A new species of *Hemicycliophora geraerti* n. sp. (Tylenchida: Hemicycliophoridae) from China

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Abstract: Hemicycliophora species are migratory root-ectoparasites of higher plants: commonly known as sheath nematodes. The present study describes a new species, *Hemicycliophora geraerti* n. sp., recovered from the rhizosphere of peach tree (*Prunus persica*) in Zhejiang Province, China. This species is characterized by a cuticular sheath loosely fitting the to body; a lip region slightly set-off with body contour bearing 3–4 annuli; oral plate narrow, ovate, and non-protruding; amphidial aperturesplugged, dorsoventrally flanked in the first lip annulus; stylet 110 (103–116) µm long and flexible, stylet knobs concave-shaped; the excretory pore is located 4–7 annuli posterior to the base of the pharyngeal bulb; vulval lips modified with a small vulval sleeve; anus located 16 (13–21) annuli posterior to vulva; tail elongated, having a narrowly rounded slightly offset spike. The molecular characterization and phylogenetic analyses based on D2–D3 expansion segments of the 28S rRNA, ITS rRNA, and COI mtDNA gene indicated the unique status and support the novelty of this population of *Hemicycliophora,* therefore herein described as *H. geraerti* n. sp.

Key words: Prunus persica, new species, morphology, D2–D3, ITS, COI, phylogeny

INTRODUCTION

China accounts for more than 60% of the world's peach production and owns more than 750,000 hectares of peach cultivated areas. Majority of this arable land is located in Hebei, Henan, Jiangsu Shandong and Zhejiang, provinces, generating $20 million/ year earnings in export (Zhang *et al*., 2019). However, research has shown that a significant number of peach orchards are increasingly affected by plant-parasitic nematodes (PPNs), resulting in loss of profitable yields and the intensification of other diseases (Gao *et al*., 1989; Xia *et al*., 2011). Therefore, nematode surveillance programs and inventory surveys are very crucial for peach orchard management strategies.

To study the wide array of PPNs associated with peach plantations, extensive samplings were carried out in Zhejiang Province. Among other PPNs, a population of the genus *Hemicycliophora* de Man, 1921 was detected. The genus is the sole member of family Hemicycliophoridae, and belongs to the superfamily Hemicycliophoroidea, due to the presence of loosened cuticular sheath, nematodes in this subfamily commonly known as 'sheath nematodes' (Chitambar & Subbotin, 2014). The genus contains over 130 nominal species, however only a few species (*H. arenaria* Raski, 1958, *H. conida* Thorne, 1955, *H. parvana* Tarjan, 1952, *H. poranga* Monteiro & Lordello, 1978, *H. onubensis* Van den Berg *et al*., 2018, *H. similis* Thorne, 1955, and *H. typica* de Man, 1921) known to

implicate in plant diseases (Subbotin *et al*., 2014; Maria *et al*., 2018; Van den Berg *et al*., 2018; Nguyen *et al*., 2021). Literature studies indicated that sheath nematodes are obligate ectoparasites of plant roots, a higher infestation of these nematodes might cause root galls and growth retardation (Chitambar & Subbotin, 2014).

Despite the wide host range of *Hemicycliophora* species, only *H. gracilis* Thorne, 1955, *H. iwia* Brzeski, 1974 and *H. similis* were currently found associated with peach trees. In China, only *H. arenaria*, *H. juglandis* Choi & Geraert, 1975 and *H. subbotini* Maria, Cai, Qu, Castillo & Zheng, 2018 were detected, but from different locations and hosts (Li, 1994; Chen & Liu, 2001; Maria *et al*., 2018). The rare occurrence and poor documentation of this genus, led us to study the newly discovered population of *Hemicycliophora* found in the peach rhizosphere in China. Detailed morphological, molecular, and scanning electron microscopy analyses of this species were performed in this study, revealing unique characters for the species, which is described herein as *H. geraerti* n. sp. The objectives of this study were: i) to provide the morphological and molecular characterization of *H. geraerti* n. sp. and ii) to study the phylogenetic relationships of this species with other *Hemicycliophora* species using rRNA and mtDNA sequences.

MATERIALS AND METHODS

Nematode population sampling, extraction and morphological identification. Nematodes were concentrated from soil samples using the modified Cobb sieving

method and finally extracted using flotation-centrifugation method (Jenkins, 1964). For morphometric studies, nematodes were killed and fixed with hot formalin and processed in glycerin (Seinhorst, 1959). The measurements and light micrographs of nematodes were completed with a compound microscope (Eclipse Ni-U 931845, Nikon, Tokyo, Japan). The drawings were made using a drawing tube attached to the microscope and were redrawn using Corel DRAW software version 16 (Corel). For the SEM examination, 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, washed three times with 0.1M phosphate buffer, dehydrated in a series of ethanol solutions, and critical point-dried with CO_2. 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 of Hitachi SU8010 (Maria *et al*., 2018).

Molecular analyses. DNA extractions were prepared from single specimens according to Zheng *et al*. (2003). Three sets of primers (synthesized by Healthy Creatures, Hangzhou, China) were used in the PCR analyses to amplify the D2-D3 expansion segments of 28S rRNA, ITS rRNA, and cytochrome c oxidase subunit 1 (COI) gene fragments. The pair of primers for amplification of ITS was F195 (5′–TCCTCCGCTAAATGATATG–3′) and V5367 (5′–TTGATTACGTCCCTGCCCTTT–3′) (Castillo *et al*., 2003). The forward D2A (5′–ACAAGTACCGTGAGGGAAAGTTG–3′) and the reverse D3B (5′–TCGGAAGGAACCAGCTACTA–3′) primers were used for amplification of D2-D3 28S (De Ley *et al*., 1999). The forward JB3 (5′–

TTTTTTGGGCATCCTGAGGTTTAT–3′) and the reverse JB4 (5′–TAAAGAAAGAACATAATGAAAATG–3′) primers were amplified for the COI gene fragments (Derycke *et al*., 2010). PCR conditions were as described by Castillo *et* al. (2003)De Ley *et al*. (1999) and Derycke *et al*. (2010), respectively. PCR products were evaluated on 1% agarose gels and stained with Gelred (Tsingke Biotechnology, TSJ003). PCR products having amplified genes of interest were sent to Healthy Creatures, Hangzhou, China for sequencing. The newly obtained sequences were submitted to the GenBank database under accession numbers indicated on the phylogenetic trees.

Phylogenetic analyses. The newly obtained sequences of D2-D3 expansion segments of 28S, ITS and COI and available sequences of other *Hemicycliophora* species obtained from GenBank were used for phylogenetic reconstructions. Outgroup taxa for the dataset were chosen according to previously published data (Maria *et al*., 2018; Miraeiz *et al*., 2020; Nguyen *et al*., 2021; Azimi *et al*., 2021). Multiple alignments of the different sequences were made using the Q-INS-i algorithm of MAFFT v.7.205 (Katoh & Standley, 2013). Sequence alignments were manually visualised using BioEdit (Hall, 1999) and edited/trimmed for the poorly aligned positions, using a light filtering strategy (up to 20% of alignment positions), which reported to has little impact on tree accuracy and may save some computation time as suggested by Tan *et al*. (2015). Since methods for automated filtering of multiple sequence alignments frequently worsen single-gene phylogenetic inference (Tan *et al.*, 2015). Percentage similarity between sequences was calculated using the sequence identity matrix using BioEdit. When the same position for

both sequences had a gap, it was not treated as a difference. Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). 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. The transition and transversion model with invariable sites and a gamma-shaped distribution of invariable sites (TIM3+I+G, TVM+I+G, TIM2+G) were selected for the D2-D3 segments of the 28S rRNA, COI, and ITS rRNA gene, respectively. These BI analyses were run separately per dataset using four chains for 2×10^6 generations for all of the molecular markers. A combined analysis of the three genes was not undertaken due to some sequences not being available for all species. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further 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 visualised using FigTree software V.1.42 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

Description of *Hemicycliophora geraerti* n. sp.
(Fig. 1−3)

Measurement. See Table 1.

Female. Body straight or ventrally arcuate after heat relaxation. Cuticular sheath detached from inner cuticle at pharyngeal, vulva, and tail regions. Annuli smooth, without ornamentation. Lateral field visible on 1/3 of the body, indistinct posterior to anus, it is mostly marked by one central longitudinal line, that forms broken annuli or anastomosis. Under SEM, lip region broadly rounded, slightly sett-off from body contour containing 3–4 annuli. The lip region arrangementconsists of ovate, non-protruding oral plate or first lip annulus. The first lip annulus is smaller and embedded in the second annulus; amphids apertures plugged, flanked dorsoventrally in the oral plate. Lateral lip sectors fused with dorsal and ventral lip sectors, which are partially fused with the labial plate. The basal lip annuli are smaller than the body annuli. Labial framework moderately sclerotized.

.. Stylet long and flexible: stylet knobs posteriorly slopping, concave-shaped. Dorsal pharyngeal gland orifice indistinct in most specimens. Isthmus narrow, short, encircled by nerve ring. Hemizonid two annuli long, 4–5 annuli posterior to basal pharyngeal bulb. Excretory pore 1–3 annuli posterior to hemizonid. Reproductive system monodelphic-prodelphic, outstretched, composed by long ovary with oocytes arranged in single file except for a short region of multiplication near anterior end. Spermatheca indistinct, spheroid sperm cells observed in few specimens. Vulval lips slightly modified with 1–3 annuli long vulval sleeve. Anus small, indistinct, located at 13th–21th annuli posterior to vulva. Tail almost cylindrical anteriorly, tapering gradually to a distal end into finely conoid

terminus or slightly offset spike with a narrowly rounded terminus. Tail annuli distinct until terminus except terminal annuli becoming irregular.

Male. Not studied

Type host and locality. The type specimens were extracted from the rhizosphere of peach tree (*Prunus persica* L.), Qiandao Lake, Hangzhou, Zhejiang Province, China, on September 29th, 2021. The geographical position of the sampling site is 119°5'6" E; 29°38'46" N.

Etymology. The species-specific epithet is named in honor of Professor Etienne Geraert for his extraordinary contribution to our knowledge of nematode taxonomy.

Type-material. Holotype female, 12 females paratypes (slide number ZJU-34-01-ZJU-34-06) were deposited in the nematode collection of Zhejiang University, Hangzhou, China. Eight female paratypes (slide number ZJU-01-ZJU-03) were deposited at the USDA nematode collection, Beltsville, Maryland, USA. The new species binomial has been registered in the ZooBank database (zoobank.org) under the identifier: urn:lsid:zoobank.org:act:A1A3BFB1-A97C-4E4E-A62A-CB179FA33943. The LSID for the publication is: urn:lsid:zoobank.org:pub:C02674FE-85EC-4304-A7B7-9B0419C67971.

Polytomous key code: According to the polytomous key of Chitambar & Subbotin. (2014), the matrix code for *H. geraerti* n. sp. is: A4, B3, C1, D1, E2, F12, G2, H1, I2, J2, K4, L3, M2, N2, O3, P1, Q1, R6, S3, T2, U3, V1, W2, X1, Y.

Diagnosis and relationships. *Hemicycliophora geraerti* n. sp. can be distinguished by the following characteristics: cuticular sheath loosely fitting to the body; lateral field with one central longitudinal line; lip region continuous with body contour bearing 3–4 annuli, non-protruding oral plate; stylet 110 (103–116) µm long; the excretory pore 4–7 annuli posterior to the base of the pharyngeal bulb; vulva with slightly modified lips and short vulval sleeve; tail 125–157 µm long, elongated, conoid or slightly offset spike with narrowly rounded terminus.

To find the closely related species, a web-based key provided by Nguyen *et al*. (2021) was consulted (available at http://nematodeidentification.mypressonline.com/news/identification-key-for-hemicycliophora-spp/). This web-based key is based on the polytomous key given by Chitamber and Subbotin (2014). In the online system, *H. geraerti* n. sp. matrix codes were entered in the respective columns and the following species appeared close to our new species: *H. andrassyi* Brzeski, 1974, *H. cardamomi* Nguyen, Trinh, Couvreur, Nguyen & Bert, 2020, *H. lutosa* Loof & Heyns, 1969, *H. postamphidia* Rahaman, Ahmad & Jairajpuri, 1996, *H. sheri* Brzeski, 1974, *H. similis*, *H. subbotini*, *H. vidua* Raski, 1958, *H. vitiensis* Orton Williams, 1978.

From *H. andrassyi*, the new species can be differentiated by the slender body, a = 21.5 (19.2–23.6) *vs.* (23.6–28.0), different type of lateral field (one line *vs.* one or two lines marked by anastomosis or breaks), lip region morphology (rounded with non-protruding labial plate *vs.* slightly elevated labial plate), lip annuli (3–4 *vs.* 2), longer stylet

110 (103–116) *vs.* 83 (71–101) µm, and more annuli between anterior end to excretory pore, Rex = 61 (57–65) *vs.* 51 (47–56).

From *H. cardamomi*, it can be differentiated by having longer body length, 1138 (1027–1270) *vs.* 890 (843–931) µm, longer pharynx length, 199 (188–210) *vs.* 170 (166–174) µm, different type of lateral field (one continues line *vs.* one line, discontinuous over short distances, indistinct additional short lines also present in some parts along body), lip region morphology (rounded with non-protruding labial plate *vs.* protruding labial plate), more lip annuli (3–4 *vs.* 3), longer stylet 110 (103–116) *vs.* 95 (90–98) µm, more annuli between anterior end to excretory pore, Rex = 61 (57–65) *vs.* 55 (48–58), and longer tail, 142 (125–157) *vs.* 95 (91–99) µm.

From *H. lutosa*, it can be differentiated by the slender body, a = 21.5 (19.2–23.6) *vs.* (26–33), different type of lateral field (one continuous line *vs.* one to three irregular longitudinal lines), lip region morphology (rounded with non-protruding labial plate *vs.* truncate lip region with rectangular, non-elevated labial plate), more lip annuli (3–4 *vs.* 2), longer stylet 110 (103–116) *vs.* 84 (74–94) µm, fewer annuli between vulva to tail terminus, RV = 58 (50–65) *vs.* 75 (61–89), and fewer annuli between anus to tail terminus, Ran = 43 (37–49) *vs.* 57 (43–70).

From *H. postamphidia*, it can be differentiated by having longer body length, 1138 (1027–1270) *vs.* 865 (794–900) µm, more lip annuli (3–4 *vs.* 3), longer stylet 110 (103–116) *vs.* 86 (82–90) µm, less annuli on the entire body, R = 289 (273–305) *vs.* 360 (340–

372), fewer annuli between anus to tail terminus, Ran = 43 (37–49) *vs.* 76 (68–82), and longer tail length, 142 (125–157) *vs.* 97 (83–107) μm.

From *H. sheri*, it can be differentiated by the slender body, a = 21.5 (19.2–23.6) vs. 32 (31–33), cuticular sheath (loosely fitting body *vs.* tightly fitting body), more lip annuli (3–4 *vs.* 3), longer stylet 110 (103–116) *vs.* 98 (92–101) μm, less annuli on the entire body, R = 289 (273–305) *vs.* 328 (320–345), and fewer annuli between vulva and anus, RVan = 16 (13–21) *vs.* 25 (21–29).

From *H. similis* (after Subbotin *et al.* 2014), it can be differentiated by the cuticular sheath (loosely fitting body *vs.* tightly fitting body), different type of lateral field (with one continuous line *vs.* without lines), more lip annuli (3–4 *vs.* 2), longer stylet 110 (103–116) *vs.* 100.3 (91–108) μm, posteriorly located vulva, V = 84 (82–84) *vs.* 79.8 (78–81) %, fewer annuli between vulva and tail terminus, RV = 58 (50–65) *vs.* 64 (57–70), fewer annuli between vulva and anus, RVan = 16 (13–21) *vs.* 21.6 (19–24), and longer tail length 142 (125–157) *vs.* 112.6 (80–125) μm.

From *H. subbotini*, it can be differentiated by lip region morphology (rounded with non-protruding labial plate *vs.* bulging, protruding labial plate), more lip annuli (3–4 *vs.* 2–3), anteriorly located vulva, V = 84 (82–86) *vs.* 91 (90–93), fewer annuli between vulva and anus, RVan = 16 (13–21) *vs.* 18 (15–22), fewer annuli between anus and tail terminus, Ran = 43 (37–49) *vs.* 28 (24–35), and longer tail length 142 (125–157) *vs.* 94 (76–111) μm.

From *H. vidua*, it can be differentiated by slender body, a = 21.5 (19.2–23.6) vs. (26–31), cephalic region morphology (rounded with non-protruding labial plate *vs.* round to truncate with rectangular labial plate), more lip annuli (3–4 *vs.* 2–3), posteriorly located vulva, V = 84 (82–86) *vs.* (78–80), and fewer annuli between vulva and anus, RVan = 16 (13–21) *vs.* 28 (24–31).

From *H. vitiensis*, it can be differentiated by the slender body, a = 21.5 (19.2–23.6) *vs.* 29.4 (25.7–31.9), cuticular sheath (loosely fitting body *vs.* tightly fitting body), lip region morphology (rounded with non-protruding labial plate *vs.* round with elevated labial plate), more lip annuli (3–4 *vs.* 2), lower c = 8.2 (7.5–9.3) *vs.* 10.6 (9.8–12.5), longer stylet 110 (103–116) *vs.* 97 (86–106) µm, and longer tail length, 142 (125–157) *vs.* (65–104) µm.

Molecular characterization and phylogenetic relationship. The sequenced fragments of D2–D3 of 28S rRNA, ITS rRNA, and COI mtDNA fragments were deposited in GenBank under the following accessions numbers OL897571–OL897574 for 28S; OL897575–OL897576 for ITS, and OL960052–OL960053 for COI. Phylogenetic relationships among *H. geraerti* n. sp., and the related *Hemicycliophora* species were inferred using the aforementioned gene sequences using BI and are given in Figures 4, 5, and 6 respectively.

The multiple sequence alignment of the D2–D3 region of 28S rDNA gene contained 133 sequences including the sequences from outgroup taxa namely: *Mesocriconema sphaerocephalum* (Taylor, 1936) Loof, 1989 (KF430522), *Xenocriconemella macrodora*

(Taylor, 1936) De Grisse & Loof, 1965 (AY780960), *Paratylenchus bukowinensis* Micoletzky, 1922 (AY780943) and *Paratylenchus nanus* Cobb, 1923 (AY780946) (Fig. 4). The D2-D3 of 28S tree showed 5 well supported molecular clades (PP=0.99–1.00), the new species grouped with *H. californica* Brzeski, 1974 (KF430518–KF430519), *H. raskii* Brzeski, 1974 (KF430520, MG019826), *H. cardamomi* (MW001620–MW001621) and *H. subbotini* (MG701275–MG701277). The sequence identity of *H. geraerti* n. sp., with the clustered species is 91%–93% with 46–61 bp and 0–6 indels difference.. Morphologically all these species share similar labial and tail morphology (i.e., lip region continuous, not offset from the body contour; tail cylindrical then tapering abruptly to a uniformly conical posterior region). In addition, the body lengths of these species are less than 1100 µm and males were absent.

The ITS tree was constructed with 130 available sequences where *Paratylenchus minutus* Linford, 1949 (EF126180), *Paratylenchus bilineatus* Brzeski, 1995 (EU247525) and *Trophurus floridensis* Tanha, Amani, Stanley, Inserra, Van den Berg & Subbotin, 2012 (JN112261) serve as outgroup taxa (Fig. 5). Likewise, the D2–D3 of 28S tree, the ITS tree also consists of 5 moderately supported molecular clades (PP=0.82–1.00), the new species grouped with *H. cardamomi* (MW001618–MW001619), *H. subbotini* (MG701272–MG701274) and *H. raskii* (KF430577). The sequences of the new species showed an 85–88% identity (differing in 92–128 bp, and 29–54 indels) with the clustered species. Morphologically, these species share similar labial morphology (i.e., lip region

continuous, not offset from the body contour), and a similar range of the number of annuli between vulva to anus, RVan = 10–20.

Not all the described *Hemicycliophora* species are characterized by COI mtDNA sequences, therefore, the COI tree was constructed with 125 available sequences. The COI sequences of the new species showed a 88–90% identity (differing in 40–46 bp, and 0 indel) with the clustered species in ribosomal genes (viz. *H. raskii*, *H. californica*, and *H. cardamomi*). The tree showed multiple clades, the new species grouped independently among other species of *Hemicycliophora* and separated from any of the known *Hemicycliophora* species. (Fig. 6).

In the D2–D3 of 28S and ITS trees, *H. geraerti* n. sp., showed close phylogenetic affinity with the *H. cardamomi*, *H. subbotini* and *H. raskii* as compared to COI tree. Morphologically, they all exhibit continuous labial region, posteriorly located vulva (87% of the entire body), modified and elongated vulval sleeve, and RVan = 10–20 annuli.

The molecular characterization and phylogenetic analyses conducted in this study indicated the unique status and supports the novelty of *H. geraerti* n. sp.

DISCUSSION

The species identification in genus *Hemicycliophora* is often problematic because of the large number of species and potential morphological characteristics to consider. To facilitate species identification Chitambar & Subbotin (2014) provided a comprehensive and systematic ‘polytomous key’ whereas more recently Nguyen *et al.* (2021) used this

polytomous key and provided a web-based method to identify this genus at the species level. Among other morphological characters, tail morphology has been vigorously used in *Hemicycliophora* species differentiation. The new species has a spike-like terminus, literature studies indicated that nearly 50 other *Hemicycliophora* species share this character and are widely distributed in North or South American, Asian, and European countries, and are known to be associated with fruits, vegetables, and grasses. Among them, *H. similis* is the only member of the genus that was found in the peach rhizosphere. Our new species is the fourth member of the genus reported from China and it is the first *Hemicycliophora* species that has been detected in the peach rhizosphere.

Phylogenetic analyses conducted in our study indicated that the genetic diversity of *Hemicycliophora* is significantly higher than morphological observations. For instance, in 28S and ITS phylogeny, *H. cardamomi*, *H. subbotini* and *H. raskii* appeared closer species with the *H. geraerti* n. sp. Morphologically, both species are different from the new species, in terms of shorter body and stylet lengths, less number of annuli (R) on the entire body, and general appearance. Whereas in COI phylogeny *H. cardamomi* and *H. raskii* grouped away from *H. geraerti* n. sp. Our results are in line with several studies that reported the usefulness of COI in species delimitation (Powers *et al*., 2014; Bai *et al*., 2020. Although, *H. geraerti* n. sp. has been characterized with ribosomal and mitochondrial markers, we anticipate that the inclusion of new sequences in the future will likely change the position of *Hemicycliophora* species. In addition to that, the differences in evolutionary patterns of ribosomal and mitochondrial genes are because

the evolutionary forces shaping the mt genome are quite different from those that affect the nuclear genome (Neiman and Taylor, 2009).

Up to now, only *H. subbotini* was reported from Zhejiang Province, China in the rhizosphere of the camphor tree. The latest reports on nematode taxonomical studies paid more attention to pathogenic nematodes such as *Bursaphelenchus*, *Heterodera*, *Meloidogyne* and *Ditylenchus* species (Kim *et al*., 2020; Peng *et al*., 1998, 2021; Wang *et al*., 2018). In the present study, we focused more on the less studied group (sheath nematodes) and presented a detailed taxonomical characterization of a new *Hemicycliophora* species found in the peach rhizosphere. Regarding *H. geraerti* n. sp. density, we detected 30 individuals/100g of soil in the studied samples. The low density suggests that it is a mild parasitic species and does not behave as a potential pest yet. However, we assert that the recognition and accurate identification of detected species are important to assess if these nematodes can pose any potential threat to stone fruits in the future and to predict if these species may eventually require appropriate control strategies and regulatory measures.

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Table 1. Female morphometric characters of *Hemicycliophora geraerti* n. sp. All measurements are in μm and in the form of mean ± SD (range).

Characters/ratio	Holotype	Paratype
n	1	20
L	1124	1138.0 ± 69 (1027–1270)
L'	1146	1165 ± 70 (1058–1293)
a	21.0	21.5 ± 1.3 (19.2–23.6)
b	5.6	5.8 ± 0.2 (5.4–6.2)
c	8.6	8.2 ± 0.5 (7.5–9.3)
c'	3.2	3.5 ± 0.4 (3.0–4.2)
V	85	84 ± 1.0 (82.5–86.5)
VL/VB	4.4	4.9 ± 0.8 (3.3–6.0)
R	280	289.0 ± 8.8 (273.0–305.0)
Rst	29	30.0 ± 1.5 (27.0–33.0)
Rex	61	61.0 ± 2.0 (57.0–65.0)

Roes	56	54.5 ± 1.9 (50.0–58.0)
RVan	16	16.0 ± 1.5 (13.0–21.0)
Ran	42	43.1 ± 2.6 (37.0–49.0)
RV	58	57.8 ± 2.7 (50.0–65.0)
Lip height	8.9	8.2 ± 0.5 (7.0-9.0)
Lip diam.	18.8	18.1 ± 0.7 (17.0–20.0)
Stylet	109	110 ± 3.6 (103–116)
Couns length	91	92 ± 3.6 (84–97)
m	83.4	83.4 ± 1.0 (81–85)
Pharynx length	205	199 ± 6.4 (188–210)
Max body diam.	55	54 ± 4.2 (48–63)
Vulva body diam. inside sheath	40	39 ± 6.0 (31–51)
Vulva body diam. outside including sheath	49	47 ± 5.7 (36–58)
Distance from vulva to tail term.	176	185 ± 15.2 (154–208)
Anal body diam. inside sheath	30	30 ± 3.5 (23–37)
Anal body diam. including sheath	42	41 ± 4.7 (32–48)
Tail length inside sheath	109	116 ± 7.7 (102–127)
Tail length including outer sheath	133	142 ± 9.9 (125.5–157.5)

Figure legends:

Figure 1: Line drawings of female *Hemicycliophora geraerti* n. sp. A: pharyngeal region; B–D: *en face* view; E: lateral field; F: SEM vulval region; G: reproductive system; H: LM vulval region; I–L: posterior body regions. (Scale bars: A, G, I–L = 50 µm; B, D = 10 µm; C = 15 µm; E–F, H =20 µm).

Figure 2: Light photomicrographs of female *Hemicycliophora geraerti* n. sp. A: Entire body; B–E: anterior pharyngeal region, arrows showing excretory pore (exp); F: lateral field; G–I: vulval regions; J-M: posterior body regions arrows showing position of anus (a). (Scale bars: A = 100 µm; B–I = 10 µm; J–M = 50 µm).

Figure 3: Scanning electron micrographs of female *Hemicycliophora geraerti* n. sp. A: Entire body; B–D: *en face* view of lip region; E: vulval region; F: midbody section; G–J: posterior body regions tail arrow showing the position of anus. (Scale bars: A = 250 μm; B–D = 10 μm; E–F = 20 μm; G–L = 50 μm).

Figure 4: Phylogenetic relationships of *Hemicycliophora geraerti* n. sp. from Bayesian analysis using the D2-D3 of 28S rRNA gene sequence data set with the TIM3+I+G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

Figure 5: Phylogenetic relationships of *Hemicycliophora geraerti* n. sp. from Bayesian analysis using the ITS rRNA gene sequence data set with the TIM2+G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

Figure 6: Phylogenetic relationships of *Hemicycliophora geraerti* n. sp. from Bayesian analysis using the COI mtDNA gene sequence data set with the TVM+I+G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.