

Morphological and molecular characterizations of *Africanema multipapillatum* sp. nov. (Nematoda, Enoplida) in intertidal sediment from the East China Sea

Benze Shi¹ · Kuidong Xu^{1,2,3}

Received: 25 February 2016 / Revised: 10 January 2017 / Accepted: 22 March 2017 / Published online: 27 April 2017
© Senckenberg Gesellschaft für Naturforschung and Springer-Verlag Berlin Heidelberg 2017

Abstract A new nematode species, *Africanema multipapillatum* sp. nov., is described from a sand beach in the East China Sea. The new species is assigned to the family Trefusiidae based on both the morphological and molecular analyses. *Africanema multipapillatum* sp. nov. is a distinct species characterized by its toothless and spacious buccal cavity, jointed labial setae, non-spiral and elongate groove-shaped amphidial fovea, faintly striated cuticle, and a single posterior ovary. Within the family Trefusiidae, the new species is most similar to the monotypic genus *Africanema*, but differs distinctly from *Africanema interstitiale* by the long and curved spicules with gubernaculum apophysis, long and slim sperm cells, and the lack of pharyngeal papillate supplements. Molecular phylogenetic analyses indicate that *Africanema multipapillatum* sp. nov. and the genera *Rhabdocoma* and *Trefusia* always fell within a single clade of the family Trefusiidae, where it is closely related to *Rhabdocoma*. Thus, we propose assigning the genus *Rhabdocoma* from the subfamily Trefusiinae to the subfamily Halanonchinae.

Keywords Marine nematode · New species · Trefusiidae · *Rhabdocoma* · Taxonomy · Phylogeny

Introduction

The taxonomic status of Trefusiidae Gerlach, 1966 has long been a controversial issue. Lorenzen (1981) elevated the family Trefusiidae to the order Trefusiida Lorenzen, 1981 which, however, was not accepted because of the lack of synapomorphic characters (Vincx and Furstenberg 1988). Trefusiidae is now considered as a member of the order Enoplida Filipjev, 1929, which is supported by morphological (Andrássy 1983; De Coninck 1965; Filipjev 1934) and molecular data (Bik et al. 2010; Holterman 2008; Rusin et al. 2001; van Megen et al. 2009). Morphological (Siddiqi 1983) and molecular analyses (Bik et al. 2010) indicate that Trefusiidae is closely related to Tripyloididae Filipjev, 1918, differing from the latter by the cephalic setae widely separated from the six outer labial setae, the two testes, and the lack of teeth in the buccal cavity.

The family Trefusiidae contains two subfamilies, Halanonchinae Wieser & Hopper, 1976 and Trefusiinae Gerlach, 1966 (Gerlach and Riemann 1974). Halanonchinae now contains two genera, *Halanonchus* Cobb, 1920 and *Africanema* Vincx & Furstenberg, 1988, both of which are monodelphic. Among the three genera, *Rhabdocoma* Cobb, 1920, *Trefusia* de Man, 1893, 1920, and *Trefusialaimus* Riemann, 1974 are contained in the subfamily Trefusiinae (Vanaverbeke 2015), *Rhabdocoma* is monodelphic, while the other genera are didelphic. *Rhabdocoma* is likely a member of Halanonchinae based on the presence of a single ovary. However, no molecular data are available for the phylogenetic analysis of Halanonchinae.

Communicated by M. Schratzberger

This article is registered in ZooBank under urn:lsid:zoobank.org:pub:EB9BA91D-A6B0-4A91-B786-6A982997CCAD

✉ Kuidong Xu
kxu@qdio.ac.cn

¹ Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

² University of Chinese Academy of Sciences, Beijing 100049, China

³ Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China

So far, the full scope of nematodes diversity in the sea areas of China is largely unknown due to insufficient investigations. During the examination of free-living nematode diversity in the East China Sea, we discovered a peculiar species of the order Enoplida. Based on the morphological and molecular analyses, we assign the species to the family Trefusiidae Lorenzen, 1981, where it is most similar to the monotypic genus *Africanema* Vincx & Furstenberg, 1988. The partial 18S rDNA sequences obtained from the new species make it possible to discuss, for the first time, the phylogenetic position of the genus *Africanema* and the subfamily Halanonchinae, as well as their relationship with the genus *Rhabdocoma*.

Materials and methods

Sampling and morphological analyses

Sediment samples were collected from the Dasha'ao Beach (27° 27.76' N, 121° 3.48' E) in the Nanji Islands National Marine Natural Reserve in the East China Sea. Samples were fixed with formalin (5% final concentration) in filtered sea water. The fixed samples were stained with 0.1% Rose Bengal for 12 h and subsequently rinsed on a 500- μ m sieve to remove large particles and on a 31- μ m sieve to retain meiofauna. Nematodes retained on the 500- μ m sieve were checked under a stereoscopic microscope and retrieved from the sieve, if present. Meiofauna were extracted from the remaining sediments using Ludox HS-40 by centrifugation and sorted to higher taxa under a stereoscopic microscope. Nematodes were transferred to 9:1 (v/v) solution of 50% alcohol-glycerol in a cavity block to slowly evaporate to pure glycerol, and then mounted into permanent slides.

Species descriptions were made from glycerol mounts (Platt and Warwick 1983) using a differential interference contrast microscope (Nikon E80i). Line drawings were made with the aid of a drawing device. Type specimens have been deposited in the Marine Biological Museum of the Chinese Academy of Sciences, Qingdao, China. All measurements are in μ m, and all curved structures are measured along the arc. The terminology used for describing the arrangement of morphological features follows Coomans (1979).

Abbreviations used in the paper are as follows: L = body length; T = tail length; abd = anal body diameter; mbd = maximum body diameter; $a = L/mbd$; $b = L/\text{pharynx length}$; $c = L/T$; $c' = T/abd$; V = distance from anterior end to vulva; and $V\% = V \times 100/L$.

DNA extraction, amplification, and sequencing

Sediment samples for molecular analyses were fixed with 100% ethanol. Nematodes were extracted from sediments by decantation. Ten specimens of the nematode species were

picked out to extract genomic DNA. Each specimen was mounted in temporary slides for morphological identification before the DNA extraction. Total genomic DNA was extracted from a single specimen using the TIANamp Marine Animals DNA Kit (TIANGEN Biotech Co., Ltd., China), following the manufacturer's instructions. Partial sequences of 18S rDNA were amplified by primers G18S4 (5'-GCTTGTCTCAAAGATTAAAGCC-3') and 26R (5'-CATTCTTGCAAATGCTTTGC-3'), 22F (5'-TCCAAGGAAGGCAGCAGGC-3') and 13R (5'-GGGCATCACAGACCTGTTA-3'), and 24F (AGRGGTGAAATYCGTGGACC-3') and 18P (5'-TGATCCWMCRGCAGGTTTCAC-3') following Blaxter et al. (1988). The 25- μ L polymerase chain reaction (PCR) contained 12.5 μ L Q5 Master Mix (New England Biolabs, USA), 8.5 μ L water, 1 μ L each of the forward and reverse primers, and 2 μ L of DNA template. The thermal cycling program was as follows: denaturation at 98 °C for 30 s, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 53 °C for 30 s, and extension at 72 °C for 30 s. A final extension was performed at 72 °C for 2 min. Sequencing reactions were conducted by the professional company TSINGKE Biological Technology Co., Ltd. (Qingdao, China) with the original PCR primers.

Sequence alignment and phylogenetic analyses

Only partial sequences of 18S rDNA (1275 bp) were successfully amplified. The DNA sequences were deposited in GenBank under the following accession numbers: KY421345 and KY421346. A total of 29 sequences of the families of Trefusiidae and Tripyloididae were downloaded from GenBank. The newly obtained sequences were aligned with the downloaded sequences by ClustalW multiple sequence alignment with default parameters using BioEdit version 7.1.3.0 (Hall 1999; Thompson et al. 1994). jModelTest2 (Darriba et al. 2012) was used to select the best Akaike information criterion (AIC) model. Bayesian inference (BI) and maximum likelihood (ML) analyses were conducted with MrBayes version 3.2.2 (Ronquist et al. 2012) and RaxML version 8.2.9 (Stamatakis 2014) on the CIPRES Gateway (Miller et al. 2010). Trees were edited in FigTree version 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>). Two sequences *Trichodorus primitivus* AJ439517 and *Tobrilus gracilis* AJ966506 of the closely related order Tripylonchida were used as outgroups in the phylogenetic analyses.

Results

Order Enoplida Filipjev, 1929

Family Trefusiidae Lorenzen, 1981

Subfamily Halanonchinae Wieser & Hopper, 1976

Genus *Africanema* Vincx & Furstenberg, 1988

Type species: *Africanema interstitiale* Vincx & Furstenberg, 1988 (= *Africanema interstitialis* Vincx & Furstenberg, 1988).

Note: Vincx and Furstenberg (1988) did not mention the etymology of *Africanema interstitialis* when they established the genus and species. The grammatical gender of *Africanema* should be neutral because it is a composite of the location Africa and the Greek noun *nema*. Thus, the ending of the specific name *interstitialis* should be changed to *interstitiale* to match the gender (neutral) of the generic name.

Emended Diagnosis of Africanema (based on Vincx and Furstenberg 1988 and the present description)

Cuticle striated. Anterior sensilla clear separated in three circles, outer labial setae jointed, inner labial setae and cephalic setae may be jointed. Amphidial fovea elongate. Pharyngeal supplements maybe present. Many papillate preloacal supplements. Gubernaculum may be present. Two opposed testes. Only a single posterior reflexed ovary. Vulva situated at anterior portion of body.

***Africanema multipapillatum* sp. nov.**

Figs. 1, 2, 3, and 4; Table 1

Diagnosis

Body long and slender, 1998–2750 μm long. Cuticle faintly striated. Head slightly set-off. Six inner labial setae stout and jointed. Six outer labial setae long, thick, and jointed. Four cephalic setae at the base of the buccal cavity, shorter than outer labial setae. Buccal cavity toothless, cylindrical and spacious, strongly sclerotized, with posterior half surrounded by pharyngeal tissue. Amphidial fovea elongate groove-shaped with transverse striation. Spicules strongly sclerotized and curved. Gubernaculum strongly sclerotized with dorso-caudally directed apophysis. About 13 papillate supplements. Two opposed testes. Only a single posterior reflexed ovary. Vulva situated at anterior fourth of body length. Tail slightly conical-cylindrical.

Holotype

One male on slide NJ-20140513-33.

Paratypes

Nine males on slides NJ-20140513-31, -32, and -33, two females on slides NJ-20140513-33 and NJ-20160809, and one juvenile on slide NJ-20140513-29.

Type locality and habitat

Intertidal sandy sediment in the Dasha'ao Beach of the Nanji Islands in the East China Sea (27° 27.76' N, 121° 3.48' E). The temperature of interstitial water was about 22 °C and salinity at 28 during sampling. The median particle diameter of sediment was about 612 μm (coarse sand) and sediment organic matter content was about 2.5%.

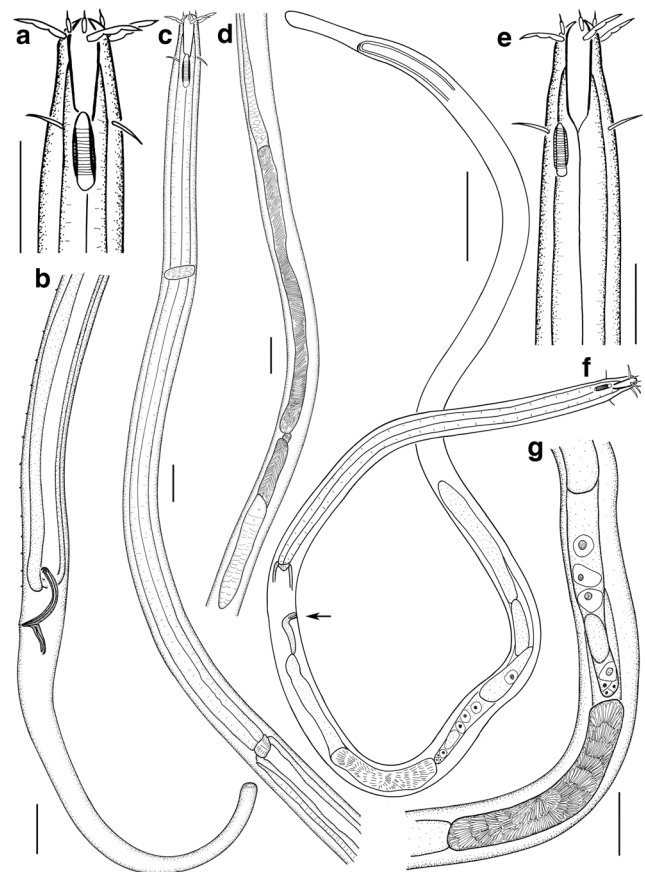


Fig. 1 *Africanema multipapillatum* sp. nov. Male holotype (a–d) and a female paratype (e–g). **a** Male head, showing the cylindrical and spacious buccal cavity without teeth, the elongate amphidial fovea with transverse straits, the jointed inner and outer labial setae, and the cephalic setae situated at the base of the buccal cavity. **b** Posterior region of male, showing the sclerotized spicule with dorso-caudally directed gubernaculum apophysis, the 13 preloacal supplements, and the long conical-cylindrical tail. **c** Anterior region of male, showing the long and cylindrical pharynx with smooth outline and the anterior testis reaching the pharyngeal region. **d** Reproductive system of male, showing the opposed testes with slim sperms and the shorter posterior testis. **e** Female head, showing similar structure to the male holotype. **f** Overview of a female specimen, showing the vulva (arrow) situated at the anterior fourth of the body, the long vagina, a single posterior reflexed ovary, a large spermatheca, and the short and rounded tail. **g** Large spermatheca filled with slim sperms. Scale bars: a–e, g = 30 μm ; f = 100 μm

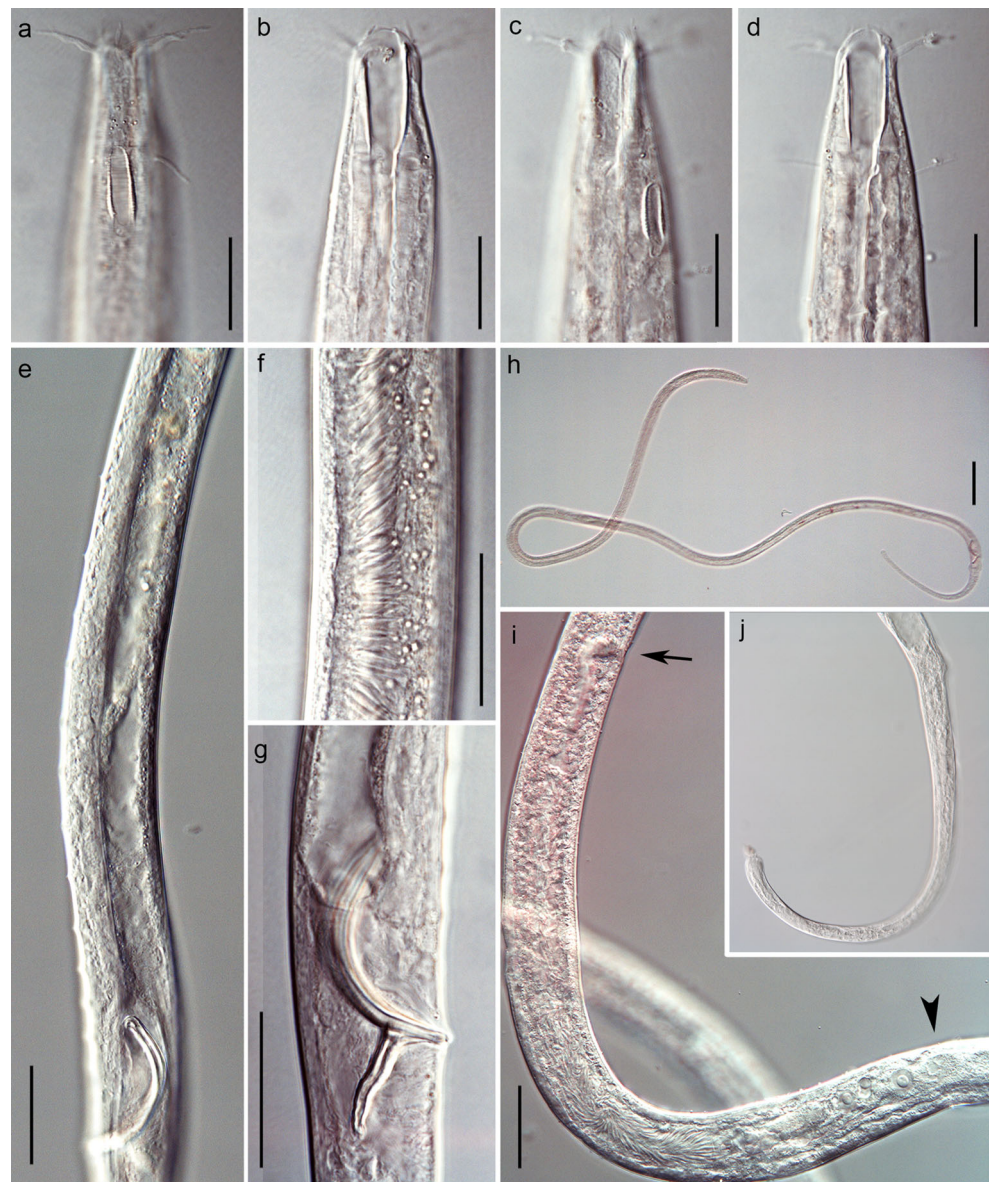
Etymology

Composite of the Latin prefix *multi* (many) and the Latin adjective *papillatus* (papillate), referring to the many papillate preloacal supplements, a main feature of the species.

Description

Body long and slender, 1998–2750 μm long (Table 1). Cuticle faintly striated. Metanemes absent. Head slightly tapering, set-

Fig. 2 *Africanema multipapillatum* sp. nov. Male holotype (a, b, e–g), female paratypes (c, d, i, j), and a male paratype (h). a, b Male head, showing the jointed inner and outer labial setae, the slender cephalic setae situated at the base of the buccal cavity, the elongate amphidial fovea, and the toothless spacious buccal cavity with only the posterior region surrounded by pharyngeal tissue. c, d Female head, showing similar structure to the male holotype. e Precloacal region of male with 13 papillate precloacal supplements. f The spermatheca is filled with slim sperms. g Strongly sclerotized spicule with dorso-caudally directed gubernaculum apophysis. h Overview of a male paratype, showing the long and slender body shape and the conical-cylindrical tail. i Reproductive system of female, showing the long vagina (arrow), large spermatheca filled with sperms, and the single posterior ovary (arrowhead). j Tail of another female specimen, showing the long conical-cylindrical tail. Scale bars = 30 μ m



off at the level of cephalic setae. Three lips. Six stout inner labial setae, jointed with two segments. Six long outer labial setae, jointed with three segments (Figs. 1a, e, 2a). Four slender cephalic setae shorter than the outer labial setae, located at the base of the buccal cavity, about 1.5 times the head diameter from the anterior end (Figs. 1a–e, 2a, d). Buccal cavity toothless, cylindrical and spacious, with walls strongly sclerotized and only posterior half surrounded by pharyngeal tissues (Figs. 1a, e, 2b, d). Cephalic capsule absent. Amphidial fovea situated at the base of the buccal cavity, elongate groove-shaped with transverse striation (Figs. 1a–e, 2a, c). Cervical setae sparse. Pharynx cylindrical with smooth outline (Fig. 1c, f). Cardia triangular. Somatic setae absent.

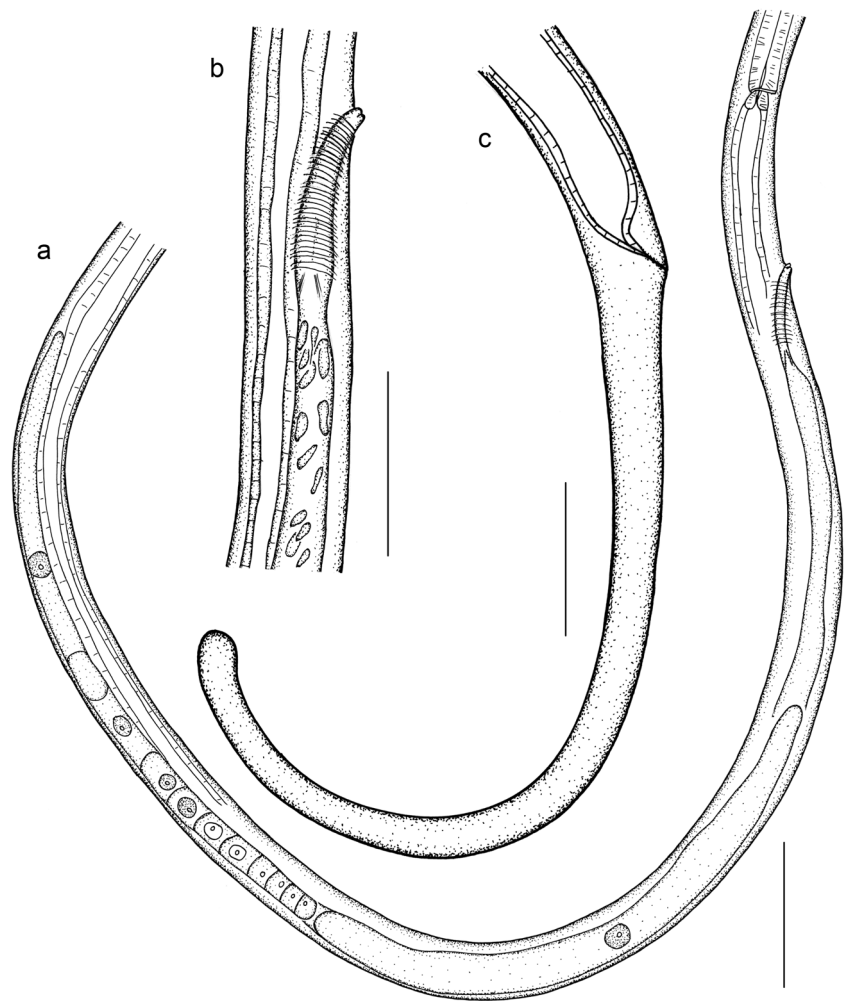
Males. Spicules strongly sclerotized and curved, length about two times the anal body diameter. Gubernaculum

strongly sclerotized with dorso-caudally directed apophysis (Figs. 1d, 2e, g). About 13 papillate precloacal supplements (Figs. 1b, 2e). Two opposed testes situated on the left side of intestine, with anterior testis longer and reaching the base of the pharynx (Fig. 1c, d). Sperm cells slim (Figs. 1d, 2f). Tail about 10 abd long and conical-cylindrical, terminal slightly swelling (Figs. 1b, 2h).

Females. Similar to male in body shape. One female with a shorter and rounded (4.3 abd) tail (Fig. 1f), likely due to fracture. Only a single posterior ovary, reflexed and situated on the left side of intestine (Figs. 1f, 2i). Vulva situated at anterior fourth of body, just posterior to pharyngeal region (Fig. 1f). Vagina long and sclerotized. Spermatheca large, filled with slim sperms (Figs. 1g, 2i).

Juvenile. Similar to male in body shape, without reproductive system.

Fig. 3 *Africanema multipapillatum* sp. nov. Another female paratype. **a** Reproductive system, showing the single posterior reflexed ovary and the cuticularized vagina. **b** Details of the cuticularized vagina. **c** The conical-cylindrical tail. Scale bars = 30 μ m



Molecular phylogenetic position

The BI and ML phylogenetic analyses closely support the monophyly of both the families Trefusiidae and Tripyloididae within the order Enoplida (Bayesian PP = 1, bootstrap value percent = 100; Fig. 4). *Africanema multipapillatum* sp. nov. is firmly supported as a member of the family Trefusiidae (Bayesian PP = 1, bootstrap value percent = 100), where it is always grouped with *Rhabdocoma* with high support values (Bayesian PP = 0.90, bootstrap value percent = 93), and are then clustered with *Trefusia* (Bayesian PP = 1, bootstrap value percent = 100). Within the family Tripyloididae, the genera *Bathylaimus* and *Tripyloides* are likely paraphyletic.

Discussion

Africanema multipapillatum sp. nov. is characterized by the long and jointed labial setae, the cephalic setae well separated from

outer labial setae, a large cylindrical and toothless buccal cavity, elongate groove-shaped amphidial fovea, and a single posterior ovary. These features match the diagnosis of the enoplid family Trefusiidae (Leduc 2013). The familial assignment in Trefusiidae is also strongly supported by the molecular phylogenetic analyses (Fig. 4). At first glance, the new species resembles the genus *Chaetonema* of the family Anoplostomatidae due to the presence of a large and toothless buccal cavity and elongate amphidial fovea (Smol and Coomans 2006; Smol et al. 2014). However, the presence of a single ovary in *Africanema multipapillatum* refutes the familial assignment to the family Anoplostomatidae, which possesses two ovaries (Smol and Coomans 2006; Smol et al., 2014). Molecular analyses also showed that *Chaetonema* occupied a clade within Enoploidea Dujardin, 1845 (Bik et al. 2010), while the new species is a member of the family Trefusiidae with high support values (Fig. 4).

Within the two subfamilies of Trefusiidae, Halanonchinae contains only the genera *Halanonchus* and *Africanema* and is characterized by possessing a large cylindrical buccal cavity and a single posterior ovary (Vinx and Furstenberg 1988). The subfamily Trefusiinae, which includes *Rhabdocoma*,

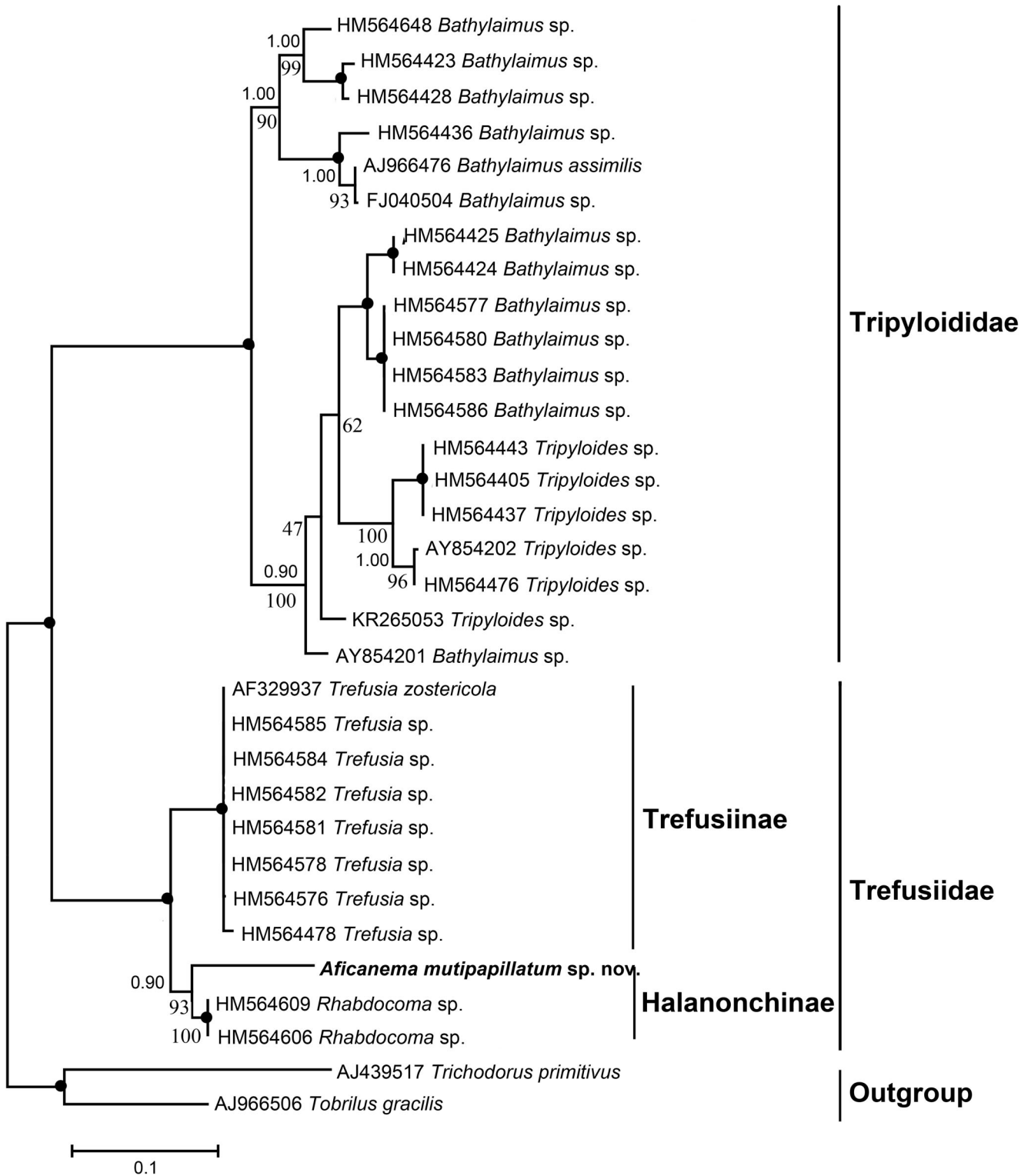


Fig. 4 Maximum likelihood (ML) tree of partial 18S rDNA sequences of the families Trefusiidae and Tripyloidea. The numbers below branches represent bootstrap support values in the ML analysis, the numbers above branches represent Bayesian posterior probabilities (BPPs) for the same clades found in the Bayesian inference (BI) analysis, the black dots on the

nodes represent BPPs in BI analysis = 1 and bootstrap support values in the ML analysis = 100. The scale bar represents the number of substitutions per site. Two nematode species of the closely related order Tripylonchida were used as outgroups: *Trichodoros primitivus* AJ439517 and *Tobrilus gracilis* AJ966506

Trefusia, and *Trefusialaimus*, differs from Halanonchinae by the possession of a minute or conical buccal cavity

(Vanaverbeke 2015; Vincx and Furstenberg 1988). Trefusiinae is likely polyphyletic because the genus

Table 1 Morphometrics (measurements in μm) of *Africanema multipapillatum* sp. nov.

Characters	Holotype	Paratypes			
		Males ($n = 9$)	Female 1	Female 2	Juvenile
Body length	2333	2100–2750	1998	3004	2083
Maximum body diameter	29	28–30	32	34	29
a	80.4	75–94.8	62.4	88	71.8
b	4.6	4.4–5.3	4.3	5	4.1
c	8.5	6.9–8.2	20	8.3	7.8
c'	9.8	11.5–12.6	4.3	13	9.9
Inner labial setae, length	4	4–5	5	5	5
Outer labial setae, length	15	14–17	15	15	15
Cephalic setae, length	9	8–10	11	11	13
Head diameter at outer labial setae	12	11–13	12	14	11
Head diameter at cephalic setae	18	19–21	20	19	18
Outer labial setae to anterior end	5	5	5	5	5
Cephalic setae to anterior end	29	25–29	30	31	25
Amphideal fovea to anterior end	22	20–28	28	30	27
Amphideal fovea length	21	20–23	15	15	15
Amphideal fovea width	5	5–6	4	5	3
Buccal cavity length	25	23–29	31	23	26
Buccal cavity width	8	7–8	8	9	7
Nerve ring to anterior end	143	127–156	130	122	152
Pharynx length	510	463–571	469	598	502
Anal body diameter	27	27–29	23	28	29
Vulva from the anterior end	–	–	530	705	–
V%	–	–	26.5	23.5	–
Supplements, number	13	13	–	–	–
Spicule length	52	50–55	–	–	–
Spicule length/abd	1.9	1.8–2	–	–	–
Gubernaculum length	21	19–24	–	–	–
Gubernaculum length/abd	0.8	0.7–0.9	–	–	–
Tail length	275	298–364	100	364	267

Rhabdocoma differs from the other members by possessing only a single posterior ovary (vs. two ovaries), a character of Halanonchinae. This is also shown by the ML and BI trees, where the genus *Rhabdocoma* is closely related to *Africanema*. Based on the morphologic and molecular characterizations, we propose assigning the genus *Rhabdocoma* from the subfamily Trefusiinae to the subfamily Halanonchinae, despite its minute or conical buccal cavity (vs. large cylindrical buccal cavity in Halanonchinae). The shape of the buccal cavity should have less important taxonomic significance than the number of ovaries at higher level taxonomy.

Within Halanonchinae, the new species is most similar to the monotypic genus *Africanema* in possessing elongate groove-shaped amphidial fovea, striated cuticle, jointed outer labial setae, cephalic setae situated at the posterior border of the buccal cavity, and a short tail (Vincx and Furstenberg

1988), by which it differs distinctly from the genus *Halanonchus* (with circular amphidial fovea). The new species differs from the only species *Africanema interstitialis* Vincx & Furstenberg, 1988 in the jointed inner labial setae and cephalic setae (vs. smooth), longer and curved spicules with gubernaculum apophysis (vs. shorter and almost straight without gubernaculum), long and slim sperm cells (vs. oval), longer tail about 10 times the anal body diameter (vs. 5–8), and the lack of pharyngeal papillate supplements in males (vs. possessing 12 supplements). These distinct differences justify the establishment of the new species within the genus: *Africanema multipapillatum* sp. nov.

Acknowledgments This work was supported by the National Programme on Global Change and Air–Sea Interaction (grant no: GASI-01-02-02-02), the China Postdoctoral Science Foundation (2016 M602201), and the Meiobenthic Community and Environmental

Assessment of Nanji Islands National Marine Natural Reserve under Human Disturbance (NJKJ2016). We thank the anonymous reviewers for their constructive suggestions and comments. Xumiao Chen, Yuhang Li, Ju Li, and Sichao Pu provided a lot of support and help in the sample collection and molecular analysis.

References

- Andrássy I (1983) A taxonomic review of the suborder Rhabditina (Nematoda: Secernentia). Office de la Recherche Scientifique et Technique Outre-Mer, Paris, 241 pp
- Bik HM, Lamshead PJD, Thomas WK, Lunt DH (2010) Moving towards a complete molecular framework of the Nematoda: a focus on the Enoplida and early-branching clades. *BMC Evol Biol* 10:1–14. doi:10.1186/1471-2148-10-353
- Blaxter ML, De Ley P, Garey JR, et al. (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* 392 (6671):71–5
- Coomans A (1979) Addendum I. A proposal for a more precise terminology of the body regions in the nematode. *Annales de la Société Royale Zoologique de Belgique* 108:155–117
- Darriba D, Taboada GL, Doallo R, Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9(8): 772–772
- De Coninck LAP (1965) Systématique des Nématodes. In: Grassé P-P (ed) *Traite de Zoologie*, vol 4. Masson et Cie, Paris, pp 586–731
- Filipjev IN (1929) Les nématodes libres de la baie de la Neva et de l'extrémité orientale du Golfe de Finlande. Première partie. *Arch Hydrobiol* 20:637–699
- Filipjev IN (1934) The classification of the free-living nematodes and their relation to the parasitic nematodes. *Smithsonian Miscellaneous Collections* 89:1–64
- Gerlach SA, Riemann F (1974) The Bremerhaven checklist of aquatic nematodes. A catalogue of Nematoda Adenophorea excluding the Dorylaimidae. *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven, Supplement* 41:1–734
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Holterman M (2008) Phylogenetic relationships within the phylum Nematoda as revealed by ribosomal DNA, and their biological implications. PhD thesis, Wageningen University
- Leduc D (2013) Two new free-living nematode species (Trefusiina: Trefusiidae) from Chatham Rise crest, Southwest Pacific Ocean. *Eur J Taxon* 55(55):1–13
- Lorenzen S (1981) Entwurf eines phylogenetischen systems der freilebenden Nematoden. *Veröff Inst Meeresforsch Breme, Supplement* 7:1–472
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, Louisiana, 14 November 2010, pp 1–8
- Platt HM, Warwick RM (1983) Freelifing marine nematodes. Part 1: British enoplids. Pictorial key to world genera and notes for the identification of British species. In: *Kermack DM, RSK B (eds) Synopses of the British Fauna (new series)*, no. 28. Cambridge University Press, Cambridge, pp 169–171
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61(3):539–542
- Rusin LY, Aleshin VV, Vladychenskaya NS, Milyutina IA, Kedrova OS, Petrov NB (2001) Trefusiidae are a subtaxon of marine Enoplida (Nematoda): evidence from primary structure of hairpin 35 and 48 loops of SSU rRNA gene. *Mol Biol* 35:778–784
- Siddiqi MR (1983) Phylogenetic relationships of the soil nematode orders Dorylaimida, Mononchida, Triplonchida and Alaimida, with a revised classification of the subclass Enoplia. *Pak J Nematol* 1:79–110
- Smol N, Coomans A (2006) Chapter 12. Order Enoplida. In: *Eyuaem-Abebe TW, Andrassy I (eds) Freshwater nematodes: ecology and taxonomy*. CABI Publishing, Wallingford, pp 225–292. doi:10.1079/9780851990095.0225
- Smol N, Muthumbi A, Sharma J (2014) Chapter 7.3. Order Enoplida. In: *Schmidt-Rhaesa A (ed) Handbook of zoology. Gastrotricha, Cycloneuralia and Gnathifera. Volume 2: Nematoda*. De Gruyter, Germany, pp 193–250. doi:10.1515/9783110274257.193
- Stamatakis A (2014) RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312–1313. doi:10.1093/bioinformatics/btu033
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- van Megen H, van den Elsen S, Holterman M, Karszen G, Mooyman P, Bongers T, Holovachov O, Bakker J, Helder J (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology* 11:927–950
- Vanaverbeke J (2015) Trefusiidae Gerlach, 1966. In: *Guilini K, Bezerra TN, Eisendle-Flöckner U, Deprez T, Fonseca G, Holovachov O, Leduc D, Miljutin D, Moens T, Sharma J, Smol N, Tchesunov A, Mokievsky V, Vanaverbeke J, Vanreusel A, Venekey V, Vincx M (2017) NeMys: World Database of Free-Living Marine Nematodes*. Accessed at <http://nemys.ugent.be/aphia.php?p=taxdetails&id=2211> on 2017-01-08
- Vincx M, Furstenberg JP (1988) *Africanema interstitialis* gen. nov., a species which indicates the relationship between the Trefusiidae (Halanonchinae) and the Tripyloididae (Nematoda). *Stylogogia* 4(1):10–16