



Three new species of *Ascarophis* van Beneden, 1871 (Nematoda: Cystidicolidae) from deep-sea hydrothermal vent fishes of the Pacific Ocean

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Abstract Examinations of some deep-sea hydrothermal vent fishes from the western and eastern regions of the Pacific Ocean revealed the presence of three new species of *Ascarophis* van Beneden, 1871 (Nematoda: Cystidicolidae), all gastrointestinal parasites, namely: *A. justinei* **n. sp.** from *Thermarces cerberus* Rosenblatt & Cohen (type host) and *Thermichthys hollisi* Cohen, Rosenblatt & Moser (both Zoarcidae, Perciformes) and *A. globuligera* **n. sp.** from *T. cerberus* from the Northern East Pacific Rise, and *A. monofilamentosa* **n. sp.** from *Pyrolicus manusanus* Machida & Hashimoto (Zoarcidae, Perciformes) from the Manus Basin near Papua New

Guinea. Specimens are described and illustrated based on light and scanning electron microscopical examinations. In addition to other morphological differences, all the three new species differ from each other by the structure of eggs: eggs bearing a lateral superficial swelling (*A. globuligera* **n. sp.**), eggs with one conspicuously long filament on one pole (*A. monofilamentosa* **n. sp.**) and eggs smooth, without any filaments or swellings (*A. justinei* **n. sp.**). The egg morphology of the two first-named species is unique within all species of *Ascarophis*, which indicates that all the three newly described species of *Ascarophis* are probably endemic to the respective hydrothermal vents as their fish hosts.

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Introduction

Hydrothermal vents are fissures on the seabed from which geothermally heated water discharges, being most commonly found near volcanically active spots in the ocean. Biological communities in venting areas occurring along oceanic ridges are composed of numerous endemic species adapted to the extreme environment (Van Dover et al., 2002). To date, very little is known about parasitism in these communities (de Buron & Morand, 2004; Dykman et al., 2023) and the only parasitic nematode so far described from a fish endemic to hydrothermal deep-sea vents is *Moravecnema segonzaci* Justine, Cassone & Petter, 2002 (Cystidicolidae),

an intestinal parasite of *Pachycara thermophilum* Geistdoerfer (Zoarcidae) from the hydrothermal sites Logatchev and Snake Pit-Moose of the Mid-Atlantic Ridge, at depths of 3,008–3,510 m (Justine et al., 2002).

Nematodes of the family Cystidicolidae Skryabin, 1946 represent an interesting and important group mainly of gastrointestinal parasites of fishes, primarily teleosts, that includes many genera and species (Moravec, 2007; Moravec et al., 2021). However, the taxonomy and classification system of these nematodes is complicated, because many cystidicolid genera have been based on cephalic structures, some of which are only evident using scanning electron microscopy (SEM) (Moravec, 2007). On the other hand, some previously described cystidicolid species, including the type species of some genera were studied solely by light microscopy (LM) and, consequently, remain inadequately described. At present, there are 27 cystidicolid genera considered to be valid (Beveridge & Moravec, 2020; Moravec et al., 2021).

Examinations of the deep-sea hydrothermal vent fishes collected by the expeditions AT03-19, AT03-33, AT03-43, AT07-05, AT15-15, RR2102, and Luk Luk from four localities in the East Pacific Rise and one locality in the Manus Basin near Papua New Guinea (other side of the Pacific Ocean) in the years 1998–2021, revealed the presence of gastrointestinal cystidicolid nematodes parasitic in three endemic fish species, *Pyrolycus manusanus* Machida & Hashimoto and *Thermarces cerberus* Roseblatt & Cohen (both Zoarcidae, Perciformes), and *Thermichthys hollisi* Cohen, Rosenblatt & Moser (Bythitidae, Ophidiiformes). Closer study of the available nematode material from these hosts showed that it included three new species of *Ascarophis* van Beneden, 1871, which are described below.

All the host species are deep-water, bathydemersal fishes: *P. manusanus* is known only from Papua New Guinea (Western Central Pacific), whereas *T. cerberus* and *T. hollisi* occur along the East Pacific Rise and the Galápagos Rift (Eastern Pacific) (Cohen & Haedrich, 1983; Froese & Pauly, 2023). *Thermarces cerberus* generally inhabits diffuse-flow areas of the active vent field, while *T. hollisi* prefers habitat peripheral to active venting (Buckman, 2009).

Materials and methods

Fish from the 9°50'N vent field on the East Pacific Rise were caught with the multi-chambered suction sampler of HOV *Alvin*. The fish specimens from the vent sites East Wall and L Vent, collected in 1998, 1999, and 2007, were immediately processed aboard ship (within 2h) and the gut contents preserved in 95% ethanol (Sancho et al., 2005; Buckman, 2009). Parasites were extracted from preserved gut contents in 2019 and stored in 95% ethanol. The fish specimens from the vent site Crab Spa, collected in 2021, was dissected and immediately examined for parasites while fresh, and the parasites were fixed and preserved in 95% ethanol (Dykman et al., 2023). Fish from South Su, Manus Basin (Papua New Guinea) were caught using a ROV suction sampler. Caught fish were fixed and preserved in 70% ethanol. The gut contents were then examined to recover nematodes. Nematodes were stored and sent for morphological examination.

For preliminary light microscopical examination, nematodes were first cleared and mounted on slides in either lactophenol or 80% phenol and afterwards transferred into 70% ethanol; later they were cleared with glycerine. Drawings were made with the aid of a Zeiss microscope drawing attachment. Subsequently, after being transferred to 4% formalin, the same specimens were used for SEM. These were post-fixed 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 at an accelerating voltage of 4 kV (GB low mode). All measurements are in micrometres unless otherwise indicated. The type and voucher specimens mounted on SEM stubs were deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS). Fish nomenclature follows FishBase (Froese & Pauly, 2023).

Family Cystidicolidae Skryabin, 1946

Ascarophis van Beneden, 1871

Type-species: *Ascarophis morrhuae* van Beneden, 1871.

***Ascarophis justinei* n. sp.**

Type-host: *Thermarces cerberus* Rosenblatt & Cohen (Zoarcidae, Perciformes).

Other-host: *Thermichthys hollisi* Cohen, Rosenblatt & Cohen (Zoarcidae, Perciformes).

Type-locality: Crab Spa, 9°50'N, Northern East Pacific Rise, 9.83993°N, 104.29156°W, depth 2511 m (collected April 15, 2021, ROV *Jason 2* dive J2-1318).

Other localities: *T. cerberus*: (1) East Wall, 9°50'N, Northern East Pacific Rise, 9.8425°N, 104.292°W, depth 2508 m (collected May 15, 1998, HOV *Alvin* dive 3214 and April 27, 1999, HOV *Alvin* dive 3388) and (2) Crab Spa, 9°50'N, Northern East Pacific Rise, 9.83993°N, 104.29156°W, depth 2511 m (collected April 14, 2021, ROV *Jason 2* dive J2-1318).

T. hollisi: L Vent, 9°50'N, Northern East Pacific Rise, 9.77100°N, 104.27900°W, depth 2528 m (collected February 2, 2007, HOV *Alvin* dive 4317).

Prevalence and intensity: *T. cerberus*: 24% (8 fish infected/34 fish examined); intensity 1–10 (mean 3) nematodes per fish. *T. hollisi*: 8% (2/24); intensity 1.

Type-material: Holotype (male) and allotype (gravid female) and paratypes mounted on SEM stubs in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS N-1285).

Site in host: Stomach and intestine.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Ascarophis justinei* n. sp. is “urn:lsid:zoobank.org:act:28F602C4-FE87-4C02-B3D4-12D82EE1DFFD”

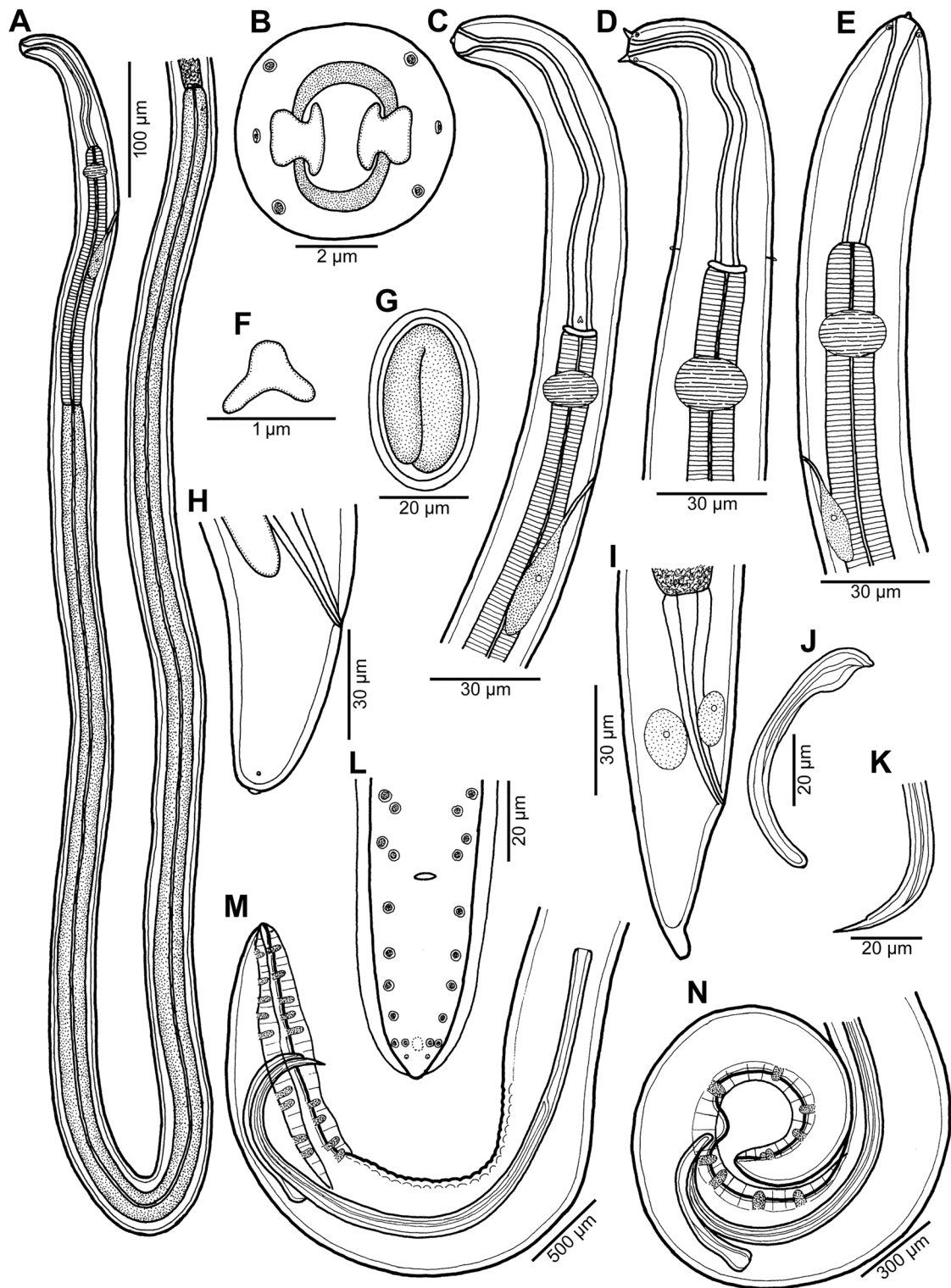
Etymology: This new species is named in honour of Prof. Jean-Lou Justine, a well-known French helminthologist, who (along with Dr. A.-J. Petter) was the first to describe a cystidicolid nematode from deep-sea hydrothermal vent fish.

Description

General. Small, whitish nematodes. Cuticle thick, with fine transverse striations (Figs 2A, 3A, E). Cephalic end rounded, with 2 conspicuous conical

pseudolabial terminal protrusions in male (Figs. 1D, 2A, B), which are considerably reduced (Fig. 3C, D) or absent (Fig. 3A, B) in gravid female. Oral aperture oval, dorsoventrally elongate (Figs. 1B, 2B, 3C, D). Submedian labia absent. Four submedian flap-like sublabia well developed, fused together dorsally and ventrally in large females (Figs. 1B, 2B, 3C, D). Lateral pseudolabia well developed, each provided with conspicuous terminal protrusion in male, this being considerably reduced or absent in large females (Figs. 1D, 2A, B, 3A–D); in apical view, flat inner parts of pseudolabia, each with distinct dorsoventral extensions (Figs. 1B, 3A, C, D), partly cover mouth. Four elongate submedian cephalic papillae and pair of lateral amphids present (Figs. 1B, 2A, B, 3A–D). Vestibule (stoma) long, cylindrical, with small funnel-shaped prostom visible in lateral view (Fig. 1C, D). Glandular oesophagus 6–11 times longer than muscular; both parts of oesophagus distinctly separated from each other (Fig. 1A). Nerve ring encircles muscular oesophagus near its anterior end; excretory pore located somewhat posterior to level of nerve ring (Fig. 1A, C, D); deirids small, bifurcated, situated somewhat anterior to end of vestibule (Figs. 1C, D, F, 2D, E).

Male [Based on 2 complete and 2 incomplete specimens; measurements of holotype in parentheses] Length of body 6.54–6.80 (6.80) mm, maximum width 54–66 (54). Height of cephalic protrusions 3 (3). Vestibule including prostom 84–90 (90) long; size of prostom 9 × 9 (-). Length of muscular oesophagus 186–195 (195), maximum width 18 (18); length of glandular oesophagus 1.78–2.00 (1.78) mm, maximum width 27–39 (27); length ratio of muscular and glandular parts of oesophagus 1:9–11 (1:9). Length of entire oesophagus and vestibule represents 30–35% (30%) of total body length. Deirids, nerve ring and excretory pore 81–84 (84), 105–129 (129) and 144–165 (165), respectively, from anterior extremity. Posterior end of body ventrally coiled, provided with narrow caudal alae. Preanal papillae: 4 pairs of subventral pedunculate papillae in groups of 2 present, of which first and second and third and fourth are shifted in relation to each other, alternating along margins of body (Figs. 1L, M, N). Postanal papillae: 6 pairs present, including 5 pairs of pedunculate subventral papillae and 1 pair of small ventral sessile papillae located at level of last pair of subventrals (Figs. 1L–N, 2C, G, 3F). Small subventral phasmids located



posterior to posteriormost pair of subventral papillae (Fig. 1L, 2G). Small ventral median protuberance

present between ventral postanal papillae (Figs. 1L, 2G). Ventral cuticular ridges (area rugosa) anterior to

◀**Fig 1** *Ascarophis justinei* n. sp. from *Thermarces cerberus*. A, Anterior (oesophageal) part of female body, lateral view; B, Cephalic end of female, apical view; C, Anterior end of female, lateral view; D, Anterior end of male, dorsoventral view; E, Anterior end of fourth-stage larva, lateral view; F, Deirid; G, Egg; H, Female tail, lateral view; I, Tail of fourth-stage larva, lateral view; J, Small spicule, lateral view; K, Distal end of large spicule, lateral view; L, M, Posterior end of male, ventral and ventrolateral views, respectively; N, Posterior end of male (another specimen), lateral view

cloaca well developed, consisting of about 5 longitudinal tessellated ornamentations (Figs. 1M, 2F, 3F). Large (left) spicule 396–426 (417) long, with sharply pointed distal tip; shaft of spicule 93–120 (120) long, forming 22–29% (29%) of overall length of spicule (Figs. 1K, M, N). Small (right) spicule boat-shaped, 60–87 (75) long, with rounded distal end (Fig. 1J, M, N). Length ratio of spicules 1:3.48–6.60 (1:3.48). Tail conical, 87–114 (108) long, with rounded tip.

Female [Based on 3 specimens with fully developed eggs; measurements of allotype in parentheses] Length of body 6.87–17.42 (15.79) mm, maximum width 69–84 (75). Height of cephalic protrusions 0 (0). Vestibule distorted (twisted), 108–132 (120) long; size of prostom 3 × 3 (3 × 3). Length of muscular oesophagus 201–249 (201), maximum width 15–21 (18); length of glandular oesophagus 1.32–1.63 (1.44) mm, maximum width 18–27 (24); length ratio of muscular and glandular parts of oesophagus 1:6–7 (1:7). Deirids, nerve ring and excretory pore 105–120 (120), 120–147 (135) and 138–168 (168), respectively, from anterior extremity. Vulva situated 4.81–11.22 (10.79) mm from anterior end of body, at 64–70% (68%) of body length; vulval lips non-elevated or slightly elevated. Vagina directed posteriorly from vulva. Amphidelphic. Uterus filled with numerous eggs. Mature eggs (containing larvae) oval, thick-walled, size 45–48 × 27–30 (45–48 × 27–30) (n=10); thickness of egg wall 3 (3); surface of eggs smooth, without filaments or swellings (Fig. 1G). Tail conical, 54–81 (60) long, with poorly developed, small terminal cuticular knob about 1 (1) high; knob lacking in smallest specimen; 2 minute pore-like phasmids located just anterior to tail tip (Figs. 1H, 3E).

Fourth-stage larva [Based on 1 specimen] Body length 2.95 mm, maximum width 36. Anterior end rounded, with minute anterior protrusion. Vestibule 96 long; prostom indistinct. Length of muscular oesophagus 171, maximum width 12; length of

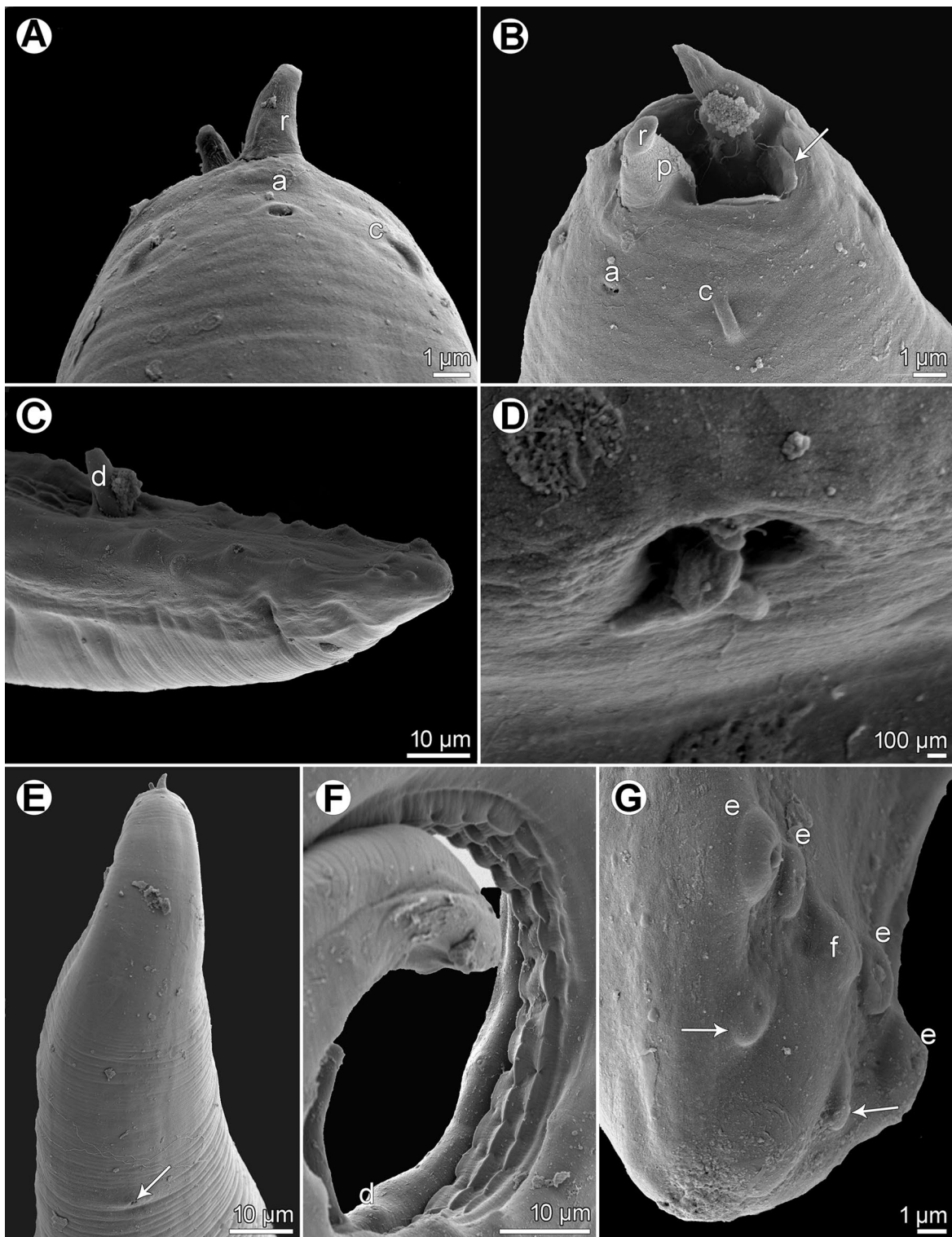
glandular oesophagus 1.22 mm, maximum width 15; length ratio of muscular and glandular parts of oesophagus 1:7. Deirids not found. Nerve ring and excretory pore 120 and 132, respectively, from anterior extremity (Fig. 1E). Small oval rectal glands present. Tail conical, 54 long including terminal projection; tail tip provided with marked terminal elongate projection with rounded end, 12 long and 6 wide (Figs. 1I, 3G).

Remarks

The general morphology of the present nematodes indicates that they belong to the genus *Ascarophis*, as defined by Ko (1986). Based on cephalic structures, Moravec & Justine (2009) recognised three subgenera in this genus: *Ascarophis* van Beneden, 1871 (well-developed pseudolabia with distinct inner dorsoventral extensions; median projections absent), *Dentiascarophis* Moravec & Justine, 2009 (well-developed pseudolabia with distinct inner dorsoventral extensions; mouth with one dorsal and one ventral median projections) and *Similascarophis* Muñoz, González & George-Nascimento, 2004 (pseudolabia considerably reduced, usually without inner extensions; median projections absent).

Ferrer et al. (2005) listed a total of 34 valid species of the genus *Ascarophis*, but two species, *A. adioryx* Machida, 1981 and *A. nasonis* Machida, 1981, were omitted (Machida 1981). However, an additional seven species of *Ascarophis* (*A. carvajali* Muñoz & George-Nascimento, 2007, *A. draconis* Muñoz & George-Nascimento, 2007, *A. richeri* Moravec & Justine, 2007, *A. patagonica* Brugnì & Viozzi, 2008, *A. longiovata* Moravec & Klimpel, 2009, *A. scatophagi* Moravec, Yooyen & Samprick, 2018 and *A. morronei* Aguilar-Aguilar, Ruiz-Campos, Martorelli, Montes & Martínez-Aquino, 2019) were described since (Moravec & Justine, 2007; Muñoz & George-Nascimento, 2007; Brugnì & Viozzi, 2008; Moravec & Klimpel, 2009; Moravec et al, 2018; Aguilar-Aguilar et al., 2019).

Moreover, two species (*S. chilensis* Muñoz, González & George-Nascimento, 2004 and *S. maulensis* Muñoz, González & George-Nascimento, 2004), originally reported in the genus *Similascarophis* Muñoz, González & George-Nascimento, 2004 (= a synonym of *Ascarophis*), were transferred to *Ascarophis* by Moravec & González-Solís (2007) and



Pseudascarophis tropica Solov'eva, 1996 was transferred to this genus by Pereira et al. (2013). However,

the cephalic structure of *Pseudascarophis genypteri* Muñoz & George-Nascimento, 2001 also seems to be

◀**Fig 2** *Ascarophis justinei* n. sp. from *Thermarces cerberus*, SEM micrographs of male. A, B, cephalic end, lateral and sub-apical views, respectively (arrow indicates sublabium); C, Tail, ventrolateral view; D, Deirid; E, Anterior end, lateral view (arrow indicates deirid); F, Ventral precloacal ridges, subventral view; G, Tail tip, ventrolateral view (arrows indicate phasmids). *Abbreviations*: a, amphid; c, cephalic papilla; d, cloacal opening; e, caudal papilla; f, ventral median protuberance; p, pseudolabium; r, anterior pseudolabial projection

very different from that in *P. kyphosi* Ko, Margolis & Machida, 1985, the type-species of *Pseudascarophis* Ko, Margolis & Machida, 1985, and in the only other congeneric species *P. brasiliensis* Pereira, Pereira, Timi & Luque, 2013 (see also Pereira et al., 2013), resembling rather the cephalic structure in *Ascarophis* (*Similascarophis*) spp.; therefore, this species should be also transferred to *Ascarophis* as *A. genypteri* (Muñoz & George-Nascimento, 2001) n. comb. Consequently, at present, 47 species of the genus *Ascarophis*, mostly parasites of marine and brackish-water fishes, are considered to be valid.

The general morphology of the present nematodes from *T. cerberus*, including their cephalic structure, shows that they belong to the nominotypical subgenus *Ascarophis* of the genus *Ascarophis*. According to Ferrer et al. (2005), all species of the genus *Ascarophis* can be divided into four groups based on the character of their fully developed (larvated) eggs: 1) two filaments on a single egg pole, 2) many filaments on a single egg pole, 3) filaments on both egg poles, and 4) filaments absent (i.e., eggs smooth, without any superficial structures). Also all later described species of *Ascarophis* or those later transferred to this genus (see above) can be assigned to these groups.

Of representatives of the genus *Ascarophis*, only the following three species have non-filamented eggs and the length of the large (left) spicule resembling that of the new species (396–426 μm): *A. brasiliensis* Pinto, Vicente & Noronha, 1984 from *Trachinotus carolinus* (Linnaeus) (Carangidae) off the Atlantic coast of Brazil (340–390 μm); *A. crassicolis* Dollfus & Campana-Rouget, 1956 from *Gadus luscus* Linnaeus (Gadidae) from off the Atlantic coast of France (Bay of Biscay) (385 μm); and *A. upenei* Parukhin, 1978 from *Upeneus vittatus* (Forsskal) (Mullidae) in the Indian Ocean near India (Bay of Monar) (450–580 μm) (Dollfus & Campana-Rouget, 1956; Parukhin, 1978; Pinto et al., 1984). It is necessary to note that all these three species were insufficiently

described based solely on LM, so that details of their mouth structure remain unknown; consequently, they cannot be assigned to any subgenus of *Ascarophis* (see above).

In contrast to *A. justinei* n. sp., *A. brasiliensis* possesses distinctly smaller eggs (32–36 \times 18 μm vs 45–48 \times 27–30 μm) and the distal end of its left spicule (erroneously reported as the right spicule) is provided with a membranous dilation (vs sharply pointed, without any membrane). The location of the excretory pore and deirids of *A. brasiliensis* were not described. Both species also differ from each other in the family and order of their fish hosts (Zoarcidae, Perciformes vs Carangidae, Carangiformes) and the region of their occurrence (Pacific Ocean vs Atlantic Ocean).

Ascarophis crassicolis differs from the new species principally in having the cuticle of the cephalic region (between nerve ring and anterior extremity) markedly thickened (inflated) (vs not inflated), the distal end of the left spicule provided with a membranous dilation (vs without any membrane) and the larvigerous eggs distinctly smaller (38 \times 30 μm vs 45–48 \times 27–30 μm); the specimens of *A. crassicolis* are smaller than those of *A. justinei* n. sp. (body length of male and female 4.30–5.12 mm and 6.65–8.00 mm, respectively, vs 6.54–6.80 mm and 6.87–17.42 mm). Both species also differ from each other in the family and order of their fish hosts (Zoarcidae, Perciformes vs Gadidae, Gadiformes) and the region of their occurrence (Pacific Ocean vs Atlantic Ocean).

Ascarophis upenei can be distinguished from the new species mainly by a somewhat longer left spicule (see above) and its distended lanceolate distal tip (vs tip sharply pointed, not distended), distinctly smaller eggs (26–33 \times 24 μm vs 45–48 \times 27–30 μm) and the presence of a conspicuous terminal spike-like projection on the female tail (vs female caudal projection very small, inconspicuous or absent). The location of the excretory pore and deirids of *A. upenei* were not described. In addition to morphological differences, both species also differ in the family and order of their fish hosts (Zoarcidae, Perciformes vs Mullidae, Mulliformes) and the region of their occurrence (Pacific Ocean vs Indian Ocean).

The morphology of the fourth-stage larva of *A. justinei* n. sp. is similar to that of the larva of *A. richeri* from the fish *Hoplichthys citrinus* Gilbert

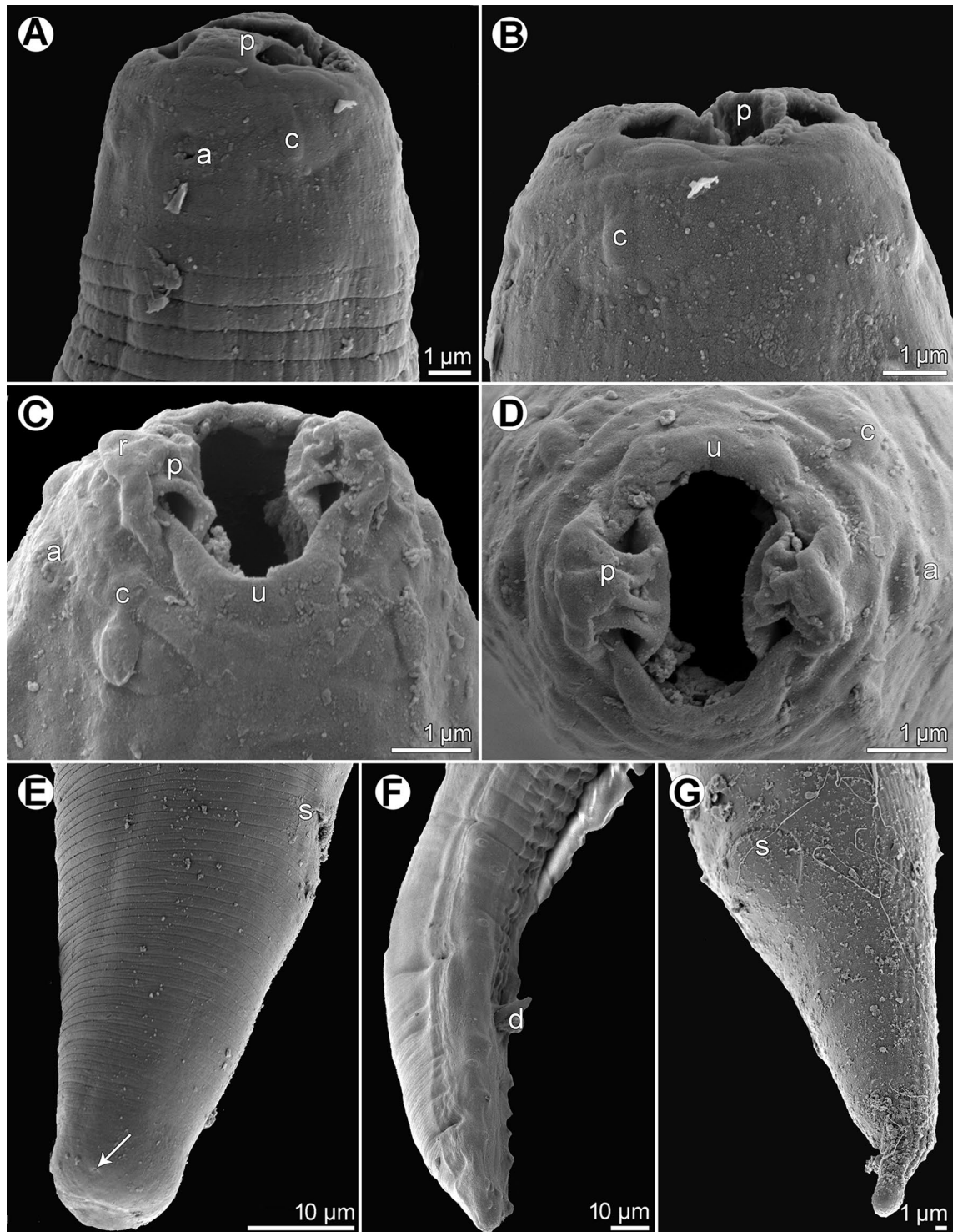


Fig 3 *Ascarophis justinei* n. sp. from *Thermarces cerberus*, SEM micrographs. A, B, Cephalic end of female, sublateral and dorsoventral views, respectively; C, D, Same, dorsoventral and apical views, respectively (another specimen); E, Female tail, lateral view (arrow indicates phasmid); F, Posterior end of

male, ventrolateral view; G, Tail of fourth-stage larva, lateral view. *Abbreviations*: a, amphid; c, cephalic papilla; p, pseudolabium; r, anterior pseudolabial projection; s, anus; u, sublabium

(Hoplichthyidae) in the South Pacific (Moravec & Justine, 2007) or of *Ascarophis* sp. described from the amphipod *Gammarus deubeni* Liljeborg in Canada (Appy & Butterworth, 2011). A characteristic feature of this larval stage is the elongate conical tail with a conspicuously long, rounded terminal projection (Fig. 1I).

Ascarophis globuligera n. sp.

Type-host: *Thermarces cerberus* Rosenblatt & Cohen (Zoarcidae, Perciformes/Zoarcoidea).

Type-locality: East Wall, 9°50'N, Northern East Pacific Rise, 9.8422°N, 104.292°W, depth 2499 m (collected December 14, 2001, HOV *Alvin* dive 3727).

Other localities: (1) Train Station, 9°50'N, Northern East Pacific Rise, 9.8274°N, 104.2892°W, depth 2491 m (collected November 16, 1999, HOV *Alvin* dive 3488); and (2) East Wall, 9°50'N, Northern East Pacific Rise, 9.84187°N, 104.29179°W, depth 2508 m (collected April 27, 1999, HOV *Alvin* dive 3488).

Prevalence and intensity: Not determined.

Type-material: Holotype (male) and allotype (gravid female) mounted on SEM stubs in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS N-1286).

Site in host: Stomach and intestine.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Ascarophis globuligera* n. sp. is “urn:isid:zoobank.org:act:167E7D01-341E-4290-9ACB-8CC52F5A41A7”

Etymology: The species name *globuligera* is the feminine form of the adjective composed of the Latin words *globulus* (= globule, button) and *gerere* (= to bear, carry, wear) and relates to the remarkable eggs of this species bearing a lateral swelling (globule).

Description

General. Small, whitish nematodes. Cuticle thick, with fine transverse striations (Fig. 6A, B, E). Cephalic end rounded, with 2 conspicuous conical pseudolabial terminal protrusions (Figs. 4A, B, F,

6A, C–E), which are considerably reduced or almost absent in largest female (Fig. 7A–C). Oral aperture oval, dorsoventrally elongate (Figs. 1C, 6B, 7A). Submedian labia absent. Four submedian flap-like sublabia well developed (Figs. 1C, 6B, C, 7A–C). Lateral pseudolabia well developed, each provided with conspicuous terminal protrusion (Figs. 4A–C, 6A, C–E), which may be considerably reduced or absent in large females (Fig. 7A–C); in apical view, flat inner parts of pseudolabia, each without dorsoventral extensions, partly cover mouth (Figs. 4C, 6B, 7A). Four elongate submedian cephalic papillae and pair of lateral amphids present (Figs. 4C, F, 5A, 6B, C, D, 7A–C). Vestibule (stoma) long, cylindrical, with small funnel-shaped prostom visible in lateral view (Fig. 4A, B). Glandular oesophagus 4–8 times longer than muscular; both parts of oesophagus distinctly separated from each other (Fig. 4A). Nerve ring encircles muscular oesophagus near its anterior end; excretory pore located somewhat posterior to level of nerve ring (Fig. 4A, B); deirids small, non-bifurcated, situated somewhat anterior to end of vestibule (Figs. 4B, J, 5D, 6E).

Male [Based on 1 specimen, holotype] Length of body 6.96 mm, maximum width 66. Height of cephalic protrusions 3. Vestibule 105 long. Length of muscular oesophagus 233, maximum width 18; length of glandular oesophagus 1.79 mm, maximum width 33; length ratio of muscular and glandular parts of oesophagus 1:8. Length of entire oesophagus and vestibule represents 30% of total body length. Deirids, nerve ring and excretory pore 108, 150 and 204, respectively, from anterior extremity. Posterior end of body ventrally coiled, provided with narrow caudal alae. Preanal papillae: 4 pairs of subventral pedunculate papillae in groups of 2 present, of which first and second and third and fourth are shifted in relation to each other, alternating along margins of body (Figs. 4M, N, 5B, G). Postanal papillae: 6 pairs present, including 5 pairs of pedunculate subventral papillae and 1 pair of minute ventral sessile papillae located at level of last pair of subventrals (Figs. 4M, N, 5B, C). Minute subventral pore-like phasmids just posterior to posteriormost pair of subventral papillae (Figs. 4N, 5C). Ventral cuticular ridges (area rugosa) anterior to cloaca well developed, consisting of about 7 longitudinal tessellated ornamentations (Fig. 5E, F). Large (left) spicule 378 long, with sharply pointed distal tip; shaft of spicule 111 long, forming 29% of

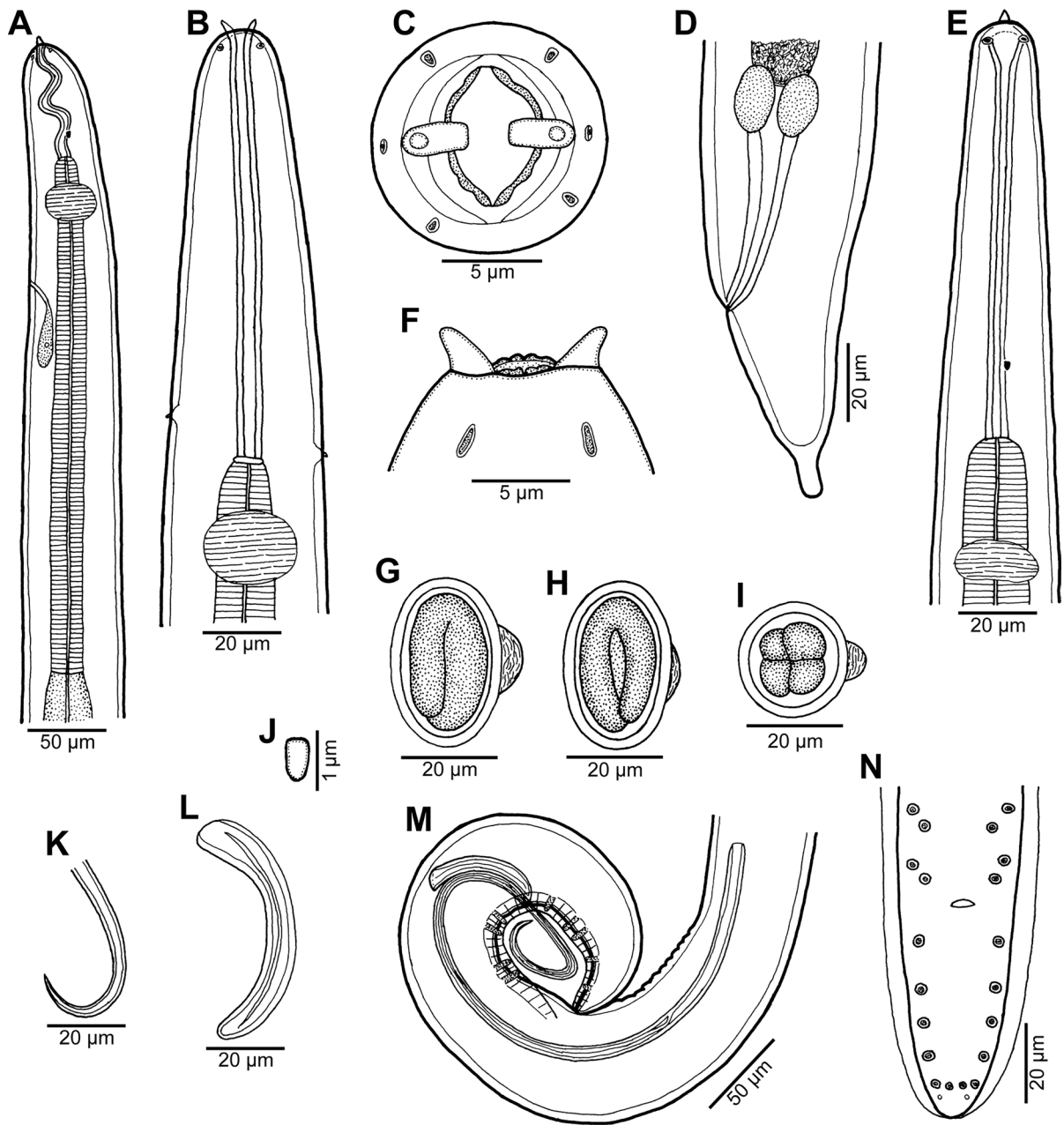


Fig 4 *Ascarophis globuligera* n. sp. from *Thermarces cerberus*. A, Anterior end of female, lateral view; B, Anterior end of male, dorsoventral view; C, Cephalic end of male, apical view; D, E, Caudal end and anterior end, respectively of fourth-stage larva, lateral views; F, Cephalic end of male, dorsoventral view; G, H, Two different larvated eggs, lateral views; I, Larvated egg, apical view; J, Deirid; K, Distal tip of large (left) spicule, lateral view; L, Small (right) spicule, lateral view; M, Posterior end of male, lateral view; N, Distribution of male caudal papillae and phasmids, ventral view

overall length of spicule (Fig. 4K, M). Small (right) spicule boat-shaped, 66 long, with rounded distal end (Fig. 4L, M). Length ratio of spicules 1:5.73. Tail conical, 75 long, with rounded tip.

Female [Based on 1 ovigerous specimen with missing posterior end, allotype, and 1 body fragment (anterior part) of ovigerous specimen, paratype. Measurements of paratype in parentheses] Length of

body fragment 15.57 (10.40) mm, maximum width 93 (95). Height of cephalic protrusions 6 (3). Vestibule distorted (twisted), 81 (129) long; size of prostom (9 × 9). Length of muscular oesophagus 363 (402), maximum width 24 (36); length of glandular oesophagus 2.86 (1.65) mm, maximum width 42 (45); length ratio of muscular and glandular parts of oesophagus 1:8 (1:4). Deirids not located. Nerve ring and excretory pore 126 (192) and 207 (205), respectively, from anterior extremity. Vulva situated 9.63 mm from anterior end of body; vulval lips not elevated. Vagina directed posteriorly from vulva. Amphidelphic. Uterus filled with numerous eggs. Mature eggs (containing larvae) oval, thick-walled, size 39 × 24 (n=10) (36–39 × 21–24, n=10); thickness of egg wall 3 (3); surface of larvated eggs with 1 conspicuous, lateral hemispherical swelling of fibrous structure 6–9 (6–9) high (Figs. 4G–I, 6G), which is lacking in some eggs (Fig. 6F). Caudal end of body missing.

Four-stage larva [Based on 3 body fragments of 1 specimen] Body length not determined, maximum width 69. Height of cephalic protrusions 3. Vestibule including funnel-shaped prostom 111 long; prostom 9 long and 6 wide. Length of muscular oesophagus 225, maximum width 18; length of glandular oesophagus 1.29 mm, maximum width 27; length ratio of muscular and glandular parts of oesophagus 1:6. Deirids, nerve ring and excretory pore 96, 168 and 219, respectively, from anterior extremity (Fig. 4E). Small oval rectal glands present. Tail conical, 144 long, with lateral pair of small phasmids situated at level of anus; tail tip provided with marked terminal elongate projection with rounded end, 12 long and 6 wide (Fig. 4D).

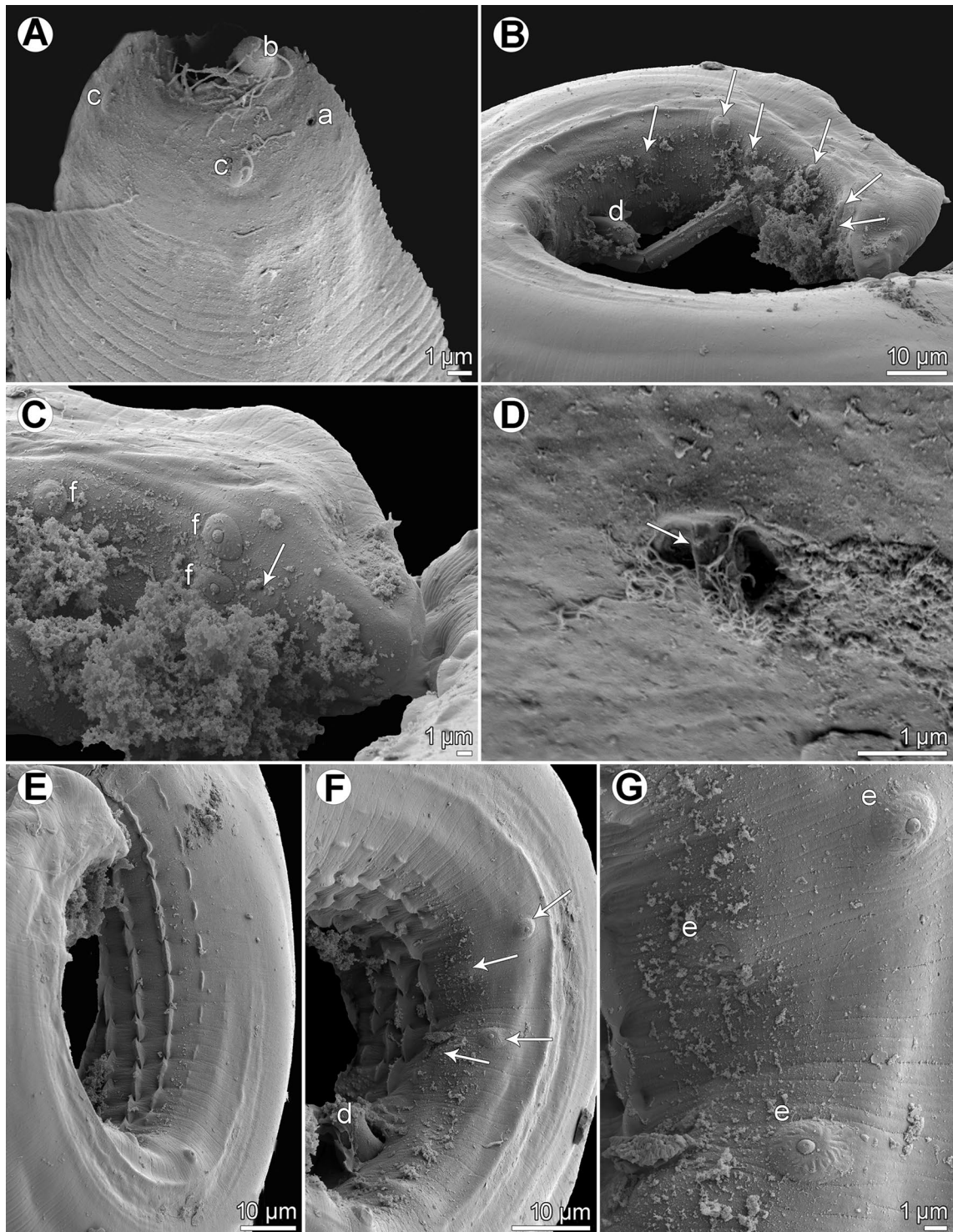
Remarks

In contrast to all known species of *Ascarophis*, the mature eggs of *A. globuligera* **n. sp.** are provided with a single lateral swelling (also called globule or flood), which is a unique feature within this genus. Considering all *Ascarophis*-like nematodes, the presence of a lateral swelling on eggs has so far been reported only for *Mastigospirura cubicipitis* Machida & Syahailatua, 1994, a species described from the intestine of the driftfish *Cubiceps* sp. (Nomeidae, Scombriformes) in Indonesia (Machida & Syahailatua, 1994). The monotypical genus *Mastigospirura* Machida &

Syahailatua, 1994 differs from the genus *Ascarophis* by the considerably different cephalic structure, a markedly short vestibule (similar to that in species of *Neoascarophis* Machida, 1976) and more numerous (14 vs 4) pairs of preanal papillae in the male.

The presence of lateral swellings (floods) on the eggs has also been reported for a few representatives of the cystidicolid genera *Cystidicola* Fischer, 1798 (*C. stigmatura* (Leidy, 1886)) and *Spinitectus* Fourment, 1884 (*S. mucronatus* Boomker & Puylaert, 1994 and *S. mirabilis* Moravec & Nagasawa, 2021), but, in contrast to *A. globuligera* **n. sp.** and *M. cubicipitis*, each egg of these species bears two or four such lateral swellings (Boomker & Puylaert, 1994; Arai & Smith, 2016; Moravec & Nagasawa, 2021); each egg of *S. mirabilis* is provided with both lateral swellings and polar filaments. Mature eggs with four lateral swellings or polar filaments also occur in some species of the spirurine genus *Rhabdochona* Railliet, 1916 (Rhabdochonidae, Thelazioidea) (Moravec, 2010).

In addition to the unusual morphology of the eggs of *A. globuligera* **n. sp.**, the shape of the deirids in this species is also remarkable. Deirids of *Ascarophis* spp. are generally very small and it is impossible to observe their shape under the LM. Mostly based on SEM examinations, the following ten species of *Ascarophis* have so far been found to possess bifurcated deirids: *A. adioryx*, *A. arctica* Polyanskiy, 1952, *A. distorta* Fusco & Overstreet, 1978, *A. longiovata*, *A. morronei*, *A. nasonis*, *A. patagonica*, *A. richeri*, *A. scatophagi* and *A. valentina* Ferrer, Aznar, Balbuena, Kostadinova, Raga & Moravec, 2005 (see Machida, 1981; Ferrer et al., 2005; Moravec & Justine, 2007, 2009; Brugni & Viozzi, 2008; Moravec & Klimpel, 2009; Moravec & Nagasawa, 2018; Moravec et al., 2018; Aguilar-Aguilar et al., 2019; Moravec et al., 2021). Bifurcated deirids are also present in *A. justinei* **n. sp.** (see above). The only exception is *A. mexicana* Moravec, Salgado-Maldonado & Vivas-Rodríguez, 1995, a parasite of *Epinephelus* spp. (Serranidae) in the Gulf of Mexico, whose deirids were found to be non-bifurcated (Moravec & González-Solís, 2007), like those in the present new species. Simple, non-bifurcated deirids were also found in the related cystidicolid *Neoascarophis mariae* Pereira, Timi, Vieira & Luque, 2012, a parasite of *Mullus argentinae* Hubbs & Marini (Mullidae) from off the Atlantic coast of Brazil (Pereira et al., 2012).



The general morphology of *A. globuligera* **n. sp.** is very similar to that of *A. justinei* **n. sp.** (see above) and both these sympatric nematodes parasitise the

same host species *T. cerberus*. However, the former species (*A. globuligera*) differs from the latter in having simple, non-bifurcated deirids (vs deirids

◀**Fig 5** *Ascarophis globuligera* n. sp. from *Thermarces cerberus*, SEM micrographs of male. A, Cephalic end (damaged), subdorsoventral view; B, Caudal end, ventrolateral view (arrows indicate postanal papillae); C, Distal end of tail with postanal papillae of three posteriormost pairs, ventral view (arrow indicates phasmid); D, Deirid (see arrow), lateral view; E, F, Precloacal region with distinct longitudinal cuticular ridges, two different ventrolateral views (arrows indicate preanal papillae); G, Distribution of preanal papillae, sublateral view. *Abbreviations*: a, amphid; b, pseudolabial projection; c, cephalic papilla; d, cloacal opening; e, preanal papilla; f, postanal papilla

bifurcated), distinctly smaller eggs ($36\text{--}39 \times 21\text{--}24 \mu\text{m}$ vs $45\text{--}48 \times 27\text{--}30 \mu\text{m}$) provided with a lateral swelling (vs lateral swelling absent), a slightly shorter left spicule ($378 \mu\text{m}$ vs $396\text{--}426 \mu\text{m}$), and the pseudolabia without dorsoventral extensions (vs pseudolabia with distinct dorsoventral extensions; compare, for example, SEM micrographs Fig. 7A and 3D).

The morphology of the fourth-stage larva of *A. globuligera* n. sp. is similar to that of the larva of *A. justinei* n. sp., as well as that of *A. richeri* or of *Ascarophis* sp. by Appy & Butterworth (2011) (see above).

Ascarophis monofilamentosa sp. n.

Type-species: *Pyrolycus manusanus* Machida & Hashimoto (Zoarcidae, Perciformes/Zoarcoidea).

Type-locality: South Su, Manus Basin (Papua New Guinea), 3.811°S , 152.104°E , depth 1399 m (collected August 4, 2007, Perry Slingby TS 200 dive 24).

Type-material: Holotype (male), allotype (gravid female) and 2 paratypes (females) mounted on SEM stubs in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic (IPCAS N-1287).

Prevalence and intensity: Not determined.

Site in host: Large intestine.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Ascarophis monofilamentosa* n. sp. is “urn:isid:zoobank.org:act:6EF136CF-62DF-4083-8621-C0CC9AAF4978”

Etymology: The species name *monofilamentosa* is the feminine form of the adjective composed of the Latin prefix *mono-* (= mono) and the adjective *filamentosa* (= with filaments, filamented) and relates to the characteristic eggs of this species bearing a single pole filament.

Description

General: Small, whitish nematodes. Cuticle thick, with fine transverse striations. Cephalic end rounded, with two conspicuous, rounded pseudolabial terminal protrusions (Figs. 8A–C, E, 9A–C). Oral aperture oval, dorsoventrally elongate, with lateral extensions (Figs. 8D, 9B, C). Four submedian labia and 4 submedian flap-like sublabia well developed. Lateral pseudolabia well developed, each provided with rounded terminal protrusion; in apical view, flat inner parts of pseudolabia partly cover mouth and are distinctly dorsoventrally expanded, forming 2 (1 dorso-lateral and 1 ventrolateral) extensions on each; inner margins of both pseudolabia dorsoventrally straight, parallel to each other (Figs. 8D, 9B). Four elongate submedian cephalic papillae and pair of lateral amphids present (Figs. 8D, 9A, C). Vestibule (stoma) long, cylindrical, with well-developed funnel-shaped prostom in lateral view (Fig. 8A–C). Glandular oesophagus 4–6 times longer than muscular; both parts of oesophagus distinctly separated from each other (Fig. 8B, C). Nerve ring encircles muscular oesophagus approximately at border of its first and second sixths; excretory pore located somewhat anterior to level of mid-length of muscular oesophagus; deirids very small (their shape could not be determined by SEM), situated somewhat anterior to end of vestibule (Fig. 8A).

Male [Based on 1 specimen, holotype] Length of body 8.41 mm, maximum width 87. Height of cephalic protrusions 3. Vestibule including prostom 153 long; prostom 9 long and 7 wide. Length of muscular oesophagus 279, maximum width 21; length of glandular oesophagus 1.48 mm, maximum width 42; length ratio of muscular and glandular parts of oesophagus 1: 5. Length of entire oesophagus and vestibule represents 23% of total body length. Deirids, nerve ring and excretory pore 138, 207 and 291, respectively, from anterior extremity. Posterior end of body ventrally curved, provided with narrow caudal alae. Preanal papillae: 4 pairs of subventral

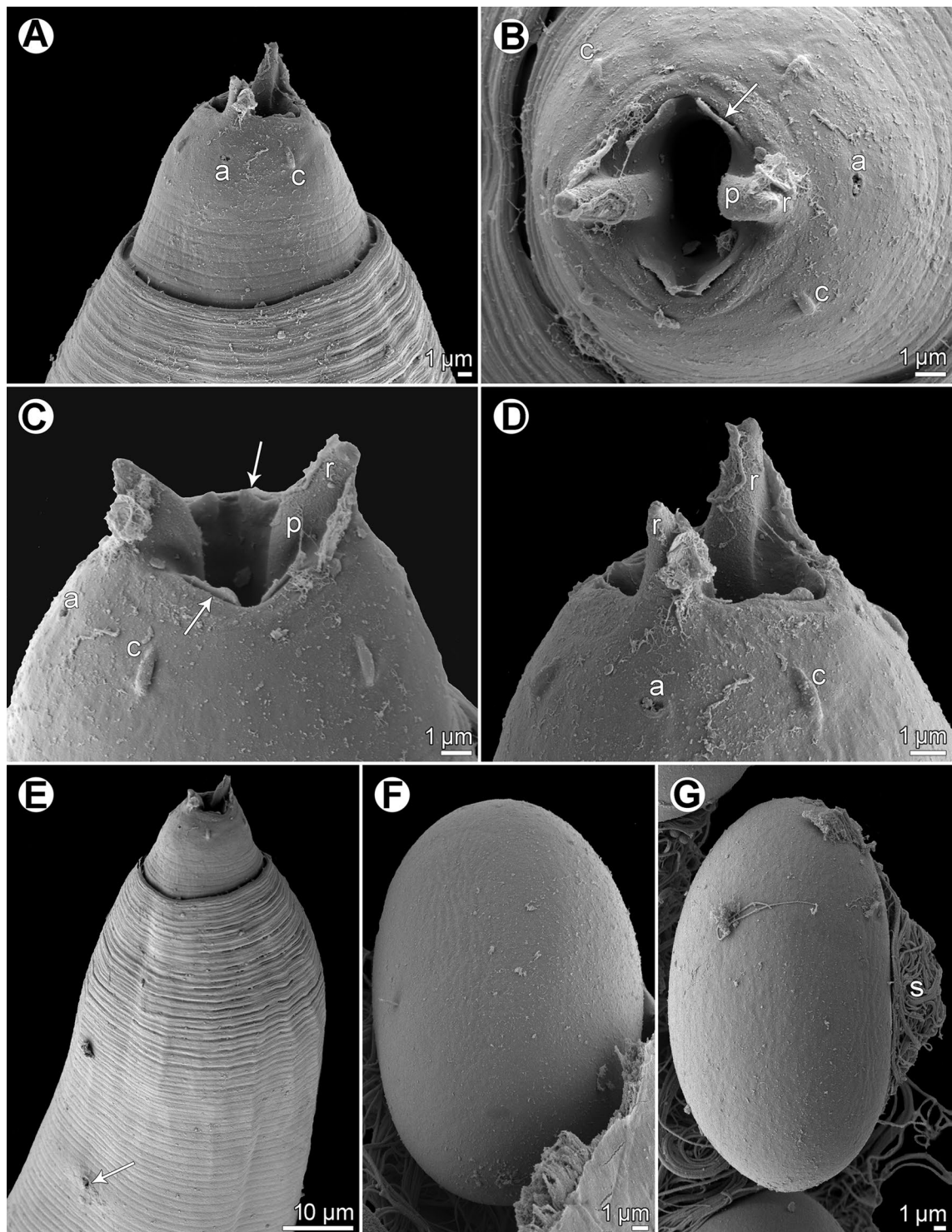


Fig 6 *Ascarophis globuligera* n. sp. from *Thermarces cerberus*, scanning electron micrographs of female. A, Anterior end, dorsoventral view; B, C, D, Cephalic end, apical, dors-oventral and sublateral views, respectively (arrows indicate sublabia); E, Anterior end of body, sublateral view (arrow

indicates deirid); F, Mature egg without lateral swelling; G, Mature egg bearing lateral swelling. *Abbreviations:* a, amphid; c, cephalic papilla; p, pseudolabium; r, anterior pseudolabial projection; s, lateral swelling

pedunculate papillae in groups of 2 present, of which first and second and third and fourth are shifted in relation to each other, alternating along margins of body (Figs. 8J, M, 9E–G). Postanal papillae: 6 pairs present, including 5 pairs of pedunculate subventral papillae and 1 pair of ventral sessile papillae located at level of last pair of subventrals (Figs. 8J, M). Small pore-like subventral phasmids located posterior to posteriormost pair of subventral papillae (Fig. 8M). Ventral cuticular ridges (area rugosa) anterior to cloaca well developed, consisting of 6 longitudinal tessellated ornamentations (Fig. 9E–G). Large (left) spicule 434 long, with narrowed, sharply pointed distal tip; shaft of spicule 132 long, forming 30% of overall length of spicule (Figs. 8K, J, 9>D). Small (right) spicule boat-shaped, 93 long, with somewhat narrowed and rounded distal end (Fig. 8L, J). Length ratio of spicules 1:4.67. Tail conical, 123 long, with rounded tip (Figs. 4J, M).

Female [Based on 2 complete and 1 incomplete ovigerous specimens; measurements of allotype in parentheses] Length of body 15.71–17.27 (17.27) mm, maximum width 111–126 (126). Height of cephalic protrusions 3–6. Vestibule including prostom 171–183 (183) long; prostom 12–15 (12) long and 12–15 (12) wide. Length of muscular oesophagus 330–345 (330), maximum width 21–24 (24); length of glandular oesophagus 1.46–1.86 (1.86) mm, maximum width 36–48 (48); length ratio of muscular

and glandular parts of oesophagus 1:4–6 (1:6). Length of entire oesophagus and vestibule represents 13–14% (14%) of total body length. Amphidelphic. Vulva postequatorial, situated 8.89–10.54 (10.54) mm from anterior end of body, at 57–61% (61%) of body length; vulval lips not elevated. Vagina directed posteriorly from vulva (Fig. 8G). Uterus filled with numerous eggs. Mature eggs (containing larvae) elongate-oval, thick-walled, size 45–48 × 27–30 (48 × 27) (n=10); thickness of egg wall 3 (3). One pole of eggs bears markedly large knob provided with 1 very long (about 680) thread-like filament of fibrous structure; opposite egg pole with minute protuberance without filament (Figs. 8H, F, 10C). Anterior ovary extending anteriorly to short distance posterior to posterior end of oesophagus, posterior ovary reaching posteriorly to rectum. Tail conical, rounded, 69–72 (72) long, with hemispherical terminal knob; knob 3 (3) long, 6–9 (9) wide (Figs. 8I, 10A, B).

Remarks

As in the previous species, the morphology of the present nematodes from *P. manusanus* shows that they belong to the nominotypical subgenus *Ascarophis* of the genus *Ascarophis*. It has already been mentioned that Ferrer et al. (2005), based on the morphology of fully mature (larvated) eggs, divided all species of

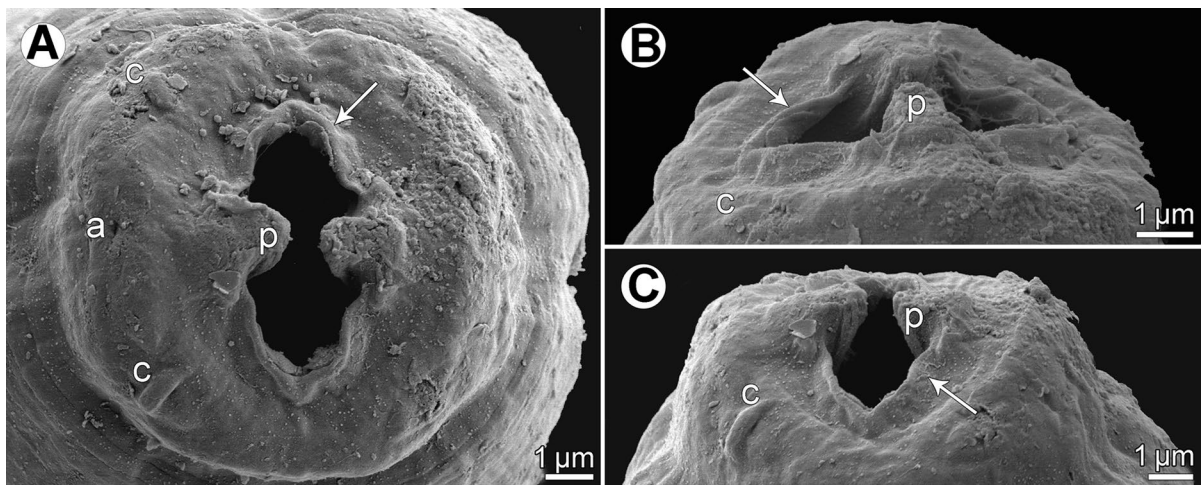
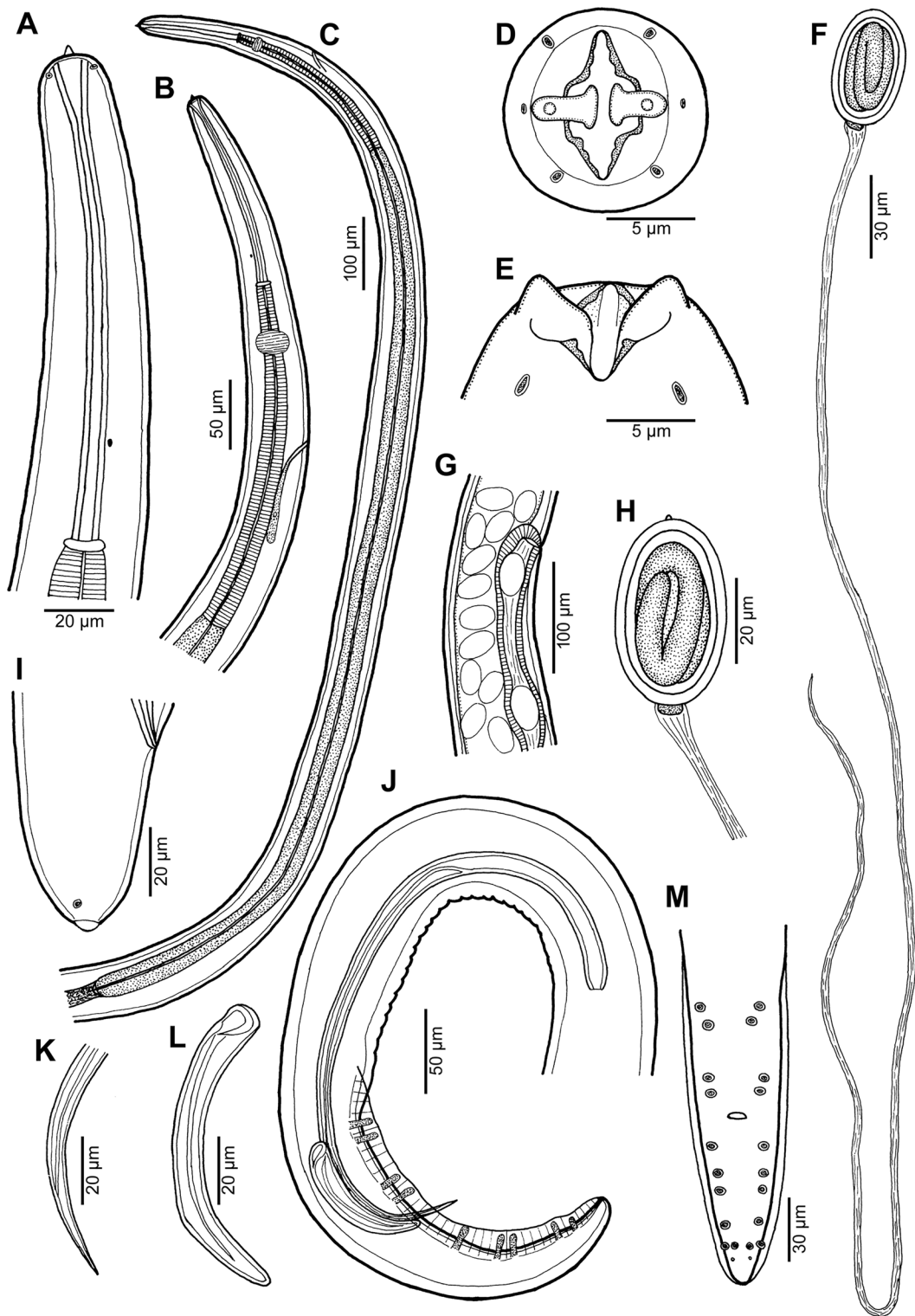


Fig 7 *Ascarophis globuligera* n. sp. from *Thermarces cerberus*, SEM micrographs of female cephalic end (larger specimen) (arrows indicate sublabia). A, B, C, Apical, lateral and

dorsoventral views, respectively, of same specimen. *Abbreviations:* a, amphid; c, cephalic papilla; p, pseudolabium



this genus into four groups (see above), to which also all later described congeneric species or those later

transferred to *Ascarophis* from other genera can be assigned. However, all species in these four groups

◀**Fig 8** *Ascarophis monofilamentosa* **n. sp.** from *Pyrolycus manusanus*. A, B, Anterior end of male (different magnifications), lateral views; C, Anterior (oesophageal) part of male body, lateral view; D, E, Cephalic end of female, apical and dorsoventral views, respectively; F, Larvated egg with polar filament; G, Region of vulva, lateral view; H, Larvated egg (higher magnification); I, Tail of female, lateral view; J, Posterior end of male, lateral view; K, Distal tip of large (left) spicule, lateral view; L, Small (right) spicule, lateral view; M, Distribution of male caudal papillae and phasmids, ventral view

are characterised either by smooth eggs without filaments or with two or many filaments on one egg pole, or many filaments on both egg poles. In contrast, the eggs of *A. monofilamentosa* **n. sp.** are provided with only one, very long filament on one pole, which is a unique feature within all *Ascarophis* spp. In addition to the remarkable egg morphology, the new species can be separated from the numerous congeneric species also by some other morphological features such as cephalic structure, the lengths and structure of spicules, the shape and location of deirids, etc.

Discussion

All new species of *Ascarophis*, *A. justinei* **n. sp.**, *A. globuligera* **n. sp.** and *A. monofilamentosa* **n. sp.**, are parasites of fishes of the family Zoarcidae. To date, there are only four other species of this genus reported as parasites of zoarcid fishes: *Ascarophis arctica* was inadequately described by Polyanskiy (1952) from the stomach of *Zoarces viviparus* (Linnaeus) in the Barents Sea (in the Baltic Sea also misidentified as *A. skryabini* (Layman, 1933) or *A. longispicula* Zhukov, 1960 – see Fagerholm & Berland, 1988); subsequently it was reported from different marine, mostly gadiform and scorpaeniform fishes in the North Atlantic region (Canada, Europe) and from the brackish-water gasterosteid *Gasterosteus aculeatus* Linnaeus in Japan (Appy, 1981; Fagerholm & Berland, 1988; Moravec & Nagasawa, 2018). *Ascarophis litoralica* Zhukov, 1960 was described by Zhukov (1960) from *Pholis picta* (Kner) (Pholidae), *Pholidapus dybowskii* (Steindachner) (Opisthocentridae) and *Zoarces elongatus* Kner (Zoarcidae) (all Perciformes/Zoarcoidei) in the South-Kurile shallow waters. *Ascarophis skryabini* (Layman, 1933), reported by Skryabin et al. (1967) from different fish species including *Z. viviparus* in eastern Russia (basins of the Sea of

Japan and the Ochotsk Sea), was designated a *species inquirenda* by Zhukov (1960). *Ascarophis carvajali* Muñoz & George-Nascimento, 2007 is an intestinal parasite of *Patagonotothen cornucola* (Richardson) (Notothentidae) (type-host) and *Austrolycus depressiceps* Regan (Zoarcidae) in the South Pacific, off Chile (Muñoz & George-Nascimento, 2007).

All the three valid species of *Ascarophis* reported from zoarcid fishes, i.e. *A. arctica*, *A. carvajali* and *A. litoralica*, have filamented eggs, but the eggs of *A. carvajali* and *A. litoralica* have two filaments on each pole (Zhukov, 1960; Muñoz & George-Nascimento, 2007), whereas those of *A. arctica* are provided by two to four thick filaments and seven to fourteen thin filaments on each pole (Appy, 1981; Fagerholm & Berland, 1988). On the contrary, the eggs of the two newly described congeneric species are characterised by features (presence of a single, very long filament on one egg pole in *A. monofilamentosa* **n. sp.** and a lateral swelling in *A. globuligera* **n. sp.**) that are unique within all species of the genus *Ascarophis*. This indicates that these two parasites are probably endemic to the respective hydrothermal vents, similarly as their fish hosts, and the same may be with the third species, *A. justinei* **n. sp.**

As mentioned above, the only *Ascarophis*-like nematode previously reported from a fish host endemic to hydrothermal deep-sea vents is *Moravecnema segonzaci* (see Justine et al., 2002). The main differences of the monotypical genus *Moravecnema* Justine, Cassone & Petter, 2002 from *Ascarophis* are the presence of rounded rudimentary pseudolabia and four submedian buccal processes on the margin of hexagonal oral opening, and the absence of area rugosa in the male. Unfortunately, cephalic structures of *M. segonzaci* were not studied by SEM. The eggs of *M. segonzaci* are provided with one to five thin filaments on each pole, by which this nematode also distinctly differs from the three newly described species of *Ascarophis*.

Some species of *Ascarophis* are reported from hosts belonging to different fish families and orders (e.g., Ferrer et al., 2005), thus appearing to show a low degree of host specificity. However, the morphology of these nematodes is mostly insufficiently known and molecular data on *Ascarophis* spp. are still rare (Černotíková et al., 2011; Choudhury & Nadler, 2018). Therefore, it cannot be excluded that at least some of such species include, in fact, more than one

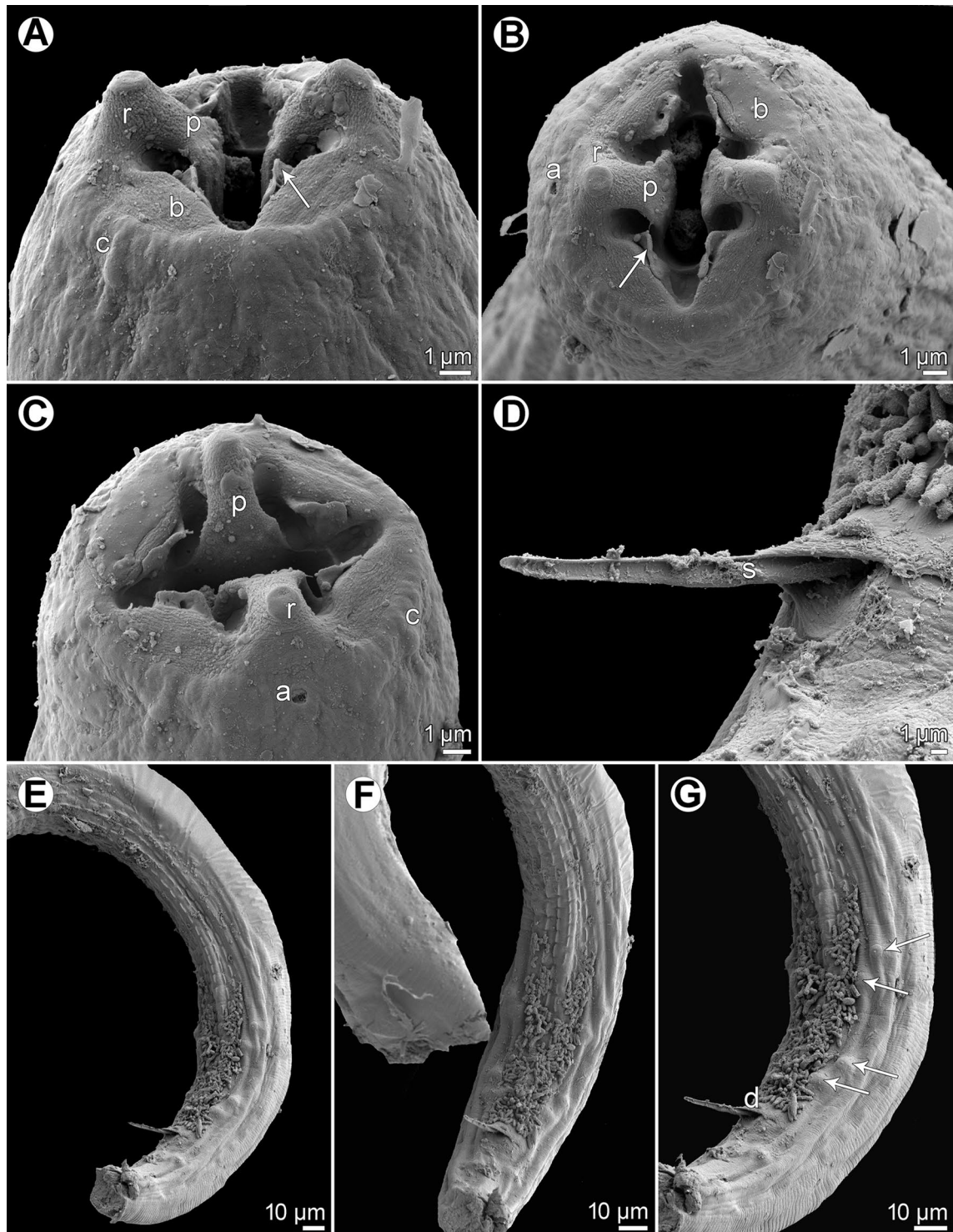


Fig 9 *Ascarophis monofilamentosa* n. sp. from *Pyrolycus manusanus*, SEM micrographs. A, B, C, Cephalic end of female, dorsoventral, apical and lateroapical views, respectively (arrows indicate sublabia); D, Distal end of longer (left) spicule, lateral view; E, F, Posterior end of male (distal end of

tail missing), ventrolateral and ventral views, respectively; G, Precloacal region (enlarged), ventrolateral view (arrows indicate preanal papillae). *Abbreviations*: a, amphid; b, submedian labium; c, cephalic papilla; d, cloaca; p, pseudolabium; r, pseudolabial projection; s, spicule

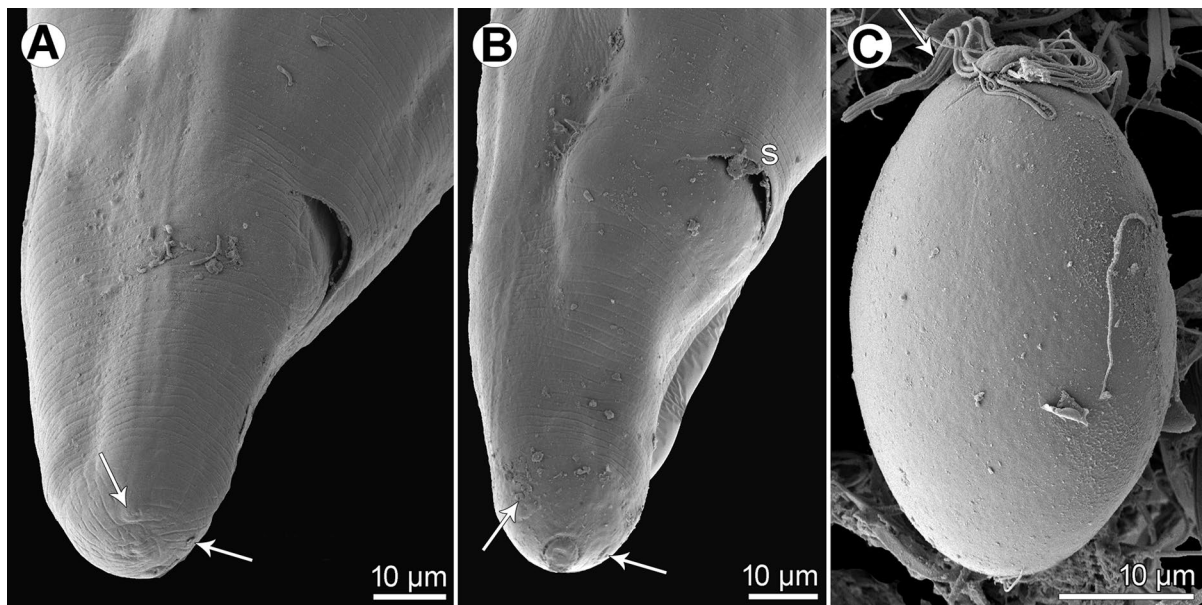


Fig 10 *Ascarophis monofilamentosa* **n. sp.** from *Pyrolycus manusanus*, SEM micrographs of female. A, B, Tail of specimen with indistinct knob at tip and of another specimen with

knob at tip, respectively, ventrolateral views (arrows indicate phasmids); C Mature egg (arrow indicates single thick polar filament)

species of *Ascarophis*. Moreover, it is also necessary to take into account that some fish species need not be the true definitive hosts of these nematodes as defined by Odening (1976), but they may probably serve as only facultative hosts (e.g., pardefinitive hosts) or the nematodes are only passaged through the fish digestive tract after the ingestion of the crustacean intermediate host. Species of *Ascarophis* are known to often exhibit precocious development in the body of the intermediate host, in which the nematodes can attain their full maturity (extreme precocity) (Anderson, 2000; Appy & Butterworth, 2011). Accordingly, the host specificity of species of *Ascarophis* is probably not as wide as believed.

The present paper shows that *T. cerberus* is the host of two species of *Ascarophis* and that *T. hollii* shares one (*A. justinei* **n. sp.**) of these species. Comparison of the data on prevalence and intensity indicates that *T. cerberus* is the primary host in the localities under investigation, but it might be different in other localities, because the presence of a parasite in the host is conditioned by many local abiotic and biotic factors. Moreover, *Ascarophis* spp. are known to frequently exhibit precocious development in their crustacean intermediate hosts, so that they may attain

full maturity in them (see below); if fish eats such a crustacean, it acquires the infection of already mature nematodes, so that, in fact, it plays a role of postcyclic (instead of definitive) host.

Species of *Ascarophis*, like other cystidicolid nematodes from marine fishes are known to generally use amphipod and decapod crustaceans as intermediate hosts in the body cavity of which their infective third-stage larvae develop (Anderson, 2000; Martorelly et al., 2000). It is likely that locally present crustaceans play a role in the life cycles of the three newly described species of *Ascarophis* and the same is presupposed for *M. segonzaci* (see Justine et al., 2002). This is supported by the fact that, for example, the stomach contents of *P. manusanus*, the type-host of *A. monofilamentosa* **n. sp.**, were reported to include bresiliid shrimps (Decapoda: Caridea) (Froese & Pauly, 2023).

To date, nobody has experimentally studied the complete life cycle and development of a species of *Ascarophis* parasitising a fish host; a heteroxenous life cycle in *Ascarophis* spp. was only deduced from the records of infective larvae in crustaceans and experimental studies on other cystidicolid nematodes (Fagerholm & Butterworth, 1988; Appy &

Butterworth, 2011). Based on frequent finds of *Ascarophis* fourth-stage larvae and adults in naturally infected crustacean intermediate hosts, *Ascarophis* spp. are considered to be capable of a precocious development in the invertebrate host (see above). Appy & Butterworth (2011) experimentally studied the complete life cycle and development of *Ascarophis* sp. parasitising the amphipod *Gammarus deubeni* Liljeborg in Canada. Depending on the water temperature, they obtained the nematode gravid females with larvigerous eggs in 69–80 days post infection. The authors speculate that a monoxenous development of this nematode is possible. Undoubtedly, the three present nematode species have heteroxenous life cycles, representing thus another example of the successful invasion of deep-sea hydrothermal vents, a patchy, ephemeral habitat, by parasites requiring multiple host species.

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Author contributions Lauren N. Dykman and Deiric B. Davis obtained the samples and undertook the processing for morphological identification of the nematodes. František Moravec carried out the identification and morphological analysis and drafted the manuscript. All authors read and approved the final manuscript.

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Data Availability All samples used in this study have been deposited in the relevant curated, internationally recognised museum collection as outlined in this paper.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that there is no conflict of interest.

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

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