

A new stunt nematode, *Geocenamus chengi* n. sp. (Nematoda: Merliniinae) in the rhizosphere of tea (*Camellia sinensis*) from Zhejiang Province, China

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Abstract

The tea plant is native to China; the country has the greatest tea production areas in the world. In an attempt to investigate the nematode biodiversity associated with the tea plantations of Hangzhou, Zhejiang Province, a population of stunt nematode was detected. This group of nematodes is comprised of migratory ecto-parasites of roots and can subsist on a variety of host plants. Therefore, the detected population was studied carefully using the integrative taxonomy approach and identified as a new species of genus *Geocenamus*. *Geocenamus chengi* n. sp. can be characterized by females having six incisures in the lateral field; labial region is dome shaped and slightly offset from the rest of the body having four to five annuli; head framework is weakly developed; deirids are absent; excretory pore is located at the anterior region of basal pharyngeal bulb. Under SEM, the vulva is a transverse slit, vulval lips are elongated and ellipsoidal with epiptygma. The tail is annulated, elongated, and conical having bluntly pointed tip and a terminal hyaline region that forms 21 to 33% of the tail length. Spicule is 22 to 25 μm long, gubernaculum is saucer shaped; bursa is crenated covering the tail until the hyaline tail region. Morphologically, the species is close to *G. circellus*, *G. joctus*, *G. loofi*, *G. ordinarius*, *G. processus*, *G. tetyllus*, and *G. tortilis*. Phylogenetic relationships of the new species based on D2-D3 expansion domains of 28S, ITS, and 18S rRNA genes indicated that *G. chengi* n. sp. clustered in a separate clade with *G. vietnamensis*.

Keywords

DNA sequencing, *Camelliae sinensis*, morphology, morphometrics, nematode, new record, species, phylogeny, scanning electron microscopy.

The tea plant is native to China; this country has the highest tea production and consumption rate in the world. More than 100,000 hectares of tea acreage are located in seven Chinese provinces: Yunnan, Sichuan, Fujian, Hubei, Zhejiang, Guizhou, and Anhui (Yao and Chen, 2012). In an attempt to investigate the nematode biodiversity associated with the tea plantations of Hangzhou, Zhejiang Province, a population of stunt nematode of subfamily Merliniinae was detected.

This family comprises migratory ectoparasites of roots; merlinid nematodes are root feeders and can subsist on a variety of host plants (Siddiqi, 2000). The detected population of stunt nematode was examined carefully, and the morphological characterization indicated that this population belongs to the genus *Geocenamus* (Thorne and Malek, 1968).

The status of genera in subfamily Merliniinae (Siddiqi, 1971) has been discussed by several nematologists,

namely Andr ssy (1977), Hooper (1978), Fortuner and Luc (1987), Siddiqi (1986, 2000), and Brzeski (1991). However, based on the recent classification (Geraert, 2011), the subfamily Merliniinae contains *Amplimerlinius* (Siddiqi, 1976), *Geocenamus*, and *Nagelus* (Thorne and Malek, 1968) species. The rest of the genera *Scutylenchus* (Jairajpuri, 1971), *Merlinius* (Siddiqi, 1979), *Hexadorus* (Ivanova and Shagalina, 1983), *Pathotylenchus* (Eroshenko and Volkova, 1987), and *Allentylenchus* (Khan and Saeed, 1988) were considered as junior synonyms of *Geocenamus* (Geraert, 2011).

Currently, the genus *Geocenamus* contains over 70 species distributed across different climatic zones and environments (Geraert, 2011; Nguyen et al., 2019). *G. brevicaudatus* (Peng and Hunt, 1995; Brzeski, 1998) is a Chinese native species, and was reported almost two decades ago from Hebei province. Other than that, *G. myunsugae* (Choi and Geraert, 1993) and *G. tenudens* (Thorne and Malek, 1968) were also reported in the rhizosphere of cultivated plants from Shandong and Liaoning provinces, respectively (Ni and Liu, 2004; Li et al., 2004). Since then, none of the *Geocenamus* have ever been reported from China; considering the scarce occurrence of the *Geocenamus* species in China, the detected species was characterized morphologically (using light and scanning microscopy) and molecularly (with 18S, 28S, and ITS genes sequences). The morphometrics and morphological characters of the detected species were compared with the related *Geocenamus* species, and it found that this species possesses unique characters and needs to be considered as a new member of the genus. Therefore, this study describes a new *Geocenamus* species with the following objectives: to provide an integrative morphological and molecular characterization of the new species; to elucidate important morphological details through SEM observations; and to study the phylogenetic relationships of these species with other merlinid and related nematodes.

Materials and methods

Nematode extraction and morphological study

Nematodes were extracted from soil and root samples using the modified Cobb sieving and flotation-centrifugation method (Jenkins, 1964). For morphometric studies, nematodes were killed and fixed in hot formalin (4% with 1% glycerol) and processed in glycerin (Seinhorst, 1959). The measurements and light micrographs of nematodes

were made with a Nikon Eclipse Ni-U 931845 compound microscope. For the SEM examination, the nematodes were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde, washed three times in 0.1M cacodylate buffer, post-fixed in 1% osmium tetroxide, dehydrated in a series of ethanol solutions, and critical point-dried with CO₂. After mounting on stubs, the samples were coated with gold at 6 to 10 nanometer thickness, and the micrographs were made at 3 to 5 kV operating system (Maria et al., 2018).

Molecular analyses

DNA was extracted by transferring individual nematodes into an Eppendorf tube containing 16 μ L ddH₂O. Nematodes were crushed using a sterilized pipette tip, and the tubes were centrifuged at 12,000 rpm for 1 min and frozen at -68°C for at least 30 min. Tubes were heated to 85°C for 2 min, and then, 2 μ L proteinase K was added in PCR buffer solution. The tubes were incubated at 56°C for 1 to 2 hr and at 95°C for 10 min. After incubation, these tubes were cooled to 4°C and used for conducting PCR analyses (Zheng et al., 2003). Several sets of primers (synthesized by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the partial 18S, ITS region and D2-D3 expansion domains of 28S of rDNA. Primers for amplification of partial 18S were 18s900-18s1713 (Olson et al., 2017). Primers for amplification of ITS were TW81-AB28 (Joyce et al., 1994). The primers for amplification of D2-D3 of 28S were D2A and D3B (De Ley et al., 1999). PCR conditions were as described by Ye et al. (2007) and Powers et al. (2010). PCR products were evaluated on 1% agarose gels stained with ethidium bromide. PCR products of sufficiently high quality were sent for sequencing by Invitrogen (Shanghai, China).

Phylogenetic analysis

The newly obtained sequences were deposited into the GenBank database, and accessions were in the phylogenetic trees. The DNA sequences were compared with those of the other merlinids and related nematodes available at the GenBank sequence database using the BLAST homology search program. Outgroup taxa for the data set were chosen according to previously published data (Handoo et al., 2014; Nguyen et al., 2019). Multiple alignments of the different sequences were made using the Q-INS-i algorithm of MAFFT v. 7.205 (Kato and Standley, 2013). The best-fit model of DNA evolution was obtained using jModelTest V.2.1.7 (Darriba et al.,

2012) with the Akaike information criterion (AIC). The best-fit model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the AIC were then given to MrBayes for the phylogenetic analyses. Transitional model and gamma-shaped distribution (TIM3ef + G) were used for the 18S; unlinked general time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) was used for D2-D3 expansion domains of 28S, and transitional model with invariable sites and a gamma-shaped distribution (TIM2 + I + G) for ITS. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck and Ronquist, 2001) with four chains for 2×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. After discarding burn-in samples of 10% and evaluating convergence, the remaining samples were retained for more in-depth analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees from all analyses were edited by FigTree software V.1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results and description

Systematics

Geocenamus chengi n. sp. (Figs. 1-4; Table 1).

Description

Female

After fixation, the body is ventrally curved or C shaped. There are six incisures on lateral field, areolation at mid-body and tail region is observed in the majority of individuals. Body annuli are clearly defined and divided into blocks (seen under SEM). Labial region is dome shaped and slightly offset from the rest of the body having four to five annuli. Irregular rounded rectangular labial disc is observed surrounded by the dorsal, ventral sectors, and amphidial apertures. The labial framework is not sclerotized. Stylet is well-developed with rounded knobs. The dorsal gland orifice is located $2.0 \mu\text{m}$ posterior to stylet knobs. Median bulb is oval with bean-shaped central valve plates; deirid is absent. Isthmus is slender, surrounded by a nerve ring; pharyngeal basal bulb is saccate and abutting intestine. Cardia is indistinct and conoid rounded. Excretory pore is located at the anterior region of basal pharyngeal bulb. Hemizonid

is 2-3 annuli long, anterior to excretory pore; vulva is a transverse slit, vulval lips are elongated and ellipsoidal with epiptygma (SEM), and vagina is "v" shaped comprising less than half of the corresponding diameter; spermatheca is rounded filled with rounded sperm cells; ovaries are outstretched with a single row of oocytes. The tail is annulated, elongated, and conical with a terminal hyaline region, which comprises 21 to 33% of the tail length, ending as bluntly pointed tip. Phasmid is small and pore like, located 10 to 19% posterior from anus.

Male

Body habitus, cuticle and anterior region of males are similar to females. Gonad is located on the right side of the intestine and outstretched. Spicule is 22 to $25 \mu\text{m}$ long with truncated head and having an abrupt depression just below the head on the dorsal side, followed by a curve that tapers till the distal end of the spicule, and distal tips are bluntly pointed; gubernaculum is saucer shaped; cloacal lips are not protuberant, and two posterior hypopygmata can be seen under SEM. Bursa crenate covers the tail until the hyaline tail region. Tail shape is similar to that of female, and terminal hyaline region comprises 20 to 28% of the tail length. Phasmid is small and located 19 to 29% posterior from cloaca.

Type host and locality

This population was detected in the rhizosphere of *Camellia sinensis* (L.) Kuntze, 1887 from Longjian Tea Village, Hangzhou City, Zhejiang Province, P. R. China, on December, 2019.

Type material

Holotype female and 16 female and 8 male paratypes (slide numbers ZJU-31-01-ZJU-31-10) were deposited in the nematode collection of Zhejiang University, Hangzhou, China. Additional 10 slides having plenty of male and female were also stored in the same collection. Also, 4 females and 2 male paratypes (slide numbers T-7378p, T-7379p) were deposited at USDA nematode collection, Beltsville, Maryland, USA.

Etymology

The species is named after late Professor Hurui Cheng, one of the famous plant nematologists in China, for his extraordinary contribution to the nematode taxonomy from China.

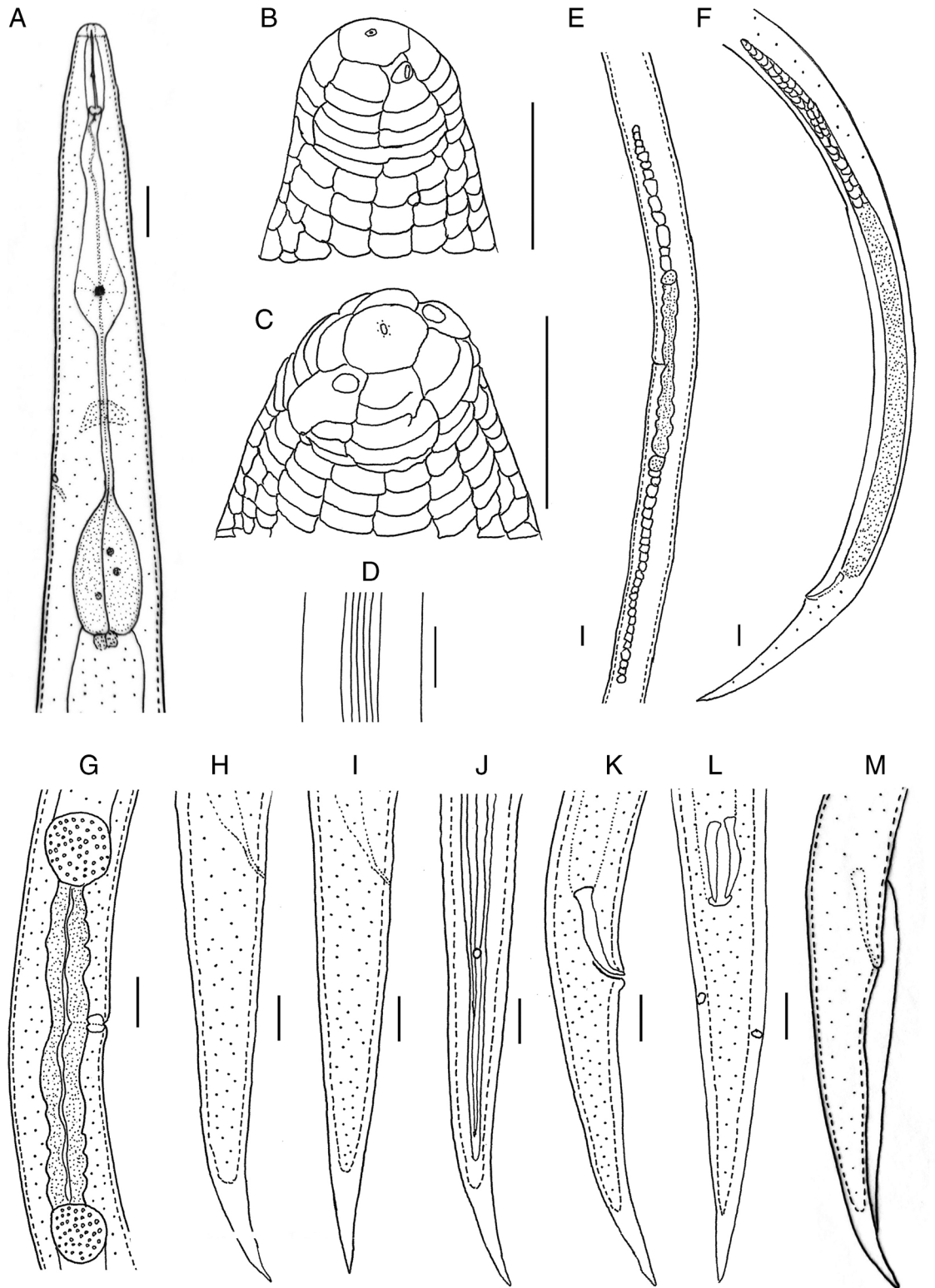


Figure 1: Line drawings of *Geocenamus chengi* n. sp. A: pharyngeal region; B, C: en face view; D: lateral lines; E: female gonad; F: male gonad; G: vulval region; H-J: female tails; K-M: male tails (Scale bars = A; D-M = 10 μ m, B, C = 5 μ m).

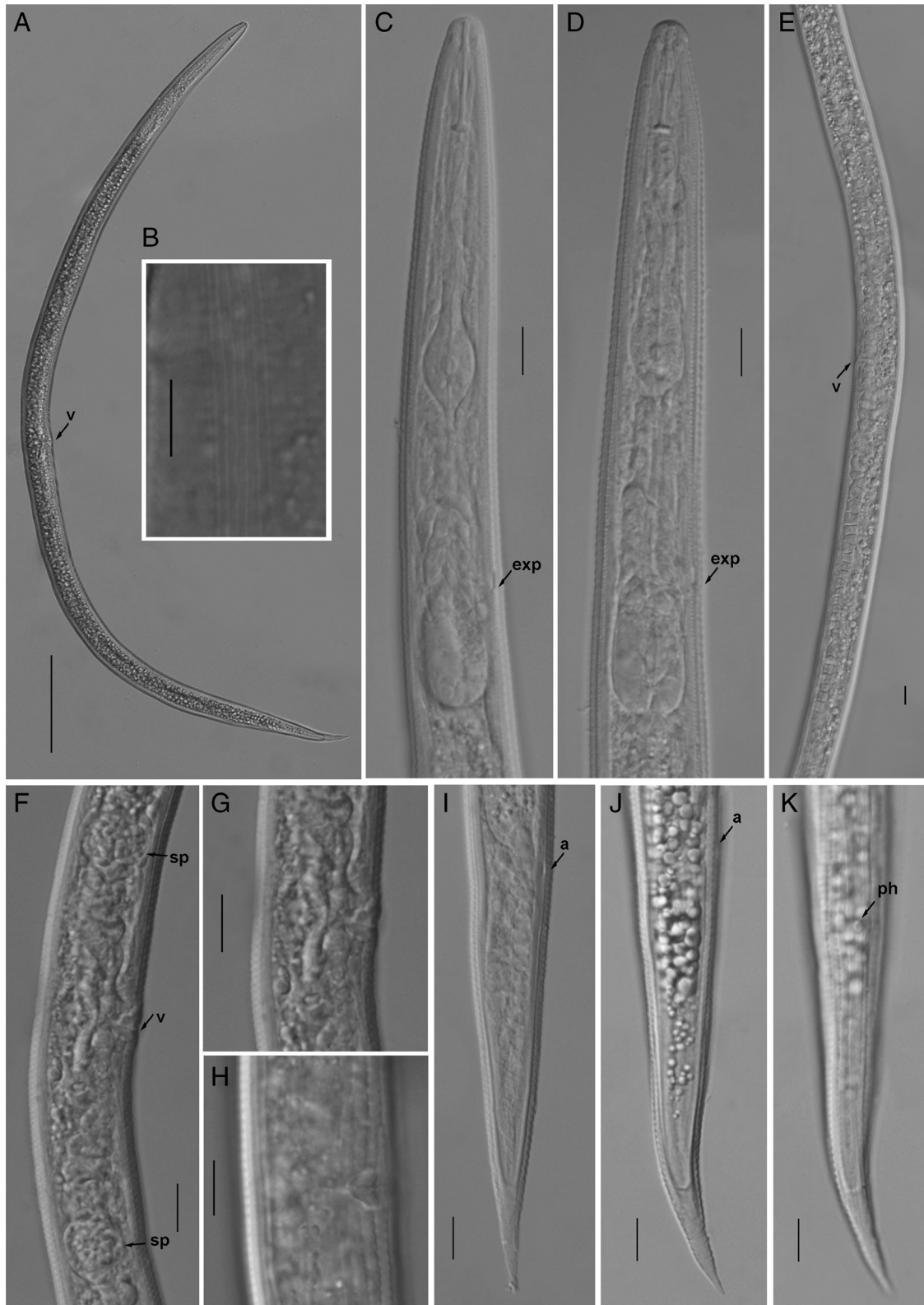


Figure 2: Light photomicrographs of *Geocenamus chengi* n. sp. Female. A: entire body; B: lateral lines; C, D: pharyngeal regions, arrow pointing on the excretory pore (exp); E: gonad; F: vulval region arrows pointing on vulva (v) and spermatheca (sp); G, H: vulval region; I-K: female tails arrows pointing on anus (a) and phasmid (ph) (Scale bars=A=100 μ m; B-K=10 μ m).

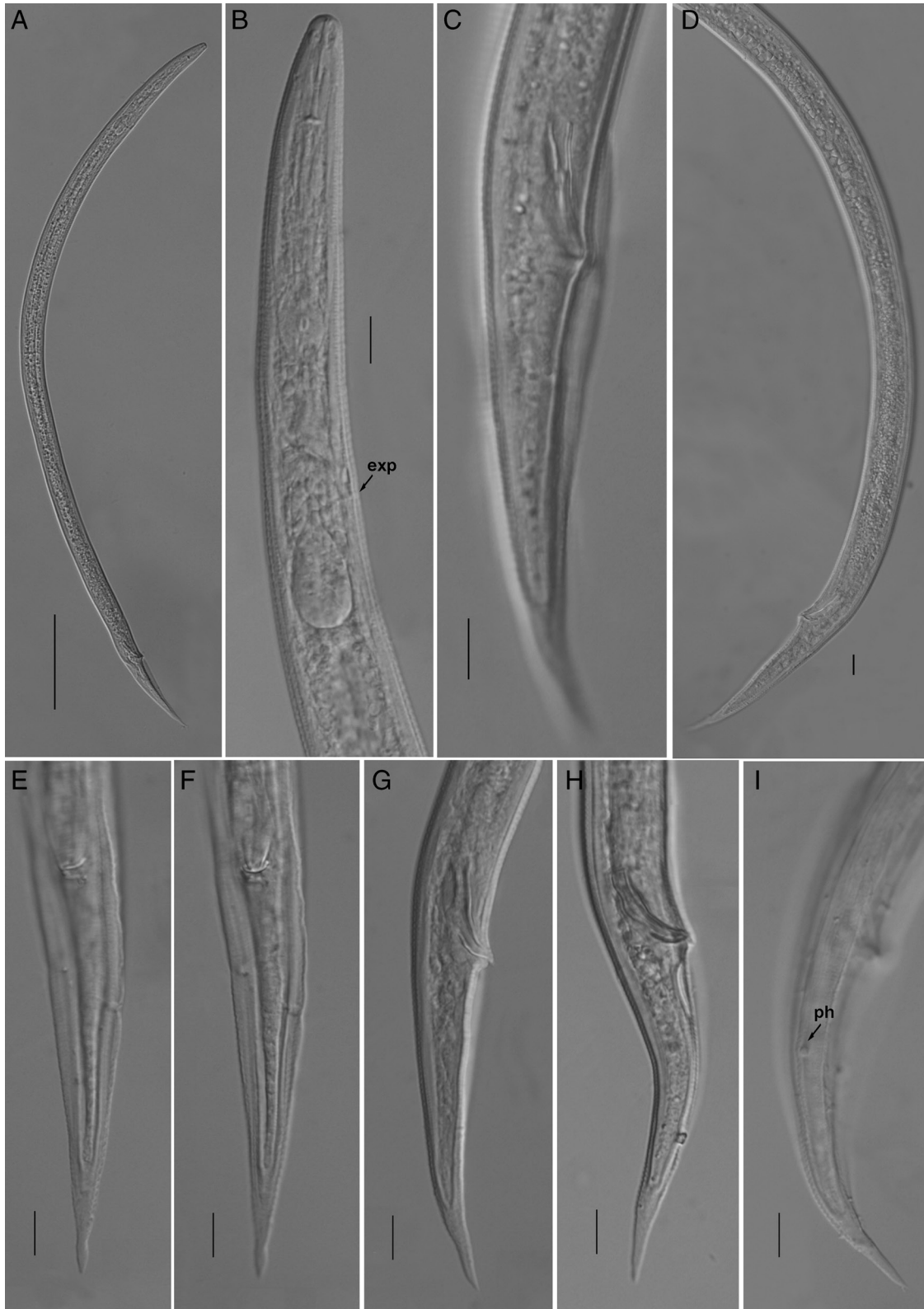


Figure 3: Light photomicrographs of *Geocenamus chengi* n. sp. Male. A: entire body; pharyngeal regions, arrow pointing on the excretory pore (exp); C: tail with bursa; D: gonad; E, F: tail in ventral view; G, I: male tail arrows pointing on phasmid (ph) (Scale bars = A = 100 μm; B-I = 10 μm).

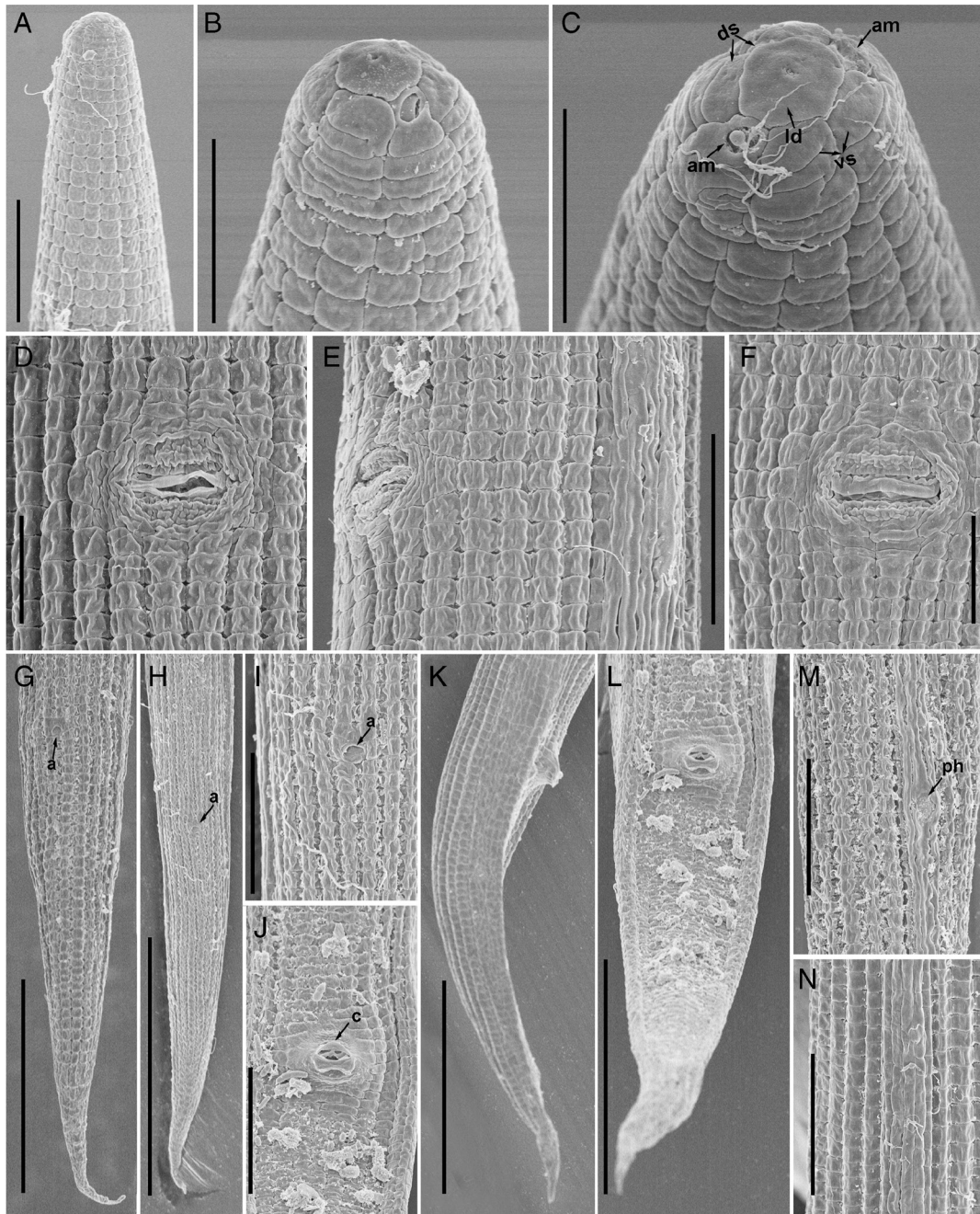


Figure 4: Scanning electron microscopy of *Geocenamus chengi* n. sp. A: anterior region; B-C: en face view; D-F: vulval regions; G, H: female tail regions arrows pointing anus (a); I: anal area at higher resolution, arrow pointing on the position of anus; J: cloacal area at higher resolution, arrow pointing on the position of cloaca (c); K, L: male tail; M: lateral field with phasmid (ph); N: lateral field. Abbreviation: am, apophid; ds, dorsal sectors; vs, =ventral sectors; ld, labial disc (Scale bars, A, E, I, J, M, N=10μm; B, C, D, E, F=5μm; G, K=30μm; H=50μm; L=20μm).

Diagnosis and relationships

Geocenamus chengi n. sp. can be characterized by females having six incisures in the lateral field; labial

region is dome shaped and slightly offset from the rest of the body having four to five annuli; deirids are absent; excretory pore is located at the anterior region of the basal pharyngeal bulb. The vulva is a transverse

Table 1. Morphometric data for *Geocenamus chengi* n. sp. All measurements are in μm and in the form of mean \pm SD (range).

	Holotype	Paratype	
		Female	Male
<i>n</i>		20	10
Body length	884.8	879.0 \pm 59.6 (783–997)	822.8 \pm 34.2 (772–892)
a	33.3	33.3 \pm 2.6 (28–39)	32.9 \pm 1.2 (31–34)
b	6.0	6.1 \pm 0.3 (5.5–6.6)	6.1 \pm 0.2 (5.8–6.6)
c	10.4	10.5 \pm 0.6 (9.2–11.7)	9.7 \pm 0.6 (8.7–10.3)
c'	4.7	4.6 \pm 0.4 (4.2–5.5)	4.8 \pm 0.3 (4.5–5.2)
V	52.8	52.5 \pm 1.6 (49.5–55.7)	39.4 \pm 5.4 (33.1–47.4)
Lip height	7.5	7.6 \pm 2.4 (7.0–8.0)	7.6 \pm 0.3 (7.0–8.0)
Lip width	3.7	3.8 \pm 0.3 (3.4–4.5)	3.7 \pm 0.3 (3.2–4.0)
Stylet length	22.3	22.2 \pm 0.6 (21–23)	21.5 \pm 0.7 (20–23)
DGO distance from stylet knobs	2.0	2.0 \pm 0.1 (1.7–2.2)	2.1 \pm 0.1 (1.9–2.3)
Excretory pore from anterior end	122.8	120.6 \pm 5.2 (109–126)	108.3 \pm 5.2 (101–116)
Hemizonid from anterior end	118.4	116.7 \pm 5.1 (104–123)	103.0 \pm 6.7 (94–113)
Pharynx length	148.4	145 \pm 5.7 (130–152)	134 \pm 3.6 (127–139)
Maximum body diam.	26.6	26.6 \pm 2.5 (22–33)	25.0 \pm 1.0 (23–27)
Vulval body diam.	25.4	26.6 \pm 2.3 (23–32)	–
Anal/cloacal body diam.	18.3	18.1 \pm 1.2 (16–21)	17.7 \pm 0.9 (16–19)
Tail length	85.2	83.8 \pm 5.4 (76–92)	85.2 \pm 5.4 (78–93)
Hyaline tail part length	25.2	22.4 \pm 2.2 (19–25)	21.3 \pm 1.9 (18–24)
Phasmid position from anus/cloaca	12.4	15.5 \pm 4.1 (10–19)	24.6 \pm 3.9 (19–29)
Phasmid % of tail	14.6	17.4 \pm 4.0 (12–20)	29.5 \pm 4.0 (24–35)
Spicule length		–	23.4 \pm 1.9 (22–25)
Gubernaculum		–	8.0 \pm 0.7 (7–9)

slit, vulval lips are elongated and ellipsoidal with epiptygma (seen under SEM). The tail is annulated, elongated and conical having bluntly pointed tip and a terminal hyaline region that comprises 21 to 33% of the tail length; phasmid is small and pore like, located 10 to 19% posterior from anus. Spicule is 22 to 25 μm long, gubernaculum is saucer shaped; bursa is crenate covering the tail until the hyaline tail region.

Based on the similar tail morphology, *G. chengi* n. sp. is characterized close to *G. circellus* (Anderson and Ebsary, 1982; Brzeski, 1991), *G. joctus* (Thorne, 1949; Brzeski, 1991); *G. loofi* (Siddiqi, 1979; Brzeski, 1991), *G. ordinarius* (Volkova, 1993), *G. processus* (Siddiqi, 1979; Brzeski, 1991), *G. tetyllus* (Anderson

and Ebsary, 1982), and *G. tortilis* (Kazachenko, 1980; Brzeski, 1991).

From *G. circellus*, it can be differentiated by longer body of females 879 (783–997) vs 520 to 650 μm , longer stylet of females 22 (21–23) vs 9 to 10 μm , longer tail of females 84 (76–92) vs 76 μm , shape of lip region (dome-shaped and slightly set off vs low, truncated, and continuous), deirids (absent vs present), location of excretory pore of females 120.6 (109–126) vs 99 μm , vulval lips (simple vs elevated), phasmid position from anus 15 (10–19) vs 23 to 27 μm , and spicule terminus (bluntly pointed vs indented).

From *G. joctus*, it can be differentiated by longer body of females 879.0 (783–997) vs 520 to 790 μm ,

longer stylet of females 22.2 (21-23) vs 15.5 to 19.5 μm , longer tail of females 84 (76-92) vs 38 to 77 μm , number of incisures in the lateral field 6 vs 10, shape of lip region (dome-shaped and slightly set off vs hemispherical set off by a constriction), deirids (absent vs present), length of hyaline tail terminus of females 22 (19-25) vs 7.5 to 11.5 μm and phasmid position on the tail of females 17.4 (12-20) vs 33 to 58%.

From *G. loofi*, it can be differentiated by longer body of females 879 (783-997) vs 510 to 620 μm , longer stylet of females 22 (21-23) vs 9 to 10 μm , longer tail of females 84 (76-92) vs 62 μm , shape of lip region (dome-shaped and slightly set off vs low, truncated, and continuous), position of excretory pore (at the anterior region of basal pharyngeal bulb vs in the isthmus region), tail terminus (bluntly pointed tip vs mucronated), and phasmid position (anterior half of the tail vs middle of the tail).

From *G. ordinarius*, it can be differentiated by longer stylet of females 22 (21-23) vs 9 to 10 μm , longer tail of females 84 (76-92) vs 60 to 70 μm , shape of lip region (dome-shaped and slightly set off vs hemispherical set off by a constriction), labial disc protruded (absent vs present), position of excretory pore (at the anterior region of basal pharyngeal bulb vs at the level of isthmus base), and phasmid position (anterior half of the tail vs middle of the tail).

From *G. processus*, it can be differentiated by longer body of females 879 (783-997) vs 500 to 630 μm , longer stylet of females 22 (21-23) vs 15.0 to 17.5 μm , longer tail of females 84 (76-92) vs 50 μm , shape of lip region (dome-shaped and slightly set off vs lip region hemispherical set off by a depression), position of excretory pore (at the anterior region of basal pharyngeal bulb vs at the level of isthmus base), tail terminus (bluntly pointed tip vs mucronated), and spicule terminus (bluntly pointed vs notched).

From *G. tetyllus*, it can be differentiated by stylet length of females 22 (21-23) vs 14 μm , shape of lip region (dome-shaped and slightly set off vs subtruncated, set off by a constriction), deirids (absent vs present), phasmid position from anus 15 (10-19) vs 42 to 46 μm , and spicule terminus (bluntly pointed vs notched).

From *G. tortilis*, it can be differentiated by longer body of females 879 (783-997) vs 480 to 540 μm , stylet length of females 22 (21-23) vs 19.5 μm , longer tail of females 84 (76-92) vs 54 to 58 μm , shape of lip region (dome-shaped and slightly set off vs rounded set off by a depression), position of excretory pore (at the anterior region of basal pharyngeal bulb vs at the level of nerve ring), and spicule length 23 (22-25) vs 27 μm .

Molecular profiles and phylogenetic status

Geocenamus chengi n. sp. was molecularly characterized using partial 18S, D2-D3 expansion domains of 28S and ITS sequences. As few species of the genus have been provided with sequence-based information, all the available sequences of subfamily Merliniinae deposited in the GenBank were included in the phylogenetic analysis.

In the 18S gene analysis (Fig. 5), the *G. chengi* n. sp. (MN983268-MN983271) forms a separate clade next to *Scutylechus rugosus* (KX789704-KX789705), *Merlinius brevidens* (KX789708), and *M. nanus* (KX789709). In the 28S gene tree (Fig. 6), *G. chengi* n. sp. (MN983258-MN983262) clustered with *G. vietnamensis* (MH191361) in a well-supported subclade (PP=1.00), whereas the other members of subfamily Merliniinae arranged in separate clades. In the ITS gene tree (Fig. 7), *G. chengi* n. sp. (MN983263-MN983267) clustered with *G. vietnamensis* (MH191362) and an unidentified *Scutylechus* (JQ069956) species from China.

In all the phylogenetic analyses, *G. chengi* n. sp. forms a separate clade and grouped with *G. vietnamensis*. The sequence identities of the new species with *G. vietnamensis* are 95% (25 nucleotide, 2 indels differences) for 28S, and 94% (39 nucleotide, 14 indels differences) for ITS. Morphologically, *G. chengi* n. sp. can be differentiated from *G. vietnamensis* by the posterior position of the excretory pore (vs at nerve ring level), short tail (vs 85-125 μm), and shorter stylet (vs 24-28 μm).

Discussion

The classification presented by Siddiqi (2000) and Andr assy (2007) distinguished *Geocenamus*, *Nagelus*, *Merlinius*, *Scutylechus*, and *Amplimerlinius* within the subfamily Merliniinae. Fortuner and Luc (1987) and Maggenti et al. (1988) placed the genus *Geocenamus* within the subfamily Belonolaiminae (Whitehead, 1959), whereas Bongers (1988) arranged *Merlinius*, *Amplimerlinius*, *Scutylechus*, *Geocenamus*, and *Nagelus* in the family Dolichodoridae (Chitwood, 1950). In a recent classification by Geraert (2011), he accepted the synonymization of *Merlinius* and *Scutylechus* with *Geocenamus* and retained only the genera *Amplimerlinius*, *Geocenamus*, and *Nagelus* in subfamily Merliniinae. On contrary to this, Sturhan (2012) agreed with Brzeski (1991) in synonymizing *Scutylechus* with the *Geocenamus*, but he considered *Merlinius* as a valid genus.

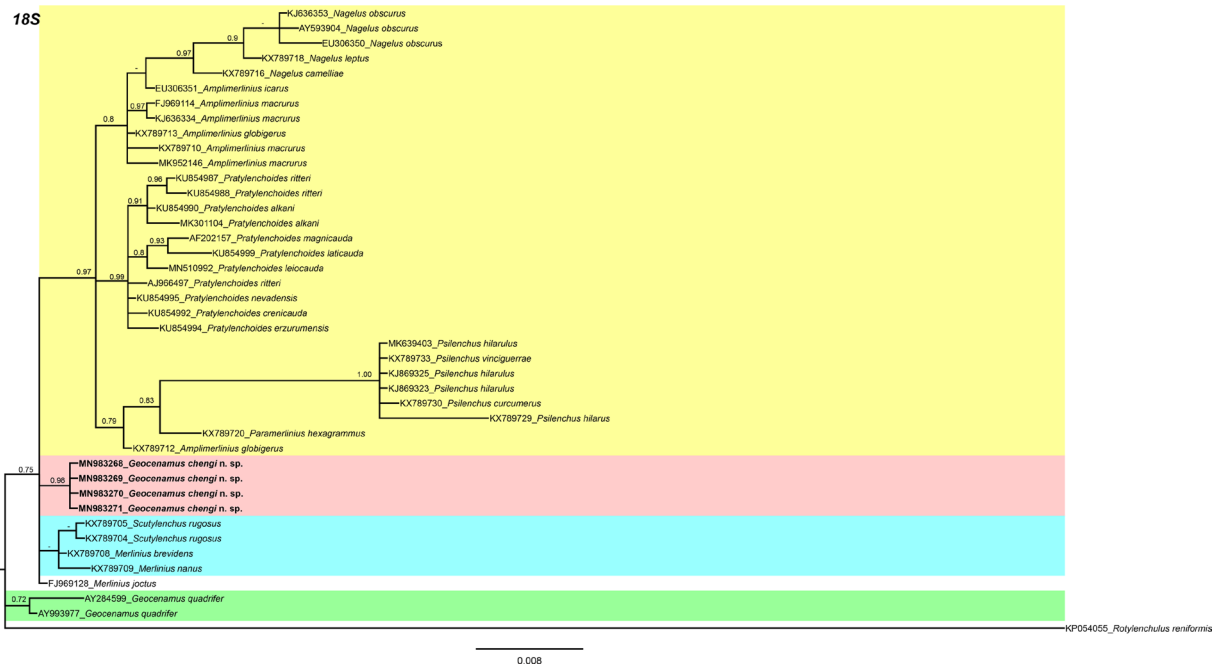


Figure 5: Phylogenetic relationships of *Geocenamus chengi* n. sp. with other merlinids as inferred from Bayesian analysis using the 18S rRNA gene sequence data set with the TIM3ef + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

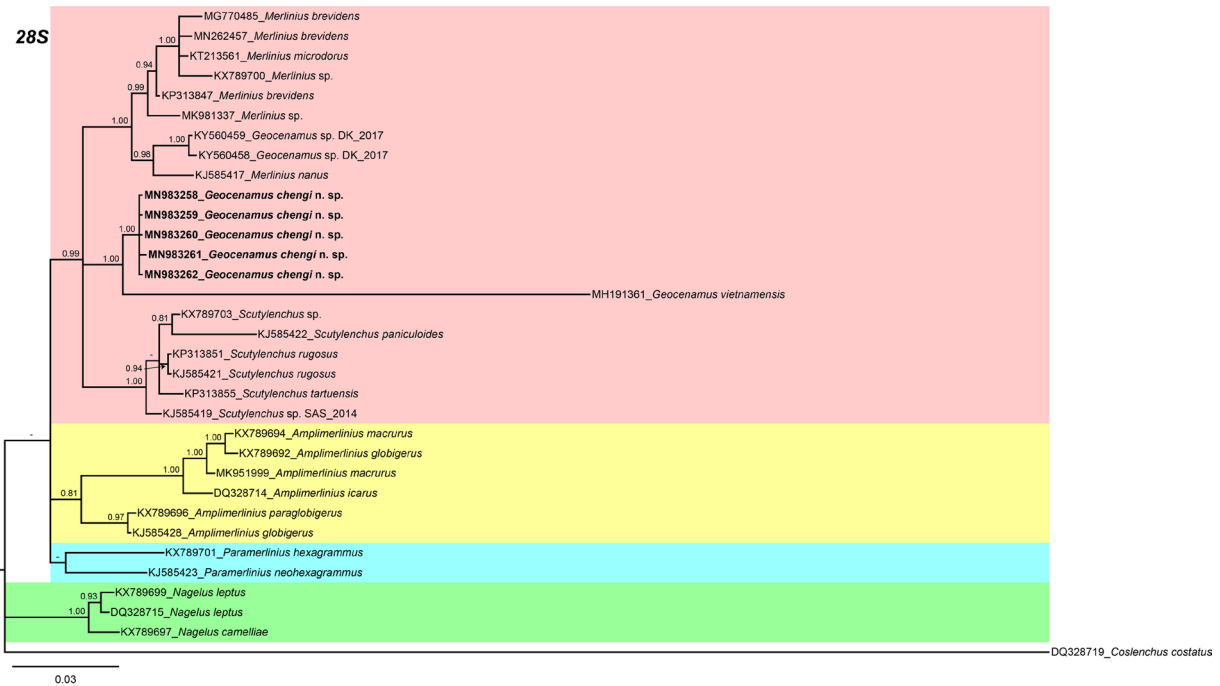


Figure 6: Phylogenetic relationships of *Geocenamus chengi* n. sp. with other merlinids as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequence data set with the GTR+I+G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

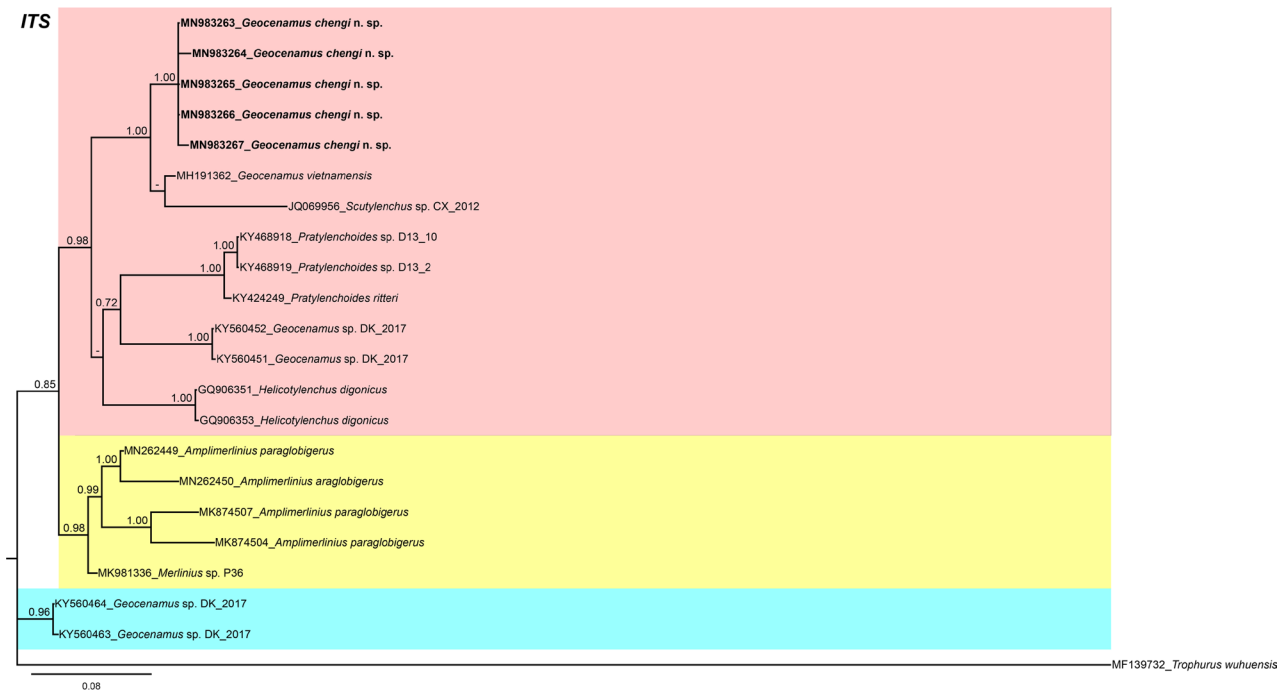


Figure 7: Phylogenetic relationships of *Geocenamus chengi* n. sp. with other merlinids as inferred from Bayesian analysis using the ITS rRNA gene sequence data set with the TIM2 + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

In our phylogenetic analysis, *Geocenamus*, *Scutylenchus*, and *Merlinius* appeared as distinct genera supporting Siddiqi's (2000) classification, similar results were obtained in several other studies (Carta et al., 2010; Ghaderi et al., 2014; Nguyen et al., 2019). It is also noted that the available *Geocenamus* species do not group with the new species and *G. vietnamensis*, and in this context, we agreed with Carta et al. (2010) who stated that greater genetic distance between species or *Geocenamus* populations possibly due to the presence of cryptic species, different haplotypes or misidentification. Other than that, the majority of Merliniinae genera and species have not yet been sequenced, and we expect, with the inclusion of additional/new sequences of Merliniinae the phylogenetic studies could provide better insights than now.

The present study does not address any taxonomic revisions or higher classification; however, it describes a new *Geocenamus* species isolated from the tea plantations of Hangzhou City. This is the first *Geocenamus* species associated with tea in China, no obvious aboveground or root symptoms were detected on the plants. Other than *G. chengi* n. sp., the most numerous plant-parasitic nematodes were the ring nematode, i.e. *Hemicricoemoides chitwoodi*. No other plant-parasitic taxa were detected, except

some predaceous, microbivores, and fungivore nematodes. Currently, *Geocenamus* contains over 70 species and 38 of them were described/reported from Asia (Geraert, 2011; Nguyen et al., 2019). The high level of *Geocenamus* diversity may indicate that a relatively rapid speciation rate of this nematode group occurred in this region; however, additional studies are required to shed light on the evolution, phylogeny, and ecological aspects of these nematodes.

Acknowledgments

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