



Nematodes from the coral reefs in Nha Trang, Khanh Hoa Province, Vietnam: *Rhinema bimorpha* sp. nov. (Nematoda, Monoposthiidae)

NGUYEN THI THU^{1,5,7}, NGUYEN THI XUAN PHUONG^{2,5,8}, PHAN KE LONG^{3,5,9}, NGUYEN THI ANH DUONG^{2,5,10}, NGUYEN NGOC ANH THU^{4,11}, TRAN THI VIET THANH^{3,12}, NGUYEN DINH TU^{2,5,13} & OLEKSANDR HOLOVACHOV^{6,14,*}

¹Center for High Technology Research and Development, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do Ward, Hanoi, Vietnam.

²Institute of Biology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do Ward, Hanoi, Vietnam.

³Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do Ward, Hanoi, Vietnam.

⁴Institute of Oceanography, Vietnam Academy of Science and Technology, 01 Cau Da Street, Nha Trang Ward, Khanh Hoa Province.

⁵Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do Ward, Hanoi, Vietnam.

⁶Department of Zoology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden.

⁷✉ nminhthu02@gmail.com; <https://orcid.org/0009-0004-7803-3133>

⁸✉ ntxphuong.iebr@gmail.com; <https://orcid.org/0000-0001-8911-014X>

⁹✉ pklong@vnmn.vast.vn; <https://orcid.org/0000-0003-4998-260X>

¹⁰✉ nad2807@gmail.com; <https://orcid.org/0000-0003-3647-6724>

¹¹✉ anhthu659144@gmail.com; <https://orcid.org/0009-0006-5643-3157>

¹²✉ thanh@vnmn.vast.vn; <https://orcid.org/0000-0002-2381-5873>

¹³✉ ngdtu@yahoo.com; <https://orcid.org/0000-0002-1829-3636>

¹⁴✉ oleksandr.holovachov@nrm.se; <https://orcid.org/0000-0002-4285-0754>

*Corresponding author

Abstract

A new species, *Rhinema bimorpha* sp. nov. is described from the coral reefs in Nha Trang, Vietnam. It is characterized by a coarsely annulated cuticle with 12 longitudinal ridges, of which middorsal and midventral extends only along the anterior part of the body; cephalic sensilla equal to 0.2–0.6 labial region diameters in length; amphideal fovea circular and occupying nearly the entire height of unstriated helmet and 0.4–0.5 of the corresponding body diameter; subventral cuticular ridges in males form bulges about 1.7–2.9 corresponding body diameters anterior to cloaca; spicules arcuate, with poorly developed manubrium and subcylindrical shaft, equal to 1.6–2.1 cloacal body diameters in length; six pairs (one pre-cloacal and five post-cloacal) of posterior setiform copulatory sensilla. The new species differs from *R. retrorsum* in having relatively larger amphids and shorter helmet (occupying the height of helmet vs. half of the height) and larger number of posterior setiform sensilla in male (one pre-cloacal and 5 post-cloacal pairs vs. 3–4 post-cloacal pairs), and from *R. longispicula* in having shorter spicules (43–82 µm vs. 120–138 µm) and larger number of posterior setiform sensilla in male (one pre-cloacal and 5 post-cloacal vs. one pre-cloacal and 3 post-cloacal pairs).

Key words: Morphology, new species, South China Sea, taxonomy, Vietnam

Introduction

Rhinema Cobb, 1920 is a small genus within itself small family Monoposthiidae Filipjev, 1934, including only three known species (Cobb 1920, Tchesunov 2014, Timm 1961, Zhai *et al.* 2020). Type species of the genus, *Rhinema retrorsum* Cobb, 1920 was originally described from the New Hebrides (now called Vanuatu) in the South Pacific Ocean (Cobb 1920) and subsequently rediscovered from the Maldive Islands in the Eastern Arabian Sea of the Northern Indian Ocean (Gerlach 1963); both descriptions included individuals of both sexes. The second species, *Rhinema exquisitum* Timm, 1961 was described from the Bay of Bengal of the Northern Indian Ocean (Timm,

1961) based on a single immature female individual. The third and last species, *Rhinema longispicula* Zhai, Huang & Huang, 2020, was described from the South China Sea, based on individuals of both sexes as well. Besides these four records, unidentified populations of *Rhinema* were recorded along the coast of Zanzibar, Western Indian Ocean (Raes *et al.* 2007) and Brazil, Western South Atlantic Ocean (Venekey *et al.* 2010). None of these recent publications included any molecular data for any of the species. In this paper we present a description of a new species of *Rhinema*, collected in the coral reefs of Nha Trang, South China Sea, including first for the genus 18S rRNA sequence data.

Material and Methods

Free-living nematodes associated with coral reef habitats were collected in Nha Trang Bay, Khanh Hoa Province, central Vietnam, in 2024. The sampling sites were located within coral reef areas dominated by scleractinian corals, including *Acropora cytherea* (Dana, 1846), *Acropora robusta* (Dana, 1846), *Pachyseris rugosa* (Lamarck, 1801), *Pachyseris speciosa* (Dana, 1846), *Echinopora lamellosa* (Esper, 1791) and *Echinopora gemmacea* (Lamarck, 1816).

Depending on depth (ranging from 4 to 16 m) and substrate characteristics, sediments were taken by professional divers using a Perspex hand corer, or a combination of a Ponar grab and Perspex hand corer. Coral gravel was carefully scooped, and coral fragments were manually selected.

For morphological study, samples were fixed in hot 10% formalin (60–70°C). Nematodes were extracted by decantation and centrifugation using Ludox HS40 (d = 1.18) following Vincx (1996), hand-picked, and transferred to glycerine using the method of De Grisse (1969). Permanent mounts were prepared in glycerol and sealed with beeswax according to Seinhorst (1959). Specimens were examined and measured using a motorized optical microscope BX63 (Olympus, Japan) equipped with a digital camera and the CS-DI image stacking software.

Samples for molecular work were fixed in DESS solution (Yoder *et al.* 2006) in the field, and extracted nematodes were likewise preserved in DESS. Specimens selected for molecular analysis were morphologically examined and documented prior to DNA extraction. Individual nematodes were washed in distilled water, temporarily mounted for imaging, and preserved in Worm Lysis Buffer (50 mM KCl; 10 mM Tris, pH 8.3; 2.5 mM MgCl₂; 0.45% NP-40; 0.45% Tween 20) at -20°C. DNA extraction followed proteinase K digestion (65°C for 1 h; 95°C for 10 min). The ~900 bp F04 region (Creer *et al.* 2010) of the 18S rDNA was amplified using primers G18S4 (5'-GCTTGTCTCAAAGATTAAGCC-3') and 4R (5'-GTATCTGATCGCCKTCGAWC-3') under standard PCR conditions (94°C for 5 min; 39 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 1 min; final extension at 72°C for 10 min). PCR products were visualized on 1% agarose gel stained with RedSafe™. Bidirectional Sanger sequencing was performed using the BigDye® Terminator v3.1 (1st BASE, Malaysia). Sequence quality was checked using Chromas 2.6.6.

Systematics

Order Desmodorida De Coninck, 1965

Family Monoposthiidae Filipjev, 1934

Genus *Rhinema* Cobb, 1920

Type species: *Rhinema retrorsum* Cobb, 1920

Other species: *Rhinema longispicula* Zhai, Huang & Huang, 2020
Rhinema bimorpha **sp. nov.**

Species inquirenda: *Rhinema exquisitum* Timm, 1961

Genus diagnosis (modified after Tchesunov 2014): Monoposthiidae. Cuticle thick and coarsely annulated; annules overlap one another slightly, resembling tiles; the annules of the anterior body have anteriorly directed margins, whereas at some point between pharynx and anterior midgut the direction of margins changes posteriorly. Longitudinal ornamentation as 12 rows of notches extends throughout the entire body or only along pharyngeal

region, Λ -shaped along the anterior end and gradually changing along the body to become V-shaped along the posterior end. Anteriormost part of the cuticle forming a helmet. Anterior sensilla pattern 6+6+4, both inner and outer labial sensilla short papilliform, located on the anterior surface of the labial region, cephalic sensilla setiform, located on the edge of the labial region. Amphideal fovea circular, located on the cephalic capsule. Cheilostom with six pairs of refractive longitudinal ribs. Gymnostom with large dorsal tooth and small denticles. Stegostom cylindrical. Pharynx muscularised, with elongate-ovoid basal bulb devoid of grinder. Ovaries antidromously reflexed, paired and opposed. Testes paired, opposed. Supplements absent. Copulatory sensilla setiform, arranged in subventral and subdorsal pairs in rows. Copulatory apparatus consists of well developed, paired and symmetrical spicules and gubernaculum. Tail conical, caudal glands and spinneret present.

***Rhinema bimorpha* sp. nov.**

(Figures 1–6, Tables 1–2)

Type material. One holotype male (L3/5), two paratype males (L2/1&L2/1) and four paratype females (L2/4, L2/8, L2/13&L2/13) on slides # SMNH-Type 10273 – SMNH-Type 10277 deposited in the invertebrate collection of the Department of Zoology, Swedish Museum of Natural History, Stockholm, Sweden. Three paratype males (CT1.1[SL19], D2.1[SL19], D2.1[SL16] on slides VNMN.0279, VNMN.0284 and VNMN.0283, and three paratype females CT2.4[SL24], D1.4[SL1], D1.1[SL1]) on slides VNMN.0280, VNMN.0282 and VNMN.0281 deposited in the collection of the Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

TABLE 1. Morphometrics of males and females of *Rhinema bimorpha* sp. nov. (in μm except for ratios a, b, c, c', T and V)

	holotype male	paratype male with long spicules	four paratype males with short spicules	seven paratype females
Body length	752	891	665±29 (638–706)	737±129 (600–945)
Body diameter	50	48	37±3 (35–41)	50±12 (36–70)
Pharynx length	153	174	141±5 (135–147)	160±20 (133–180)
Tail length	76	100	72±3 (69–75)	69±11 (54–84)
Cloacal or anal body diameter	35	38	27±3 (24–30)	24±3 (20–28)
a	15.1	18.7	17.9±1.7 (15.4–18.8)	15.0±1.1 (13.4–16.8)
b	4.9	4.9	4.7±0.1 (4.5–4.8)	4.6±0.4 (4.2–5.3)
c	9.9	8.9	9.3±0.1 (9.1–9.4)	10.7±1.1 (9.3–12.2)
c'	2.2	2.6	2.7±0.2 (2.5–2.9)	2.9±0.5 (2.4–3.8)
T (%) or V (%)	66.1	74.7	64.2±2.5 (61.5–66.4)	53.7±3.0 (51.4–60.4)
Labial region diameter (widest)	15.0	19.0	12.1±0.8 (12.0–13.0)	14.7±1.9 (12.0–17.0)
Body diameter at middle of amphid	18.5	21.0	16.3±0.7 (16.0–17.5)	19.1±2.0 (15.5–21.5)
Helmet length	14.5	13.5	13.8±0.6 (13.0–14.5)	13.5±2.9 (10.0–17.0)
Helmet base diameter	23.0	24.5	21.1±3.6 (18.0–26.0)	22.0±2.0 (19.0–24.0)
Inner labial sensilla length	2.0	2.0	1.5	1.6±0.3 (1.5–2.0)
Outer labial sensilla length	2.0	2.0	1.5	1.8±0.5 (1.5–3.0)
Cephalic sensilla length	5.5	6.0	6.1±1.2 (5.5–8.0)	4.8±1.2 (3.0–6.0)
Amphideal fovea width	9.0	9.5	8.9±0.4 (8.0–9.0)	8.6±0.8 (7.5–9.5)

.....continued on the next page

TABLE 1. (Continued)

	holotype male	paratype male with long spicules	four paratype males with short spicules	seven paratype females
Amphid anterior edge from anterior end	3.5	5.0	3.7±0.2 (3.5–4.0)	4.4±1.7 (2.0–7.0)
Buccal cavity (stoma) length	34	35	28±2 (25–30)	33±5 (25–37)
Cheilostom length	4.0	3.5	3.6±0.3 (3.5–4.0)	4.2±0.7 (3.0–5.0)
Gymnostom length	3.5	5.5	3.8±0.3 (3.5–4.0)	3.8±0.5 (3.5–5.0)
Gymnostom width	10.5	7.0	7.0	8.6±1.6 (6.0–10.5)
Stegostom length	19	26	21±2 (19–23)	25±4 (18–29)
Stegostom width	5.0	4.0	3.5	4.2±0.9 (3.0–5.5)
Pharyngeal corpus length	77	93	72±7 (64–81)	75±10 (61–87)
Pharyngeal bulbus length	42	52	40±3 (37–43)	50±8 (40–64)
Spicules arc or vulva length	71	82	45±2 (43–48)	13±2 (10–15)
Gubernaculum or rectum length	37	43	28±1 (27–30)	24±3 (20–28)
Precloacal swelling from cloaca	57	74	63±5 (58–69)	–
Unstriated part of tail length	19	26	18±2 (17–21)	35±5 (28–42)
Cephalic sensilla / labial region diameter	0.4	0.3	0.5±0.1 (0.5–0.6)	0.3±0.1 (0.2–0.4)
Cephalic capsule base width / length	1.6	1.8	1.5±0.2 (1.3–1.8)	1.7±0.3 (1.4–2.2)
Spicules / anal body diameter	2.1	2.1	1.7±0.1 (1.6–1.9)	–
Vagina length / Body diameter	–	–	–	0.3±0.1 (0.2–0.4)
Rectum length / anal body diameter	–	–	–	1.0±0.2 (0.8–1.3)
Unstriated part of tail length / tail length (%)	25	26	26±2 (24–28)	52±7 (46–63)

Type locality. Vietnam, Khanh Hoa Province, Hon Mun Island, Nha Trang Bay, shallow coral reef area off the coast; stations L (12°10'28.668"N, 109°17'53.304"E), CT (12°10'28.992"N, 109°18'05.7594"E), and D (12°10'49.44"N, 109°14'51.9354"E); depth 4–16 m; salinity 31.7–34.0‰, collected on 11.03.2024 and 23.11.2024.

GenBank accession number. PZ112143

Etymology. The species epithet reflects the dimorphism in spicules length.

Descriptions

Adults: Body fusiform, gradually narrowing towards truncate anterior end and tapering along the tail (Figures 2D; 4F; 5A). Cuticle with distinct transverse annules and twelve longitudinal ridges, originating at the helmet and extending posteriorly towards the tip of the tail (Figure 2C). Each annule has highly refractive margins and internal granulations, more distinct along the pharyngeal region (Figure 5D) and near tail tip (Figure 2H). Longitudinal ridges start as narrow groves on the helmet, becoming rows of Λ -shaped notches of each annule along the anteriormost part of the pharyngeal region, with each individual segment nested in the inverted V of the preceding one (Figures 1A; 3E; 5D); further posterior along the body this pattern becomes less defined, gradually changing along the body to become V-shaped along the posterior end, while the precise point of inversion is too subtle to pinpoint. Middorsal cuticular ridge extends to middle of pharyngeal region length; midventral cuticular ridge extends towards basal

bulb (Figure 1D); remaining ridges extend towards the tail tip, including midlateral ones. Somatic sensilla absent, except for the caudal region in both males and females. Body pores absent. Anterior body end in a shape of a well defined truncate-conoid helmet. Labial region distinctly offset from the helmet by a double constriction, lips small and partially fused. Inner labial sensilla small papilliform, located on the anterior surface of lips and projecting forward. Outer labial sensilla small papilliform, located on the outer edges of the lips, directed outward. Cephalic

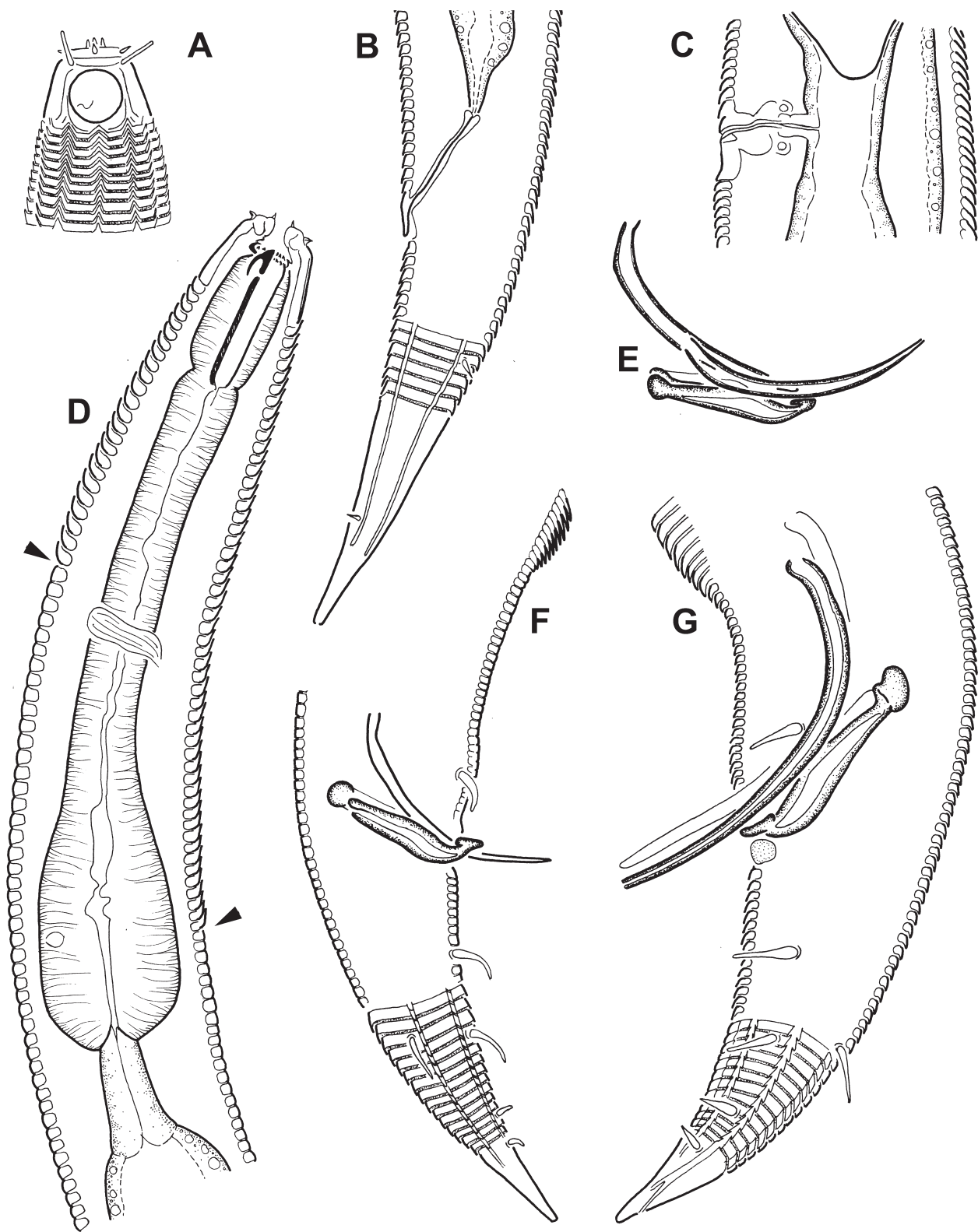


FIGURE 1. Line drawings of *Rhinema bimorpha* sp. nov. A. Male anterior end, surface view. B. Female tail. C. Female vulval region. D. Male pharyngeal region. E. Spicules and gubernaculum of a male with “short” spicules. F. Posterior region of a male with “short” spicules. G. Posterior region of a male with “long” spicules. Scale bar: A–G = 20 µm.

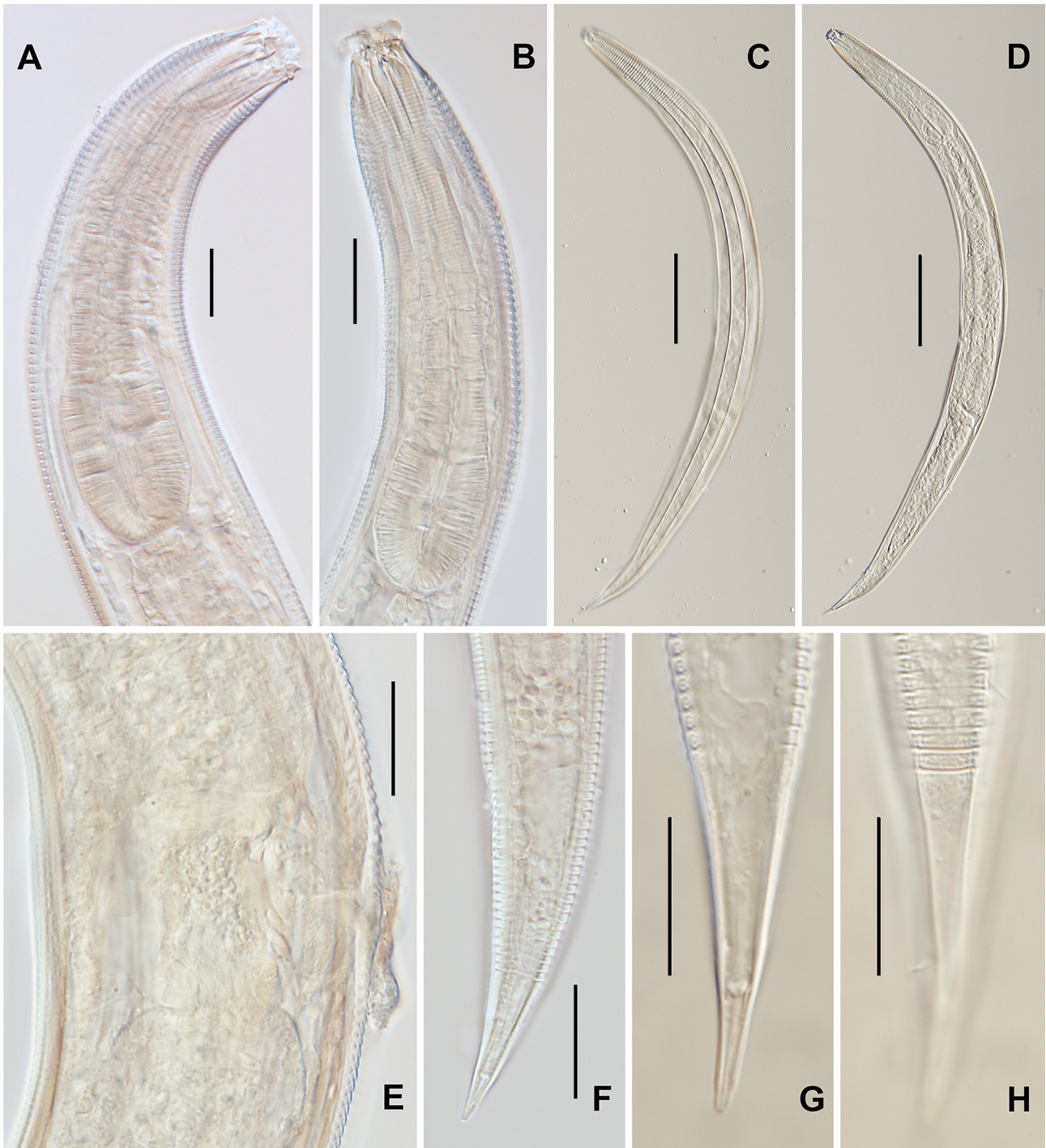


FIGURE 2. Photomicrographs of *Rhinema bimorpha* sp. nov. female. A–B. Pharyngeal region. C. Entire body, surface view showing cuticular ornamentation. D. Entire body. E. Vulval region showing vulva covered with copulatory plug. F. Tail. G. Posterior part of tail, median section. H. Posterior part of tail, surface view. Scale bars: A–B, E–H = 20 μ m, C–D = 100 μ m.

sensilla setiform, equal to 0.2–0.4 in females and 0.3–0.6 in males labial region diameters in length, located at the base of the labial region, immediately behind the constriction, projecting outward. Amphideal fovea nearly circular, similar in shape and size in males and in females, occupying nearly the entire height of helmet (below the bases of cephalic setae) and 0.4–0.5 of the corresponding body diameter (Figures 3F, 5B–C). Buccal cavity (stoma) clearly divided into cheilostom, gymnostom and stegostom (following definitions in Decraemer *et al.* 2014). Cheilostom (vestibulum) narrow cylindrical, with six pairs of refractive longitudinal ribs (Figures 3B, D; 5G). Gymnostom ovoid in shape, with large strongly cuticularised protrusible dorsal tooth with solid tip, and with two transverse

rows of small denticles, encircling the gymnostom from one side of the dorsal tooth to the other (Figures 3A–D; 4A; 5E–G). Stegostom cylindrical, with strongly cuticularised walls, more so along the dorsal side. Muscular tissue enveloping the stegostom is thicker on the dorsal side, displacing the lumen of stegostom towards the ventral body side; it is also separated from the rest of the pharynx by a distinct constriction. Pharynx muscularised, gradually expanding into elongate-ovoid basal bulb, with weakly cuticularised thin lumen along its entire length (Figures 1D; 2A–B; 4B; 5J). Cardia present, cylindrical. Secretory-excretory system difficult to observe due to preservation, but renette cell is visible on the right hand side of the basal bulb. Nerve ring at middle of the pharyngeal region length. Tail conoid and ventrally curved (Figures 1B; 2F). Caudal glands present, opening via a common spinneret. Non-annulated part of the tail encompassing caudal gland ampulla and spinneret 24–28 μm long in males and 46–63 μm long in females (Figure 2G), with strongly cuticularised walls.

TABLE 2. Comparison between different species and populations of *Rhinema* using key morphological characters, excluding *R. exquisitum*.

Characters	<i>R. retrorsum</i>	<i>R. retrorsum</i>	<i>R. longispicula</i>	<i>R. bimorpha</i> sp. nov.
Reference	Cobb, 1920	Gerlach, 1963	Zhai <i>et al.</i> 2020	this paper
Body length (μm)	1100–1200	960–1012	840–886	600–945
Labial region diameter (μm)	not described	17–18	16–20	12–19
Amphideal fovea diameter (μm)	not described	9	9–12	7.5–9.5
Stoma length (μm)	not described	36	31–36	25–37
Spicules length (μm)	?	45	120–138	71–82 and 43–48
Subventral denticles	not described	numerous, in two rows	two	numerous, in two rows
Spicules length / cloacal body diameter	≈ 1.5	1.5	3.5–3.9	2.1 and 1.6–1.9
Cuticular bulges in males	not described	absent	visible in photographs	present
Non-annulated part of the tail in males	not described	30–40% tail length	short	24–28% tail length
Non-annulated part of the tail in females	not described	not described	short	46–63% tail length
Posterior setiform sensilla (males)	not described	3 pairs ?	4 pairs	6 pairs
Caudal setiform sensilla (females)	not described	not described	not described	2 pairs

Males (general): Subventral cuticular ridges form thicker and more refractive bulges about 1.7–2.9 corresponding body diameters anterior to cloaca (Figures 1F–G; 4C; 5H). Reproductive system diorchic, with outstretched anterior testis located on the left-hand side of the intestine and reflexed posterior testis located on the right-hand side of the intestine. Gubernaculum similar in shape to those in some species of *Monoposthia* de Man, 1889, claw-like but with longer terminally bilobed proximal part, each lobe ending in a round thick “handle”; distal part of the gubernaculum with disk-like tip (Figures 1E–G; 5I). Supplements absent. Copulatory sensilla present, setiform, arranged as follows: one subventral pair about 6–8 annules anterior to cloaca, one subventral pair about 10 annules posterior to cloaca, one subdorsal pair about 14–16 annules posterior to cloaca, one subventral pair about 20 annules posterior to cloaca, one subventral pair about 3–5 annules anterior to unstriated part of the tail enveloping the spinneret, and one subventral pair on the unstriated part of the tail (Figures 1F–G).

Males with long spicules: Spicules paired and symmetrical, arcuate, with poorly developed manubrium and subcylindrical shaft, narrowing to a sharp tip; equal to 2.1 cloacal body diameters in length (Figures 1G; 4C–E), more robust in appearance.

Males with short spicules: Spicules paired and symmetrical, arcuate, with poorly developed manubrium and subcylindrical shaft, narrowing to a sharp tip; equal to 1.6–1.9 cloacal body diameters in length (Figures 1E–F; 5H–I), more delicate in appearance.

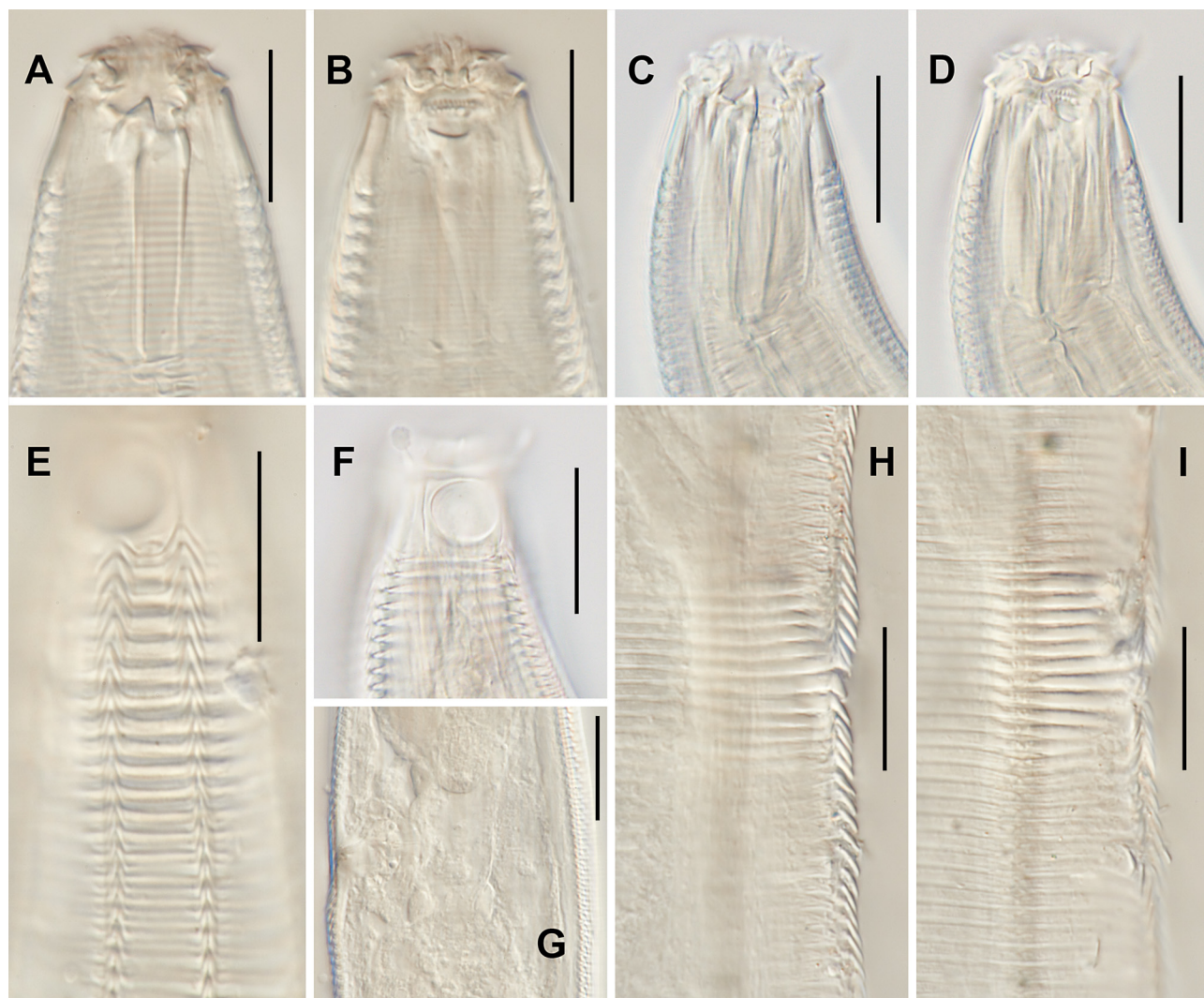


FIGURE 3. Photomicrographs of *Rhinema bimorpha* sp. nov. female. A, C. Stoma, median section. B, D. Stoma, submedian section. E. Cuticular ornamentation along the anterior body part. F. Amphid. G. Vulval/vaginal region, median section. H–I. Cuticular ornamentation along the vulval region at different focal point. Scale bars: A–I = 20 μ m.

Females: Middorsal cuticular ridge extends toward anterior part of the intestine; midventral cuticular ridge extends towards the middle of the pharyngeal region length; midlateral ridges end some distance before the anal opening; remaining ridges extend towards the tail tip. Cuticular annules and V-shaped elements of cuticular ridges are thicker and more refractive along the ventral/ventrosublateral sides of the vulval region (Figures 3H–I). Reproductive system didelphic, amphidelphic, ovary branches reflexed antidromously. Anterior ovary on left-hand side of intestine, posterior ovary on right-hand side of intestine. Vulva located at midbody or slightly posterior to it. Vagina with refractive walls, slightly angled, vulval opening covered with copulatory plug in some specimens (Figure 2E). Tail with one pair of subdorsal setiform sensilla five or so annules anterior to the unstriated part of the tail enveloping the spinneret and one pair of subventral caudal setiform sensilla located along the middle of the unstriated part of the tail enveloping the spinneret (Figures 1B; 2H).

Diagnosis. *Rhinema bimorpha* sp. nov. is particularly characterised by: a coarsely annulated cuticle with 12 longitudinal ridges, of which middorsal and midventral extends only along the anterior part of the body; cephalic sensilla equal to 0.2–0.6 labial region diameters in length; amphideal fovea circular and occupying nearly the entire height of helmet and 0.4–0.5 of the corresponding body diameter; subventral cuticular ridges in males form bulges

about 1.7–2.9 corresponding body diameters anterior to cloaca; spicules arcuate, with poorly developed manubrium and subcylindrical shaft, equal to 1.6–2.1 cloacal body diameters in length; copulatory sensilla setiform, arranged in five (one pre-cloacal and four post-cloacal) subventral and one subdorsal (post-cloacal) pairs.

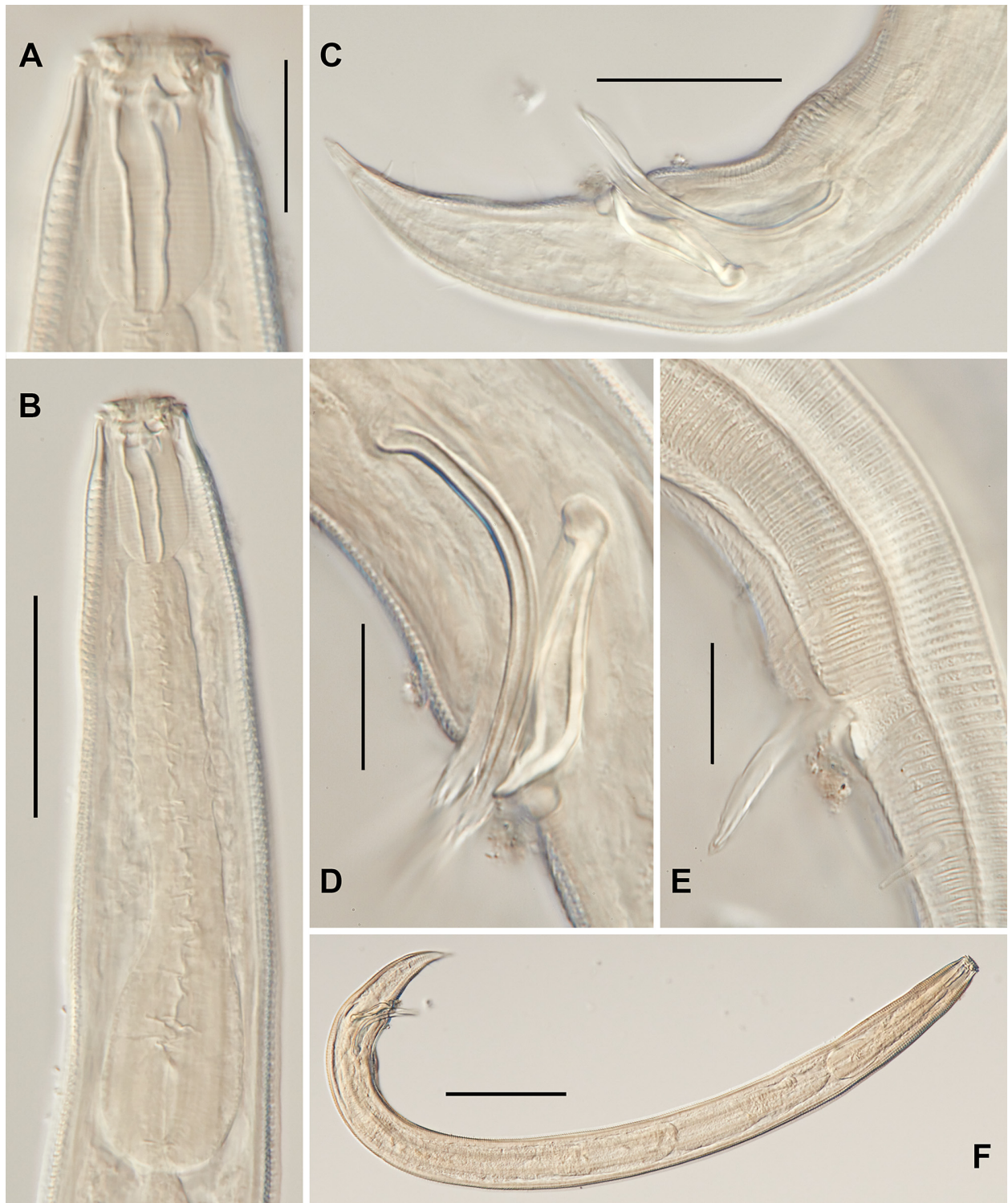


FIGURE 4. Photomicrographs of *Rhinema bimorpha* **sp. nov.** males with “long” spicules. A. Stoma, median section. B. Pharyngeal region, median section. C. Caudal region. D. Spicules and gubernaculum. E. Cloacal region, surface view. F. Entire body. Scale bars: A, D–E = 20 μ m, B–C = 50 μ m, F = 100 μ m.

Relationships. Among the tree currently described nominal species of *Rhinema*, the taxonomic position of *R. exqisitum* can not be ascertained with confidence – this species was described based on a single immature female, and the morphology of the male copulatory apparatus, the major character used in the classification of the family Monoposthiidae remains unknown. Although, Timm (1961) stated that the species has “rudiments of 2 ovaries”, there is no illustration to confirm and the absence of a helmet in this species suggest it to be more closely related to monodelphic genera of the family. Thus, it is considered *species inquirenda* and is excluded from subsequent comparison.

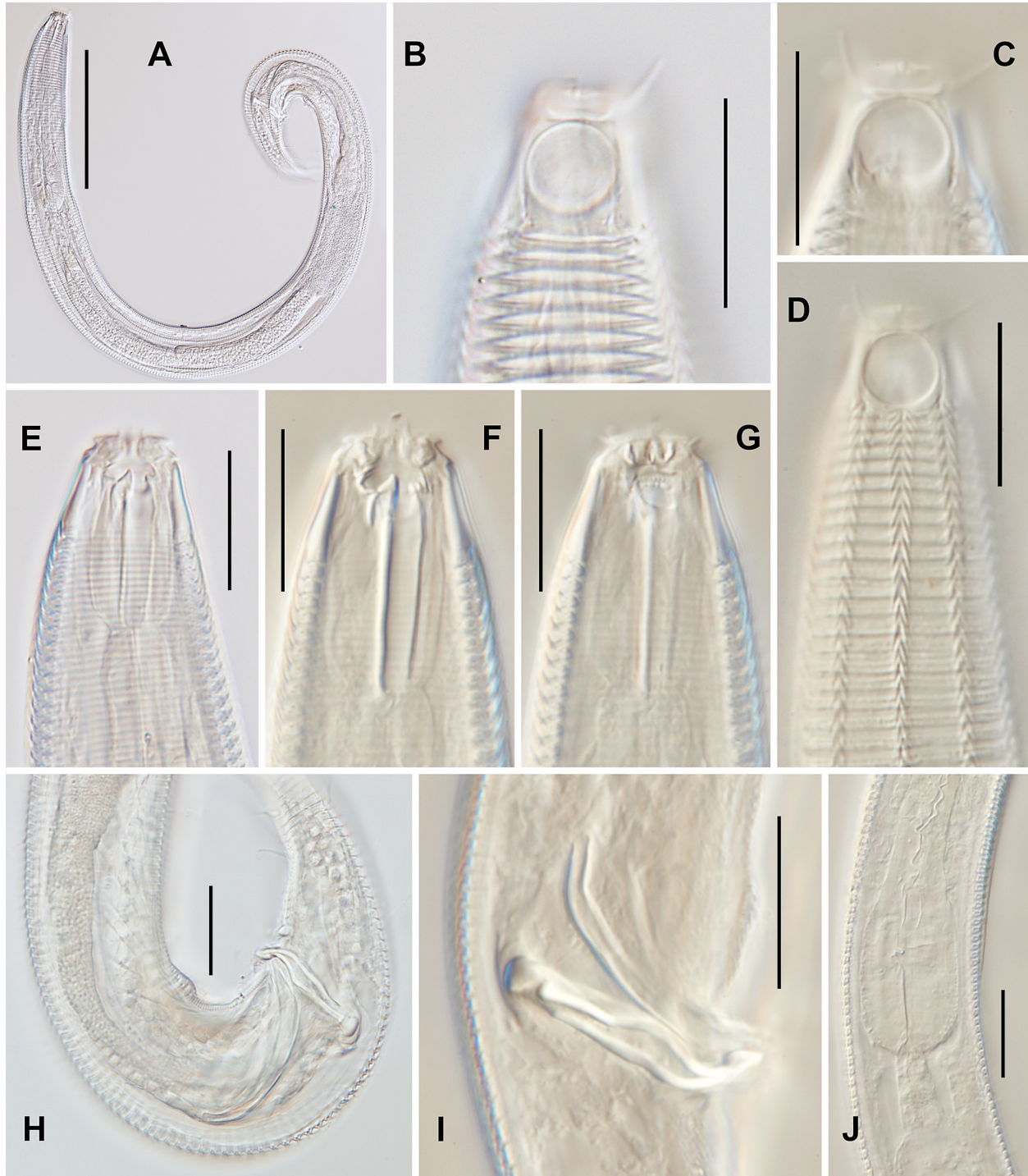


FIGURE 5. Photomicrographs of *Rhinema bimorpha* sp. nov. males with “short” spicules. A. Entire body. B–C. Amphid. D. Cuticular ornamentation along the anterior body part. E–F. Stoma, median section. G. Stoma, submedian section. H. Cloacal region. I. Spicules and gubernaculum. J. base of the pharynx. Scale bars: A = 50 μ m, B–J = 20 μ m.

The new species differs from *R. retrosum* in having relatively larger amphideal fovea and shorter helmet (occupying the height of a helmet vs. half of the height in *R. retrosum*) and larger number of posterior setiform sensilla in males (one pre-cloacal and 5 post-cloacal pairs vs. 3–4 post-cloacal pairs in *R. retrosum*). Cobb (1920) describes “submedian longitudinal striations extending to the middle of the tail”, which are not the same as “distinct longitudinal wings”, as he describes longitudinal ridges; these are not present in current specimens. Neither Cobb (1920) nor Gerlach (1963) describe anything resembling a cuticular bulge in front of the cloaca.

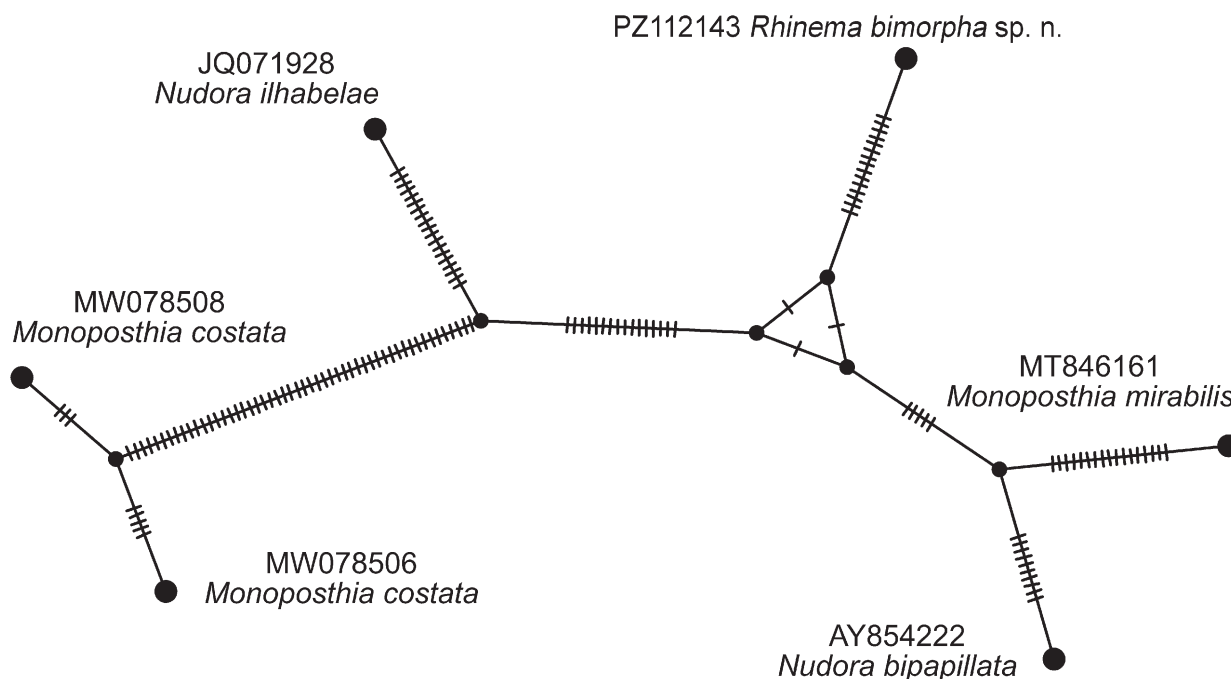


FIGURE 6. Haplotype network based on partial 18S rRNA sequence of members of the family Monoposthiidae.

The new species differs from *R. longispicula* mainly in having much shorter spicules (43–82 μm vs. 120–138 μm in *R. longispicula*) and larger number of posterior setiform sensilla in male (one pre-cloacal and 5 post-cloacal pairs vs. one pre-cloacal and 3 post-cloacal pairs in *R. longispicula*). There are only two small subventral denticles in *R. longispicula*, while *R. bimorpha* has two distinct rows of numerous denticles occupying subventral and lateral walls of the buccal cavity. Moreover, females of *R. longispicula* were described as having “vulva with slightly raised lips”, without mentioning of any specific morphology in the vulval region, while vulval region of new species is specifically characterised by thicker and more refractive V-shaped elements of cuticular ridges along the ventral/ventrosublateral sides of the vulval region. Figure 1d in Zhai *et al.* (2020) shows female of *R. longispicula* with relatively short non-annulated terminal part of the tail, while in the females of *R. bimorpha* **sp. nov.** non-annulated part of the tail surrounding the spinneret comprises about half of the tail length.

Molecular characterisation. Out of seven *R. bimorpha* **sp. nov.** specimens (two males, two females and three juveniles) used for PCR and sequencing, only one sample (female) was successful in producing partial (939 bases) 18S rRNA sequence. Haplotype network comparison between all existing, identified to species level, sequences of the family Monoposthiidae (Figure 6) based on the 948 bases long alignment agrees with previously published phylogeny (Ahmed & Holovachov, 2020), suggesting that the genus *Monoposthia* de Man, 1889 is not monophyletic. It also suggests that the genus *Nudora* Cobb, 1920 may not be monophyletic either. Thus, only broader taxonomic sampling of Monoposthiidae, targeting multiple loci, can provide a better understanding of the phylogeny and taxonomy within this family of marine nematodes.

Remarks on male dimorphism. Out of six males found in the samples, two have markedly longer spicules (71–82 μm) than the remaining four (43–48 μm). However, no other morphological differences could be found between these morphotypes. Moreover, all examined females are morphologically very similar, and could not be divided by any observable morphological features. For this reason, we consider all specimens to belong to the same species. The dimorphism in the length of spicules is most likely random and caused by rather limited number of investigated specimens.

Sampling and field studies

All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities. The study is compliant with CBD and Nagoya protocols.

Funding

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number: 106.05-2021.85.

Ethical approval

Not applicable

References

- Ahmed, M. & Holovachov, O. (2020) Description of a new marine predatory nematode *Latronema dyngi* sp. nov. (Nematoda, Chromadorida, Selachinematidae) from the west coast of Sweden and an updated phylogeny of Chromadoria. *Marine Biodiversity*, 50, 113.
<https://doi.org/10.1007/s12526-020-01129-w>
- Cobb, N.A. (1920) One hundred new nemas (type species of 100 new genera). *Contributions to a Science of Nematology*, 9, 217–343.
- Creer, S., Fonseca, V.G., Porazinska, D.L., Giblin-Davis, R.M., Sung, W., Power, D.M., Packer, M., Carvalho, G.R., Blaxter, M.L., Lamshead, P.J. & Thomas, W.K. (2010) Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. *Molecular Ecology*, 19 (Supplement 1), 4–20.
<https://doi.org/10.1111/j.1365-294X.2009.04473.x>
- Dana, J.D. (1846) Zoophytes. *United States Exploring Expedition during the years 1838–1842*, 7, 1–740.
<https://doi.org/10.5962/bhl.title.70845>
- De Coninck, L.A. (1965) Classe des Nématodes - Systématique des Nématodes et sous-classe des Adenophorea. *Traité de Zoologie*, 4, 586–681.
- De Grisse, A.T. (1969) Redescription ou modification de quelques techniques utilisées dans L'étude des nematodes phytoparasitaires. *Mededelingen Rijksfaculteit Landbouwwetenschappen*, 34, 351–369.
- Esper, E.J.C. (1791) *Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet nebst Beschreibungen*. Raspischen Buchhandlung, Nuremberg, 320 pp.
<https://doi.org/10.11588/diglit.2885>
- Filipjev, I.N. (1934) The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithsonian Miscellaneous Collection*, 89, 1–63.
- Gerlach, S.A. (1963) Freilebende Meeresnematoden von den Malediven II. *Kieler Meeresforschungen*, 19, 67–103.
- Lamarck, J.-B. (1801) *Système des animaux sans vertèbres, ou tableau général des classes, des ordres et des genres de ces animaux*. Deterville, Paris, 432 pp.
- Lamarck, J.-B. (1816) *Histoire naturelle des animaux sans vertèbres. Tome second*. Verdière, Paris, 566 pp.
- de Man, J.G. (1889) Espèces et genres nouveaux de Nématodes libres de la mer du Nord et de la Manche. *Meimoires de la Sociètiè zoologique de France*, 2, 1–10.
- Raes, M., De Troch, M., Ndarò, S.G.M., Muthumbi, A., Guilini, K. & Vanreusel, A. (2007) The structuring role of microhabitat type in coral degradation zones: a case study with marine nematodes from Kenya and Zanzibar. *Coral Reefs*, 26, 113–126.
<https://doi.org/10.1007/s00338-006-0184-8>
- Seinhorst, J.V. (1959) A rapid method to the transverse of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4, 67–69.
<https://doi.org/10.1163/187529259X00381>
- Tchesunov, A.V. (2014) Order Desmodorida De Coninck, 1965. In: Schmidt-Rhaesa A. (Ed.), *Handbook of Zoology. Gastrotricha, Cycloneuralia, Gnathifera. Vol. 2. Nematoda*. de Gruyter, Berlin, pp. 399–434.
<https://doi.org/10.1515/9783110274257.399>
- Timm, R.W. (1961) The marine nematodes of the Bay of Bengal. *Proceedings of the Pakistan Academy of Sciences*, 1, 1–88.

- Venekey, V., Fonseca-Genevois, V.G. & Santos, P.J.P. (2010) Biodiversity of free-living marine nematodes on the coast of Brazil: a review. *Zootaxa*, 2568 (1), 39–66.
<https://doi.org/10.11646/zootaxa.2568.1.2>
- Vincx, M. (1996) Meiofauna in marine and freshwater sediments. *In*: Hall, G. (Ed.), *Methods for the Examination of Organismal Diversity in Soils and Sediments*. CABI Publishing, Wallingford, pp. 187–195.
- Yoder, M., De Ley, I.T., King, I.W., Mundo-Ocampo, M., Mann, J., Blaxter, M., Poiras, L. & De Ley, P. (2006) DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology*, 8, 367–376.
<https://doi.org/10.1163/156854106778493448>
- Zhai, H., Huang, M. & Huang, Y. (2020) A new free-living marine nematode species of *Rhinema* from the South China Sea. *Journal of Oceanology and Limnology*, 38, 545–549.
<https://doi.org/10.1007/s00343-019-9169-7>