

Two new species of free-living nematodes of genus *Tripyloides* (Nematoda: Enoplida: Tripyloididae) from mangrove wetlands in the Xiamen Bay, China

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Abstract

Two new species of free living nematodes *Tripyloides mangrovensis* n. sp. and *Tripyloides amoyanus* n. sp. of genus *Tripyloides* de Man, 1886 are described from mangroves of the Tong'an Bay and Haimen Island in the Xiamen Bay, China. The two species are both characterized by six stout outer labial setae, male without preanal papillae, tail conico-cylindrical without swollen terminally. *Tripyloides mangrovensis* n. sp. can be additionally by its buccal cavity consisting of two parts, amphid circular, distinct proximal conical and distal slender cylindrical portions, 2/3 of the tail cylindrical. *Tripyloides amoyanus* n. sp. is distinguished by buccal cavity consisting of three parts with small tooth, amphid small, rounded, like a comma-shaped loop, 1/3 of the tail cylindrical, gubernaculum with four denticles.

Key words: *Tripyloides mangrovensis* n. sp., *Tripyloides amoyanus* n. sp., mangrove, free living marine nematodes, China

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1 Introduction

About 7 000 species of free-living nematodes have been described so far (Appeltans et al., 2012), but only 300 species have been described in detail in China, among them 90 species are newly found (Shi, 2016). Mangroves are one of the most productive ecosystems in the world, an important ecological asset, and economic resource of the coastal environment (Kathiresan et al., 1996). Investigations on free-living marine nematodes were done in mangrove wetlands of the Tong'an Bay in 2014 and Haimen Island in 2016. Two new species of the genus *Tripyloides* de Man, 1886 are found and described here.

The genus *Tripyloides* was established by de Man in 1886. Only 15 species of the genus *Tripyloides* have been described but 14 species of them can be regarded as valid (<http://nemys.ugent.be>). Tchesunov et al. (2010) provided annotated list of 11 valid species. The genus is characterized by buccal cavity which divided into several stoma, outer labial setae may be jointed, amphid situated posterior to the buccal cavity, spicule wide, gubernaculum with small teeth, tail conico-cylindrical, male reproductive system single testis, female didelphic with reflexed ovaries. So far, only ten nematode species have been published in Xiamen (Zou, 2000, 2001; Huang and Liu, 2002; Chen and Guo, 2015; Guo et al., 2015; Li and Guo, 2016), and the species of genus *Tri-*

pyloides have never been described in China.

2 Materials and methods

Sediment samples were collected from mangroves of the Tong'an Bay and Haimen Island in the Xiamen Bay. Meiofauna samples were obtained using a modified syringe with 2.9 cm inner diameter, then the samples were fixed with 5% formalin seawater solution for permanent preservation. In the laboratory, samples were sieved using two mesh size (0.5 mm and 0.042 mm), sediment retained on 0.042 mm mesh size was extracted using the floatation technique in Ludox TM-50 solution (Heip et al., 1985). Samples were washed into petri dishes, nematodes were picked out and transferred into 9:1 (v:v) solution of 50% ethanol/glycerol under the stereoscopic microscope. Then they were placed in anhydrous glycerol, mounted on permanent slides after ethanol evaporated (Zhang and Platt, 1983). Photographs were taken using a differential interference contrast microscope (Nikon 50i). Drawings were made using a high magnification microscope (Olympus CX41) which with a camera lucida. Holotype and paratype specimens are deposited in College of the Environment and Ecology, Xiamen University, China.

Abbreviations are as follows: *a*=body length/maximum body diameter, *b*=body length/oesophagus length, *c*=body length/tail

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length, abd=anal body diameter, c' =tail length/abd, cbd=corresponding body diameter, $V\%$ =distance from vulva opening to the anterior end as a percentage of body length, cbd =corresponding body diameter, Outer labial setae%=outer labial setae length as percentage of corresponding body diameter, and Amphid%=amphid diameter as percentage of corresponding body diameter.

3 Results

Description

Order Enoplida Lorenzen, 1981

Suborder Tripyloidina Lorenzen, 1981

Family Tripyloididae Filipjev, 1918

Genus *Tripyloides* de Man, 1886

Diagnosis

(Modified from Tchesunov et al. (2010)) Thick outer labial setae, buccal cavity consists of 2–4 separate chambers. Stoma configuration varies because of the angle of the specimen under microscope, and the degree of stoma compression make the stoma more difficult for discrimination of *Tripyloides* species. The characters of anterior setae, body width and the position of the amphideal fovea are the most valuable diagnostic features in *Tripyloides*. Other characters such as tail shape and the arrangement of preanal and postanal supplementary papillae are also suitable for species discrimination.

Type species

Tripyloides caudaensis Tchesunov, Mokievsky and Nguyen Vu Thanh, 2010

Other species

Tripyloides acherusius Gerlach, 1952

Tripyloides amazonicus (Gerlach, 1957) Riemann, 1970

Tripyloides amoyanus n. sp.

Tripyloides brevis Gerlach, 1958

Tripyloides gracilis (Ditlevsen, 1918) Filipjev, 1927

Tripyloides granulatus (Cobb, 1913) Wieser, 1956

Tripyloides imitans Wieser, 1959

Tripyloides mangrovensis n. sp.

Tripyloides marinus (Bütschli, 1874) de Man, 1886

Tripyloides omblaica Micoletzky, 1924

Tripyloides pallidus Tchesunov, 1981

Tripyloides septentrionalis Schuurmans Stekhoven and De Coninck, 1933

Tripyloides soyeri de Bovée, 1977

Tripyloides taafi de Bovée, Coineau, Soyer and Travé, 1973

Tripyloides undulatus Gerlach, 1962

Tripyloides mangrovensis n. sp.

(Figs 1, 2 and Table 1)

Type material

Two males and four females were measured and studied. Male holotype, slides XTW201402B208. Paratypes, male slides, HMD201609BSA106, females, slides XTW201402B103, XTW-201402B204, XTW201402B204 and XTW201402G201.

Etymology

The species name is derived from mangrove wetlands where meiofauna samples were collected.

Measurements

Morphometric characteristic of the holotype and paratypes are given in Table 1.

Description

Male. Body 1 219–1 725 μm long. Body cylindrical, cuticle smooth. Mouth opening wide. Inner labial sensilla as minute papillae, six stout outer labial setae about 7 μm long, counting 31% of corresponding body diameter. Four cephalic setae, 5–7 μm long,

slightly shorter and thinner than the outer labial setae. Amphid small, circular, situates at level of posterior pockets of buccal cavity. Buccal cavity with strongly cuticularised walls, divided by distinct transversal cuticular ring into two chambers, anterior chamber irregularly cup-shaped and armed with a triangular, solid, dorsal tooth. Posterior chamber includes an irregularly cylindrical-conical part and two lateral hemispherical pouches. Pharynx posteriorly enlarged, not forming a true bulb. Cardia small. Nerve ring near middle of pharynx. Secretory-excretory pore situated posteriorly to nerve ring. Intestine with numerous sausage-like granules.

Anterior testis single, outstretched, situates ventrally of intestine. Spicules short and strong, slightly curved, distally pointed, proximally with a ring-shaped structure, strengthened by longitudinal cuticularisations. Gubernaculum parallel to the spicule, slightly sclerotized, with solid semi-rectangular structure distally. Tail conical proximally, 2/3 of the tail cylindrical, slender cylindrical distally without swollen terminally, three preanal setae. Three caudal glands.

Female. Body 1 095–1 763 μm in length, outer labial 7–9 μm long, four cephalic setae 4–6 μm long. Amphid diameter 7–8 μm , about 17%–19% of corresponding body diameter. Reproductive system didelphic with reflexed ovaries, 17 mature eggs were observed in uteri of a paratype female.

Diagnosis and relationships

Body length 1 095–1 763 μm , a 17–23. Outer labial and cephalic setae smooth, amphid small, circular, situated at level of posterior lateral pouches of buccal cavity. Tail 1/3 proximal conical and 2/3 distal cylindrical portions, three preanal setae present, slender cylindrical distally without swollen terminally.

Tripyloides mangrovensis n. sp. is close to a group of species of *T. caudaensis* Tchesunov, Mokievsky and Nguyen Vu Thanh, 2010, *T. gracilis* (Ditlevsen, 1918) Filipjev, 1927, *T. marinus* (Bütschli, 1874) de Man, 1886 and *T. pallidus* Tchesunov, 1981 with similar morphometric characteristics, and most resembles *T. caudaensis* which was also collected from mangrove habitat in terms of buccal cavity and tail with distinct proximal conical and distal slender cylindrical portion. *Tripyloides mangrovensis* n. sp. can be distinguished from *T. caudaensis* by circular amphid (comma-shaped loop in *T. caudaensis*) and presence of ring-shaped structure in spicules. *Tripyloides mangrovensis* n. sp. can be distinguished from other three species *T. gracilis*, *T. marinus* and *T. pallidus* by tail with distinctly set-off cylindrical portion.

Tripyloides amoyanus n. sp.

(Figs 3, 4 and Table 2)

Type material

One male and six females were measured and studied. Male holotype, slide XTW201402B211. Female paratypes, slides XTW201402B103, XTW201402G201, XTW201402G202, XTW201402G202, XTW201402G202 and XTW201402G204.

Etymology

The species name is derived from Amoy which city *T. amoyanus* n. sp. has been collected.

Measurements

Morphometric characteristic of the holotype and paratypes are given in Table 2.

Description

Male. Body length 1 578 μm , cylindrical, stout, cuticle smooth. Mouth opening narrow, 30 μm long. Buccal cavity with distinctly sclerotised walls, cylindrical-conoid with small denticle and could be divided into three parts. Inner labial sensilla as minute papillae. Six outer labial setae 9 μm long, about 42% of

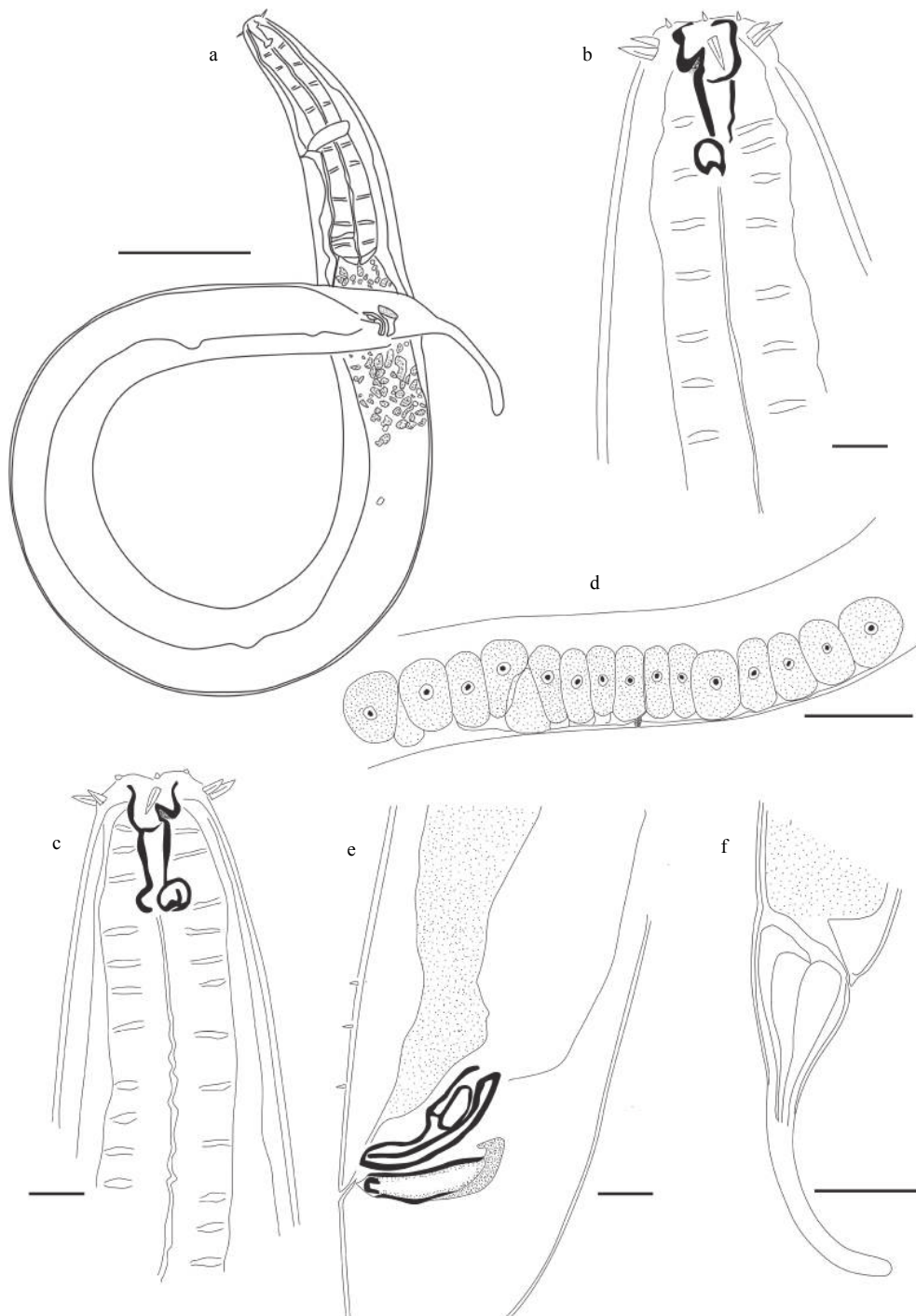


Fig. 1. *Tripylodes mangrovensis* n. sp.. a. Entire male, b. head of female, c. head of male, d. reproductive system of female, e. spicular area of male tail, f. tail of female. Scale bar(s) for a and d are 100 μm , for b, c and e are 10 μm and for f is 50 μm .

corresponding body diameter. Four cephalic sensilla 6 μm long. Outer labial setae stout, composes of two-joints and longer than cephalic setae, arranged in one circle with the cephalic setae. Amphid small and rounded, as a comma-shaped loop, situates behind the buccal cavity with diameter 7 μm long, counting about 19% of corresponding body diameter. Pharynx gradually swelling, not forming a distinct bulb. Cardia small. Nerve ring not distinct. Intestine wall consisting of cells containing colourless granules.

Anterior testis single, outstretched, situates ventrally of intestine. Spicules short, 39 μm long, slightly curved, with some longit-

udinal ribs, distally acute and proximally non-knobbed. Gubernaculum not sclerotized, with four denticles pointing to the ventral side. 2/3 of the tail conical proximally and 1/3 of the tail slender cylindrical distally without swollen terminally. The length of tail is 3.5 times of anal corresponding body diameter.

Female. Body 1 410–1 813 μm in length, outer labial setae 6–9 μm long, four cephalic setae 4–7 μm long. Amphid diameter 7–9 μm , about 15%–21% of corresponding body diameter. Reproductive system didelphic with reflexed ovaries.

Diagnosis and relationships

Tripylodes amoyanus n. sp. is characterized by body cyl-

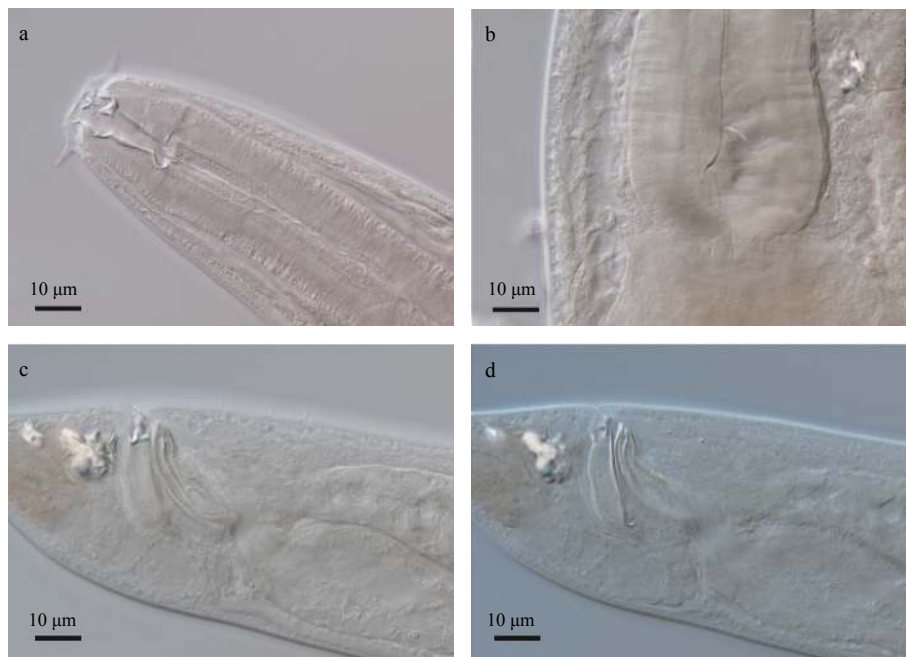


Fig. 2. *Tripyloides mangrovensis* n. sp.. a. Head of the male: buccal cavity, amphid, b. oesophagus of female, c. spicules, and d. gubernaculum.

Table 1. Morphometrics of *Tripyloides mangrovensis* n. sp. (in µm)

Characters	Holotype	Paratype		
		Male (n=1)	Female (n=4)	
			Min-Max	Mean
Body length	1 725	1 219	1 095–1 763	1 466
<i>a</i>	22	19	17–23	19
<i>b</i>	7	6	5–8	6
<i>c</i>	13	10	9–11	10
<i>c'</i>	3	3	3–4	3
V%	—	—	50–53	51
Head diameter	28	23	19–28	23
Amphid cbd	39	36	39–42	39
Nerve ring cbd	45	45	43–55	48
Cardia cbd	72	75	63–70	67
Maximum body diameter	80	66	66–102	78
abd	43	39	40–47	44
Outer labial setae length	7	7	7–9	8
Outer labial setae%	31%	30%	30%–43%	38%
Cephalic setae length	7	5	4–6	4
Amphid diameter	7	8	7–8	7
Amphid%	18%	20%	17%–19%	18%
Amphid from the anterior end	29	26	24%–29	27
Maximum buccal cavity width	13	11	7–11	9
Buccal cavity length	32	33	24–35	29
Spicule length as chord	32	31	—	—
Spicule length as arc	42	37	—	—
Gubernaculum length	30	30	—	—
Tail length	129	123	124–170	143

indrical, stout, body length 1 410–1 813 µm, *a* 19–24, outer labial setae stout, two-jointed, amphid rather small, comma-shaped loop, male without preanal papillae, gubernaculum with four denticles in the ventral side, and tail conico-cylindrical without swollen terminally.

Tripyloides amoyanus n. sp. differs from most other species of the genus, except *T. gracilis* (Ditlevsen, 1918) Filipjev, 1927, *T. marinus* (Bütschli, 1874) de Man, 1886 and *T. pallidus* Tchesunov, 1981 in the presence of conico-cylindrical tail, not with distinctly set-off cylindrical portion. *Tripyloides amoyanus* n. sp. can

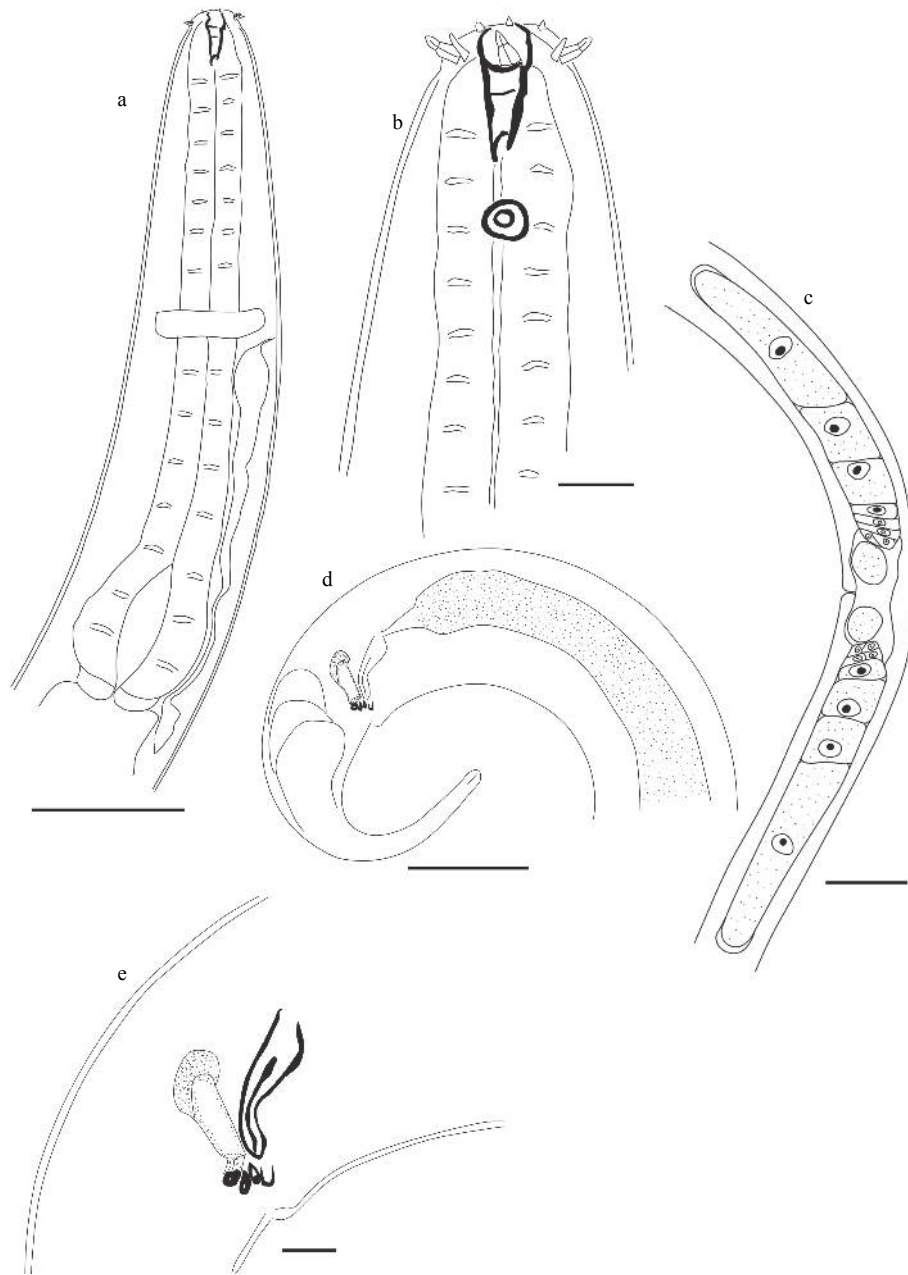


Fig. 3. *Tripyloides amoyanus* n. sp.. a. Anterior body region of female, b. head of male, c. reproductive system of female, d. tail of male, and e. spicular area of male tail. Scale bar(s) for a and d are 50 μ m, for c is 100 μ m, and for b and e are 10 μ m.

be distinguished from *T. marinus* by three-chambered buccal cavity (four-chambered in *T. marinus*) and gubernaculum with four denticles. *Tripyloides amoyanus* n. sp. differs from *T. pallidus* by oviparity vs. viviparity. *Tripyloides amoyanus* n. sp. can be distinguished from *T. gracilis* by comma-shaped loop amphid (spiral amphid in *T. gracilis*) and gubernaculum with four denticles.

Emended identification key to valid species of the genus *Tripyloides* de Man, 1886 (emended after Tchesunov et al. 2010)

- 1. Tail not consisting of distinct proximal conical and distal slender cylindrical portions.....2
- Tail consisting of distinct proximal conical and distal slender cylindrical portions.....5
- 2. Large multi-spiral amphideal fovea.....

-*T. acherusius* Gerlach, 1952
- Non-spiral amphideal fovea.....3
- 3. Supplement present.....4
- Supplement absent.....*T. brevis* Gerlach, 1958
- 4. Prominent pre- and postanal mid-ventral supplementary papillae.....*T. undulates* Gerlach, 1962
- Only pre-ventral supplementary papillae.....
-*T. imitans* Wieser, 1959
- 5. Relatively long and stout three-jointed outer labial setae.....
-*T. soyeri* de Bovée, 1977
- Short, not three-jointed outer labial setae.....6
- 6. Preanal midventral papillae minute.....
-*T. amazonicus* (Gerlach, 1957) Riemann, 1970
- Preanal midventral papillae absent.....7
- 7. Short outer labial (3–5 μ m), tail with longer cylindrical portion

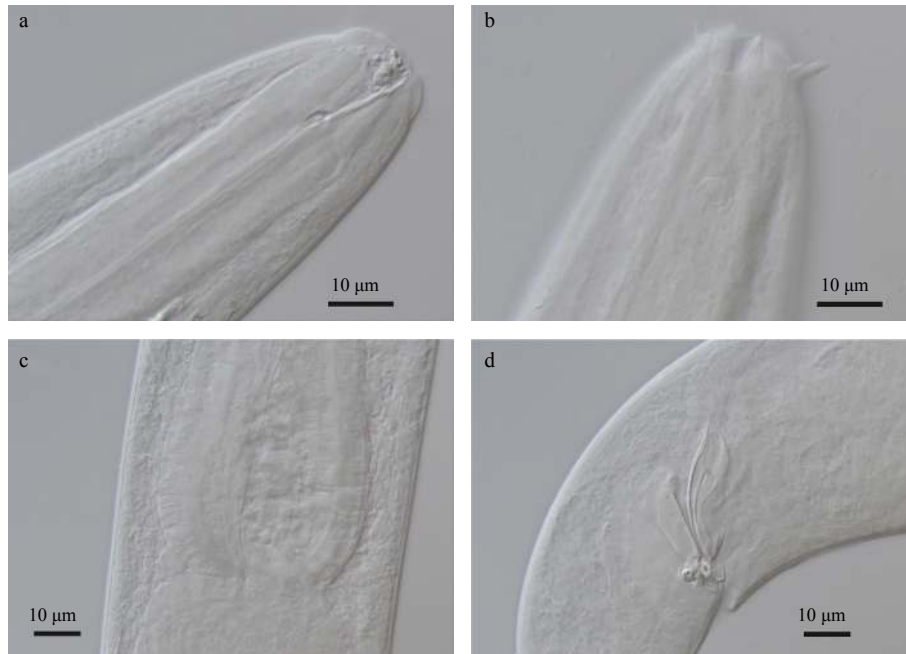


Fig. 4. *Tripylloides amoyanus* n. sp.. a. Head of the female: buccal cavity, b. head of male: amphid, cephalic setae and outer labial setae, c. oesophagus of male, and d. spicules and gubernaculum.

Table 2. Morphometrics of *T. amoyanus* n. sp. (in μm)

Character	Holotype	Paratype female (n=6)	
		Min-Max	Mean
Body length	1 578	1 410–1 813	1 696
<i>a</i>	24	19–24	22
<i>b</i>	7	7–8	7
<i>c</i>	12	11–13	12
<i>c'</i>	4	3	3
V%	—	46–48	47
Head setae cbd	19	20–23	22
Amphid cbd	38	37–43	40
Cardia cbd	66	58–80	71
Maximum body diameter	66	58–94	78
abd	39	38–52	47
Outer labial setae length	9	6–9	8
Outer labial setae%	42%	27%–40%	36%
Cephalic setae length	6	4–7	6
Amphid diameter	7	7–9	7
Amphid%	19%	15%–21%	18%
Amphid from the anterior end	30	27–33	30
Maximum buccal cavity width	7	7–9	8
Buccal cavity length	30	20–24	22
Spicule length as chord	35	—	—
Spicule length as arc	39	—	—
Gubernaculum length	46	—	—
Tail length	137	128–149	139

- (*c* = 5.6).....*T. granulatus* (Cobb, 1913) Wieser, 1956
- Longer outer labial setae, tail with shorter cylindrical portion (*c* > 5.6).....8
- 8. Distinct proximal conical and distal slender cylindrical portions.....9
- Not distinct proximal conical and distal slender cylindrical portions.....10

- 9. Single loop amphideal fovea, a ring-shaped structure in spicules.....*T. mangrovensis* n. sp.
- Comma-shaped loop amphideal fovea, no ring-shaped structure in spicules.....
-*T. caudaensis* Tchesunov, Mokievsky and Nguyen Vu Thanh, 2010
- 10. Four-chambered buccal cavity.....
-*T. marinus* (Bütschli, 1874) de Man, 1886
- Two or three chambered buccal cavity.....11
- 11. Viviparity.....*T. pallidus* Tchesunov, 1981
- Oviparity.....12
- 12. Gubernaculum with four denticles, tail tip without swollen terminally.....*T. amoyanus* n. sp.
- Gubernaculum without denticles and tail tip is swollen terminally*T. gracilis* (Ditlevsen, 1918) Filipjev, 1927

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References

Appeltans W, Ahyong S T, Anderson G, et al. 2012. The magnitude of global marine species diversity. *Current Biology*, 22(23): 2189–2202

Chen Yuzhen, Guo Yuqing. 2015. Two new species of *Lauratonema* (nematoda: Lauratonematidae) from the intertidal zone of the East China Sea. *Journal of Natural History*, 49(29–30): 1–12

de Man J G. 1886. Anatomische Untersuchungen über freilebende Nordsee-nematoden. Leipzig (Verlag von Paul Froberg), 1886: 1–82

Guo Yuqing, Chang Yu, Chen Yuzhen, et al. 2015. Description of a marine nematode *Hopperia sinensis* sp. nov. (Comesomatidae) from mangrove forests of Quanzhou, China, with a pictorial key to *Hopperia* species. *Journal of Ocean University of China*, 14(6): 1111–1115

Heip C, Vincx M, Vranken G. 1985. The ecology of marine nematodes.

- Oceanography and Marine Biology: an Annual Review, 23: 399–489
- Huang Hongliang, Liu Shengfa. 2002. One new species of free-living marine nematodes from southeastern beach of Xiamen Island. *Journal Oceanography in Taiwan Strait (in Chinese)*, 21(2): 177–180
- Kathiresan K, Rajendran N, Thangadurai G. 1996. Growth of mangrove seedlings in the intertidal area of Vellar estuary, south-east coast of India. *Indian Journal of Marine Sciences*, 25: 240–243
- Li Yongxiang, Guo Yuqing. 2016. Two new free-living marine nematode species of the genus *Anoplostoma* (Anoplostomatidae) from the mangrove habitats of Xiamen Bay, East China Sea. *Journal of Ocean University of China*, 15(1): 11–18
- Shi Benze. 2016. Taxonomy of nematodes and community structure of meiofauna in various marine habitats. Qingdao: University of Chinese Academy of Sciences
- Tchesunov A V, Mokievsky V O, Thanh N V. 2010. Three new free-living nematode species (Nematoda, Enoplida) from mangrove habitats of Nha Trang, Central Vietnam. *Russian Journal of Nematology*, 18(2): 155–173
- Zhang Zhinan, Platt H M. 1983. New species of marine nematodes from Qingdao, China. *Bulletin of the British Museum (Natural History) Zoology*, 45(5): 253–261
- Zou Chaozhong. 2000. Research on free-living marine nematodes near the Xiamen Island—description of two species of the family Axonolaimidae (Filipjev, 1918). *Journal of Xiamen University (Natural Science in Chinese)*, 6: 862–868
- Zou Chaozhong. 2001. Research on free living marine nematodes near Xiamen Island—new and known species of family Comesomatidae (Nematoda). *Journal of Oceanography in Taiwan Strait (in Chinese)*, 1: 48–53