Journal of Helminthology

cambridge.org/jhl

Description of *Paravulvus zhongshanensis* sp. nov. (Dorylaimida: Nygolaimidae) from Nanjing, China

S. Sun[1], Q.L. Zeng[2], X. Qing[1], H. Li[1] and S. Álvarez-Ortega[3]

[1]Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China; [2]Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, China and [3]Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, Campus de Móstoles, 28933-Madrid, Spain

Research Paper

Cite this article: Sun S, Zeng QL, Qing X, Li H, Álvarez-Ortega S (2023). Description of *Paravulvus zhongshanensis* sp. nov. (Dorylaimida: Nygolaimidae) from Nanjing, China. *Journal of Helminthology* **97**, e19, 1–9. https://doi.org/10.1017/S0022149X23000032

Received: 20 September 2022
Revised: 6 December 2022
Accepted: 7 January 2023

Keywords:
Molecular; morphology; new species; Nygolaimina; phylogeny; taxonomy

Author for correspondence:
X. Qing,
E-mail: qingxue@njau.edu.cn

Abstract

Paravulvus zhongshanensis sp. nov., isolated from soil in a location at Jiangsu Province, China, is described and illustrated based on morphological, morphometric and molecular characterizations. The new species is characterized by its body 1.17–1.53 mm long, lip region offset by marked constriction and 12.1–13.8 µm broad, mural tooth deltoid and 9.6–11.7 µm long, neck 278–360 µm long, pharyngeal expansion 164–208 µm long or occupying more than one-half (54–62%) of total neck length, uterus 32.5–35.3 µm long or 1.0–1.1 times the corresponding body diameter, $V = 47.8–53.4$, paravulvae absent, female tail subcylindrical conoid (30.5–39.5 µm, $c = 36.0–45.5$, $c' = 1.7–2.2$) with widely rounded end, and male unknown. The new species was compared with six known species of the genus including *Paravulvus acuticaudatus*, *Paravulvus confusus*, *Paravulvus hartingii*, *Paravulvus iranicus*, *Paravulvus loofi* and *Paravulvus microdontus* mainly by similarities in having conical tail and c' value larger than 1.3. The rRNA and mitochondrial cytochrome oxidase subunit 1 genes of the new species were obtained and were used for reconstructing the phylogenetic relationships of the new species.

Introduction

The genus *Paravulvus* Heyns, 1968 is an interesting and widely distributed nygolaimid taxon. Its taxonomy was revised in 2002 by Lazarova *et al.* (2002) and posteriorly by Gilarte *et al.* (2013). Currently, *Paravulvus* contains 16 valid species (Peña-Santiago, 2021).

Leaving aside the plant parasitic forms of the family Longidoridae Thorne, 1935, the study of dorylaims in China is in its infancy despite the great extent of the country and the abundance and diversity of these nematodes. The matter, however, has received more attention in recent years resulting in the discovery of several new and known species in Dorylaimina Pearse, 1936 (Wu *et al.*, 2016, 2017, 2018, 2019) and demonstrating that Chinese dorylaims are likely as diverse as those from other regions of the world. Nygolaims are especially poorly known in China, with only three records, for example, *Aquatides aquaticus* Thorne, 1930 found in bottom mud of the Baoan Lake in Wuhan of Hubei Province (Wu, 1999), *Laevides laevis* (Thorne, 1939) Thorne, 1974 found in soil of grassland and bottom mud of the Baoan Lake, but also in soil on the shore of Taiping Lake in Anhui Province and the soil of Xianhua Mountain in Jinhua of Zhejiang Province (Wu, 1999) and *Laevides rapax* (Thorne, 1939) Ahmad & Jairajpuri, 1982 found in bottom mud of the Poyang Lake in Jiangxi Province and the Baoan Lake in Hubei Province (Wu & Liang, 1997).

A general nematode survey was conducted in several provinces of China during 2020–2021. Six nygolaimid populations from genera *Clavicaudoides* Heyns, 1968, *Paravulvus* and *Solididens* Heyns, 1968, were recovered from moss, grass and forest soil samples (table 1). All these populations were morphologically and molecularly identified, and three populations of the genus *Paravulvus* were identified to species level, including one population of *Paravulvus hartingii* (de Man, 1880) Heyns, 1968 recovered from grassland in the Tibet Autonomous Region, another population from mossy soil in the campus of Nanjing Agricultural University and one undescribed *Paravulvus* species from the rhizospheric soil of several grasses at the Zhongshan Mountain region in Nanjing. In the present study, we described this new species with a detailed morphological and molecular characterization, and we provided the first sequences for internal transcribed rRNA gene spacer (ITS) sequence as well as the mitochondrial cytochrome oxidase subunit 1 (*COI*) gene in suborder Nygolaimina Ahmad & Jairajpuri, 1979.

Materials and methods

Nematodes' extraction and morphological observations

In April 2021, soil samples were collected from the rhizosphere of grass species including *Digitaria sanguinalis* L., *Cynodon dactylon* (L.) Pers., *Erigeron acer* Linn and *Hemarthria*

Table 1. The origin and molecular information of nygolaimid species in this study.

Species	Locality	Global Positioning System coordinates	Habitat	18S rRNA gene accession number/ length (nt)	28S rRNA gene accession number/ length (nt)	Internal transcribed rRNA gene spacer accession number/length (nt)	Mitochondrial cytochrome oxidase subunit 1 mtDNA gene accession number/ length (nt)
Paravulvus zhongshanensis sp. nov.	Zhongshan Mountain, Nanjing, Jiangsu	32°03′15″N, 118°51′46″E	grassland	OP218880/ 1600	OP221721/ 853	OP251343/747	OP218836/403
				OP218881/ 1598	OP221722/ 851	OP251344/759	OP218837/401
				OP218882/ 1598	OP221723/ 850	OP251345/765	OP218838/404
Paravulvus hartingii	Nanjing Agricultural University (NAU), Nanjing, Jiangsu	32°19′04″N, 118°49′48″E	mossy soil	OP218883/ 814	–	–	–
P. hartingii	Linzhi, Tibet	29°39′43″N, 91.08′02″E	grassland	OP218885/ 1763	OP221726/ 578	–	–
Clavicaudoides sp.	NAU, Nanjing, Jiangsu	32°19′04″N, 118°49′48″E	mossy soil	–	OP221724/ 798	–	–
Clavicaudoides sp.	Qiqihar, Heilongjiang	47°21′35″N, 123°55′28″E	forest soil	OP218884/ 826	OP221725/ 738	–	–
Solididens sp.	Linzhi, Tibet	29°39′43″N, 91.08′02″E	grassland	OP218886/ 1611	OP221727/ 988	–	–

altissima (Poir.) Hubb. at the Zhongshan Mountain region in Nanjing of Jiangsu Province, China (table 1). The nematodes were extracted using the modified Baermann tray method (Whitehead & Hemming, 1965). The fresh nematodes were killed by heat, fixed with formaldehyde solution at 60 °C and processed to ethanol–glycerine dehydration according to Seinhorst (1959) as modified by De Grisse (1969), and mounted on permanent slides. Specimens were examined, photographed and measured using an Olympus BX51 microscope equipped with an Olympus DP72 camera (Olympus Corporation, Tokyo, Japan). Morphometrics included de Man's indices and standard measurements. The location of the pharyngeal gland nuclei was expressed according to Loof & Coomans (1968).

Table 2. Information about primers used in this study. F = forward, R = reverse

Target gene	Primer name	Direction (F/R)	Sequence (5'–3')	Tm/°C	Size /base pairs	Reference
18S rRNA	18S 39	F	AAAGATTAAGCCATGCATG	58	560	Mullin *et al.*, 2005
	18S 599R	R	ATCCAACTACGAGCTTTTTAA			
	18S 550	F	GCAGCCGCGGTAATTCCAGCT	58	427	
	18S 977R	R	TTTACGGTTAGAACTAGGGCG			
	18S 965	F	GGCGATCAGATACCGCCCTAGTT	58	608	
	18S 1573R	R	TACAAAGGGCAGGGACGTAAT			
28S rRNA	28–61 for	F	GTCGTGATTACCCGCTGAACTTA	54	945	Holterman *et al.*, 2008
	28–1006 rev	R	GTTCGATTAGTCTTTCGCCCCT			
internal transcribed rRNA gene spacer	Nygo-F	F	ATTACGTCCCTGCCCTTTGTAC	55	900	present study
	Nygo-R	R	GCTTAAATTCGGCGGGTAGTCA			
mitochondrial cytochrome c oxidase subunit 1	JB3	F	TTTTTTGGGCATCCTGAGGTTTAT'	51	500	Kanzaki & Futai, 2002
	JB5	R	AGCACCTAAACTTAAAACATAATGAAAATG			

https://doi.org/10.1017/S0022149X23000032 Published online by Cambridge University Press

DNA extraction and polymerase chain reaction (PCR) amplification

The DNA samples were extracted from nematode individuals according to Li *et al.* (2008). The fragments of 18S and 28S rRNA genes and the ITS, as well as that of the *COI* gene, were amplified with the corresponding primer pairs listed in table 2.

The PCR reaction was carried out in a total volume of 25 µl containing 1 µl of DNA template, 2 µl of each primer (10 µM), 12.5 µl of Ex Taq DNA Polymerase Mix (Bioscience, Shanghai, China), and 7.5 µl double-distilled water. The thermal cycle programme was as follows: initial step of 95 °C for 4 min; 35 cycles of 30 s at 95 °C; 30 s at annealing temperature corresponded to the primer pair and 2 min at 72 °C; and finished at 72 °C for 10 min. PCR products were separated on 1% agarose gels and visualized by staining with ethidium bromide. PCR products of sufficiently high quality were purified for cloning and sequencing by Sangon Corporation (Sangon Biotech, Shanghai, China).

Phylogenetic analysis

The newly obtained sequences of all nygolaims (table 1) were subjected to a Basic Local Alignment Search Tool to check for closely related species on GenBank. The sequences of relevant species in Nygolaimina, representative species in Dorylaimina, typical species in Mononchida Jairajpuri, 1969 or Mermithida Hyman, 1951, were downloaded from the GenBank. All the collected sequences for each gene were aligned by using MAFFT v. 7.205 with the G-INS-i algorithm (Katoh & Standley, 2013), except the *COI* gene was aligned using TranslatorX (Abascal *et al.*, 2010) under the invertebrate mitochondrial genetic code. The sequence dataset was analysed with Bayesian inference (BI) and maximum likelihood (ML) on CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010) using MrBayes 3.2.3 (Ronquist *et al.*, 2012) and RAxML 8.1.11 (Stamatakis *et al.*, 2008), respectively. BI analyses of all genes were performed under the GTR + I + G evolutionary model. The Markov chains were set with 1×10^7 generations, two runs and 25% burn-in and the sampling frequency was one per 100 generations. ML analysis included 1000 bootstrap (BS) replicates under the GTRCAT model. Trees were visualized and modified by using the FigTree v. 1.4.3 (Rambaut, 2016) and the Adobe Illustrator CS5.

Results

Paravulvus zhongshanensis sp. nov.

ZooBank identifier: 1160E054-AC24-47F1-9491-D92A904CA653

Description

(Figures 1 and 2, table 3).

Female: Slender to very slender nematodes of medium size. Body cylindrical, tapering towards both extremities, but more so towards posterior end. Habitus curved ventrad after fixation, especially in posterior body region, to C-shaped. Cuticle smooth, two-layered, its thickness 1.5 µm on mid-body and 2.0 µm on tail tip. Lateral, dorsal and ventral body pores indistinct. Lip region offset by depression as a marked constriction (a deep furrow or groove separating two parts), with smooth or slightly angular contour, 2.0–2.5 times as broad as high and 41.1% (37.5–48.5%) of body diameter at neck base. Amphidial fovea cup-shaped, located at approximately 1.0 µm from lip region, its aperture 6.9 µm (6.5–7.5 µm) or 53.6% (50.4–56.9%) of lip region diameter. Cheilostom nearly cylindrical, lacking any differentiation. Mural tooth deltoid, with visible lumen throughout its length, 0.8–0.9 times as long as lip region diameter. Guiding ring simple, situated at 6.0–7.5 µm from anterior end. Odontophore well developed, 0.9–1.1 times the odontostyle length. Anterior region of pharynx weakly muscular and enlarging gradually, basal expansion occupying 56.6% (53.9–62.0%) of total neck length. Expanded part of pharynx differentiated into two parts of different texture, with anterior two-thirds part having a much coarser granular appearance. Pharynx gland nuclei located as follows: DO (orifice of dorsal gland)= 66.8% (66.1–68.8%); DN (dorsal gland nucleus at center of nucleolus) = 71.1% (70.8–72.6%); DO–DN = 3.95% (3.6–4.3%); S_1N_1 (anterior nucleus of the first pair of ventrosublateral glands)= 76.4% (74.7–78.9%); S_1N_2 (posterior nucleus of the first pair of ventrosublateral glands)= 77.0% (75.1–78.9%); S_2N_1 (anterior nucleus of the second pair of ventrosublateral glands)= 87.1% (85.9–88.5%); S_2N_2 (posterior nucleus of the second pair of ventrosublateral glands) = 87.7% (86.6–88.8%). Cardia rounded-conoid, 10.2 µm (9.5–11.5) long; cardiac glands longitudinal (longer than wide), 9.0 × 4.5–5.0 µm. A dorsal cell mass present at level of anterior end of intestine. Nerve ring located at 81.6 µm (71.0–91.0 µm) from anterior end or 26.8% (22.6–30.8%) of total neck length. Genital system didelphic–amphidelphic, with both branches equally and well-developed, anterior branch 173 µm (135–207 µm) or 13% (11–16%) of body length, and posterior one 172 µm (147–202 µm) or 13% (11–16%) of body length. Ovaries usually small, reflexed, not surpassing the sphincter level, anterior ovary 61.1 µm (52.3–72.9 µm) and posterior one 65.3 µm (59.0–80.0 µm) long; oocytes arranged first in two or more rows, then in a single row. Oviduct 63.8 µm (61.0–69.0 µm) long or 1.7–2.1 times corresponding body diameter, consisting of slender part made of prismatic cells and well-developed *pars dilatata* without distinct lumen. Oviduct–uterus junction barely marked. Uterus a short, simple, tube-like structure 34.1 µm (32.5–35.5 µm) long or 1.0–1.1 times corresponding body diameter. Vagina extending inwards for 14.6 µm (13.5–16.0 µm) or 46.3% (40.2–50.4%) of body diameter, *pars proximalis* 17–23 × 14–18 µm, with sigmoid walls and surrounded by weak musculature, *pars refringens* with (in lateral view) two slender pieces measuring 3.5–4.0 × 2.0–2.5 µm and separated by an intermediate hyaline area resulting in a combined width of 9.0–9.5 µm, and *pars distalis* 3.2 µm (3.0–3.5 µm) long (according to nomenclature proposed by De Ley *et al.*, 1993). Vulva longitudinal, slit 11.4 µm (11.0–11.5 µm) long. Paravulvae absent. Prerectum 1.7–2.2 times and rectum 0.9–1.0 times anal body' diameters long. Tail subcylindrical conoid with broadly rounded terminus, ventrally concave, and dorsally convex, with thickened cuticle.

Male: Not found.

Diagnosis and relationship

The most characteristic feature of *P. zhongshanensis* sp. nov. is the combination of paravulvae absent and comparatively longer subcylindrical conoid female tail ($c' = 1.9$ (1.7–2.2)), and none of the 16 currently valid species of the genus bears these kind of traits. The new species is further characterized by the female body length of 1.34 mm (1.17–1.53 mm), lip region offset by a marked constriction, mural tooth deltoid with 10.0 µm (9.5–11.5 µm) long, pharyngeal expansion occupying 56.6% (54.0–62.0%) of total neck length, dorsal cell mass present at level of anterior

https://doi.org/10.1017/S0022149X23000032 Published online by Cambridge University Press

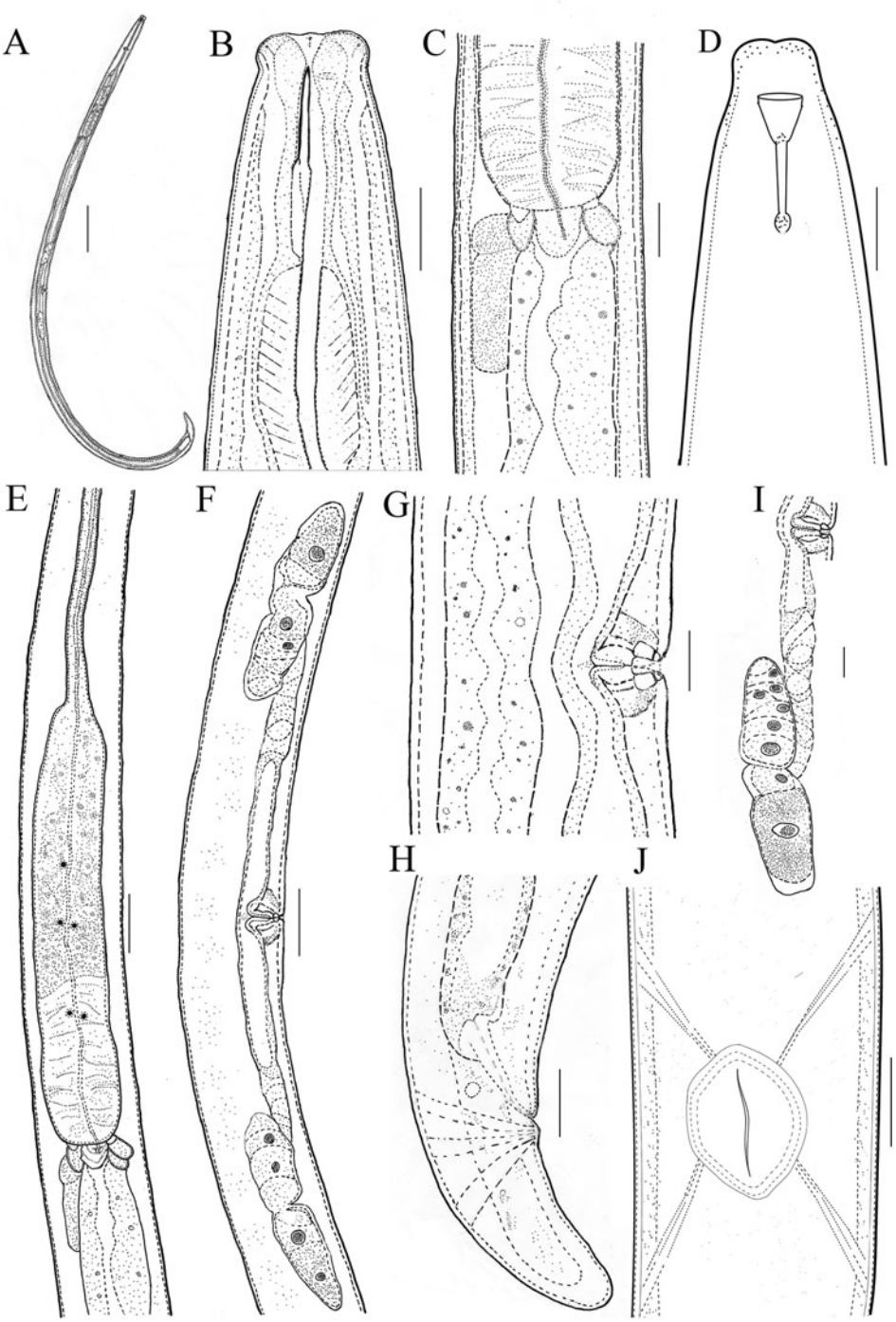

Fig. 1. Line drawings of *Paravulvus zhongshanensis* sp. nov. female: (a) entire body; (b) anterior body region; (c) pharynx–intestinal junction; (d) amphidial fovea; (e) posterior part of pharynx; (f) genital system; (g) vulval region; (h) tail region; (i) posterior branch of genital system; and (j) ventral view of vulval region. Scale bars: (a) = 50 µm; (b–d) = 10 µm; (e, f) = 20 µm; (g–j) = 10 µm.

end of intestine, $V = 49.3$ (47.8–53.4), vulva slit longitudinal 11.4 µm (11.0–11.5 µm) long, paravulvae absent, and male unknown.

According to the key to species identification to the genus *Paravulvus* (Gilarte *et al.*, 2013), *P. zhongshanensis* sp. nov. is similar to six species by having conical tail, curved ventrad and c' value between 1.3 and 2.8, which includes: *Paravulvus acuticaudatus* (Thorne, 1930) Heyns, 1968; *Paravulvus confusus* Akthar *et al.*, 1994; *Paravulvus hartingii*; *Paravulvus iranicus* Olia *et al.*, 2004; *Paravulvus loofi* Ahmad *et al.*, 2003; and *Paravulvus microdontus* Olia *et al.*, 2004.

However, the new species differs from *P. acuticaudatus* by the shorter female body length (1.17–1.53 vs. 1.61–2.01 mm), mural tooth shape (deltoid vs. solididentoid–deltoid) and longer length (9.5–11.5 vs. 5.0–7.0 µm), larger pharyngeal expansion (53.9–62.0% vs. 46–52% of total length), comparatively shorter prerectum length (1.7–2.2 vs. 2.7–3.7 times of anal body diameter), and female tail shape (subcylindrical conoid with broadly rounded terminus vs. short conical with finely rounded tip); from *P. confusus* by the female mural tooth length (9.6–11.7 µm vs. 7.5–10.0 µm), dorsal cell mass at level of anterior end of

https://doi.org/10.1017/S0022149X23000032 Published online by Cambridge University Press

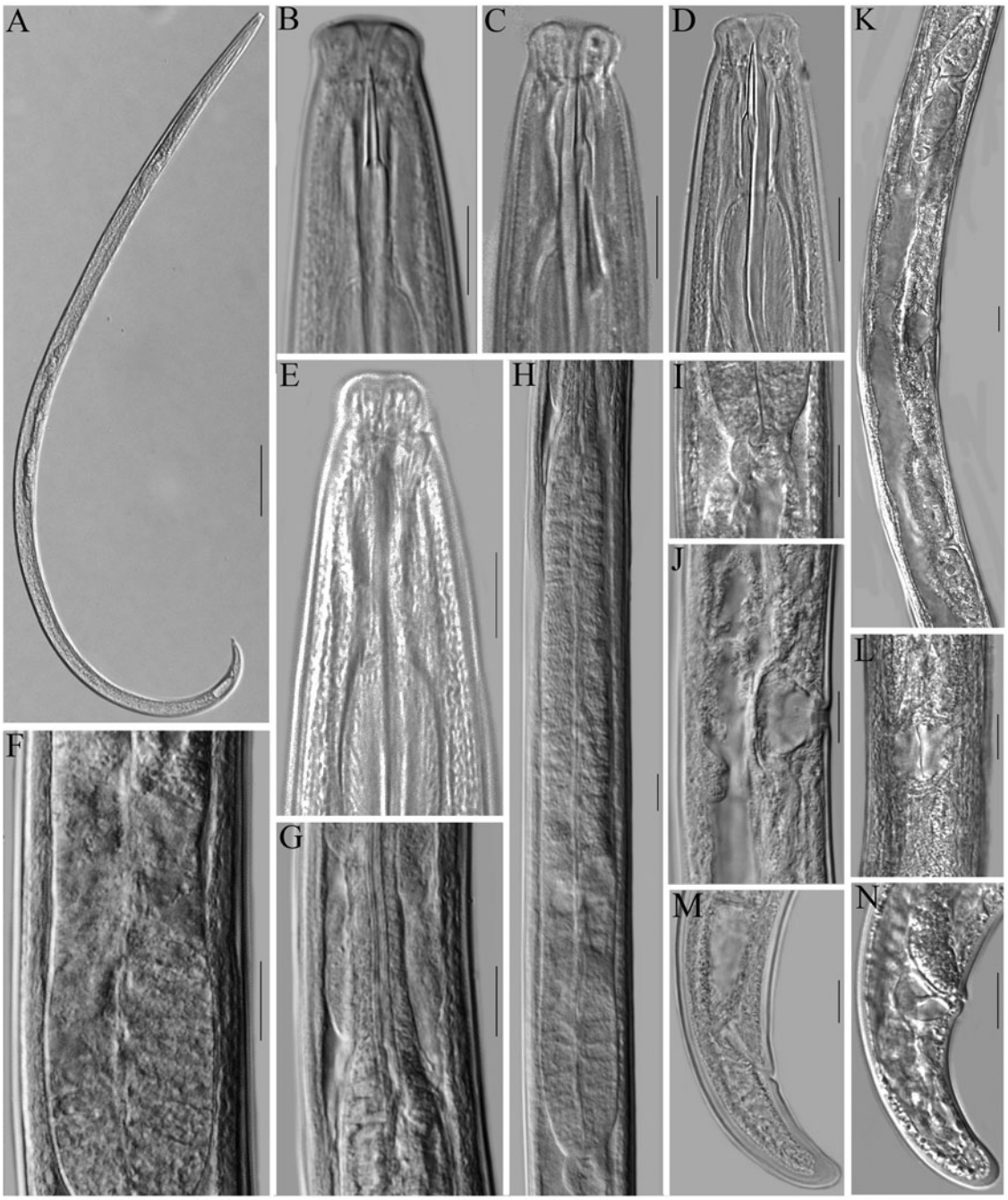

Fig. 2. Photomicrographs of *Paravulvus zhongshanensis* sp. nov. female: (a) entire body; (b–d) anterior body region; (e) amphidial fovea; (f) basal part of pharynx; (g) anterior part of pharyngeal bulb; (h) entire expanded part of pharynx; (i) pharynx–intestinal junction; (j) vulval region; (k) genital system; (l) longitudinal opening of vulva; and (m, n) tail region. Scale bars: (a) = 100 μm; (b–n) = 10 μm.

intestine (present vs. absent), DN position (at 70.8–72.6% vs. 52–55% of total pharynx length), S_1N_1 position (at 74.7–78.9% vs. 64–67% of total pharynx length), prerectum length (29–34 μm vs. 39–45 μm, or 1.7–2.2 vs. 2–3 times of anal body diam.), *V* value (47.8–53.4 vs. 44–47), a value (38.0–48.3 vs. 31–39), *c* value (36.0–45.4 vs. 25–35), and tail shape (subcylindrical conoid with broadly rounded terminus vs. elongated-conoid, ventrally arcuate with finely rounded tip); from *P. hartingii* by lip region (offset by a marked constriction vs. continuous with body), S_1N_1 position (at 74.7–78.9% vs. 80–83% of total pharynx length), c' value of female (1.7–2.2 vs. 1.9–3.8), and tail shape (subcylindrical conoid with broadly rounded terminus vs. elongated-conoid, ventrally arcuate with finely rounded tip) according to Gilarte *et al.* (2013); from *P. iranicus* by mural tooth length (9.6–11.7 μm vs. 7.5–8 μm), DN position (at 70.8–72.6% vs. 63–64% of total pharynx length), S_1N_1 position (74.7–78.9% vs. 73–74% of total pharynx length), *V* value (47.8–53.4 vs. 39–45), prerectum length (1.7–2.2 vs. 2.7–3.2 times of anal body diam.), female tail shape (subcylindrical conoid with broadly rounded terminus vs. conoid, with finely rounded terminus), and shorter female tail length (30.5–39.5, *c* = 36.0–45.4, c' = 1.7–2.2 μm vs. 43.0–58.0 μm, *c* = 28.0–33.0, c' =

https://doi.org/10.1017/S0022149X23000032 Published online by Cambridge University Press

Table 3. Morphometric data of *Paravulvus zhongshanensis* sp. nov. All measurements are in µm except L in mm, and in the form: mean ± standard deviation (range).

Character	Holotype	Paratype
n	♀	16♀♀
L	1.32	1.34 ± 0.10 (1.17–1.53)
a	39.6	42.4 ± 3.0 (38.0–48.3)
b	4.3	4.4 ± 0.2 (4.1–4.7)
c	37.2	40.2 ± 2.8 (36.0–45.4)
c'	1.9	1.9 ± 0.1 (1.7–2.2)
V	50.1	49.3 ± 1.3 (47.8–53.4)
lip region diameter	13.0	12.9 ± 0.5 (12.1–13.8)
mural tooth length	11.2	9.9 ± 0.7 (9.6–11.7)
odontophore length	10.4	10.6 ± 0.4 (9.9–11.1)
guiding ring from anterior end	6.7	6.7 ± 0.3 (6.1–7.4)
neck length	304	305 ± 19.0 (278–361)
pharyngeal expansion length	177	173 ± 10.1 (164–208)
diameter at neck base	30.6	31.4 ± 2.9 (25.5–40.1)
at midbody	33.3	31.5 ± 2.7 (25.9–39.1)
at anus	17.9	17.2 ± 0.8 (14.7–18.4)
prerectum length	33.6	31.9 ± 1.4 (29.0–34.0)
rectum length	18.1	16.9 ± 0.9 (14.7–18.2)
tail length	35.4	33.5 ± 2.6 (30.5–39.5)

♀, female.

2.3–2.6); from *P. loofi* by the longer female mural tooth length (9.5–11.5 vs. 6.5–7.5 µm), larger pharyngeal expansion (53.9–62.0% vs. 44.0–51.0% of total length), DN position (at 70.8–72.6% vs. 68–69% of total pharynx length), S_1N_1 position (at 74.7–78.9% vs. 72–74% of total pharynx length), tail shape (subcylindrical conoid with broadly rounded terminus vs. conoid with peg-like terminus), longer tail length (30.5–39.5 µm vs. 21.0–34.0 µm), and absence of male (vs. presence); and finally from *P. microdontus* by the longer female mural tooth length (9.5–11.5 µm vs. 4.0–5.0 µm), DN position at (70.8–72.6% vs. 56–57% of total pharynx length), S_1N_1 position at (74.7–78.9% vs. 67–68% of total pharynx length), comparative prerectum length (1.7–2.2 vs. 2.5–3.0 times of anal body diameter), female tail shape (subcylindrical conoid with broadly rounded terminus vs. short conoid ventrally arcuate with sharply-rounded tip), and lower *c'* value (1.7–2.2 vs. 2.2–2.6).

Type host and locality

Rhizosphere of grasses including *Digitaria sanguinalis*, *Cynodon dactylon*, *Erigeron acer* and *Hemarthria altissima* at the Zhongshan Mountain region in Nanjing, China (Global Positioning System coordinates: 32°03′15″N, 118°51′46″E).

Type material

Holotype female and additional paratypes (six females and three juveniles in two slides) are deposited in the collection of the Nematology Laboratory of Nanjing Agricultural University. Four paratype females in two slides (collection numbers PTP16236A and PTP16236B), are deposited in the nematode collection of the National Museum of Natural Sciences, Madrid, Spain.

Etymology

The species epithet refers to the mountain where the species was recovered (Zhongshan Mountain in Nanjing, China).

Molecular characterization and phylogeny

The GenBank accession number and information on the length of the sequences of rRNA genes and *COI* mtDNA gene of *P. zhongshanensis* sp. nov. are listed in table 1. The molecular information of the Jiangsu and Tibet populations of *P. hartingii*, Jiangsu and Heilongjiang populations of *Clavicaudoides* sp. and Tibet population of *Solididens* sp., are also listed in table 1.

The phylogenetic relationships of *P. zhongshanensis* sp. nov. were revealed by the BI and ML analyses based on the sequences of the 18S and 28S genes. The 18S tree (fig. 3) showed the new species grouped with *P. hartingii* (AY146537, AY552976, AY284774 and AY284775) and two Chinese populations of *P. hartingii* obtained in this study (OP218883 and OP218885), with posterior probability (PP) and BS values as 0.97 and 67, respectively. This clade forms a monophyletic group with *Nygolaimus* cf. *parvus* Thorne, 1974 (AY552974) (PP = 1, BS = 100). The aforementioned clade is in sister relation with the clade including species of genera *Aquatides* Heyns, 1968, *Clavicaudoides*, *Nygolaimus* Cobb, 1913 and *Solididens*, representatives of the superfamily Nygolaimoidea Thorne, 1935 (PP = 0.97, BS = 96). The clade of Nygolaimoidea has separated from the clade including all other superfamilies of the order Dorylaimida Pearse, 1942. Intraspecific variation among the 18S gene of the new species is 0–1 nucleotide (99.9–100.0% identity, 1598–1600 base pairs (bp)). The new species (OP218880) differs from the Tibet population of *P. hartingii* (OP218885) by 51 nucleotides (97.4% identity, 1579 bp/1630 bp), and from Jiangsu population of *P. hartingii* (OP218883) by 14 nucleotides (98.3% identity, 800 bp/814 bp), and from *P. hartingii* PVulHar2 isolate (AY284775) by 67 nucleotides (96.6% identity, 1568 bp/1618 bp), and from *Nygolaimus* cf. *parvus* by 71 nucleotides (96.1% identity, 1559 bp/1630 bp).

The 28S tree (fig. 4) showed similar relationships as that revealed by the 18S phylogeny. The new species grouped with the Tibet population of *P. hartingii* into a maximally supported clade (PP = 1, BS = 100). This clade was in sister relationship with a fully supported branch (PP = 1, BS = 100) containing species of *Clavicaudoides* and *Nygolaimus*, forming Nygolaimoidea clade (PP = 0.96, BS = 54) together. Intraspecific variation among the 28S gene of the new species is 2–19 nucleotides (97.8–99.8% identity, 850 bp–853 bp), while the new species (OP221721) differs from the Tibet population of *P. hartingii* (OP221726) by 141 nucleotides (78.4% identity, 437 bp/578 bp). Because only very few reference sequences are available in GenBank, ITS and *COI* genes were not used for phylogeny reconstruction. The intraspecific variation among the ITS sequences of the new species were 35–96 nucleotides (87.7–95.8% identity, 747–765 bp). Moreover, for the *COI* gene, the intraspecific variation of the new species is 1–4 nucleotides (99.1–99.7% identity, 401–404 bp).

https://doi.org/10.1017/S0022149X23000032 Published online by Cambridge University Press

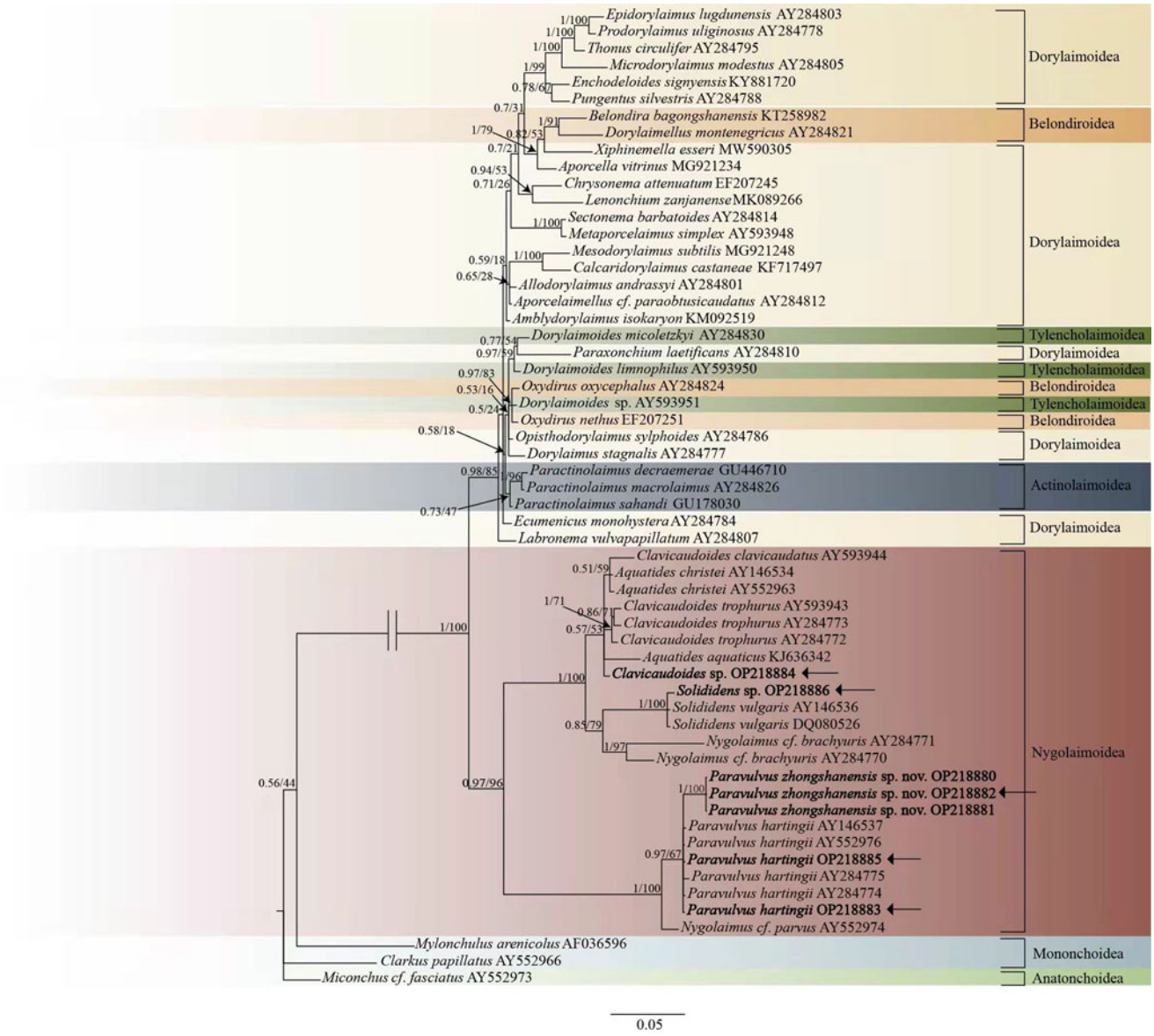

Fig. 3. Bayesian 50% majority-rule consensus tree of *Paravulvus zhongshanensis* sp. nov. and other related nematodes in the order Dorylaimida Pearse, 1942 inferred from 18S rRNA gene. Dataset was aligned with G-INS-i implemented in MAFFT. The values at clade node indicate posterior probability/bootstrap. Newly obtained sequences are indicated in boldface typ. The scale bar indicates expected changes per site.

Discussion

Paravulvus zhongshanensis sp. nov. was characterized through morphological observation and molecular analysis in the present study. The new species fits the typical pattern of genus *Paravulvus* by having the longitudinal vulva, unusual arrangement of pharyngeal gland nuclei, presence of large dorsal cell mass(es) at level of anterior end of intestine and *pars refringens vaginae* present (Ahmad & Jairajpuri, 1982; Jairajpuri & Ahmad, 1992; Lazarova *et al.*, 2002; Gilarte *et al.*, 2013).

According to intrageneric morphology variability in *Paravulvus*, this new species is easily distinguished from other species of the genus by its deltoid mural tooth, absence of paravulvae and subcylindrical conoid with broadly rounded terminus, although it is so similar to *P. hartingii* and *P. confusus*, two very similar species (Gilarte *et al.*, 2013).

The molecular data in genus *Paravulvus* are rather deficient. Currently molecular data available in GenBank for 18S rRNA gene are limited to *P. hartingii* and an unidentified *Paravulvus* species, whereas for 28S rRNA gene, there is only one, assigned to *P. hartingii*, and neither ITS rRNA gene nor *COI* mtDNA gene have been sequenced for the genus yet. Therefore, the molecular data, herein obtained, represent the first molecular information for the ITS rRNA and *COI* mtDNA genes for the suborder Nygolaimina. Based on the molecular data of the 18S and 28S marker genes, we resolved the evolutionary status of *P. zhongshanensis* sp. nov. by phylogenetic analysis. In this contribution, 20 sequences from six nygolaim populations were newly obtained, which enriched the nygolaim molecular database. The 18S and 28S phylogenetic trees in our study perfectly fit with previous results (Mullin *et al.*, 2005; van Megen *et al.*, 2009) and show that

https://doi.org/10.1017/S0022149X23000032 Published online by Cambridge University Press

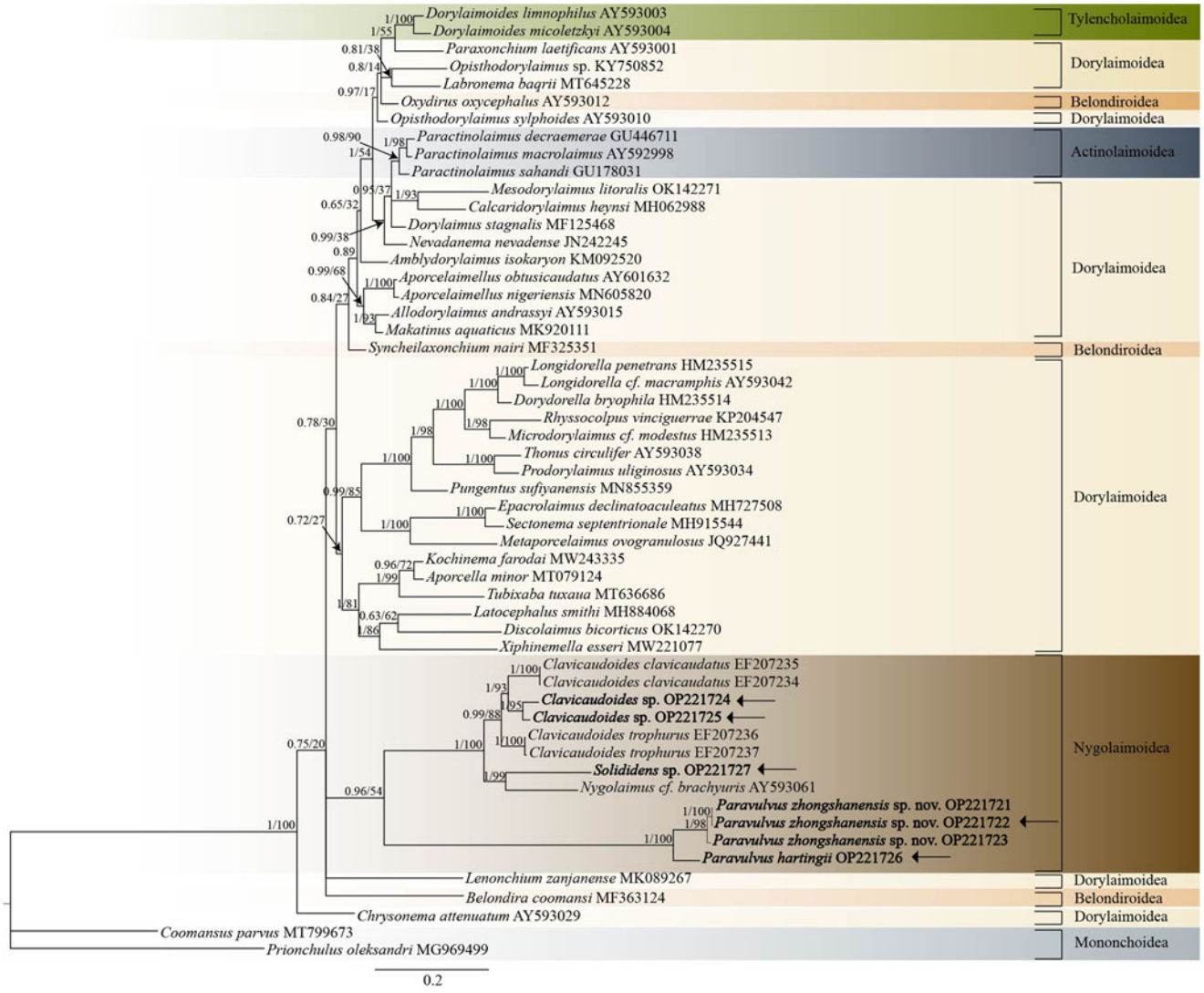

Fig. 4. Bayesian 50% majority-rule consensus tree of *Paravulvus zhongshanensis* sp. nov. and other related nematodes in Dorylaimida Pearse, 1942 inferred from 28S rRNA gene. Dataset was aligned with G-INS-i implemented in MAFFT. The values at clade node indicate posterior probability/bootstrap. Newly obtained sequences are indicated in boldface type. The scale bar indicates expected changes per site.

the members of the suborder Nygolaimina clustered together in a well-supported clade, comprising two subgroups, one clade including the sequences of the genera *Solididens*, *Aquatides* and *Clavicaudoides* or the genera *Solididens* and *Clavicaudoides*, respectively. in the 18S and 28S trees, and the other clade including sequences of the genus *Paravulvus*. Besides, the 18S tree shows that the sequences of the genus *Nygolaimus* have placed in two aforementioned clades, which is in accordance with a previous study (van Megen *et al.*, 2009). Further analyses with broader sampling and more species of the members of Nygolaimina are needed to clarify the phylogenetic relationships between the families and genera of this suborder.

The recovery of six nygolaimid populations of the genera *Clavicaudoides*, *Paravulvus* and *Solididens* from moss, grass and forest soil samples indicated their wide distribution in different ecosystems on Chinese land. Further samplings for nygolaimid nematodes together with morphological and molecular analyses may present more useful and interesting information on diversity of this group in China.

Financial support. This research was supported by the National Natural Science Foundation of China (Grant number 32001876). S. Álvarez-Ortega thanks 'AYUDA PUENTE 2020, URJC' of the Universidad Rey Juan Carlos for financial support.

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

References

Abascal F, Zardoya R and Telford MJ (2010) Translator X: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Research* **38(Server issue)**, W7–W13.

Ahmad M and Jairajpuri MS (1979) On the systematic position of Nygolaimina new suborder (Nematoda: Dorylaimida). pp. 29 (Abstracts) *Second National Congress of Parasitology*. India, BHU.

Ahmad M and Jairajpuri MS (1982) Nygolaimina of India. *Records of the zoological survey of India, Calcutta* **34(1)**, 1–70.

Ahmad W, Araki M and Kaneda S (2003) Two new species of the genus *Paravulvus* Heyns, 1968 (Nematoda: Nygolaimidae) from Japan. *International Journal of Nematology* **13(1)**, 57–64.

Akthar Y, Ahmad W and Jairajpuri MS (1994) Two new species of Dorylaimida (Nematoda) from Kashmir Valley, India. *Records of the Zoological Survey of India* **94(1)**, 87–92.

Cobb NA (1913) New nematode genera found inhabiting fresh water and non-brackish soils. *Journal of the Washington Academy of Sciences* **3(16)**, 432–444.

De Grisse AT (1969) Redescription ou modiphications de quelques techniques utilisées dans l'édude de nématodes phytoparasitaires [Redescription or modifications of some techniques used in the study of phytoparasitic nematodes]. *Mededelingen Rijksfaculteit Landbouwwetenschappen Gent* **34(2)**, 351–366. [In French.]

De Ley P, Loof PAA and Coomans A (1993) Terrestrial nematodes from the Galápagos Archipelago II: redescription of *Aporcelaimellus obtusicaudatus* (Bastian, 1865) Altherr, 1968, with review of similar species and a nomenclature for the vagina in Dorylaimida (Nematoda). *Bulletin de L'Institut Royal des Sciences Naturelles de Belgique* **63(1)**, 13–34.

de Man JG (1880) Die einheimischen, frei in der reinen erde und im süssen wasser lebenden nematoden [The native nematodes living freely in pure earth and fresh water]. *Tijdschrift Nederlandsche Dierkundige Vereeniging* **5(1)**, 1–104. [In German.]

Gilarte P, Carreira JA and Peña-Santiago R (2013) Description of *Paravulvus moroccanus* sp. n. (Nematoda, Dorylaimida, Nygolaimidae) from the High Atlas Mountains, Morocco, with notes on the taxonomy of the genus. *Zootaxa* **3734(5)**, 545–558.

Heyns J (1968) A monographic study of the nematode families Nygolaimidae and Nygolaimellidae. *Entomology Memoirs Plant Protection Research Institute Pretoria, South Africa* **19**(1), 1–144.

Holterman M, Rybarczyk K, Van Den Elsen S, van Megen H, Mooyman P, Santiago RP, Bongers T, Bakker J and Helder J (2008) A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* **8(1)**, 23–34.

Jairajpuri MS and Ahmad W (1992) *Dorylaimida: free-living, predaceous and plant-parasitic nematodes*. New Delhi, India, Oxford & IBH Publishing Co, p. 458.

Kanzaki N and Futai K (2002) A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within the *xylophilus* group. *Nematology* **4(1)**, 35–41.

Katoh K and Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**(4), 772–780.

Lazarova S, Peneva V and Loof PAA (2002) *Paravulvus rhodopensis* sp. n. (Nematoda: Nygolaimidae) and three known species from Bulgaria, with notes on the taxonomy of the genus. *Nematology* **4 (4)**, 502–520.

Li H, Trinh PQ, Waeyenberge L and Moens M (2008) *Bursaphelenchus chengi* sp. n. (Nematoda: Parasitaphelenchidae) isolated at Nanjing, China, in packaging wood from Taiwan. *Nematology* **10(3)**, 335–346.

Loof PAA and Coomans A (1968) On the development and location of the oesophageal gland nuclei in Dorylaimina. *Nematologica* **14**(4), 596–597.

Miller MA, Pfeiffer W and Schwartz T (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. pp. 1–8. In *Proceedings of the Gateway Computing Environments Workshop (GCE) LA*, New Orleans, LA, USA, 14 November 2010. Institute of Electrical and Electronics Engineers, Piscataway, NJ.

Mullin P, Harris T and Powers T (2005) Phylogenetic relationships of Nygolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. *Nematology* **7**(1), 59–79.

Olia M, Ahmad W, Choudhary M and Jairajpuri MS (2004) Studies on nematodes of the suborder Nygolaimina (Dorylaimida) from Iran with descriptions of three new species. *International Journal of Nematology* **14(1)**, 91–98.

Pearse AS (1936) Parasites from Yucatan. *Carnegie Institute of Washington Publications* **457**, 45–59.

Pearse AS (1942) *An Introduction to parasitology*. lll., Baltimore, MD, Springfield, p. 357.

Peña-Santiago R (2021) Dorylaimida Mundi (Nematoda): checklist of genera and species, with their records. In *Monographic papers on nematology 7*. Jaén, University of Jaén.

Rambaut A. (2016) FigTree v1.4.3. Available at http://tree.bio.ed.ac.uk/software/figtree/ (accessed 11 September 2021).

Ronquist F, Teslenko M, van der Mark P, *et al.* (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**(3), 539–542.

Seinhorst JW (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* **4(1)**, 67–69.

Stamatakis A, Hoover P and Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**(5), 758–771.

Thorne G (1930) Predaceous nemas of the genus *Nygolaimus* and a new genus *Sectonema*. *Journal of Agricultural Research*, *U.S.D.A* **41(6)**, 445–466.

Thorne G (1935) Notes on free-living and plant-parasitic nematodes. II. Higher classification groups of Dorylaimoidea. *Proceedings of the Helminthological Society of Washington* **2(1)**, 96–98.

Thorne G (1939) A monograph of the nematodes of the superfamily dorylaimoidea. *Capita Zoologica* **8**(6), 261.

Thorne G (1974) Nematodes of the northern great plains. Pari II. Dorylaimoidea in part (nemata: Adenophorea). *Technical Bulletin of Agricultural Experiment Station, South Dakota State University* **41**, 1–120.

Van Megen H, Van Den Elsen S, Holterman M, Karssen G, Mooyman P and Helder J (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology* **11 (6)**, 927–950.

Whitehead AG and Hemming JR (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* **55(1)**, 25–38.

Wu JH (1999) *Studies of freshwater and soil nematodes of China. PhD dissertation*, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan. [In Chinese.]

Wu JH and Liang YL (1997) Preliminary study on free living nematodes of Changjiang River basins of China. *Acta Hydrobiologica Sinica* **21** (Supplement), 114–122. [In Chinese.]

Wu WJ, Yan L, Xu CL, Yu L, Wang K, Jin SY and Xie H (2016) Morphology and morphometrics of *Heterodorus qinghaiensis* n. sp. (Dorylaimida, Nordiidae) from soil samples in China. *Journal of Helminthology* **90(4)**, 385–391.

Wu WJ, Huang X, Xie H, Wang K and Xu CL (2017) Morphometrics and molecular analysis of the free-living nematode, *Belondira bagongshanensis* n. sp. (Dorylaimida, Belondiridae), from China. *Journal of Helminthology* **91(1)**, 7–13.

Wu WJ, Yu L, Xu CL, Wang DW and Xie H (2018) A new species of the genus *Eudorylaimus* Andrássy, 1959 (Nematoda: Dorylaimida: Qudsianematidae) associated with *Picea crassifolia* in China. *Zootaxa* **4526**(4), 576–588.

Wu WJ, Xu CL, Xie H and Wang DW (2019) Three new species, one new genus and subfamily of Dorylaimida (de Man, 1876) Pearse, 1942, and revisions on the families Tylencholaimellidae Jairajpuri, 1964 and Mydonomidae Thorne, 1964 (Nematoda: Dorylaimida). *Peer J* **7(1)**, e7541.

https://doi.org/10.1017/S0022149X23000032 Published online by Cambridge University Press