

Research Article

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Corresponding author:

Yong Huang;

Email: huangy@lcu.edu.cn

Two new species of genus *Tripyloides* (Nematoda, Enoplida, Tripyloididae) from the Chinese sea area

Mengdi Chu¹, Yingdong Hao¹ and Yong Huang^{1,2} 

¹College of Life Sciences, Liaocheng University, Liaocheng 252059, People's Republic of China and ²Key Laboratory of Ecological Conservation and Innovative use of Biological Resource of Shandong College and University, Liaocheng 252059, People's Republic of China

Abstract

Two new free-living marine nematode species of genus *Tripyloides* were discovered in intertidal sediments along Rudong coast of the Yellow Sea and on Qi'ao Island of the South China Sea, respectively. They are described here as *Tripyloides conicus* sp. nov. and *Tripyloides boucheri* sp. nov. *T. conicus* sp. nov. is characterized by outer labial setae two-segments, amphideal fovea circular, buccal cavity with four chambers and with a distinct tooth at the bottom, spicules slender and straight, gubernaculum kidney-like with two lateral denticles at distal end, tail sexual dimorphism (elongated conical in males, conico-cylindrical in females). *T. boucheri* sp. nov. is characterized by body size small, outer labial setae stout, two-segments, amphideal fovea small, circular, narrow conical buccal cavity without distinct tooth, male with papilliform preloacal supplements, spicules slender, gubernaculum with two lateral denticles at distal end, tail conico-cylindrical and not swollen terminally. An updated dichotomous key for fifteen species of the genus is also given.

Introduction

Free-living marine nematodes are the most dominant and diverse meiofauna in marine benthic habitats. They have strong adaptability and wide distribution, and play a very important role in the material circulation and energy flow in the benthic ecosystem (Heip *et al.*, 1985; Lamshead and Boucher, 2003). More than 7 000 species of free-living marine nematodes have been described around the world currently (Appeltans *et al.*, 2012; Nemys, 2022), but only 500 species have been identified in China, and there are still a lot of taxa need to be identified to the species level. Among the known species, 168 species were new to science (Huang and Zhang, 2019; Huang *et al.*, 2021; Sun *et al.*, 2021).

In order to investigate the diversity of free-living nematodes along the coast of the Yellow Sea and the South China Sea, sediment samples were collected in a number of intertidal sites in recent years. More than 300 species have been discovered in the sea area (Hao *et al.*, 2021). The main dominant species were *Daptonema parabreviseta* Huang & Sun, 2018, *Parodontophora deliensis* Zhang, 2005, *Bathylaimus huanghaiensis* Huang & Zhang, 2009, *Thalassomonhystera siamensis* Kito & Aryuthaka 1998 and *Leptolaimus* spp. Among the species in the sea area, two species belonging to genus *Tripyloides* de Man, 1886 were identified as new to science and described here as *Tripyloides conicus* sp. nov. and *T. boucheri* sp. nov., respectively.

The genus *Tripyloides* was erected by de Man in 1886. Tchesunov *et al.* (2010) reviewed the genus and proposed an annotated list of eleven valid species and a pictorial key for species identification. The last review to the genus was conducted by Fu *et al.* (2018), who provided a list of sixteen species including two new species described by them in the same paper and an updated key for thirteen species without *T. omblaica* Micoletzky, 1924, *T. septentrionalis* Schuurmans Stekhoven & De Coninck, 1933 and *T. taafi* de Bovée, Coineau, Soyer & Travé, 1973. So far, sixteen valid species in the genus have been recorded in the world (Nemys, 2022). The genus is characterized by buccal cavity consisting of 2–4 chambers with teeth or cuticular projections; outer labial setae usually thick and jointed; cephalic setae short and fine, arranged in same circle with outer labial setae; amphids spiral or circular, situated posterior to the buccal cavity; spicules wide or slender, gubernaculum large with denticles at distal end; tails conical or conico-cylindrical, male reproductive system monorchic with an anterior testis, female didelphic with two reflexed ovaries (Smol *et al.*, 2014).

Materials and methods

In January 2019 and February 2021, meiofaunal samples were obtained using a sawn-off syringe with a 2.6 cm inner diameter at an intertidal gravel beach along Qi'ao Island of the South China Sea and an intertidal silt beach along the Rudong coast of the Yellow Sea, respectively. Samples were taken from the sediment layer 0–8 cm and divided into three sections, (i.e. 0–2, 2–5 and 5–8 cm), then fixed with 10% formalin in filtered seawater for long-term preservation. Stained samples by 0.1% Rose Bengal were poured into two sieves (500 and 42 µm mesh sizes, respectively), and washed with tap water to remove silt and separate macrofauna from



meiofauna. Heavier sediment particles were removed using centrifugation in Ludox-TM with a specific gravity of 1.15 g ml^{-1} (de Jonge and Bouwman, 1977). Each sample was washed into a Petri dish with distilled water and meiofauna was sorted under a stereoscopic microscope. Nematodes were transferred into a cavity block containing a solution of 5% glycerol, 5% pure ethanol, 90% freshwater by volume (McIntyre and Warwick, 1984). After ethanol slowly evaporated, the specimens were mounted in glycerine on permanent slides. The descriptions were made from glycerine mounts using a differential interference contrast microscope (Leica DM 2500). The photos were taken with Leica DMC 4500. Line drawings were made with the aid of a camera lucida. All measurements were taken using Leica LAS X version 3.3.3, and all measurements are in μm . All curved structures were measured along the curved median line. Type specimens were deposited in the Marine Biological Museum of Chinese Academy of Sciences, Qingdao.

Abbreviations used in the table are as follows: a- the ratio of body length to maximum body diameter; a.b.d.- body diameter at cloaca or anus; b- ratio of body length to pharynx length; c- ratio of body length to tail length; c'- ratio of tail length to cloacal or anal body diameter; c.b.d.- corresponding body diameter; V%- position of vulva from anterior end expressed as a percentage of total body length.

Results

Systematics

Order ENOPLIDA Filipjev, 1929
 Family TRIPYLOIDIDAE Filipjev, 1918
 Genus *Tripyloides* de Man, 1886
Tripyloides conicus sp. nov.
 (Figures 1–3)

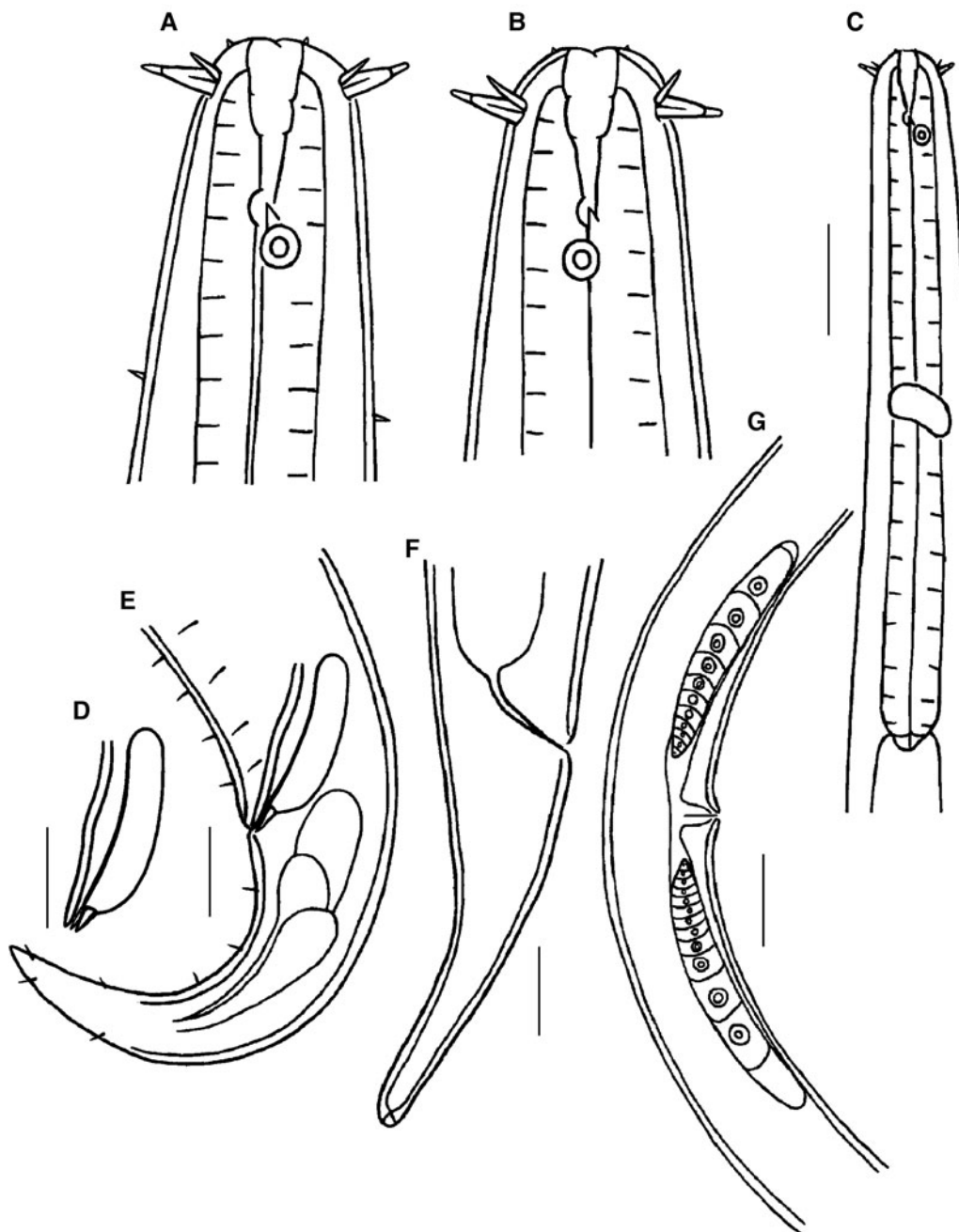


Figure 1. *Tripyloides conicus* sp. nov. (A) anterior end of male; (B) anterior end of female; (C) pharyngeal region of female; (D) spicule and gubernaculum; (E) posterior end of male; (F) tail of female; (G) middle region of female, showing vulva and ovaries. (Scale bars: A, B = $10 \mu\text{m}$; C, G = $40 \mu\text{m}$; D, E, F = $20 \mu\text{m}$).

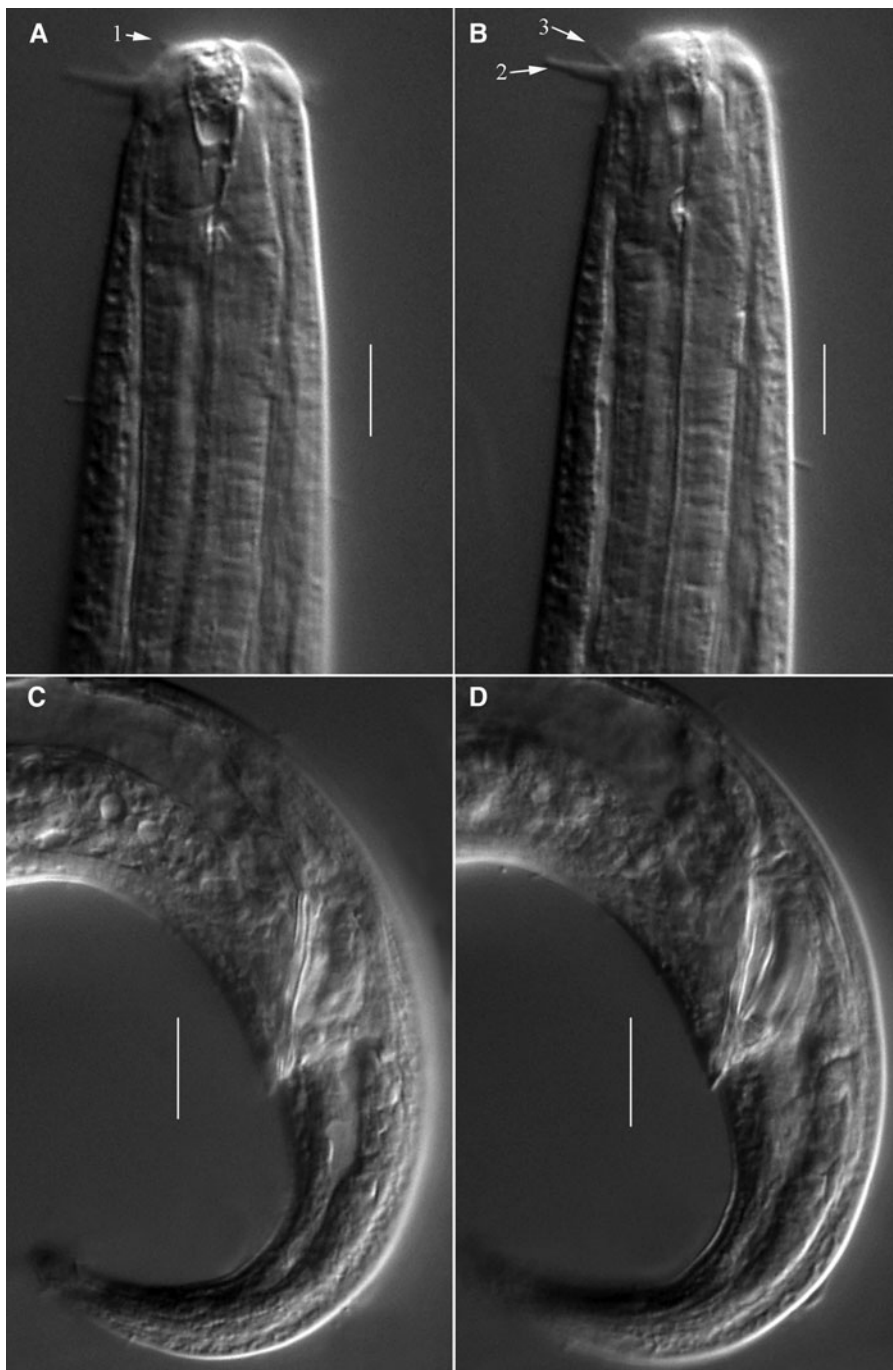


Figure 2. *Tripolyoides conicus* sp. nov. (A) anterior end of male, showing inner labial sensilla (arrow 1), buccal cavity and teeth; (B) anterior end of holotype, showing outer labial setae (arrow 2) and cephalic setae (arrow 3); (C) posterior end of holotype, showing spicules and tail; (D) cloacal region of holotype, showing spicule and gubernaculum. (Scale bars: A, B = 10 μ m; C, D = 20 μ m).

Type and additional material

Three males, two females and a juvenile were obtained. Holotype male on slide RDX-N33-1. Paratype male 2 on slide RDX-N33-5, male 3 on slide RDX-N33-3, female 1 on slide RDX-N33-14, female 2 on slide RDX-N33-3 and juvenile on slide RDX-N33-4.

Type locality and habitat

Type and all the additional specimens were collected from the surface layer of an intertidal muddy sediment along Rudong coast of the Yellow Sea, China (32°36'2"N, 121°0'2"E).

Etymology

The species name is derived from the Latin word *conicus*, refers to males with conical tail.

Measurements

All measurement data are given in Table 1.

Description

Holotype: Body cylindrical with conical tail. Cuticle smooth. Inner labial sensilla as minute papillae. Six outer labial setae jointed and divided into two segments (Figure 2B, arrow 2), 9 μ m long, about 50% of corresponding body diameter. Four cephalic setae are short and fine (Figure 2B, arrow 3), 5 μ m long, arranged in the same circle of outer labial setae. Amphideal fovea is circular with a double contour, 5 μ m in diameter (corresponding to 20% of corresponding body diameter), situated just behind the base of buccal cavity (20 μ m from the anterior end). Buccal cavity conical with strongly sclerotized walls, divided into four chambers by cuticular rings. Anterior chamber is irregularly cup-shaped without tooth. The last chamber looks like hemispherical pouches, with a distinct tooth at the bottom. Pharynx is gradually swelled, not forming a distinct bulb. Cardia small. Nerve ring is situated at the middle of pharyngeal length. Excretory pore is not observed.

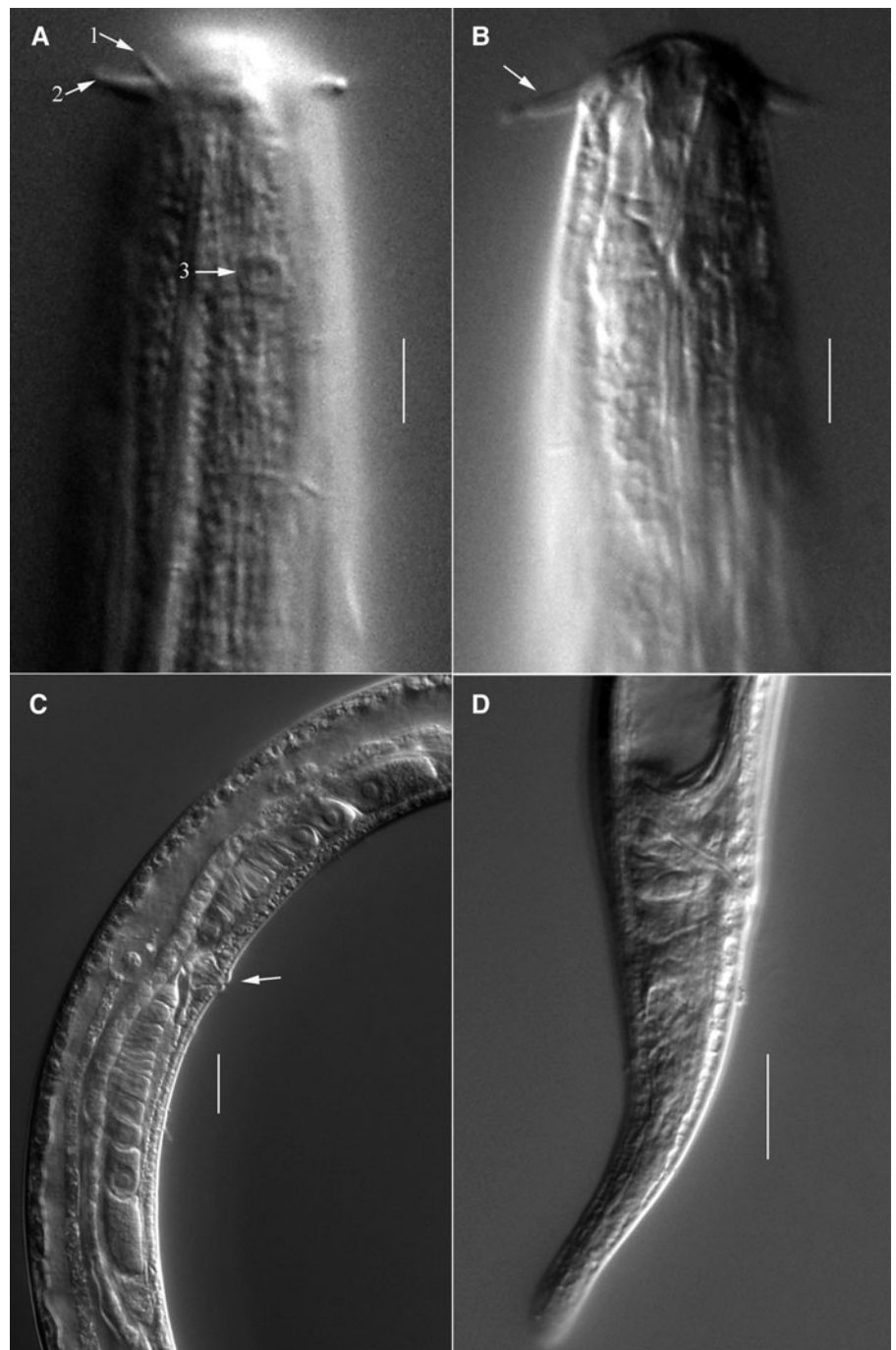


Figure 3. *Tripylloides conicus* sp. nov. (A) anterior end of female, showing cephalic setae (arrow 1), outer labial setae (arrow 2) and amphidial fovea (arrow 3); (B) anterior end of female, showing outer labial seta (arrow) and buccal cavity; (C) vulva region of female, showing vulva (arrow) and ovaries; (D) tail of female. (Scale bars: A, B = 10 μm ; C, D = 20 μm).

Reproductive system is monorchic with an anterior outstretched testis, situated at ventral side of intestine. Spicules slender, 31 μm long, almost straight. Gubernaculum is kidney shaped with thickened ventral rib and two lateral denticles at distal end. Precloacal supplement is absent. Tail is conical, gradually tapered, 2.9 times of body diameter at cloaca. There are two longitudinal rows of pre- and postcloacal lateroventral setae, about 5 μm long, four pairs in front of cloaca and five pairs on tail.

Females: Similar to males in most morphological characteristics except tail conico-cylindrical, without seta. Reproductive system didelphic with two opposed, reflexed and very narrow ovaries, located at ventral side of intestine (Figure 3C). Vagina straight, 0.3 times vulval body diameters long. Vulva raised, located at 53–54% of total body length from anterior end.

Juvenile: Similar to female in shape except slightly smaller body size. Tail conico-cylindrical without seta.

Differential diagnosis and discussion

Tripylloides conicus sp. nov. is characterized by outer labial setae of two-segments, amphidial fovea circular, buccal cavity divided into four chambers and with a distinct tooth at the bottom, spicules slender and straight, gubernaculum kidney-like with two lateral denticles at distal end, tail sexual dimorphism (elongated conical with two longitudinal rows of subventral setae in males, conico-cylindrical without setae in females).

Tripylloides conicus sp. nov. is similar to *T. imitans* Wieser, 1959 and *T. undulatus* Gerlach, 1962 in having conical tail. However, the new species differs from *T. imitans* and *T. undulatus* by small body size shorter than 1300 μm , tail without ventral papillae. The latter both species longer than 2100 μm , tail with ventral papillae. In body size and four chambers of buccal cavity, the new species is also similar to *T. marinus* (Bütschli, 1874) de Man, 1886. But the new species can be distinguished from *T.*

Table 1. Individual measurements of *Tripyloides conicus* sp. nov. (in μm except a, b, c, c', number and V%)

Characters	Holotype			Paratypes		
	♂1	♂2	♂3	♀1	♀2	Juvenile
Total body length	1273	1280	1204	1154	1203	1025
Maximum body diameter	42	47	42	47	50	37
Head diameter	18	18	19	21	20	17
Length of outer labial setae	9	8	8	8	9	6
Length of cephalic setae	5	4	4	4	4	3
Depth of buccal cavity	22	25	23	24	22	19
Amphidial width	5	4.5	5	5	5	4
Amphid from anterior end	20	22	21	20	30	15
Nerve ring from anterior	106	115	112	110	99	91
Pharynx length	210	215	209	212	192	180
c.b.d. at pharyngeal base	38	38	38	41	43	34
Spicule length	31	31	30	–	–	–
Gubernacular length	34	32	32	–	–	–
Body diameter at cloaca or anus	32	30	30	29	29	23
Vulva from anterior end	–	–	–	611	646	–
V%	–	–	–	53	54	–
Body diameter at vulva	–	–	–	47	50	–
Tail length	92	88	85	91	95	94
a	30.3	27.2	28.7	24.6	24.1	27.7
b	6.1	6.0	5.8	5.4	6.3	5.7
c	13.8	14.5	14.2	12.7	12.7	10.9
c'	2.9	2.9	2.8	3.1	3.3	4.1

Table 2. Individual measurements of *Tripyloides boucheri* sp. nov. (in μm except a, b, c, c', number and V%)

Characters	Holotype	Paratypes		
	♂1	♀1	♀2	Juvenile
Total body length	831	991	794	468
Maximum body diameter	34	45	41	20
Head diameter	10	10	11	8
Length of outer labial setae	3	3	3	2
Length of cephalic setae	–	–	–	–
Depth of buccal cavity	17	17	17	10
Amphidial width	6	–	5	3
Amphid from anterior end	15	–	19	16
Nerve ring from anterior	86	83	81	66
Pharynx length	136	154	133	113
c.b.d. at pharyngeal base	30	39	33	18
Spicule length	25	–	–	–
Gubernacular length	27	–	–	–
Body diameter at cloaca or anus	23	24	19	13
Vulva from anterior end	–	475	392	–
V%	–	48	49	–
Body diameter at vulva	–	45	39	–
Tail length	95	108	97	72
a	24.4	22.0	19.4	23.4
b	6.1	6.4	6.0	4.1
c	8.7	9.2	8.2	6.5
c'	4.1	4.5	5.1	5.5

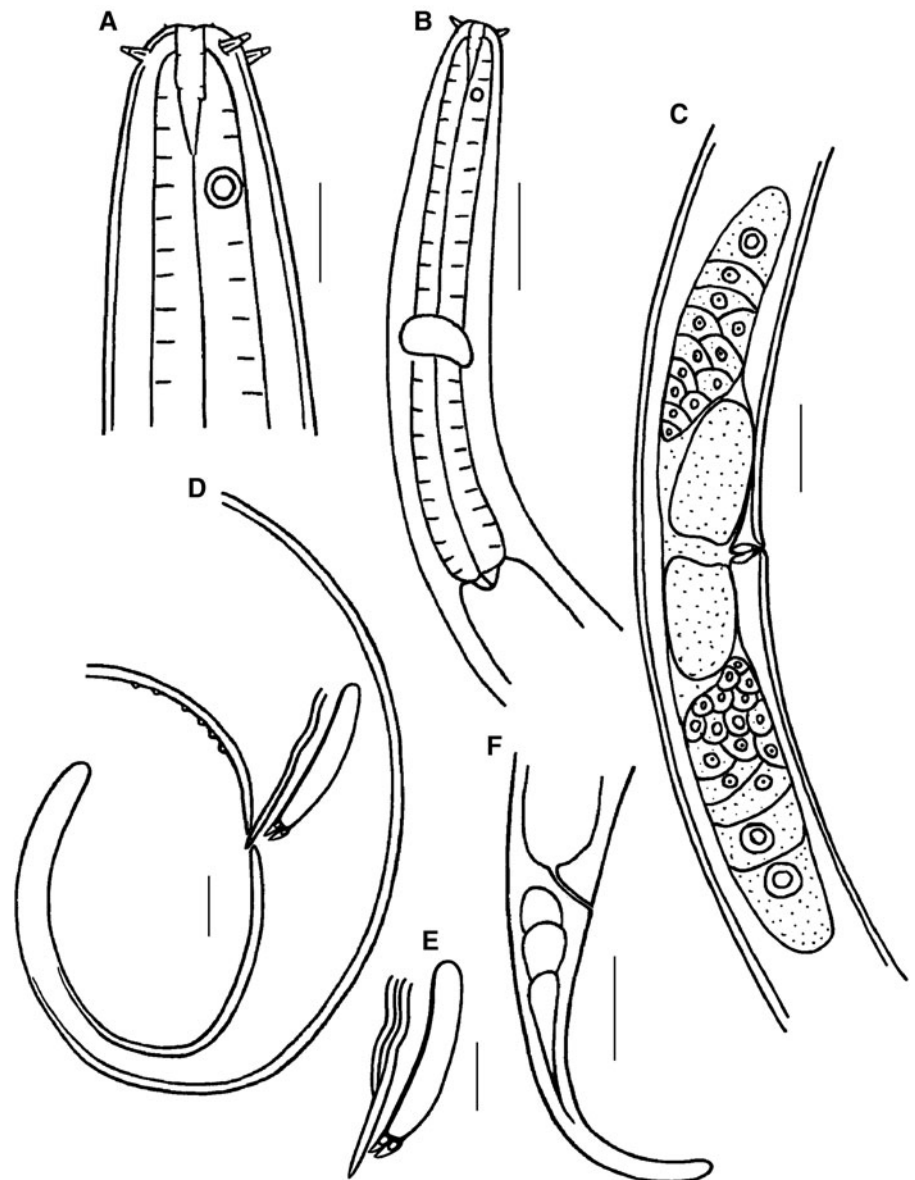


Figure 4. *Tripylloides boucheri* sp. nov. (A) anterior end of male; (B) Pharyngeal region of female; (C) middle region of female, showing vulva and ovaries; (D) posterior end of male, showing spicule, gubernaculum and prelocaal supplements; (E) spicule and gubernaculum; (F) tail of female. (Scale bars: A, D, E = 10 μ m; B, C, F = 30 μ m).

marinus by tails conical in males (*vs* conico-cylindrical), spicules slender and simple, 30–31 μ m long (*vs* broad and complex, 23 μ m long in *T. marinus*). Further differences between *T. conicus* sp. nov. and its congeners can be inferred from the key below.

Tripylloides boucheri sp. nov.
(Table 2, Figures 4–6)

Type and additional material

One male, two females and one juvenile were obtained. Holotype male on slide NH-QANT-94. Paratype female 1 on slide NH-QANT-108 and female 2 on slide NH-QANT-83 and juvenile on slide NH-QANT-94.

Type locality and habitat

Type and all the additional specimens were collected from the surface layer of an intertidal gravel beach on Qi'ao island of the South China Sea (22°14'1"N-113°21'53"E).

Etymology

The species is named in honour of Dr Guy Boucher, a well-known French nematologist, in recognition of his contributions to nematode taxonomy.

Measurements

All measurement data are given in Table 2.

Description

Holotype: Body cylindrical, tapered towards both ends. Cuticle smooth. Inner labial sensilla as minute papillae, six outer labial setae stout, 3 μ m long, corresponding to 30% of corresponding body diameter, jointed with two segments. Cephalic setae not observed. Small amphideal fovea circular with a double contour, situated posterior to the base of buccal cavity, 15 μ m from the anterior end. Buccal cavity conical with cuticularized walls, divided into three chambers by cuticular ring, without distinct tooth. Pharynx posteriorly enlarged, not forming a true bulb. Cardia small. Nerve ring situated posterior to middle of pharynx, 63% of pharyngeal length from the anterior end. Secretory-excretory pore situated posterior to nerve ring. Tail conical proximal half, then transforming to slender cylindrical portion, and not swollen terminally. Caudal setae absent.

Reproductive system with single anterior outstretched testis, located at ventral side of intestine. Spicules slender and slightly straight, 25 μ m long, distally pointed, proximal half waved bending. Gubernaculum parallel to the spicule, broad, 27 μ m long, with thickened ventral rib and two cuticularized lateral teeth at

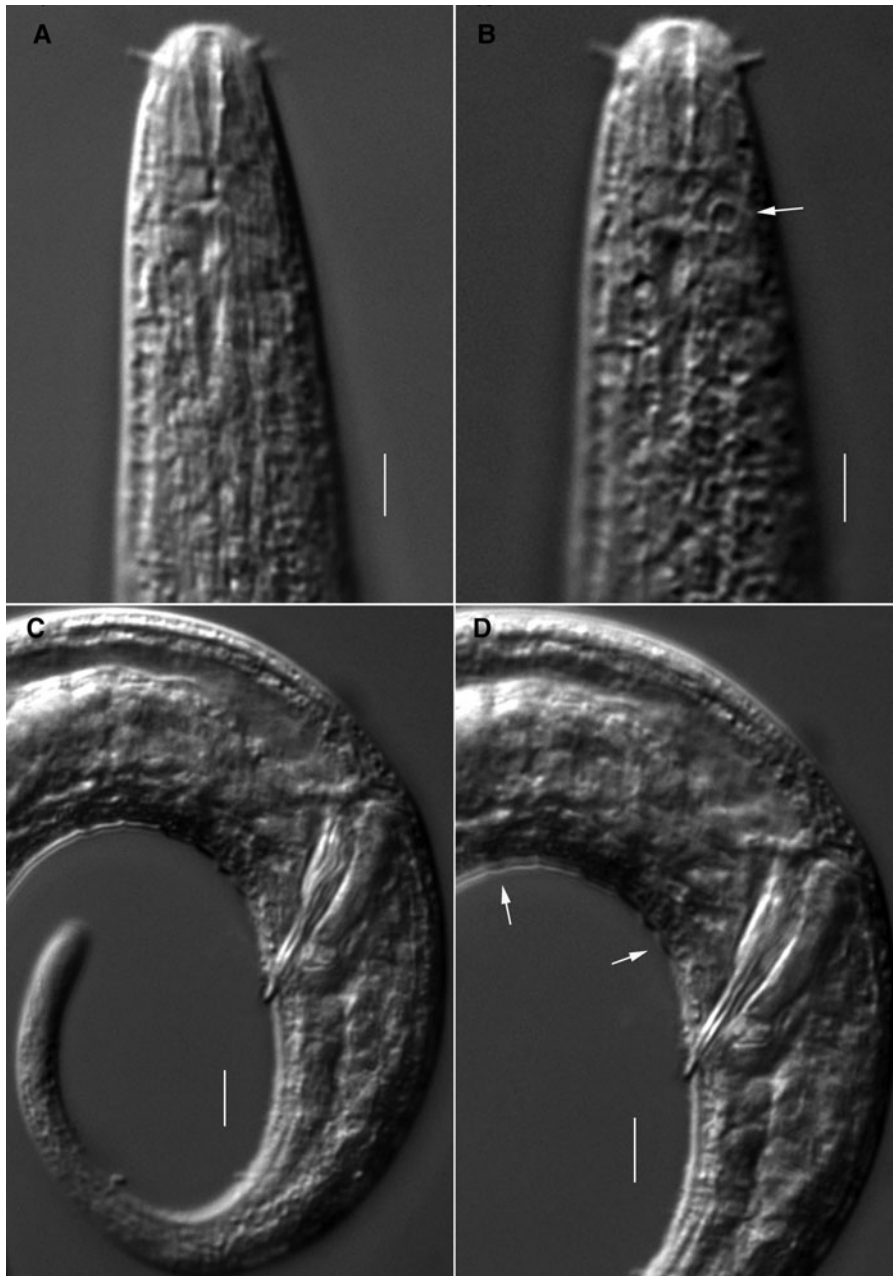


Figure 5. *Tripylloides boucheri* sp. nov. (A, B) anterior end of male, showing buccal cavity, outer labial setae and amphid (arrow); (C) posterior end of holotype; (D) cloacal region of holotype, showing spicules, gubernaculum and precloacal supplements (arrow). (Scale bars: A–D = 10 μm).

distal end. Apophysis absent. Six small papilliform ventral supplements in front of cloaca.

Females: Similar to male in most respects except the body slightly plumper and tail slightly longer. Reproductive system didelphic with two opposed, reflexed ovaries, located at left side of intestine. Vulva slightly raised, located at about middle of body length, 48–49% of body length from the anterior end.

Juvenile: Body is half as small as an adult, similar to female in shape with relatively longer conico-cylindrical tail.

Differential diagnosis and discussion

Tripylloides boucheri sp. nov. is characterized by body relatively small, outer labial setae stout, two-segments, amphidial fovea circular with a double contour, buccal cavity without distinct tooth, male with papilliform precloacal supplements, spicules slender, gubernaculum with two lateral denticles at distal end, tail conico-cylindrical, not swollen terminally.

Tripylloides boucheri sp. nov. most resembles *T. amazonicus* in having papilliform precloacal supplements and is similar to *T.*

granulatus in having short outer labial setae (3–5 μm). *T. boucheri* sp. can be distinguished from *T. amazonicus* by shorter body length and less *a* value (794–991 μm, *a* = 19.4–24.4), narrow buccal cavity divided into three chambers and without distinct tooth, tail tip not swollen. However, the latter species with longer body and greater *a* value (1510–1640 μm, *a* = 42.4–46), broad buccal cavity divided into two chambers with large dorsal tooth, tail tip swollen. The new species differs from *T. granulatus* by short tails (4.1–5.1 a.b.d. vs 7.2 a.b.d. long in *T. granulatus*), buccal cavity without distinct tooth (vs with distinct tooth), amphideal fovea rounded with a double contour (vs single contour). Further differences between *T. boucheri* sp. nov. and its congeners can be specified in the key below.

Updated identification key to valid species of the genus *Tripylloides* de Man, 1886 (emended after Tchesunov *et al.*, 2010 and Fu *et al.*, 2018)

- 1. Body longer than 2100 μm, tail with ventral papillae . . . 2
 - Body usually shorter than 2100 μm, tail without ventral papillae 3



Figure 6. *Tripylloides boucheri* sp. nov. (A) anterior end of female; (B) vulva region of female, showing ovary, eggs and vulva (arrow); (C) posterior end of female, showing posterior ovary and tail. (Scale bars: A = 10 μ m; B, C = 30 μ m).

2. Amphideal fovea large, 40% c.b.d., located at base of buccal cavity...*T. undulatus* Gerlach, 1962
 - Amphideal fovea small, 23% c.b.d., situated posterior to buccal cavity.....*T. imitans* Wieser, 1959
3. Outer labial setae longer than 16 μ m 4
 - Outer labial setae shorter than 13 μ m 6
4. Outer labial setae not jointed, amphidial fovea multispiral *T. acherusius* Gerlach, 1952
 - Outer labial setae jointed with three-segments, amphidial fovea not multispiral 5
5. Tails stout, nearly cylindrical, 2.2 a.b.d. long *T. brevis* Gerlach, 1958
 - Tails conico-cylindrical, 3.6–4 a.b.d. long ... *T. soyeri* de Bovée, 1977
6. Ventral preloacal papillae present 7
 - Ventral preloacal papillae absent 8
7. Buccal cavity broad with two chambers and a dorsal tooth, outer labial setae 8–13 μ m long *T. amazonicus* (Gerlach, 1957) Riemann, 1970
 - Buccal cavity narrow with three chambers without distinct tooth, outer labial setae 3 μ m long *T. boucheri* sp. nov.
8. Outer labial setae 3–5 μ m long, tails 7.2 a.b.d. long... *T. granulatus* (Cobb, 1913; Wieser, 1956)
 - Outer labial setae longer than 5 μ m, tails shorter than 4.5 a.b.d. 9
9. Outer labial setae two-segments 10
 - Outer labial setae smooth, not jointed 14
10. Viviparity *T. pallidus* Tchesunov, 1981
 - Oviparity 11
11. Buccal cavity with four chambers 12
 - Buccal cavity with two or three chambers 13
12. Tails conical in males, spicules slender, 30–31 μ m long ... *T. conicus* sp. nov.
 - Tails conico-cylindrical, spicules broad, 23 μ m long... *T. marinus* (Bütschli, 1874) de Man, 1886
13. Buccal cavity without tooth, gubernaculum 46 μ m long with four obtuse denticles ... *T. amoyanus* Fu, Zeng, Zhou, Tan and Cai, 2018
 - Buccal cavity with distinct tooth, gubernaculum 32–34 μ m long with pointed teeth ... *T. gracilis* (Ditlevsen, 1918) Filipjev, 1927
14. Amphidial fovea single loop, spicule with a ring-shaped structure *T. mangrovensis* Fu, Zeng, Zhou, Tan and Cai, 2018
 - Amphideal fovea rounded as a comma shaped loop with a double contour, spicule without ring-shaped structure *T. caudaensis* Tchesunov, Mokievsky & Nguyen Vu Thanh, 2010

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