup>2</sup>Institut Systématique Évolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, Paris, France

**Abstract:** Based on light and electron microscopical studies, a new nematode parasite, *Echinocephalus inserratus* sp. n. (Spirurida: Gnathostomatidae), is described from the spiral valve of the broad cowtail stingray *Pastinachus ater* (Macleay) (Dasyatidae, Myliobatiformes) from off New Caledonia. The new species is morphologically and biometrically most similar to *Echinocephalus overstreeti* Deardorff et Ko, 1983, differing from it mainly in the absence of serrations on the posterior parts of pseudolabia and on interlabia, and in having a longer gubernaculum (150–299 µm long). Morphologically unidentifiable, mostly encapsulated larvae of *Echinocephalus* spp. were recorded from the following six species of teleost fishes collected in New Caledonian waters, serving as paratenic hosts: Perciformes: *Acanthopagrus berda* (Forsskal) (Sparidae) and *Nemipterus furcosus* (Valenciennes) (Nemipteridae); Tetraodontiformes: *Abalistes stellatus* (Anonymous), *Pseudobalistes fuscus* (Bloch et Schneider) (both Balistidae), *Lagocephalus sceleratus* (Gmelin) (Tetraodontidae) and *Aluterus monoceros* (Linnaeus) (Monacanthidae). Co-parasitising larvae of *Ascarophis* sp. and *Hysterothylacium* sp. were also collected from *P. fuscus*. All these findings represent new host and geographical records. A key to valid species of *Echinocephalus* Molin, 1858 is provided.

**Keywords:** Parasitic nematode, Gnathostomatoidea, elasmobranchs, marine fish, paratenic host, South Pacific.

As mentioned by Moravec and Justine (2006), the taxonomy of nematodes of the gnathostomatid genus *Echinocephalus* Molin, 1858 remains rather confused, mainly because of the inadequate descriptions of many species, often based on larval forms collected from teleost fishes or some marine invertebrates, which probably serve only as paratenic or second intermediate hosts for these parasites (Ivashkin and Khromova 1976, Anderson 2000). The revision of the respective literature shows that only elasmobranchs, mainly rays and less often sharks, are the definitive hosts (as defined by Odening 1976) of *Echinocephalus* spp. To date, two species of *Echinocephalus*, *Echinocephalus overstreeti* Deardorff et Ko, 1983 and *Echinocephalus sinensis* Ko, 1975, have been recorded from New Caledonian waters, in the rays *Taeniurops meyeri* (Müller et Henle) and *Aetobatus ocellatus* (Kuhl) (reported as *Aetobatus* cf. *narinari* (Euphrasen)), respectively (Moravec and Justine 2006). Other nematodes from elasmobranchs (sharks) off New Caledonia were reported by Moravec and Justine (2020).

The recent examination of nematodes collected from the stingray *Pastinachus ater* (Macleay) (Dasyatidae) and

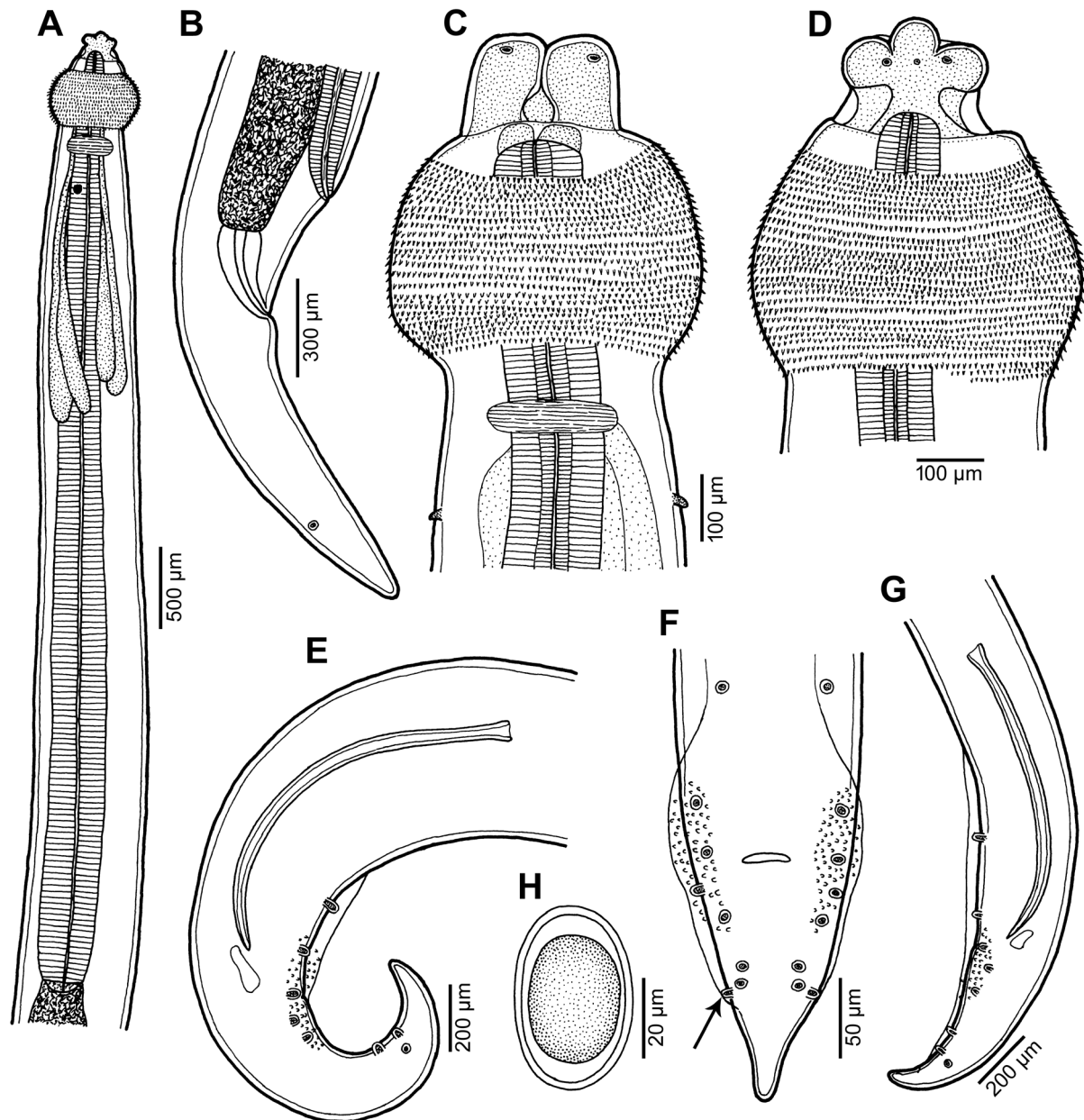
some teleosts of the perciform and tetraodontiform families Balistidae, Monacanthidae, Nemipteridae, Sparidae and Tetraodontidae off New Caledonia during 2005–2009, revealed the presence of adult nematodes representing a new species of *Echinocephalus* in the ray and congeneric larvae (*Echinocephalus* spp.) in teleosts. In addition to *Echinocephalus* larvae, co-infecting third-stage larvae of *Ascarophis* van Beneden, 1871 (Cystidicolidae) and *Hysterothylacium* Ward et Magath, 1917 (Anisakidae) were recorded from *Pseudobalistes fuscus* (Bloch et Schneider) (Balistidae). The results of detailed studies of these parasites are presented herein. This study adds new records of fish parasites off New Caledonia, already abundantly documented (Justine 2010, Justine et al. 2010a,b, 2012a).

## **MATERIALS AND METHODS**

Fish were caught off New Caledonia by various means. Nematodes were generally obtained by the wash method (Justine et al. 2012b), followed by examination of organs for encapsulated nematodes. The nematodes were fixed in 70% ethanol. For light microscopical examination (LM), they were cleared with glycerine.

Address for correspondence: F. Moravec, Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czech Republic. Phone: +420 38 777 5432; E-mail: moravec@paru.cas.cz

Zoobank number for article: [urn:lsid:zoobank.org:pub:5FB5DD1B-7511-4A22-BDFD-4F4550CE1A9A](http://zoobank.org/pub:5FB5DD1B-7511-4A22-BDFD-4F4550CE1A9A)



**Fig. 1.** *Echinocephalus inserratus* sp. n. ex *Pastinachus ater* (Macleay). **A** – anterior end of female, lateral view; **B** – posterior end of female, lateral view; **C** – cephalic end of male, dorsoventral view; **D** – cephalic end of female, lateral view; **E** – posterior end of larger male, lateral view; **F** – male tail, ventral view (arrow indicates phasmid); **G** – posterior end of smaller male, lateral view; **H** – egg.

Drawings were made with the aid of a Zeiss microscope drawing attachment. Specimens used for scanning electron microscopical examination (SEM) were postfixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical-point-dried in CO<sub>2</sub> and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV (GB low mode). All measurements are in micrometres unless otherwise indicated. The type and voucher specimens were deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS) and in Muséum National d'Histoire Naturelle, Paris, France (MNHN). The nematode classification system adopted follows Key to Nematode Parasites of Vertebrates (An-

derson et al. 2009, Gibbons 2010). The fish nomenclature follows FishBase (Froese and Pauly 2020).

## RESULTS

### Family Gnathostomatidae Railliet, 1895

#### *Echinocephalus inserratus* sp. n.

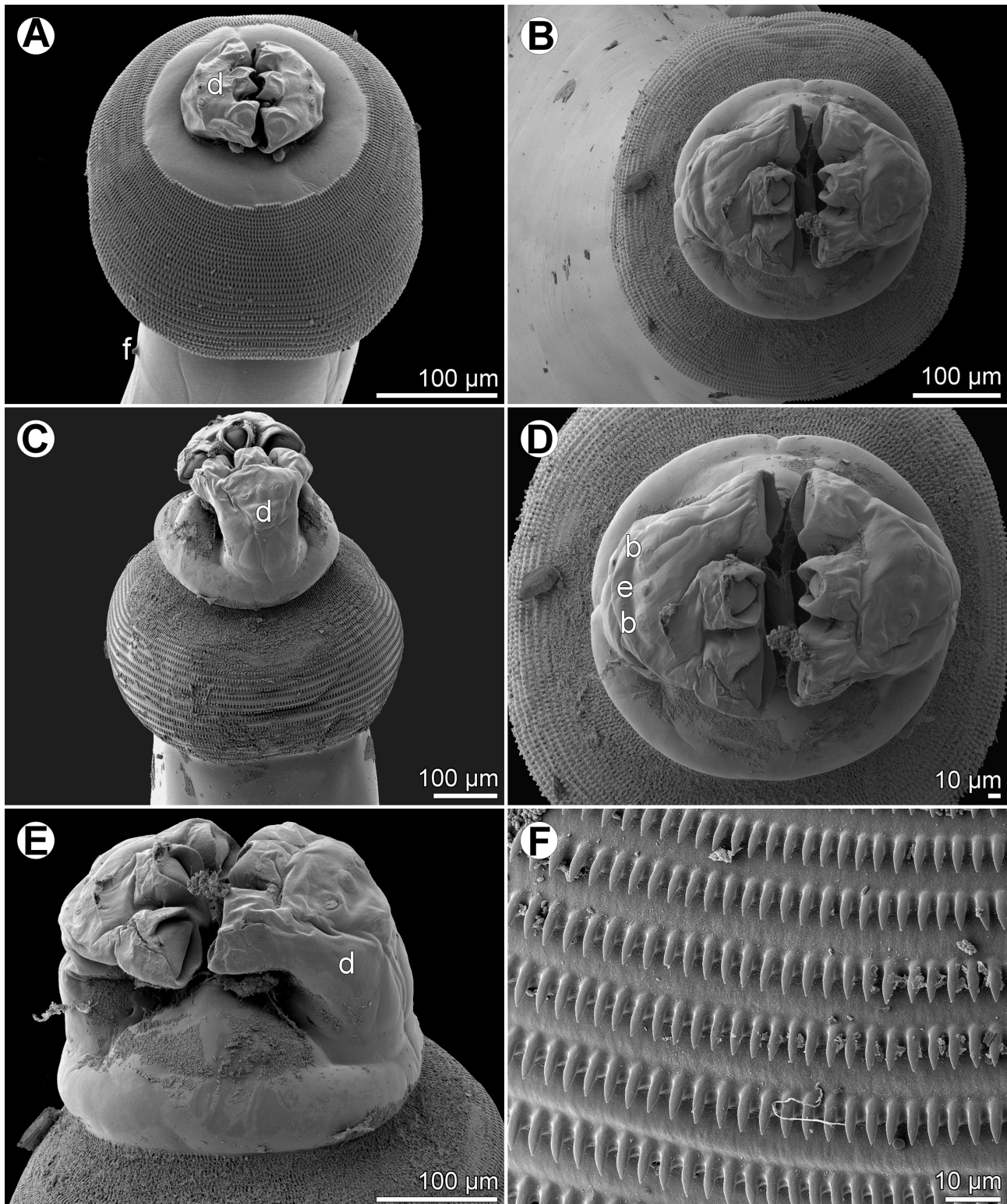
Figs. 1–3

ZooBank number for species:

[urn:lsid:zoobank.org:act:E1640959-B489-4A11-96E8-5FE203A105FE](https://zoobank.org/act:E1640959-B489-4A11-96E8-5FE203A105FE)

**General:** Large nematodes with finely transversely striated cuticle (Fig. 3B,D,G). Cephalic end provided with 2 large lateral pseudolabia with trilobed anterior portions;





**Fig. 2.** *Echinocephalus inserratus* sp. n. ex *Pastinachus ater* (Macleay), scanning electron micrographs. **A, B** – anterior end of male, dorsoventral and apical views, respectively; **C** – anterior end of female, lateral view; **D, E** – region of pseudolabia of male, apical and dorsoventral views, respectively; **F** – detail of spines on cephalic bulbous inflation. *Abbreviations:* b – labial papilla; d – pseudolabium; e – amphid; f – deirid.

each lobe bears 2 cuticular thickenings along external edges, which interlock with those of opposite pseudolabium; medial part of each pseudolabium dorsoventrally elongated, provided with pair of submedian papillae and lateral amphid between them in region posterior to lobes (Figs.

1A,C,D, 2A–E, 3A,E). Small triangular interlabia (1 dorsal and 1 ventral) present between pseudolabia. Posterodorsal and posteroventral parts of each pseudolabium and interlabia without cuticular serrations (Figs. 2C,E, 3A,E). Cephalic bulb prominent, armed with 32–33 transverse rows



of small, elongate spines; some rows discontinuous; rows of spines close to each other but non-overlapping; approximately 10 anteriormost rows formed by distinctly smaller spines as compared with those on middle portion of cephalic bulb and posteriormost row of spines consisting of conspicuously very small and thin spines (Figs. 1A,C,D, 2A–D,F, 3A,E). Oesophagus long (17–20%) of body length, widest near its posterior end, not clearly divided into anterior muscular and posterior glandular portions. Four cervical sacs present, extending posteriorly to about 1–2 thirds of oesophagus length (Fig. 1A). Deirids well developed, situated symmetrically somewhat posterior to level of nerve ring (Figs. 1A,C, 3E).

**Male** (2 specimens; holotype; measurements of paratype in parentheses): Length of body 33.12 (12.21) mm, maximum width 789 (286). Length of pseudolabia 177 (68). Cephalic bulb 408 (272) long, 571 (354) wide. Transverse rows of spines 32 (33) in number; length of spines (including roots) 3–12 (3–9); distance between rows 0–12 (3–9). Length of entire oesophagus 5.74 (2.39) mm, 17% (20%) of body length, maximum width 408 (163). Nerve ring and deirids 639 (408) and 898 (517), respectively, from anterior extremity. Cervical sacs ending 3.28 (1.67) mm from anterior end of body. Posterior end of body curved ventrally. Spicules almost equal, 1.48 (1.10) mm long, 4.5% (9.02%) of body length. Gubernaculum poorly developed, about 299 (150) long. Simple (unilobed) vesicular caudal alae present; area rugosa composed of minute bosses extending in lateral regions approximately between 3rd to 5th pairs of caudal papillae. Caudal papillae 7 pairs: 2 subventral pairs preanal, 1 subventral pair adanal and 4 pairs (3 subventral and 1 lateral) postanal; pair of papilla-like phasmids located laterally posterior to posteriormost pair of caudal papillae (Figs. 1E–G, 3B,D,F,G). Tail 857 (258) long.

**Female** (1 ovigerous specimen, allotype): Length of body 35.61 mm, maximum width 816. Length of pseudolabia 163. Cephalic bulb 476 long and 598 wide. Transverse rows of spines 33 in number; length of spines (including roots) 3–12; distance between rows 0–18. Length of entire oesophagus 6.16 mm (17% of body length), maximum width 422. Nerve ring and deirids 707 and 938, respectively, from anterior extremity. Cervical sacs ending 2.48 mm from anterior end of body. Vulva in posterior part of body, 33.46 mm from anterior extremity (at 94% of body length), 680 anterior to anus; vagina directed anteriorly from vulva (Fig. 1B). Uterus containing numerous eggs. Eggs oval, thin-walled, unembryonated, size 48–51 × 33–36 (Fig. 1H). Tail conical, 1.36 mm long, with pair of lateral phasmids located short distance anterior to tail tip (Figs. 1B, 3C).

Type host: Broad cowtail stingray *Pastinachus ater* (Macleay) (Dasyatidae, Myliobatiformes).

Details of fish: One ray, obtained from the fishmarket in Nouméa, New Caledonia, 27 November 2009, length 1,420 mm, span 640 mm.

Site of infection: Spiral valve.

Type locality: Off New Caledonia.

Prevalence and intensity: 1 fish infected/1 fish examined; 3 nematodes.

Deposition of type specimens: IPCAS N-1250 (anterior and

posterior body ends of holotype, allotype and paratype mounted on SEM stub) and MNHN JNC 3139 (middle body parts of holotype, allotype and paratype preserved in a vial with 70% ethanol for possible future sequencing).

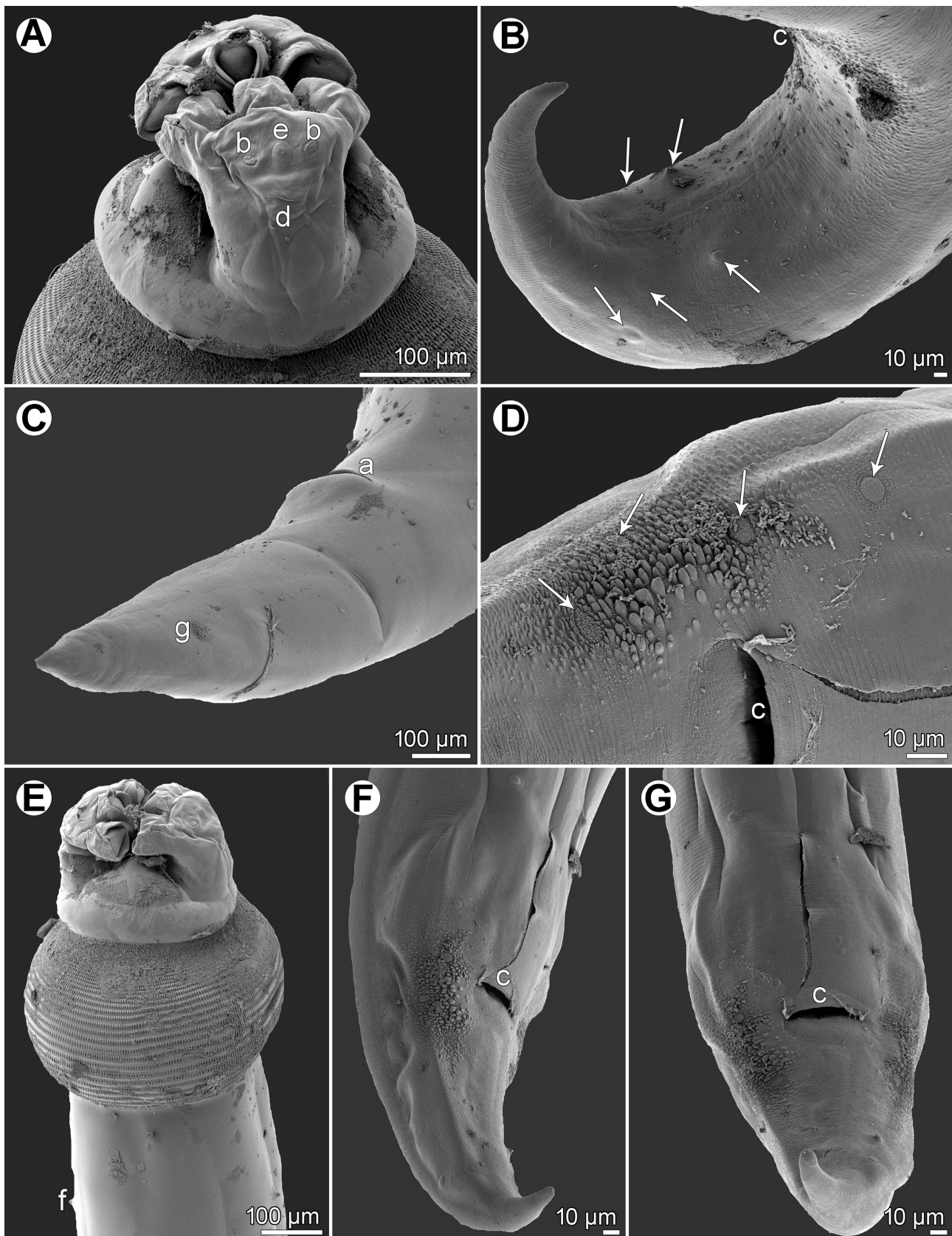
**Etymology:** The specific name *inserratus* (= non-serrated) of this nematode is a Latin adjective relating to the fact that there are no cuticular serrations on the interlabia and the posterior parts of pseudolabia in this species.

**Remarks.** At present, the genus *Echinocephalus* contains the following 11 valid species: *E. daileyi* Deardorff, Brooks et Thorson, 1981; *E. diazi* Troncy, 1969; *E. janzeni* Hoberg, Brooks, Molina-Ureña et Erbe, 1998; *E. multidentatus* Baylis et Lane, 1920; *E. overstreeti* Deardorff et Ko, 1983; *E. pseudouncinatus* Millemann, 1951; *E. pteroplatae* Wang, Zhao et Chen, 1978; *E. sinensis* Ko, 1975; *E. southwelli* Baylis et Lane, 1920; *E. spinosissimus* (Linstow in Shipley et Hornell, 1905); and *E. uncinatus* Molin, 1858 (see Shipley and Hornell 1905, Baylis and Lane 1920, Millemann 1951, 1963, Troncy 1969, Ko 1975, Wang et al. 1978, Deardorff et al. 1981, Deardorff and Ko 1983, Beveridge 1985, Hoberg et al. 1998).

On the contrary, the six poorly described species of this genus from India, all with descriptions based on adults, *Echinocephalus chengii* Singh, Chauhan et Khare, 2010, *E. mastacembeli* Begum et Gupta, 2012; *E. mobulae* Kalyankar, 1971, *E. scoliodonti* Lakshmi, 1994, *E. unispiculus* Arya, 1982 and *E. waltairensis* Lakshmi, Rao et Shyamasundari, 1984 (see Arya 1982, Begum and Gupta 2012, Kalyankar 1971, Lakshmi et al. 1984, Lakshmi 1994, Singh et al. 2010, Sood 2017), should be considered *species inquirendae*. Of them, *E. mastacembeli* was apparently established on misidentified specimens of *Spinitectus* Fourment, 1884, probably *S. mastacembeli* Karve et Naik, 1951.

The species of *Echinocephalus* described solely from larvae should also be considered as *species inquirendae* or *species dubiae*, because the morphology of larvae is considerably different from that of conspecific adults and, therefore, any interspecific comparison based on morphology is impossible. This concerns *Echinocephalus carpiæ* Abdel-Ghaffar, Bashtar, Mehlhorn, Abdel-Gaber, Al Quraishy et Saleh, 2013, *E. crassostreai* Cheng, 1975, *E. muraenesocis* Bilqees, Khanum et Jehan, 1971 and *E. oligocanthus* Arya, 1977 (see Bilqees et al. 1971, Cheng 1975, Arya 1977, Abdel-Ghaffar et al. 2013, Sood 2017).

Of the above-mentioned valid species of *Echinocephalus*, the new species is most similar in nearly all morphological features to *E. overstreeti*, which was originally described from the ray *Taeniurops meyeri* (syn. *Taeniura melanospilos* Bleeker) (Dasyatidae) from the Marquesas Islands, South Pacific (Deardorff and Ko 1983) and later reported (but not described) from the ray *Urogymnus asperrimus* (Bloch et Schneider) (Dasyatidae) from Eniwetok (also spelled Enewetak) Atoll in the Marshall Islands of the eastern Pacific (Brooks and Deardorff 1988) and from the type host (*T. meyeri*) off New Caledonia (Moravec and Justine 2006). Beveridge (1987) reported *E. overstreeti* from the shark *Heterodontus portusjacksoni* (Meyer) (Heterodontidae, Heterodontiformes) (see also Andrews et al.



**Fig. 3.** *Echinocephalus inserratus* sp. n. ex *Pastinachus ater* (Macleay), scanning electron micrographs. **A** – region of pseudolabia of female, sublateral view; **B** – male tail of larger specimen, ventrolateral view (arrows indicate caudal papillae); **C** – tail of female, sublateral view; **D** – region of cloaca with lateral cuticular ornamentation (*area rugosa*), ventral view (arrows indicate caudal papillae); **E** – anterior end of male, dorsoventral view; **F, G** – posterior end of smaller male, sublateral and ventral views, respectively. *Abbreviations:* a – anus; b – labial papilla; c – cloaca; d – pseudolabium; e – amphid; f – deirid; g – phasmid.



1988) and an additional 11 elasmobranch and chimeriform fishes belonging to the orders Chimaeriformes, Myliobatiformes, Orectolobiformes, Rajiformes, Rhinopristiformes and Torpediniformes (but gravid nematode females were found only in *H. portusjacksoni*). Nevertheless, further studies, including molecular methods, should confirm such a low degree of host specificity in *E. overstreeti*.

The new species mainly differs from *E. overstreeti* in having no cuticular serrations on the base of each pseudolabium and on interlabia. Although these serrations have not been mentioned in the original description of *E. overstreeti* (mouth structures were probably examined only by LM), they are well visible and very distinct on SEM micrographs of this nematode from the type host off New Caledonia, provided by Moravec and Justine (2006). Their presence in *E. overstreeti* was also described by Beveridge (1987).

*Echinocephalus inserratus* sp. n. also differs from *E. overstreeti* in that its gubernaculum is longer (150–299  $\mu\text{m}$  vs 63–101  $\mu\text{m}$ ). The number and distribution of caudal papillae seem to be identical in both these species, corresponding practically to those illustrated for *E. overstreeti* by Beveridge (1987). Observations of Deardorff and Ko (1983) on the distribution of papillae in *E. overstreeti* and of Moravec and Justine (2006) on the number of postanal papillae in the same species, were apparently inaccurate. It is necessary to remark that the caudal papillae are not readily visible in these fairly large nematodes under the LM and sometimes even when using SEM (because of the ventrally curved tail).

Distinction of *E. inserratus* sp. n. from other congeners is apparent from the key to *Echinocephalus* spp. at the end of the Discussion.

#### *Echinocephalus* spp. larvae

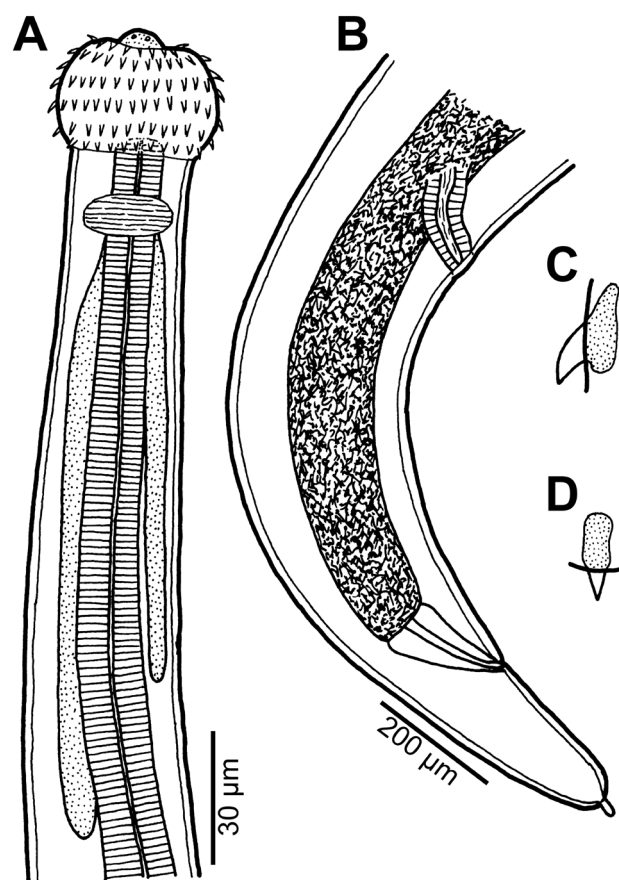
Figs. 4, 5

**Description** (2 specimens from *Acanthopagrus berda*; measurements of 4 specimens from *Pseudobalistes fuscus* in parentheses): Body 15.44–15.55 (10.91–12.51) mm long, maximum width 408 (340–408). Deirids and excretory pore not found. Pseudolabia simple, 27–41 (27) long; each with 2 well-developed papillae and amphid (Fig. 5A–C). Small rounded interlabia present. Cephalic bulb 258–272 (190–218) long, 367–408 (272) wide, armed with 6 transverse rows of claw-shaped spines 9–15 (9–15) long (including their roots); size of spines gradually increasing from first to sixth row (Figs. 4A, 5A–C). In addition, 2 groups of minute spines, 1 dorsal and 1 ventral, present between interlabia and first anterior ring of larger spines on cephalic bulb; each group consisting of spines arranged in 3 rows of 2, 2 and 3 spines; 1 or 2 larger spine-like formations present laterally to third row of 3 spines (Fig. 5C). Four cervical sacs present (Fig. 4A). Oesophagus 2.99–3.41 (2.67–2.79) mm long, representing 19–22% (22–24%) of body length; its maximum width 190–272 (258–313). Nerve ring 381 (313) from anterior extremity (Fig. 4A). Tail conical, 258–272 (313–408) long; tail tip of 2 larger female larvae with distinct terminal mucron 3 long (Figs. 4B, 5D). These larger female larvae possess vulva still covered by cuticle, situated 14.38–14.42 mm from an-

terior extremity (at 93% of body length); vagina directed anteriorly from vulva (Fig. 4A).

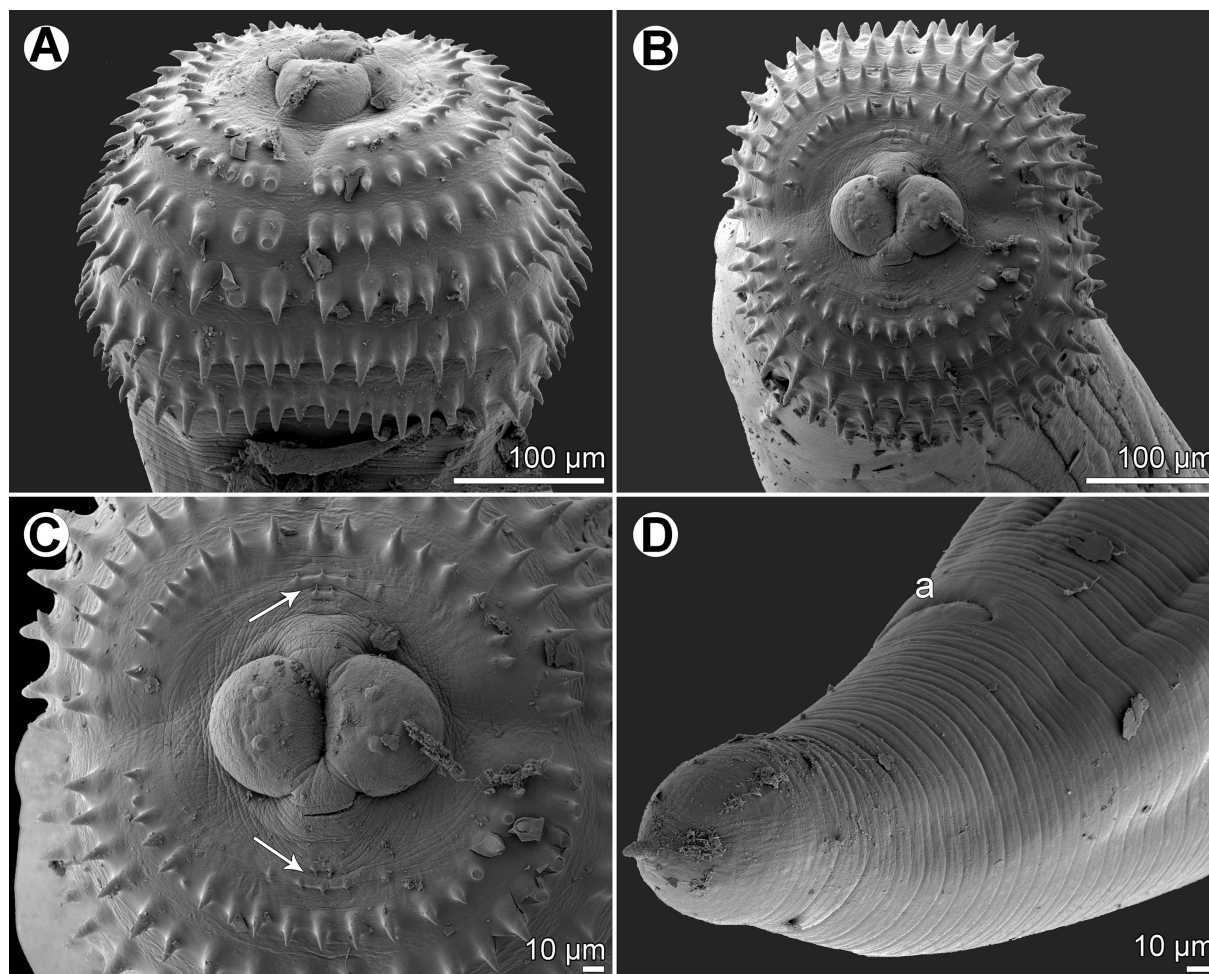
**Hosts:** Perciformes: Goldsilk seabream *Acanthopagrus berda* (Forsskål) (Sparidae) and fork-tailed threadfin bream *Nemipterus furcosus* (Valenciennes) (Nemipteridae); Tetraodontiformes: *Abalistes stellatus* (Anonymous), yellow-spotted triggerfish *Pseudobalistes fuscus* (Bloch et Schneider) (both Balistidae), silver-cheeked toadfish *Lagocephalus sceleratus* (Gmelin) (Tetraodontidae) and unicorn leatherjacket filefish *Aluterus monoceros* (Linnaeus) (Monacanthidae).

**Details of infected fishes:** *A. berda*, fish specimen JNC2224, 6 July 2007, fishmarket, Nouméa; *N. furcosus*, fish specimen JNC2258, fork length 330 mm, 21 July 2007, Baie des Citrons, off Nouméa; JNC2272, fork length 251 mm, 4 August 2007, Baie des Citrons, off Nouméa; JNC2272, fork length 251 mm, 4 August 2007, Baie des Citrons, off Nouméa; JNC2287, JNC2288, fork length 230–252 mm, weight 207–294 g, 30 August 2007, Baie Maa, near Nouméa; JNC2504, fork length 210 mm, weight 158 g, Baie des Citrons; JNC3017, 3018, fork length 239 mm, weight 226 g, 10 August 2009, Pointe Bovis, near Nouméa; *A. stellatus*, fish specimen JNC2271, 4 August 2007, fork length 380 mm, Baie des Citrons, off Nouméa. *P. fuscus*, fish specimen JNC1680, 13 December 2005, fork length 550 mm, weight 4,700 g, inside Récif Toombo, off Nouméa; *L. sceleratus*, fish specimen JNC2298, 4 August 2007, fork



**Fig. 4.** Female larva of *Echinocephalus* sp. ex *Acanthopagrus fuscus*. **A** – anterior end, lateral view; **B** – posterior end, lateral view; **C**, **D** – cuticular spine including root on cephalic bulb, lateral and dorsal views, respectively.





**Fig. 5.** Larva of *Echinocephalus* sp. ex *Acanthopagrus fuscus*, scanning electron micrographs. **A, B** – cephalic end, dorsoventral and apical views, respectively; **C** – region of pseudolabia, apical view (arrows indicate two rows of minute spines); **D** – tail, sublateral view. Abbreviation: a – anus.

length 172 mm, weight 76 g, Baie des Citrons, off Nouméa; *A. monoceros*, fish specimen JNC2262, male, fork length 545 mm, weight 1,600 g, Reef near Passe de Dumbéa, off Nouméa. Site of infection: Surface of inner organs (mostly encapsulated). Locality: Off Nouméa, New Caledonia. Prevalence and intensity: *A. berda*: 1 fish infected/10 fish examined; 1 nematode. *N. furcosus*: 6/160; 1–4. *A. stellatus*: 1/6; 1. *P. fuscus*: 1/12; 4. *L. scleratus*: 1/23; 1. *A. monoceros*: 1/1; 1. Deposition of specimens: MNHN JNC1680, JNC2258, JNC2262H, JNC2271, JNC2272, JNC2287, JNC2288, JNC2298, JNC2504, JNC3017 and JNC3018.

**Remarks.** In having the cephalic bulb armed with six rows of spines, these nematodes represent larvae of *Echinocephalus*. They were mostly found encapsulated in their fish hosts. Since some of them already possessed the vulva (still covered by the cuticle) and the developing vagina, it is apparent that they represented the fourth larval stage. Nevertheless, the presence of third-stage larvae in this material cannot be excluded, because some specimens fixed inside capsules could not be studied in detail.

To date, no developmental cycle has been studied in any species of *Echinocephalus*, but congeneric larvae were often recorded (in addition to elasmobranchs serving as the definitive hosts) from marine invertebrates, mainly

molluscs and echinoderms, and from a variety of teleost fishes (see Ivashkin and Khromova 1976, Anderson 2000). In contrast to adult forms of *Echinocephalus* from elasmobranchs, larger congeneric larvae differ considerably in having their cephalic bulb armed with only six major transverse rows of spines and two groups (dorsal and ventral) of few minute spines, arranged in two or three rows (Millemann 1963, Ko 1975, Beveridge 1987, Moravec and Justine 2006). Unfortunately, several nominal species of *Echinocephalus* have been established based solely on such larvae (see above), which contributed to taxonomic confusions in this genus.

Since the life cycles and morphogenesis of larvae of *Echinocephalus* spp. remain unknown, at present the species identification of congeneric larvae from marine invertebrates and teleost fishes based on morphological features is impossible. Nevertheless, Moravec and Justine (2006) showed some differences in the number and arrangement of the minute spines in dorsal and ventral groups among *Echinocephalus* larvae from different hosts, which might be of taxonomic importance. However, to date, only the larvae of *E. pseudouncinatus*, *E. overstreeti* and *E. sinensis* have been described in some detail, of which only those of

*E. overstreeti* were studied by SEM (Millemann 1963, Ko 1975, Moravec and Justine 2006).

Whereas the third-stage larva of *E. pseudouncinatus* was illustrated to have dorsal and ventral groups of minute spines in two rows, each consisting of two and four spines (Millemann 1963) and that of *E. sinensis* possessing three rows of minute spines in each of the dorsal and ventral group, consisting of two, two and three spines (Ko 1975); the same number and arrangement of minute spines was observed in an *Echinocephalus* larva from a Mexican teleost (Moravec and Justine 2006); the third-stage larva from scallops (molluscs) in South Australia, considered to belong to *E. overstreeti*, had the dorsal and ventral groups of minute spines in two rows each, formed by two and three spines (Beveridge 1987). However, in contrast, the larvae of *E. overstreeti* collected along with adults from the type host species (*T. meyeri*) in New Caledonia had dorsal and ventral groups of minute spines arranged in three rows each, with two, two and six spines, and with two larger spine-like formations laterally to the third row of six spines (Moravec and Justine 2006).

In having three rows of minute spines in each dorsal and ventral groups, consisting of two, two and three spines and one or two larger spine-like formations laterally to the third row of three spines, the present *Echinocephalus* larvae examined by SEM (from *A. berda*) evidently differ from all the above-mentioned congeneric larvae studied to date. Consequently, only future studies with the use of molecular methods may enable the species identification of these larvae. Nevertheless, the present findings of *Echinocephalus* larvae in all the above-mentioned species of teleosts in New Caledonia represent new host records.

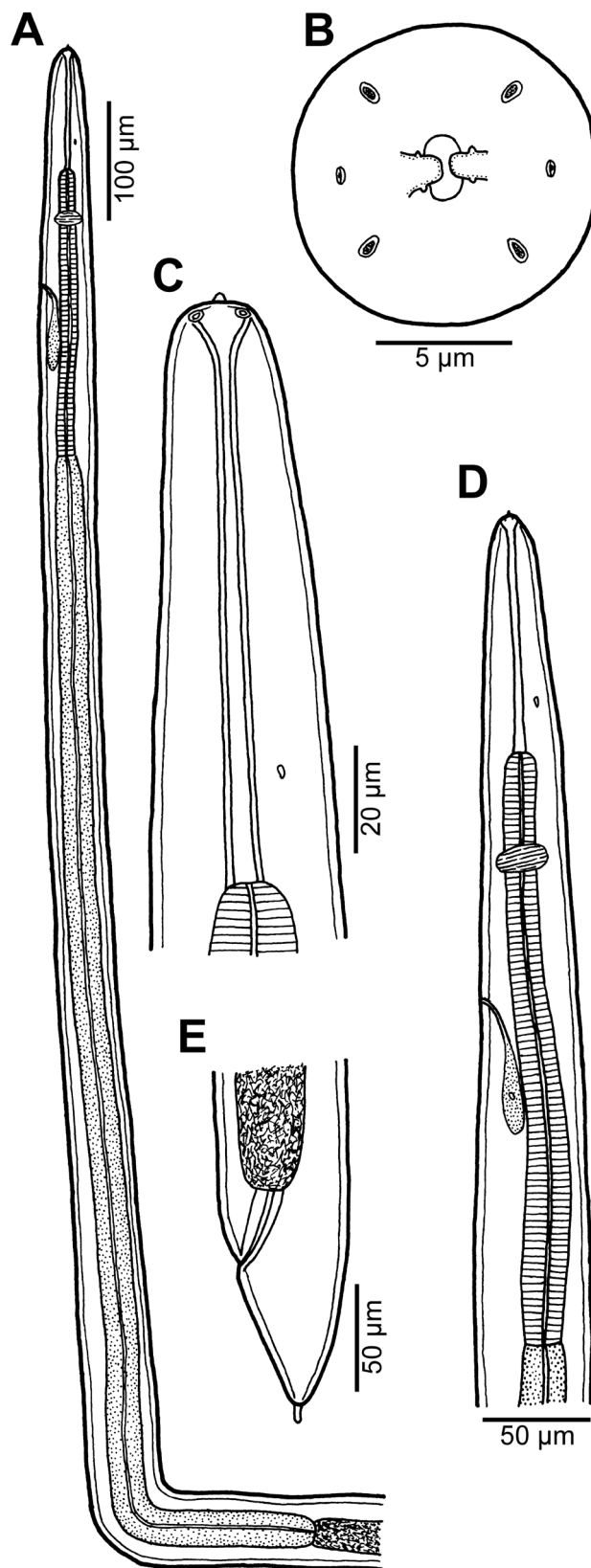
It is already known that *N. furcosus* is a fish with a rich parasite diversity (Justine et al. 2012a); this report adds yet another species to the total.

#### Family Cystidicolidae Skryabin, 1946

##### *Ascarophis* sp. third-stage larva

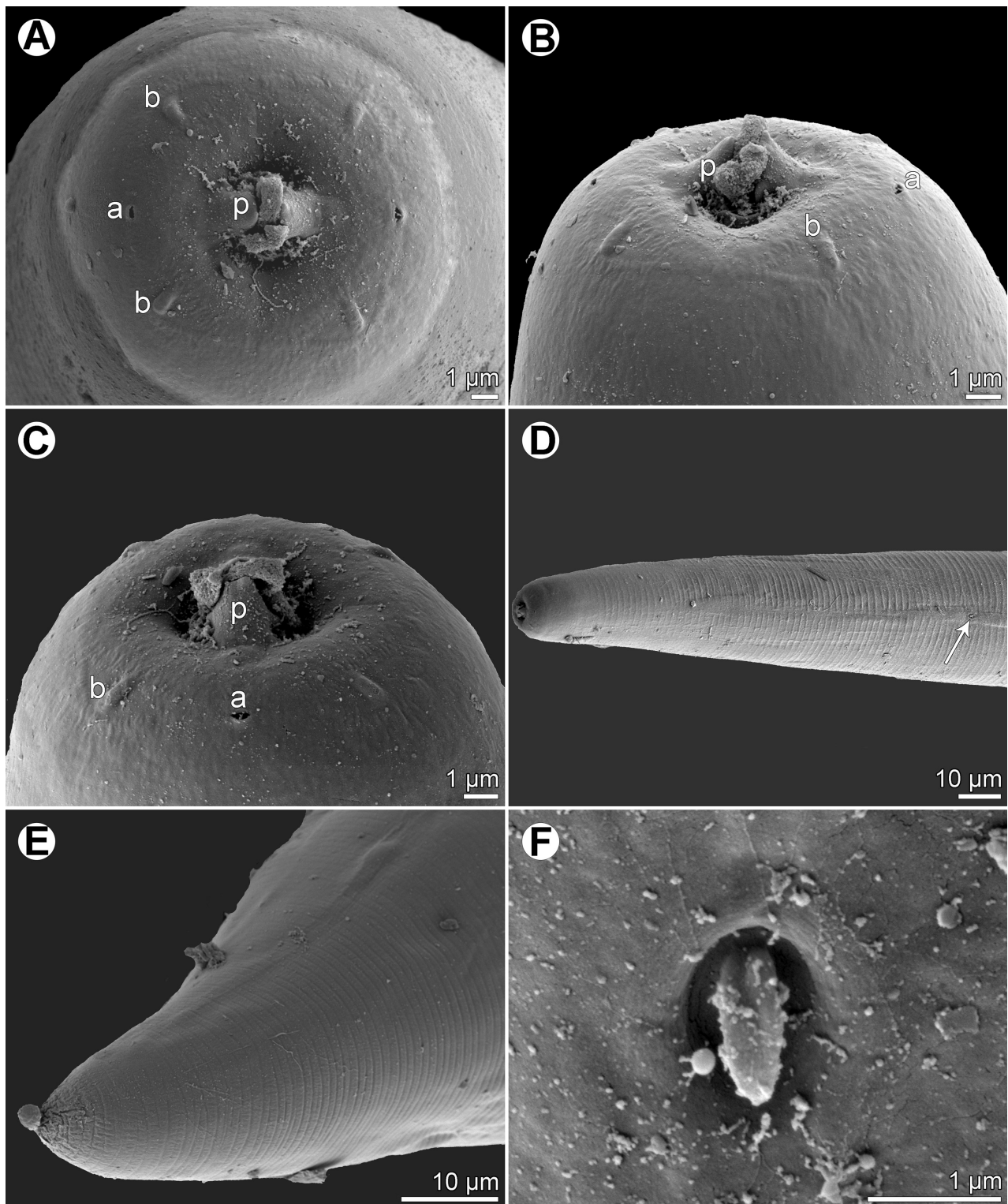
Figs. 6, 7

**Description** (1 specimen): Body small, filiform, whitish, 6.15 mm long, maximum width 66. Cuticle thin, densely transversely striated (Fig. 7D,E); striation more apparent in middle part of body; width of striae at this body portion 6. Cephalic end rounded, with small anteriorly protruding pseudolabia. Oral aperture oval, surrounded by 4 submedian cephalic papillae and pair of lateral amphids (Figs. 6B, 7A–C). Lateral pseudolabia small, rounded in apical view, partly covering oral aperture, each with pair (1 dorsal and 1 ventral) of minute round extensions; in lateral view pseudolabia form anterior projections. Sublabia absent (Figs. 6B, 7A–C). Deirids small, simple, situated at short distance anterior to anterior end of muscular oesophagus, at 108 from anterior extremity (Figs. 6C,D, 7D,F). Vestibule (stoma) long, with small funnel-shaped prostom at anterior end in lateral view (Fig. 6C,D); length of vestibule including prostom 120. Muscular oesophagus 243 long, maximum width 21, well separated from glandular oesophagus; glandular oesophagus 1.29 mm long, maximum width 36; length ratio of both oesophageal portions



**Fig. 6.** Larva of *Ascarophis* sp. ex *Pseudobalistes fuscus* (Bloch et Schneider). A – anterior (oesophageal) part of body, lateral view; B, C – cephalic end, apical and lateral views, respectively; D – anterior end, lateral view; E – caudal end, lateral view.





**Fig. 7.** Larva of *Ascarophis* sp. ex *Pseudobalistes fuscus* (Bloch et Schneider), scanning electron micrographs. **A** – cephalic end, apical view; **B**, **C** – cephalic end, dorsoventral and lateral views, respectively; **D** – anterior end of body, lateral view (arrow indicates deirid); **E** – tail tip with terminal mucron, lateral view; **F** – deirid. *Abbreviations:* a – amphid; b – cephalic papilla; p – pseudolabium.

1 : 5.31 (Fig. 6A,D). Length of vestibule and entire oesophagus represents 27% of body length. Intestine narrow, pale-coloured. Nerve ring and excretory pore 180 and 240, respectively, from anterior extremity (Fig. 6A,D). Genital primordium very elongated, inconspicuous, situated ventrally at approximately 2/3 of body length. Tail conical, 72

long, with small terminal digitiform mucron 6 long (Figs. 6E, 7E).

Host: Yellow-spotted triggerfish *Pseudobalistes fuscus* (Bloch et Schneider) (Balistidae, Tetraodontiformes).

Details of fish: Fish specimen JNC2164, fork length 437 mm,



weight 2,000 g.

Site of infection: Intestine.

Locality: Near Récif Toombo, off Nouméa, New Caledonia, 7 May 2007.

Prevalence and intensity: 1 fish infected/12 fish examined; 1 nematode.

Deposition of specimen: Not deposited, used for SEM.

**Remarks.** The general morphology and measurements of the only available specimen indicate that this belongs to the cystidicolid genus *Ascarophis*, including parasites of the digestive tract of marine and estuarine fishes (Ko 1986). The presence of a moderately developed genital primordium and a characteristic mucron on the tail tip show that they are at the third larval stage. Since no species of *Ascarophis* has so far been described from fishes of the family Balistidae, it is probable that these larvae belong to a new species for which *P. fuscus* serves as the definitive host.

The life cycles of *Ascarophis* spp. include crustaceans (e.g., gammarids) as intermediate hosts, in which the nematode larvae can even attain sexual maturity (Fagerholm and Butterworth 1988, Appy and Butterworth 2011).

#### Family Anisakidae Railliet et Henry, 1912

##### *Hysterothylacium* sp. third-stage larva Figs. 8, 9

**Description** (2 specimens): Length of body 8.73–10.31 mm, maximum width 272–340. Cephalic end with rounded formation 39–51 long, representing primordium of developing lips; oral aperture triangular, surrounded by 4 (2 dorsal and 2 ventrolateral) poorly visible cephalic papillae (Figs. 8A, 9A–C). Two very narrow lateral alae extend along body (Fig. 9C,D). Oesophagus 680–748 long and 68–82 wide. Ventriculus spherical, 68–95 in diameter; ventricular appendix markedly broad, 653–680 long, 163–177 wide. Intestinal caecum short, 340–354 long, 68–82 wide, extending anteriorly to about mid-way between nerve ring and ventriculus. Caecum to ventricular appendix length ratio 1 : 1.8–2.0. Nerve ring and excretory pore 313–340 and 313–354, respectively, from anterior extremity (Fig. 8A). Coils of developing genital tract located in posterior part of body. Tail conical, with its approximately last third abruptly narrowed to conspicuously digitiform appendix (Figs. 8B, 9D,E).

Host: Yellow-spotted triggerfish *Pseudobalistes fuscus* (Bloch et Schneider) (Balistidae, Tetraodontiformes).

Details of fish: fish specimen JNC1680, fork length 550 mm, weight 4,700 g.

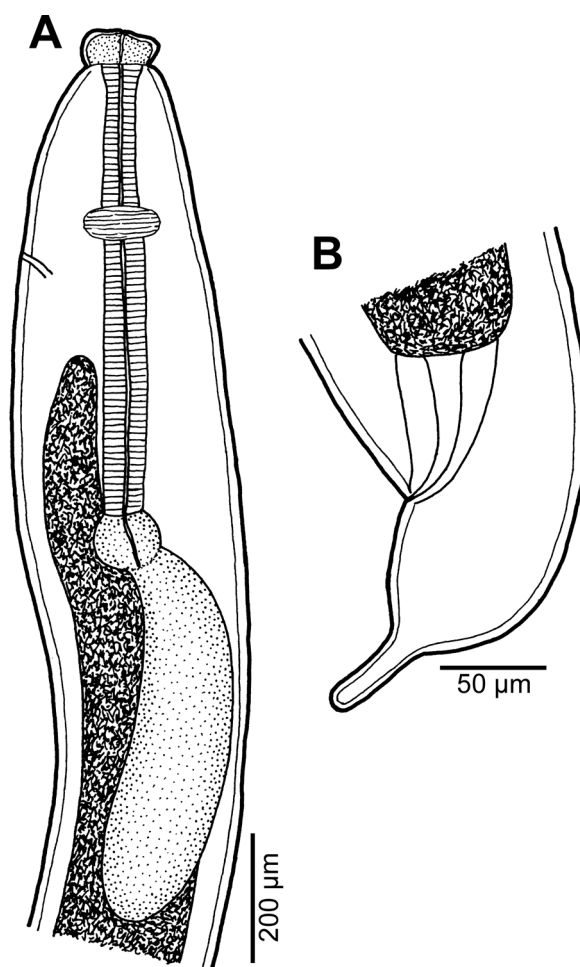
Site of infection: Intestine.

Locality: Inside Récif Toombo, off Nouméa, New Caledonia, 13 December 2005.

Prevalence and intensity: 1 fish infected/12 fish examined; 2 nematodes.

Deposition of specimens: Not deposited, used for SEM.

**Remarks.** The morphology of the present larvae of *Hysterothylacium* sp. is interesting especially by the shape of the tail. Larvae of this genus are unidentifiable to species



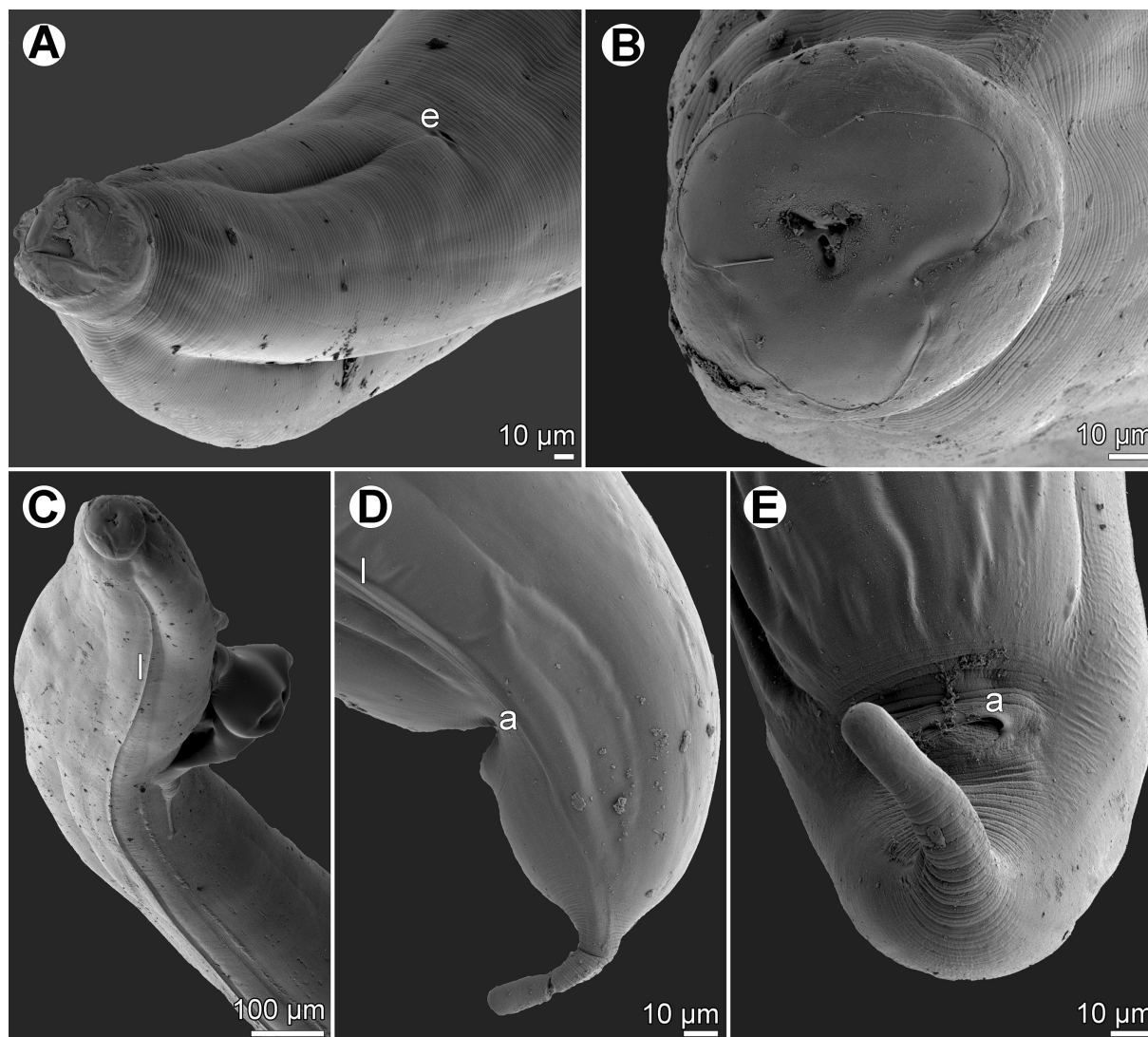
**Fig. 8.** Third-stage larva of *Hysterothylacium* sp. ex *Pseudobalistes fuscus* (Bloch et Schneider). A – anterior end, lateral view; B – caudal end, lateral view.

by morphological features. Nevertheless, judging from the host type, it cannot be excluded that these larvae belong to an undescribed species maturing in *P. fuscus*.

#### DISCUSSION

The nematode family Gnathostomatidae comprises three genera with adults parasitising elasmobranch and teleost fishes: *Ancyracanthus* Diesing, 1838, *Echinocephalus* and *Mooleptus* Özdikmen, 2010 (syn. *Metaleptus* Machida, Ogawa et Okiyama, 1982) (see Moravec 2007). Whereas *Ancyracanthus* includes one species parasitic in Neotropical characids and another one parasitising chelonians, and *Mooleptus* is represented by a single species from sharks, many nominal species of *Echinocephalus* were described from elasmobranchs and teleosts (Moravec 2007, Gibbons 2010, Özdikmen 2010). However, as mentioned above, the validity of numerous species of *Echinocephalus* cannot be accepted and those considered valid are limited in host distribution to marine and freshwater elasmobranchs, primarily rays.

The taxonomy of *Echinocephalus* is practically based on morphological features. Nevertheless, there is a need for the use of SEM, especially for the study of the structure of pseudolabia, spines on the cephalic bulb, ge-



**Fig. 9.** Third-stage larva of *Hysterothylacium* sp. ex *Pseudobalistes fuscus* (Bloch et Schneider), scanning electron micrographs. **A** – anterior end, subventral view; **B** – cephalic end, apical view; **C** – anterior end, lateral view; **D, E** – posterior end, lateral and ventral views, respectively. Abbreviations: a – anus; e – excretory pore; l – lateral ala.

nital papillae and ventral cuticular ornamentations on the male caudal end, features that are not always readily visible under LM. A phylogenetic analysis of *Echinocephalus* spp. based on morphological features was provided by Hoberg et al. (1998). However, the use of molecular methods of these nematodes in future studies is highly needed. To date, only three nominal species, *E. carpioe* (*species inquirenda*), *E. overstreeti* and *E. pseudouncinatus*, have been sequenced (e.g., Abdel-Ghaffar et al. 2013).

Encapsulated larvae of *Echinocephalus* were recorded from the muscles of some molluscs by Shipley and Harnell (1905), Stossich in Shipley and Harnell (1906) and Baylis and Lane (1920); the last-named authors considered marine bivalves to serve as the intermediate hosts for these nematodes. Marine molluscs (e.g., oysters and scallops) and echinoderms (sea urchins) were designated as possible intermediate hosts of *Echinocephalus* spp. by subsequent authors (e.g., Millemann 1951, 1963, Ko 1975, Beveridge 1987).

Larvae of *Echinocephalus* were also recorded from teleost fishes (e.g., Johnston and Mawson 1945a,b, Ivashkin and Khromova 1976). However, Ivashkin and Khromova (1976) showed that the intermediate hosts of nematodes of two other gnathostomatid genera, *Gnathostoma* Owen, 1836 and *Spiroxys* Schneider, 1866, are crustaceans (copepods), whereas molluscs were found to serve as reservoir (= paratenic) hosts. Therefore, these authors presumed that the true intermediate hosts of *Echinocephalus* spp. are not molluscs but marine crustaceans and that molluscs, echinoderms and teleost fishes play a role of reservoir (= paratenic) hosts. Anderson (2000) also considered arthropods, probably marine crustaceans such as copepods, as possible intermediate hosts of *E. sinensis* and *E. pseudouncinatus*, and molluscs, echinoderms and other marine organisms as paratenic or second intermediate hosts in which growth occurs.

We agree with Ivashkin and Khromova (1976) that marine invertebrates, excluding crustaceans, and teleost fishes serve probably only as paratenic hosts (in the conception of

Odening 1976) for *Echinocephalus* spp. In contrast to the second intermediate hosts, which are obligatory for completing the parasite's life cycle, paratenic hosts need not participate in the cycle, even though they may represent an important source of infection for the definitive host. The presence of four-stage larvae of *Echinocephalus* in some teleosts of the present material indicates that the echinocephalid larvae may not only grow in the body of the paratenic host, but they may even attain their next larval stage (= metaparatenic hosts according to Odening 1976).

As mentioned above, no developmental cycle of any species of *Echinocephalus* has been studied experimentally. Millemann (1963) considered larvae of *E. pseudouncinatus* from molluscs to be at the second larval stage, whereas the congeneric larvae of *E. sinensis* from oysters were taken by Ko (1975) for second-stage (smaller) or third-stage (larger) larvae. However, the cephalic morphology of both these larval forms was much the same (presence of six rows of larger cephalic spines), indicating that these were most probably third- and fourth-stage larvae; the presence of the developing vagina in female larvae (designated as third-stage larvae) is, in fact, typical of the fourth larval stage. A microphotograph of second-stage larvae of *E. overstreeti* hatched from eggs after 10 days of cultivation in sea-water was provided by Beveridge (1987). This clearly shows that the morphology of these larvae and their body size (length 108–122 µm) are very different from those of the larvae from molluscs reported by Millemann (1963) and Ko (1975).

The present finding of *Echinocephalus* fourth-stage larvae in teleost fishes and those collected by Ko (1975) in oysters (see above) indicate that larvae may moult in paratenic hosts to attain the fourth larval stage. Nevertheless, it is necessary to confirm this experimentally. Apparently, there is very low host specificity of infective larvae ( $L_3$  and  $L_4$ ) of *Echinocephalus* spp. at the level of paratenic hosts and, consequently, one paratenic host may harbour the larvae of different species of *Echinocephalus*. At present, these larvae could be identified to species only by molecular methods.

#### Key to the valid species of *Echinocephalus*:

1. Complete transverse rows of spines on cephalic bulb 11–29 in number ..... 2
  - Complete transverse rows of spines on cephalic bulb usually 30–43 in number..... 7
2. Length of spicules 800–840 µm. Deirids considerably asymmetrical. Transverse rows of spines on bulb 24–26. Allegedly 5 (2 preanal and 3 postanal) pairs of caudal papillae; anterior 2 pairs of preanals at mid-length of tail, last pair of preanals just anterior to tail tip. Parasitic in Gymnuridae (*Gymnura japonica* (Temminck et Schlegel)) (butterfly rays); Taiwan Strait ..... ***E. pteroplatae***
  - Spicules longer than 1 mm. Deirids symmetrical. Parasitic in hosts of other families ..... 3
3. Caudal alae markedly bilobed. Males with modified annules in ventral region anterior to caudal alae. Cephalic bulb with 25 transverse rows of spines. Spicules 2 mm long. Eight (2 preanal and 6 postanal) pairs of caudal papillae. Parasitic in Potamotrygonidae (*Potamotrygon hystrix* (Müller et Henle)) (river stingrays); Venezuela ..... ***E. diazi***
  - Caudal alae unilobed. Modified annules in ventral region anterior to caudal alae lacking ..... 4
4. Rugose areas located on ventrolateral regions adjacent to cloaca present. Parasites of bullhead sharks (Heterodontidae) or eagle rays (Myliobatidae) ..... 5
  - Rugose areas in males not observed. Parasites of whiptail stingrays (Dasyatidae) ..... 6
5. Gubernaculum present. Six (3 preanal and 3 postanal) pairs of caudal papillae. Length of spicules 1.39–2.12 mm (0.51–1.08 mm in juvenile forms). Parasitic in bullhead sharks (*Heterodontus Blainville*, Heterodontidae), juvenile forms also in eagle rays (*Myliobatis* Cuvier, Myliobatidae); eastern North Pacific (California, USA) ..... ***E. pseudouncinatus***
  - Gubernaculum not observed. Seven (2 preanal, 1 adanal and 4 postanal) pairs of caudal papillae and pair of phasmids present. Length of spicules 1.01–1.40 mm. Parasitic in Aetobatidae (eagle rays) (*Aetobatus* Blainville); South China Sea (off southern China) and South Pacific off Australia and New Caledonia ..... ***E. sinensis***
6. Length of spicules 1.40–1.49 mm. Transverse rows of spines on bulb 11–13 in number. Caudal papillae: 3 preanal and 5 postanal pairs (adanal papillae absent). Parasitic in Dasyatidae (whiptail stingrays) (*Urogymnus* Müller et Henle); Sri Lanka ..... ***E. multidentatus***
  - Length of spicules 2 mm. Transverse rows of spines on bulb 15–18 in number. Eight (3 preanal, 1 adanal and 4 postanal) pairs of caudal papillae. Parasitic in Dasyatidae (whiptail stingrays) (*Urogymnus*); Sri Lanka ..... ***E. southwelli***
7. Six postanal papillae (excluding phasmids). Males with modified annules in ventral region anterior to caudal alae. Parasites of Potamotrygonidae and Dasyatidae in Central and South America ..... 8
  - Four postanal papillae (excluding phasmids). Modified annules in ventral region anterior to caudal alae lacking. Parasites of Dasyatidae, Myliobatidae and possibly some other elasmobranch families except for Potamotrygonidae in other regions ..... 9
8. Male caudal alae markedly bilobed. Transverse rows of spines on bulb 30–38 in number. Spicules 1.18–2.00 mm long. Nine (3 preanal and 6 postanal) pairs of caudal papillae. Parasitic in Dasyatidae (*Himantura pacifica* (Beebe et Tee-Van)) (whiptail stingrays); East Pacific (Costa Rica and Chiapas, Mexico) .... ***E. janzeni***
  - Male caudal alae unilobed. Transverse rows of spines on bulb 30–32 in number. Spicules 1.40–2.40 mm long. Nine (3 preanal and 6 postanal) pairs of caudal papillae. Parasitic in Potamotrygonidae (*Potamotrygon* spp.) (river stingrays); northern South America (Colombia, Brazil and Venezuela; Amazon and Orinoco Rivers basins) ..... ***E. daileyi***
9. Lateral rugose regions adjacent to cloaca lacking.



- Transverse rows of spines on bulb 30–40 in number. Spicules 1.55–1.90 mm long. Seven (3 preanal and 4 postanal) pairs of caudal papillae and pair of phasmids. Parasitic in Myliobatidae (*Aetomylaeus* Garman) and Dasyatidae (*Brevitrygon* Last, Naylor et Manjaji-Matsumoto); off India and Sri Lanka .. *E. spinosissimus*
- Lateral rugose regions adjacent to cloaca present. Parasites of elasmobranchs in East Atlantic or South Pacific regions ..... 10
  - 10. Posterodorsal and posteroventral part of base of each pseudolabium and interlabia with distinct cuticular serrations ..... 11
  - Posterodorsal and posteroventral part of each pseudolabium and interlabia without cuticular serrations. Transverse rows of spines on bulb 32–33. Spicules 1.10–1.48 mm long. Seven (2 preanal, 1 adanal and 4 postanal) pairs of caudal papillae and pair of phasmids. Parasitic in Dasyatidae (*Pastinachus ater*) (whiptail stingrays); off New Caledonia ..... *E. inserratus* sp. n.
  - 11. Caudal papillae of fourth to sixth pairs arranged in cluster. Transverse rows of spines on bulb 31–40. Spicules 1.00–1.25 mm long. Size of eggs  $40 \times 35 \mu\text{m}$ . Parasitic in Dasyatidae (*Bathytoshia* Whitley, *Dasyatis* Rafinesque) (whiptail stingrays); eastern North Atlantic region (Adriatic and Black Seas) ..... *E. uncinatus*
  - Caudal papillae of sixth pair located distinctly more

posterior to those of fourth and fifth pairs. Transverse rows of spines on bulb 31–43 (less often 23–35). Size of eggs  $20\text{--}40 \times 10\text{--}30 \mu\text{m}$ . Parasitic in Dasyatidae (*Taeniurops* Garman, *Urogymnus*), possibly also in some other elasmobranchs; Pacific Ocean (Marquesas Islands, South Australia, New Caledonia, Eniwetog Atoll) ..... *E. overstreeti*

**Acknowledgements.** Most colleagues, fishermen and students involved in the catching of fishes and the work of the parasitological survey were listed in Justine et al. (2012a); some echinocephalid larvae were found by undergraduate students during practical courses taught by JLJ in 2007 at the University of Nouméa on the basis of freshly caught fish. Bernard Séret (IRD, France) identified our specimen of *Pastinachus ater* from photographs. The late Jack Randall (Bishop Museum, Hawaii) identified several fish from photographs, including our specimen of *Aluterus monoceros* for which he commented that this was probably the first confirmed record for the South Pacific. Thanks are also due to the Laboratory of Electron Microscopy, Institute of Parasitology, Biology Centre CAS, institution supported by the MEYS CR (LM2015062 Czech-Biolmaging) and ERDF (No. CZ.02.1.01/0.0/0.0/16\_013/0001775), for their support with obtaining the scientific data presented in this paper, and to Blanka Škoríková of the same Institute for help with the illustrations. The English translation of the original Chinese description of *E. pteroplatae* was kindly provided by Liang Li, Hebei Normal University, China. This study was partly supported by the institutional support of the Institute of Parasitology, BC AS CR (RVO: 60077344).

## REFERENCES

- ABDEL-GHAFFAR F., BASHTAR A.-R., MEHLHORN H., ABDEL-GABER R., AL QURAIISHY S., SALEH R. 2013: Morphological and phylogenetic analysis of *Echinocephalus carpioe* n. sp. (Nematoda: Gnathostomatidae) infecting the common carp *Cyprinus carpio* inhabiting Burullus Lake – a new host record in Egypt. *Parasitol. Res.* 112: 4021–4028.
- ANDERSON R.C. 2000: Nematode Parasites of Vertebrates. Their Development and Transmission. Second Edition. CABI Publishing, Wallingford, 650 pp.
- ANDERSON R.C., CHABAUD A.G., WILLMOTT S. (Eds.) 2009: Keys to the Nematode Parasites of Vertebrates. Archival Volume. CAB International, Wallingford, 463 pp.
- ANDREWS R.H., BEVERIDGE I., ADAMS M., BAVERSTOCK P.R. 1988: Identification of life cycle stages of the nematode *Echinocephalus overstreeti* by allozyme electrophoresis. *J. Helminthol.* 62: 153–157.
- APPY R.G., BUTTERWORTH E.W. 2011: Development of *Ascarophis* sp. (Nematoda: Cystidicolidae) to maturity in *Gammarus deubeni* (Amphipoda). *J. Parasitol.* 97: 1035–1048.
- ARYA S.N. 1977: A new species of the genus *Echinocephalus* Molin, 1858 from a fish, with a key to the species of *Echinocephalus* (Gnathostomatidae). *Ind. J. Helminthol.* 29: 42–45.
- ARYA S.N. 1982: A new species of the genus *Echinocephalus* Molin, 1858 from a fish *Trichiurus muticus* (Nematoda: Gnathostomatidae). *Riv. Parassitol.* 43: 443–446.
- BAYLIS H.A., LANE C. 1920: A revision of the nematode family Gnathostomidae. *Proc. Zool. Soc., London*, Sept.: 245–310.
- BEGUM I., GUPTA P.C. 2012: Morphotaxonomic Studies of Nematode Parasites of Fishes from India: Morphology and Taxonomy of Nematode Parasites. LAP Lambert Academic Publishing, Saarbrücken, 376 pp.
- BEVERIDGE I. 1985: A redescription of *Echinocephalus uncinatus* Molin, 1858 (Nematoda, Gnathostomatoidea) from European rays, *Dasyatis pastinaca* (Linnaeus, 1758). *Bull. Mus. Nat. Hist. Nat.*, 4<sup>e</sup> sér., 4: 781–790.
- BEVERIDGE I. 1987: *Echinocephalus overstreeti* Deardorff & Ko, 1983 (Nematoda: Gnathostomatoidea) from elasmobranchs and molluscs in South Australia. *Trans. Roy. Soc. S. Austr.* 111: 79–92.
- BILQUEES F.M., KHANUM Z., JEHAN Q. 1971: Marine fish nematodes of West Pakistan I. Description of seven new species from Karachi coast. *J. Sci., Univ. Karachi* 1: 175–184.
- BROOKS D.R., DEARDORFF T.L. 1988: *Rhinebothrium devaneyi* n. sp. (Eucestoda: Tetrathyrididae) and *Echinocephalus overstreeti* Deardorff and Ko, 1983 (Nematoda: Gnathostomatidae) in a thorny back ray, *Urogymnus asperrimus*, from Enewetak Atoll, with phylogenetic analysis of both species groups. *J. Parasitol.* 74: 459–465.
- CHENG T.C. 1975: *Echinocephalus crassostreai* sp. nov., a larval nematode from the oyster *Crassostrea gigas* in the Orient. *J. Invert. Pathol.* 26: 81–90.
- DEARDORFF T.L., BROOKS D.R., THORSON T.B. 1981: A new species of *Echinocephalus* (Nematoda: Gnathostomidae) from Neotropical stingrays with comments on *E. diazi*. *J. Parasitol.* 67: 433–439.
- DEARDORFF T.L., KO R.C. 1983: *Echinocephalus overstreeti* sp. n. (Nematoda: Gnathostomatidae) in the stingray, *Taeniura melanopilos* Bleeker, from the Marquesas Islands, with comments on *E. sinensis* Ko, 1975. *Proc. Helminthol. Soc. Wash.* 50: 285–293.
- FAGERHOLM H.-P., BUTTERWORTH E.W. 1988: *Ascarophis* sp. (Nematoda: Spirurida) attaining sexual maturity in *Gammarus* spp. (Crustacea). *Syst. Parasitol.* 12: 123–139.
- FROESE R., PAULY D. (Eds) 2020: FishBase. World Wide Web electronic publication. <http://www.fishbase.org>, version 12/2020.
- GIBBONS L.M. 2010: Keys to the Nematode Parasites of Vertebrates. Supplementary Volume. CABI, Wallingford, 216 pp.

- HOBERG E.P., BROOKS D.R., MOLIAN-UREÑA H., ERBE E. 1998: *Echinocephalus janzeni* n. sp. (Nematoda: Gnathostomatidae) in *Himantura pacifica* (Chondrichthyes: Myliobatiformes) from the Pacific coast of Costa Rica and Mexico, with historical biogeographic analysis of the genus. *J. Parasitol.* 84: 571–581.
- IVASHKIN V.M., KHROMOVA L.A. 1976: [Cucullanata and Gnathostomata of Animals and Man and the Diseases Caused by Them.] *Osnovy Nematologii* 27. Nauka, Moscow, 436 pp. (In Russian.)
- JOHNSTON T.H., MAWSON P.M. 1945a: Parasitic nematodes. Rep. B. A. N. Z. Antarct. Res. Exped. 1929–1931, 5 (Pt. 2): 73–159.
- JOHNSTON T.H., MAWSON P.M. 1945b: Some parasitic nematodes from South Australian marine fish. *Trans. Roy. Soc. S. Austr.* 69: 114–117.
- JUSTINE J.-L. 2010: Parasites of coral reef fish: how much do we know? With a bibliography of fish parasites in New Caledonia. *Belg. J. Zool.* 140 (Suppl.): 155–190.
- JUSTINE J.-L., BEVERIDGE I., BOXSHALL G.A., BRAY R.A., MILLER T.L., MORAVEC F., TRILLES J.-P., WHITTINGTON I.D. 2010a: An annotated list of parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected in groupers (Serranidae, Epinephelinae) in New Caledonia emphasizes parasite biodiversity in coral reef fish. *Folia Parasitol.* 57: 237–262.
- JUSTINE J.-L., BEVERIDGE I., BOXSHALL G.A., BRAY R.A., MILLER T.L., MORAVEC F., TRILLES J.-P., WHITTINGTON I.D. 2012a: An annotated list of fish parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected from snappers and bream (Lutjanidae, Nemipteridae, Caesionidae) in New Caledonia confirms high parasite biodiversity on coral reef fish. *Aquat. Biosyst.* 8: 22.
- JUSTINE J.-L., BEVERIDGE I., BOXSHALL G.A., BRAY R.A., MORAVEC F., WHITTINGTON I.D. 2010b: An annotated list of fish parasites (Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected from emperors and emperor bream (Lethrinidae) in New Caledonia further highlights parasite biodiversity estimates on coral reef fish. *Zootaxa* 2691: 1–40.
- JUSTINE J.-L., BRIAND M.J., BRAY R.A. 2012b: A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies. *Parasitol. Res.* 111: 341–351.
- KALYANKAR S.D. 1971: On a new species of *Echinocephalus* Molin, 1858 (Nematoda: Gnathostomatidae: Echinocephalinae) from India, with a key to the species. *Zool. Anz.* 187: 317–320.
- KO R.C. 1975: *Echinocephalus sinensis* n. sp. (Nematoda: Gnathostomatidae) from the ray (*Aetabatus flagellum*) in Hong Kong, Southern China. *Can. J. Zool.* 53: 490–500.
- KO R.C. 1986: A Preliminary Review of *Ascarophis* (Nematoda) of fishes. *Occas. Publ. Dept. Zool., Univ. Hong Kong, Hong Kong*, 54 pp.
- LAKSHMI R.I. 1994: *Echinocephalus scoliodonti* n. sp. (Nematoda: Gnathostomatidae) from elasmobranch fish *Scoliodon sorakowah* (Cuvier). *Bol. Chil. Parasitol.* 49: 66–68.
- LAKSHMI R.I., RAO K.H., SHYAMASUNDARI K. 1984: *Echinocephalus waltainrensis* n. sp. (Nematoda: Gnathostomatidae) from the stomach of *Rhinobatus granulatus* Cuvier. *Ind. J. Parasitol.* 8: 235–238.
- MILLEMANN R.E. 1951: *Echinocephalus pseudouncinatus* n. sp., a nematode parasite of the abalone. *J. Parasitol.* 37: 435–439.
- MILLEMANN R.E. 1963: Studies on the taxonomy and life history of echinocephalid worms (Nematoda: Spiruroidea) with a complete description of *Echinocephalus pseudouncinatus* Millemann, 1951. *J. Parasitol.* 49: 754–764.
- MORAVEC F. 2007: Some aspects of the taxonomy and biology of adult spirurine nematodes parasitic in fishes: a review. *Folia Parasitol.* 54: 239–257.
- MORAVEC F., JUSTINE J.-L. 2006: Three nematode species from elasmobranchs off New Caledonia. *Syst. Parasitol.* 64: 131–145.
- MORAVEC F., JUSTINE J.-L. 2020: Erection of *Euterranova* n. gen. and *Neoterranova* n. gen. (Nematoda, Anisakidae), with the description of *E. dentiduplicata* n. sp. and new records of two other anisakid nematodes from sharks off New Caledonia. *Parasite* 27: 58.
- ODENING K. 1976: Conception and terminology of hosts in parasitology. *Adv. Parasitol.* 14: 1–93.
- ÖZDIKMEH H. 2010: Substitute names for some Nematoda. *Mun. Ent. Zool.* 5: 780–784.
- SHIPLEY A.E., HORNELL J. 1905: Further report on parasites found in connection with the pearl oyster fishery at Ceylon. In: Herdman W.A. (Ed.), Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Manaar. Pt. 3. Roy. Soc., London, pp. 49–56 + 1 pl.
- SHIPLEY A.E., HORNELL J. 1906: Report on the cestode and nematode parasites from the marine fishes of Ceylon. In: Herdman W.A. (Ed.), Report to the Government of Ceylon on Pearl Oyster Fisheries of the Gulf of Manaar. Pt. 5. Roy. Soc., London, pp. 43–96.
- SINGH N., CHAUHARO D.K., KHARE A.K. 2010: Diversity of fish nematode *Echinocephalus chengii* n. sp. (Nematoda: Spirurida, Gnathostomatidae) from Meerut-Delhi region. *J. Exp. Zool.* 13: 587–591.
- SOOD M.L. 2017: Fish Nematodes from South Asia. Second Edition. Kalyani Publishers, Ludhiana, 1039 pp.
- TRONCY P.-M. 1969: Description de deux nouvelles espèces de nématodes parasites de poissons. *Bull. Mus. Nat. Hist. Nat.*, 2<sup>e</sup> sér., 41: 598–605.
- WANG P.Q., ZHAO Y.R., CHEN C.C. 1978: [On some nematodes from vertebrates in South China.] *Fujian Shida Xuebao* 2: 75–90 (In Chinese.)

Received 8 January 2021

Accepted 22 February 2021

Published online 25 May 2021

**Cite this article as:** Moravec F., Justine J.-L. 2021: *Echinocephalus inserratus* sp. n. (Nematoda: Gnathostomatidae) from the stingray *Pastinachus ater* (Dasyatidae) and new records of congeneric and some other nematode larvae from teleost fishes off New Caledonia. *Folia Parasitol.* 68: 014.