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Characterisation of two Chinese native *Hemicriconemoides* species (Nematoda: Criconematidae) with updated descriptions of *H. chitwoodi* Esser, 1960 and *Criconemoides myungsugae* Choi & Geraert, 1975

Munawar MARIA[1], Ruihang CAI[1], Pablo CASTILLO[2], Carolina CANTALAPIEDRA-NAVARRETE[2] and Jingwu ZHENG[1,3*]

[1] *Laboratory of Plant Nematology, Institute of Biotechnology, College of Agriculture & Biotechnology, Hangzhou 310058, Zhejiang, P.R. China*

[2] *Institute for Sustainable Agriculture (IAS), Spanish National Research Council (CSIC), Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain*

[3] *Ministry of Agriculture Key Lab of Molecular Biology of Crop Pathogens and Insects, Hangzhou 310058, P.R. China*

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*Corresponding author, e-mail: jwzheng@zju.edu.cn

Summary – Populations of *Hemicriconemoides chitwoodi*, *H. fujianensis*, *H. parasinensis*, and *Criconemoides myungsugae* isolated in China from the rhizosphere soil of woody perennials were characterised molecularly, important morphological details being elucidated by SEM observations. The morphometric data of the Chinese populations were compared with all previously reported populations. The SEM observations of *en face* views indicated that *H. chitwoodi* and *H. parasinensis* have lip patterns belonging to type 1 and phylogenetically both species clustered with other *Hemicriconemoides* species that showed the same kind of lip pattern. On the other hand, *H. fujianensis* showed a lip pattern belonging to type 2 and phylogenetically appears basal to the above-mentioned clade. SEM observation of *C. myungsugae* showed that the first lip annulus forming a non-projecting uninterrupted disc and the labial annulus is rectangular shaped with slight dorsal and ventral indentations. Phylogenetic relationships among *Criconemoides* spp. are apparently not well resolved. The present study provides updated morphological descriptions, molecular diagnostics and phylogenetic relationships of *H. chitwoodi*, *H. fujianensis*, *H. parasinensis*, and *C. myungsugae*, last species being the first report from China.

Keywords – *Criconemoides myungsugae*, description, first record, *Hemicriconemoides chitwoodi*, *Hemicriconemoides fujianensis*, *Hemicriconemoides parasinensis*, host association, molecular, morphology, phylogeny, SEM, taxonomy.

Sheathoid nematodes of genus *Hemicriconemoides* Chitwood & Birchfield, 1957 are generally found inhabiting warmer areas of the world, particularly in Africa, the Americas, Australia, South and Southeast Asia and southern Europe (Van den Berg *et al*., 2014). The genus contains around 55 species and out of these, five have been described from China (Geraert, 2010; Maria *et al*., 2018a). In an attempt to document criconematid species occurring in China, three *Hemicriconemoides* and one *Criconemoides* Taylor, 1936 populations were recovered from soil samples obtained from different ecological locations.

After preliminary examination, these species were identified as *H. chitwoodi* Esser, 1960, *H. fujianensis* Zhang, 1998, *H. parasinensis* Chen & Liu, 2003, and *C. myungsugae* Choi & Geraert, 1975. Literature studies revealed that the *H. chitwoodi* was previously reported from Shaanxi (Wang, 1993) and Yunnan (Lin *et al*., 2014) Provinces. *Hemicriconemoides parasinensis* was described from Liaoning Province (Chen & Liu, 2003) and also reported from Taiwan (Chen *et al*., 2008). In addition, *H. fujianensis* was described from Fujian Province and has not been reported thereafter.

As these species were reported almost a decade ago, there is a lack of molecular sequencing data and SEM observations. *Hemicriconemoides fujianensis* and *H. parasinensis* are Chinese native species and the details mentioned in the original description are insufficient to use for integrative molecular taxonomy. Moreover, *C. myungsugae* is the first report from China. This led us to perform detailed morphological, molecular and scanning electron microscopy analyses for these four criconematids. Thus, the specific objectives of this study were: *i*) to provide a morphological and molecular characterisation of these species; *ii*) to elucidate important morphological details through SEM observations; and *iii*) to study the phylogenetic relationships of these species with other *Hemicriconemoides* and *Criconemoides* species.

In this study, *Mesocriconema* Andrássy, 1965 is regarded as a junior synonym of *Criconemoides* (Hunt *et al*., 2005; Eskandari *et al*., 2010; Van den Berg *et al*., 2012).

Materials and methods

Nematode population sampling, extraction and morphological identification

Nematodes were extracted from soil samples using the modified Baermann funnel method.

For morphometric studies, the nematodes were killed and fixed with hot formalin and processed to glycerin (Seinhorst, 1959) as modified by De Grisse (1969). The drawings, measurements and light micrographs of nematodes were completed with the help of a Zeiss compound microscope (Stemi 2000-C).

For the SEM examination, the nematodes were fixed in a mixture of 2.5% paraformaldehyde and 2.5% glutaraldehyde, washed three times in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide, dehydrated in a series of ethanol solutions and critical point-dried with CO_2. After mounting on stubs, the samples were coated with gold (Maria *et al*., 2018a)

MOLECULAR ANALYSES

DNA samples were prepared according to Zheng *et al*. (2003). Five sets of primers (synthesised by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the nearly full-length 18S, D2-D3 expansion segments of 28S, ITS and *cox*I regions. Nearly full length 18S region was amplified with two sets of primers 18s39F (5'-AAAGATTAAGCCATGCATG-3') and 18s977R (5'-TTTACGGTTAGAACTAGGGCGG-3'), the second set was 18s900F (5'-AAGACGGACTACAGCGAAAG-3') and 18s1713R (5'-TCACCTACAGCTACCTTGTTACG-3') (Olson *et al*., 2017). Primers for amplification of ITS were TW81 (5'-GTTTCCGTAGGTGGTGAACCTGC3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Joyce *et al*., 1994), the forward D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and the reverse D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers for amplification of D2-D3 28S (De Ley *et al*., 1999), and finally the primers used for *cox*I amplification were COI-F5 (5'-AATWTWGGTGTTGGAACTTCTTGAAC-3') and COI-R9 (5'-CTTAAAACATAATGRAAATGWGCWACWACATAATAAGTATC-3') (Mullen *et al*., 2014). PCR conditions were as described by Ye *et al*. (2007), Powers *et al*. (2010) and Mullen *et al*. (2014). 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. The newly obtained sequences were submitted to the GenBank database under accession numbers indicated on the phylogenetic trees.

PHYLOGENETIC ANALYSES

Newly obtained sequences of D2-D3 expansion segments of 28S, ITS, partial 18S and *cox*I and available sequences of other nematodes obtained from GenBank were used for phylogenetic reconstructions of *Hemicriconemoides* and *Criconemoides* species. Outgroup taxa for the dataset were chosen according to previously published data (Van den Berg *et al*., 2014, 2015; Maria *et al*., 2018a). 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 by Gblocks ver. 0.91b (Castresana, 2000) in a Castresana Laboratory server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using options for a less stringent selection (minimum number of sequences for a conserved or a flanking position: 50% of the number of sequences +1; maximum number of contiguous non-conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half). Percentage similarity between sequences was calculated using the sequence identity matrix using BioEdit. For that, the score for each pair of sequences was compared directly and all gap or place-holding characters were treated as a gap. 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. An unlinked general time-reversible model with a gamma-shaped distribution (GTR + G) for the 18S rRNA and D2-D3 expansion segments of 28S rRNA datasets of *Hemicriconemoides* spp.: an unlinked general time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) for ITS and *cox*I dataset of *Hemicriconemoides* spp.; a GTR + G model for the D2-D3 expansion segments of 28S rRNA and ITS datasets of *Criconemoides* spp.; and an unlinked general time-reversible model with invariable sites and a gamma-shaped distribution (GTR + I + G) for *cox*I dataset of *Criconemoides* spp. These BI analyses were run separately per dataset using four chains for 2×10^6 generations for all of 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 visualized using FigTree software V.1.42 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Hemicriconemoides chitwoodi Esser, 1960

(Figs 1, 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body slightly curved ventrad. Sheath closely fitting except in lip region. Lip region angular, with two annuli, labial plate appearing elevated or slightly dome-shaped in some individuals. Labial annuli set off from rest of body, first annulus wider than second. SEM observation showing oral aperture with slit-like opening and somewhat rectangular labial plate composed of semi-globular shaped projections embedded in labial plate. Lip pattern matching that of type 1 proposed for *Hemicriconemoides* species by Decraemer & Geraert (1992). Stylet long and slender. Stylet knobs indented anteriorly and rounded posteriorly. Dorsal pharyngeal gland opening slightly posterior to stylet base. Pharynx typical of genus. Excretory pore conspicuous, situated four or six annuli posterior to base of pharynx. Sheath annuli flattened to mostly indented over whole length of body, single anastomoses observed in posterior half of body. Vulva a distinct slit without vulval flaps. Vagina straight, extending for half of body diam. Spermatheca

oblonged and rounded, filled with sperm cells, generally situated on left side of distal end of uterus. Ovary single, prodelphic. Anus small, located 4-5 annuli posterior to vulva. Body gradually tapering posterior to vulval region, presenting pronounced dorsal curve to tail tip. Tail, conoid, narrowing down to form a rounded terminus. Terminal tail annuli sometimes curved dorsally or ventrally.

Male

Not detected.

HOST AND LOCALITY

This population was found in the rhizosphere of *Camellia sinensis* (L.) O. Kuntz from a Longwu tea village, Hangzhou, Zhejiang Province, China. The geographical position of the sampling site is E:120°01'20" N:30°10'27.

REMARKS

Hemicriconemoides chitwoodi was found in the rhizosphere of *Camellia* sp. and described from Florida, USA (Esser, 1960). Recently Van den Berg *et al*. (2014) re-characterised the topotypes of *H. chitwoodi* and presented some additional morphometric details. From China, this species was reported from Shaanxi (Wang, 1993) and Yunnan (Lin *et al*., 2014) Provinces in the rhizosphere of *Juglans* sp. and *Ziziphus jujuba* Mill., respectively. It has now been found in Zhejiang Province. The three Chinese populations matched well with original description except for the Shaanxi population, which presents slightly shorter body lengths. Additionally, all Chinese populations have slightly shorter stylet lengths as compared to the original and topotype populations. We consider that these small morphometric differences are due to intraspecific geographical variability.

Hemicriconemoides fujianensis Zhang, 1998

(Figs 3-6)

MEASUREMENTS

See Table 2.

DESCRIPTION

Female

Body, slightly curved ventrad. Sheath closely fitting except in a few individuals where it is extended anteriorly over lip region and appearing slightly loosened at tail terminus. Lip region continuous, with two annuli, first annulus slightly wider or equal in size than second. SEM observation showing an expanded first annulus having a somewhat ellipsoidal labial plate, oral aperture with slit-like opening and surrounded by small elevated globular pieces. Lip pattern matching that of type 2 proposed for *Hemicriconemoides* species by Decraemer & Geraert (1992). Stylet small and robust. Stylet knobs indented anteriorly and rounded posteriorly. Dorsal pharyngeal gland opening slightly posterior to stylet base. Pharynx typical of genus. Excretory pore situated 4-5 annuli posterior to base of pharynx. Sheath annuli retrorse, mostly indented over whole length of body, no anastomoses present. Vulva a distinct slit without vulval flaps. Vagina straight, extending less than half of body diam. Spermatheca spherical, generally situated on left side of distal end of uterus, ovary single, prodelphic. Anus small, located 4-5 annuli posterior to vulva. Tail broadly conoid, ending in a bluntly rounded tip.

Male

Not detected.

HOST AND LOCALITY

Two populations were detected for *H. fujianensis*, the first in the rhizosphere of *Phyllostachys* sp. from Huangshan Mountain, Anhui Province; the second in the rhizosphere of

Quercus acutissima Carruth from Longwu tea village, Hangzhou, Zhejiang, Province. The geographical position of the sampling site E: 120°01'18" N:30°10'27.

REMARKS

Hemicriconemoides fujianensis was described from Fujian Province from the rhizosphere of *Litchi chinensis* Sonn. (Zhang, 1998), and has not been reported thereafter. In this study, it was found from Anhui and Zhejiang Provinces. Morphometrically, both populations are indistinguishable except that the Zhejiang population is slightly shorter than the Anhui population. Slight intra-species variation was detected in both populations in terms of *en face* view, the labial plate of the Zhejiang population being more pronounced than that of the Anhui population. However, the rest of the morphological characters are similar for both populations. The two populations correspond well with the original description except for slightly longer body lengths of 545 (465-583) *vs* 470 (380-510) μm and fewer body annuli: 103 (100-107) *vs* 124 (118-130). We consider these small morphometric differences are due to geographical variability.

Hemicriconemoides parasinensis Chen & Liu, 2003

(Figs 7, 8)

MEASUREMENTS

See Table 3.

DESCRIPTION

Female

Body, slightly curved ventrad. Sheath closely fitting except in a few individuals where it is extended anteriorly over lip region. Lip region continuous, with two annuli, first annulus slightly narrower than second. SEM observation showing lip region composed of a dorsoventrally orientated oral disc with a slit-like opening and labial plate extending as two lateral semi-globular

shaped projections on lateral sides of oral disc. Lip pattern matching that of type 1 proposed for *Hemicriconemoides* species by Decraemer & Geraert (1992). Stylet long and slender mostly straight. Stylet knobs anchor-shaped. Dorsal pharyngeal gland opening slightly posterior to stylet base. Pharynx typical of genus. Excretory pore situated 1-3 annuli anterior or posterior to base of pharynx. Sheath annuli retrorse, mostly indented over whole length of body, no anastomoses present. Vulva a distinct slit without vulval flaps. Vagina straight, extending for less than half of body diam. Spermatheca rounded, filled with sperm cells, generally situated on left side of distal end of uterus, ovary single, prodelphic. Anus small located 3-5annuli posterior to vulva. Tail tapering gradually to a slightly narrower, rounded tip.

Male

Not detected.

HOST AND LOCALITY

This population was found in the rhizosphere of *Osmanthus fragrans* Loureiro from a Hushan garden, Hangzhou, Zhejiang Province, China. The geographical position of the sampling site is; E:120°11'53" N:30°21'45".

REMARKS

Chen & Liu, 2003 found *H. parasinensis* in the rhizosphere of *Syringa oblate* Lindley and *Ligustrum quihoui* Carrière from Liaoning and Henan Provinces respectively and cited both populations as paratypes. Later it was reported from Taiwan by Chen *et al*. (2008) and has now been found in Zhejiang Province. The morphology of the Zhejiang population match well with the original description, although the morphometric values are closer to the paratype population from Henan Province. The paratype population described from Liaoning Province and the population from Taiwan are slightly shorter than the Zhejiang population. We consider these minor morphometric differences to be due to geographical variability.

***Criconemoides myungsugae* Choi & Geraert, 1975**

(Figs 9, 10)

MEASUREMENTS

See Table 4.

DESCRIPTION

Female

Body cylindrical, ventrally arcuate after heat relaxation. First lip annulus forming a non-projecting uninterrupted disc. SEM observation showing rectangular labial annulus with slight dorsal and ventral indentations. Labial plate somewhat ellipsoidal with slit-like oral aperture. Stylet small and robust. Stylet knobs anchor-shaped. Dorsal pharyngeal gland opening slightly posterior to stylet base. Pharynx typical of genus. Excretory pore situated 3-4 annuli posterior to base of pharynx. Sheath annuli retrorse with numerous anastomoses and margins finely crenated. Vulva a distinct slit, vulval lips protruding, without vulval flaps. Vagina straight, extending for half of body diam. Spermatheca rounded, generally situated on left side of distal end of uterus, ovary single, prodelphic. Anus small, distinctly located 4-5 annuli posterior to vulva. Tail cylindrical, ending in a squared terminus, corners of which appearing lobed in lateral view.

Male

Not detected.

HOST AND LOCALITY

This population was found in the rhizosphere of *Altingia gracilipes* Hemsl, from the Botanical Garden, Hangzhou, Zhejiang Province, China. The geographical position of the sampling site is E: 120°07'01" N:30°15'19".

REMARKS

This is the first report of *C. myungsugae* from China. It was described from Korea in the rhizosphere of *Indigofera kirilowii* Linnaeus by Choi & Geraert (1975). Later, Choi *et al*. (2000) re-characterised some of the paratypes and supplemented the description with additional morphometrics. Another population of the same nematode was reported from Iran in the rhizosphere of herbaceous plants (Eskandari *et al*., 2010). Now it has been reported from Zhejiang Province. The Chinese population matches well with the original description except for a slightly longer body of 459-574 *vs* 445-500 μm and fewer body annuli: R = 104.0-115.0 *vs* 122-127. Morphometrically the Chinese population has shorter body lengths compared to the Iranian population: 527 (459-574) *vs* 546 (455-630) μm and more annuli between vulva and anus: Rvan = 4.6 (4.0-5.0) *vs* 2 (1-3). We consider these minor morphometric differences to be due to intraspecific geographical variability.

MOLECULAR CHARACTERISATION AND PHYLOGENETIC RELATIONSHIPS OF *HEMICRICONEMOIDES* AND *CRICONEMOIDES* SPECIES

All four species were molecularly characterised using D2-D3 expansion segments of 28S, ITS, 18S and *cox*I fragments and sequences were deposited in GenBank. *Hemicriconemoides fujianenesis* and *C. myungsugae* have been molecularly characterised for the first time in this study. Nine new sequences of the D2-D3 of 28S rRNA gene were obtained from *Hemicriconemoides* species in the present study. D2-D3 expansion segments of 28S rRNA sequences of *H. chitwoodi* (MH444611-MH444613) matched well with other accessions from the same species deposited in GenBank (KF856532-KF856535), differing by 4-6 nucleotides. *Hemicriconemoides parasinensis* (MH444632-MH444634) showed sequence identity values of 94% (40-41 bp difference) and 95% (30-34 bp difference) with *H. paracamelliae* Maria, Cai, Castillo & Zheng 2018 (MG0295569-MG0295571) and *H. parataiwanesis* Decraemer & Geraert 1992 (MG0295572-MG0295574), respectively. *Hemicriconemoides fujianensis* (MH444624, MH444625, MH444619) showed 88-90% (60-88 bp difference) sequence identity with several accessions, such as *H. strictathecatus* Esser, 1960 (MF363013, KM516172-KM516178), *H.*

kanayaensis Nakasono & Ichinohe 1961 (MG029576) and *Hemicriconemoides* sp. 1 VSS 2017 (MF438042). Finally, the closest related species to *C. myungsugae* (MH444641-MH4446443) for the D2-D3 expansion segments of 28S rRNA was *C. brevistylus* Singh & Khera, 1976 (KC937033), being 85% similar (116 different nucleotides and 11 indels).

Ten new ITS sequences were obtained for *Hemicriconemoides* species. *Hemicriconemoides chitwoodi* (MH444609-MH444610) showed 95-99% identity (2-24 bp difference) with *H. chitwoodi* (KF856543-44, KJ934162, EU180057, JQ708140) deposited in GenBank. *Hemicriconemoides parasinensis* (MH444629-MH444631) showed 96% identity (31 bp difference) with *H. parasinensis* (EU664601) from Taiwan. While *H. fujianenesis* (MH444622-MH444623, MH444616-MH444618) showed 86% sequence identity (86-87 bp difference) with *H. alexis* Vovlas 1980 (KF856561-KF856562), 83-87% (60-76 bp difference) with *H. chitwoodi* (KF856543-44, KJ934162, EU180057, JQ708140), 86% (84 bp difference) with *H. parasinensis* (EU664601) and 87% (71 bp difference) identity with *H. silvaticus* Eroshenko & Volkova 1985 (KF856542). For the 18S, ten sequences were obtained in this study, *H. chitwoodi* (MH444614-MH444615), *H. parasiensis* (MH444635-MH444636), *H. fujianensis* (MH444626-MH444628, MH444620-MH444621) and *C. myungsugae* (MH444644- MH444645), all showing very high similarity values with the other accessions from *Hemicriconemoides* spp. and *Criconemoides* spp. deposited in GenBank, being 98-99% similar to all.

Phylogenetic relationships among *Hemicriconemoides* species inferred from analyses of D2-D3 expansion segments of 28S rRNA, ITS, the partial 18S rRNA, and the partial *cox*I mtDNA gene sequences using BI are given in Supplementary Figures S1, S2, S3 and S4, respectively. The phylogenetic trees generated with nuclear markers, D2-D3 expansion segments of 28S rRNA gene, ITS and the partial 18S rRNA including 70, 25 and 73 sequences with 668, 768 and 1686 bp in length, respectively. The D2-D3 of 28S gene tree (Supplementary Fig. S1), revealed four major low to well supported clades (PP = 0.72, PP = 1.00, respectively). All D2-D3 expansion segments of 28S gene accessions obtained in this study clustered within clade I. *Hemicriconemoides chitwoodi* and *H. parasinensis* formed a well-supported subclade with *H. paracamelliae*, *H. parataiwanensis*, *H. silvaticus*, and *H. gaddi* (Loos 1949) Chitwood & Birchfeild 1957, all with lip patterns belonging to type 1, whereas *H. fujianenesis* clustered alone, occupying a basal position within this superior major clade. The intraspecific variability between the *H. fujianensis* Zhejiang and Anhui populations was very low, both populations showing 99%

(2 bp difference) identities.

For ITS gene tree (Supplementary Fig. S2), the 50% majority-rule BI tree also showed four major low to well-supported clades (PP = 0.76, PP = 1.00, respectively). Likewise, in the former tree, *H. chitwoodi* from China (MH444609-MH444610) clusters with *H. chitwoodi* (KF856543-KF856544, KJ934162, EU180057, JQ708140) present in the GenBank with 100% support. In this tree, *H. parasinensis* (MH444629-MH444631) clusters with the sole sequence of *H. parasinesis* from Taiwan (EU664601), showing 96% (31 bp difference) sequence identity. Morphologically, *H. parasinensis* from Zhejiang Province and Taiwan are indistinguishable. This difference in the ITS sequences of both populations could be due to variations among copies of the ITS within an individual (Pokharel *et al*., 2007).*Hemicriconemoides fujianensis* (MH444622-MH444623, MH444616-MH444618) clusters within *Hemicriconemoides* species but appears as an independent subclade. The intraspecific variability between the *H. fujianensis* Zhejiang and Anhui populations are very low, both populations showing 99% (8 bp difference) identities. A similar topology was found with the partial 18S (Supplementary Fig. S3), although fewer sequences were available for this fragment in GenBank. The well-supported subclade (PP = 1.00) formed by *H. chitwoodi*, *H. parasinensis*, *H. paracamelliae*, and *H. parataiwanensis* was maintained as in the 28S and ITS trees and *H. fujianenesis* did not form any sublcade with other species. Another population of *H. chitwoodi* (JQ708170) from the USA does not cluster with the Chinese *H. chitwoodi* population, sharing a branch with *H. wessoni* (JF972467, HM116035). *Hemicriconemoides wessoni* has a type 2lip pattern, vulva with flaps and a tail with rounded terminus, indicating that the USA population of *H. chitwoodi* (JQ708170) may be a misidentified species. In this tree, *H. fujianensis* (MH444626-MH444628, MH444620-MH444621) still occupy the individual subclade position within *Hemicriconemoides* species. The intraspecific variability between the *H. fujianensis* Zhejiang and Anhui populations are very low, both populations showing 99% (2-5 bp difference) identities with each other. Finally, the tree inferred with the *cox*I gene (Supplementary Fig. S4), showed a different topology to the other trees obtained with nuclear markers; in this case *H. chitwoodi* (MH478584-MH478585) clustered with *H. macrodorus* Vovlas, Troccoli & Castillo, 2000 (KM577166-KM577168) and *H. promissus* Vovlas, 1980 (KM577164-KM577165) in a well-supported clade (PP = 0.96) and *H. fujianensis* clustered separately to this clade. The intraspecific variability of the *H. fujianensis* Zhejiang and Anhui populations is comparatively higher in this gene, *i.e*., 93% (difference of 52 bp).

Twelve sequences were obtained for *C. myungsugae* in the present study, three for 28S (MH444641-MH444643), four for ITS (MH444637-MH444640), two for 18S (MH444644-MH444645), and three for *cox*I (MH496163-MH496165). Most of the *Criconemoides* species are not fully characterised using all the rDNA fragments. The 28S, ITS and *cox*I phylogenetic trees of *C. myungsugae* are presented in Supplementary Figures S5-S7, respectively. In all the analyses, *C. myungsugae* clustered separately, except for 28S where it shared a branch with *C. solivagus* (AY780969) and *C. informis* (KU722386, AY780970), although showing 82% (102 bp difference) and 81% (110 bp difference) sequence identity, respectively. Phylogenetic analysis based on near full-length 18S was not done since only three species have been characterised for this gene.

Discussion

Species of *Hemicriconemoides* are widely distributed in China, with 18 species described/reported so far (Maria *et al*., 2018a). *Hemicriconemoides parasinensis* and *H. fujianensis* are Chinese native species, both being originally described from flowering and fruit trees, respectively. In the present study, *H. parasinensis* was isolated from *Osmanthus fragrans*, a native plant species of southern China. This plant is regarded as the city flower of Hangzhou, the flowers of the tree being used to make a herbal tea that is known to have some medicinal effect (Zhou, 2008). *Hemicriconemoides fujianensis* was isolated from *Quercus acutissima*, it is also a native plant species of China, mostly grown for its dense shade and forestry purposes (Edward & Dennis, 1994).

The genus *Criconemoides* has not been well explored in China, until now only three species (*C. informis* (Micoletzky, 1922) Taylor, 1936; *C. parvus* Raski, 1952, and *C. zavadskii* (Tulaganov, 1941) Raski, 1958) being reported (Maria *et al*., 2018c). In the present study, another *Criconemoides* species, *i.e*., *C. myungsugae*, was found in the rhizosphere of *Altingia gracilipes* and was characterised molecularly for the first time. Our phylogenetic analysis place *C. myungsugae* within species of *Criconemoides* but, due to a lack of sequences, phylogenetically close species cannot be determined. The 28S tree places *C. solivagum* Andrassy, 1962 and *C. informis* (Micoletzky, 1922), Taylor, 1936 in one clade with *C. myunsugae*, however, morphologically *C. myunsugae* can be differentiated from both species by having a discoid lip

region, longer stylet, and broadly rounded to cylindrical tail.

The phylogenetic analyses of *Hemicriconemoides* spp. obtained in the present study agree with previous studies of this genus (Van den Berg *et al*., 2014, 2015, Maria *et al*., 2018a) although small differences were found, probably due to the inclusion of new sequences. In the 28S, ITS and 18S trees, the species having lip patterns of type 1 grouped within the same clade, including *H. chitwoodi* and *H. parasinensis*. However, *H. fujianensis* did not seem to be phylogenetically related with the other species belonging to type 2 lip pattern. The phylogenetic position of *H. fujianensis* suggesting a possible transition of lip morphology from type 1 to type 2. The phylogenetic relationships of *H. chitwoodi*, *H. parasinensis*, and *H. fujianensis* were consistent in all nuclear markers, although these relationships were unresolved when the *cox*I gene analysis was used. In this tree, *H. chitwoodi* shares a branch with *H. macrodorus* and *H. promisus* (species with unknown lip patterns), *H. fujianensis* continuing to cluster alone. More molecular information about other species from this genus is necessary in order to establish the phylogenetic relationship within the group better.

Overall, this study provides the molecular characterisation together with morphological and SEM observation of native *Hemicriconemoides* and one *Criconemoides* species from China. The addition of these species from China to a reference dataset will assist to provide insights into the phylogeny and biogeography of criconematid nematodes. Several studies have emphasised the need for an integrative taxonomical approach (Van den Berg *et al*., 2014, 2015; Maria *et al*., 2018a, b). We also agreed that molecular diagnostics or phylogenetics should be combined with morphological and SEM observations in order to avoid errors in accurate species identification. However, we recommend the need for future research to supplement additional molecular data in order to clarify the complex taxonomic status of criconematid species.

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References

Andrássy, I. (1965). Verzeichnis und Bestimmungsschlüssel der Arten der Nematoden-Gattungen *Criconemoides* Taylor, 1936 und *Mesocriconema* n. gen. *Opuscula Zoologica Instituti Zoosystematici Universitatis Budapestinensis* 5, 153-171.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17, 540-552. DOI: 10.1093/oxfordjournals.molbev.a026334

Chen, D.Y., Ni, H.F., Tsay, T.T. & Yen, J.H. (2008). Identification of a new recorded sheath nematode *Hemicriconemoides parasinensis* (Nematoda: Criconematoidea, Criconematidae) from grape plantation in Taiwan. *Plant Pathology Bulletin* 17, 315-320.

Chen, D.Y., Ni, H.F., Tsay, T.T. & Chen, R.S. (2011). Differentiation of *Hemicriconemoides mangiferae* and *H. litchi* (Nematoda: Criconematina) based on morphometrics and nuclear ribosomal DNA sequences. *Nematology* 13, 165-175.DOI: 10.1163/138855410X508714

Chen, L.J. & Liu, W.Z. (2003). Description of three new recorded species of *Hemicriconemoides* in China. *Journal of Shenyang Agricultural University* 33, 416-419.

Chitwood, B.G. & Birchfield, W. (1957). A new genus, *Hemicriconemoides* (Criconematidae: Tylenchina). *Proceedings of the Helminthological Society of Washington* 24, 80-86.

Choi, Y.E. & Geraert, E. (1975). Criconematids from Korea with the description of eight new species (Nematoda: Tylenchida). *Nematologica* 21, 35-52. DOI: 10.1163/187529275X00338

Choi, Y.E., Brzeski, M.W. & Kim, J.I. (2000). Observations of some species of *Criconemoides* Taylor, 1936 (Nematoda: Criconematidae) with proposals of new synonyms. *Nematology* 2, 273-284. DOI: 10.1163/156854100509141

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9, 772. DOI: 10.1038/nmeth.2109

De Grisse, A.T. (1969). Redescription ou modification de quelques techniques utilisées dans l'étude des nematodes phytoparasitaires. *Mededelingen van de Rijksfaculteit der Landbouwwetenschappen Gent* 34, 351-369.

De Ley, P., Félix, M.-A., Frisse, L.M., Nadler, S.A., Sternberg, P.W. & Thomas, W.K. (1999). Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). *Nematology* 1, 591-612. DOI:

10.1163/156854199508559

Decraemer, W. & Geraert, E. (1992). Description of *Hemicriconemoides parataiwanensis* sp. n. (Criconematidae) and four other *Hemicriconemoides* species from Papua New Guinea with consideration of variability in the genus. *Nematologica* 38, 267-295.Eskandari, A., Karegar, A., Pourjam, E., Van den Berg, E. & Tiedt, R. (2010). Additional data on some poorly known species of *Criconemoides* Taylor, 1936 (Nematoda: Criconematidae). *Nematology* 12, 505-518. DOI: 10.1163/138855409X12506855979596

Esser, R.P. (1960). Three additional species in the genus *Hemicriconemoides* Chitwood & Birchfield, 1957 (Nemata: Tylenchida). *Nematologica* 5, 64-71 DOI: 10.1163/187529260X00271

Geraert, E. (2010). *The Criconematidae of the world: identification of the family Criconematidae (Nematoda)*. Ghent, Belgium, Academia Press.

Hall, T.A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/ NT. *Nucleic Acids Symposium Series* 41, 95-98.

Hunt, D.J., Luc, M. & Manzanilla-López, R.H. (2005). Identification, morphology and biology of plant parasitic nematodes. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*, 2nd edition. Wallingford, UK, CABI Publishing.

Joyce, S., Reid, A., Driver, F. & Curran, J. (1994). Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. In: Burnell, A.M, Ehlers, R.-U. & Masson, J.P. (Eds). *COST 812 Biotechnology*: *Genetics of Entomopathogenic Nematode-bacterium Complexes*, *Proceedings of Symposium & Workshop*, St. Patrick's College, Maynooth, Co. Kildare, Ireland, Luxembourg, European Commission, DG XII, pp 178-187.

.

Katoh, K. & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30, 772-780. doi: 10.1093/molbev/mst010

Lin, L., Li, W., Hong, L., Zhou, Y. & Hu, X. (2014). Description of five species of

Hemicriconemoides nematodes from rhizosphere of *Ziziphus jujube* in Yunan. *Plant Protection* 40, 154-158.

Maria, M., Cai, R., Castillo, P. & Zheng, J. (2018a) Morphological and molecular characterisation of *Hemicriconemoides paracamelliae* sp. n. (Nematoda: Criconematidae) and two known species of *Hemicriconemoides* from China. *Nematology* 20, 403-422. DOI 10.1163/15685411-00003147

Maria, M., Cai, R., Nan, Q., Castillo, P. & Zheng, J. (2018b). Morphological and molecular characterization of *Hemicycliophora subbotini* n. sp. (Tylenchida: Hemicycliophoridae) from China. *Zootaxa* 4433, 161-173. DOI: 10.11646/zootaxa.4433.1.10

Maria, M., Powers, T.O., Tian, Z., Harris, T., Higgins, R. & Zheng, J. (2018c). Description and distribution of three Criconematid nematodes from Hangzhou, Zhejiang Province, China. *Journal of Nematology* 50. DOI: 10.21307/jofnem-2018-010

Mullen, P., Sutton, L., Powers, K.S., Powers, T.O., Bernard, E.C., Harris, T., Higgins, R., Olson, M. & Lodema, M. (2014). COI haplotype groups in *Mesocriconema* (Nematoda: Criconematidae) and their morphospecies associations. *Zootaxa* 3827, 101-146. DOI: 10.11646/zootaxa.3827.2.1

Olson, M., Harris, T., Higgins, R., Mullin, P., Powers, K., Olson, S. & Powers, T.O. (2017). Species delimitation and description of *Mesocriconema nebraskense* n. sp. (Nematoda: Criconematidae), a morphologically cryptic, parthenogenetic species from North American Grasslands. *Journal of Nematology* 49, 42-66.

Pokharel, R.R., Abawi, G.S., Zhang, N., Duxbury, J.M. & Smart, C.D. (2007). Characterization of isolates of *Meloidogyne* from rice-wheat production fields in Nepal. *Journal of Nematology* 39, 221-230.

Powers, T.O., Harris, T., Higgins, R., Sutton, L. & Powers, K.S. (2010). Morphological and molecular characterization of *Discocriconemella inarata*, an endemic nematode from North American native tallgrass prairies. *Journal of Nematology* 42, 35-45.

Ronquist, F. & Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574. DOI: 10.1093/bioinformatics/btg180

Seinhorst, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4, 67-69. DOI: 10.1163/187529259X00381

Subbotin, S.A., Vovlas, N., Crozzoli, R., Sturhan, D., Lamberti, F., Moens, M. & Baldwin, J.G.

(2005). Phylogeny of Criconematina Siddiqi, 1980 (Nematoda: Tylenchida) based on morphology and D2-D3 expansion segments of the 28S-rRNA gene sequences with application of a secondary structure model. *Nematology* 7, 927-944. DOI: 10.1163/156854105776186307Taylor, A.L. (1936). The genera and species of the Criconematinae, a sub-family of the Anguillulinidae (Nematoda). *Transactions of the American Microscopical Society* 55, 391-421.

Van den Berg, E., Tiedt, L.R. & Subbotin, S.A. (2012). Morphological and molecular characterisation of *Criconemoides brevistylus* Singh & Khera, 1976 and *C. obtusicaudatus* Heyns, 1962 from South Africa (Nematoda: Criconematidae) with first description of a male *C. obtusicaudatus* and proposal of new synonyms. *Nematology* 14, 961-976. DOI: 10.1163/156854112X640304

Van den Berg, E., Tiedt, L.R., Inserra, R.N., Stanley, J.D., Vovlas, N., Palomares-Rius, J.E., Castillo, P. & Subbotin, S.A. (2014). Morphological and molecular characterisation of some *Hemicriconemoides* species (Nematoda: Criconematidae) together with a phylogeny of the genus. *Nematology* 16, 519-553. DOI: 10.1163/15685411-00002786

Van den Berg, E., Tiedt, L.R., Inserra, R.N., Stanley, J.D., Vovlas, N., Palomares-Rius, J.E, Castillo, P. & Subbotin, S.A. (2015). Characterisation of a topotype and other populations of *Hemicriconemoides strictathecatus* Esser, 1960 (Nematoda: Criconematidae) from Florida with description of *H. phoenicis* sp. n. from the USA. *Nematology* 17, 265-300. DOI: 10.1163/15685411-00002866

Wang, R. (1993). Species identification of parasitic nematodes of fruit trees in Shaanxi Province. *Acta Agriculturae Boreali Occidentalis Sinica* 2, 81-86.

Ye, W., Giblin-Davis, R.M., Braasch, H., Morris, K. & Thomas, W.K. (2007). Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution* 43, 1185-1197. DOI: 10.1016/j.ympev.2007.02.006

Zhang, S. (1998). Two new species of *Hemicriconemoides* (Nemata: Criconematidae). *Acta Phytopathologica Sinica* 28, 367-375.

Zheng, J., Subbotin, S.A., He, S., Gu, J. & Moens, M. (2003). Molecular characterisation of some Asian isolates of *Bursaphelenchus xylophilus* and *B. mucronatus* using PCR-RFLPs and sequences of ribosomal DNA. *Russian Journal of Nematology* 11, 17-22.

Zhou, S. (2008). Flower herbal tea used for treatment of menopathies. *Journal of Traditional Chinese Medicine* 3, 202-204.

Figure legends

Fig. 1. Light photomicrographs of female *Hemicriconemoides chitwoodi* Esser, 1960. A: Entire body (v = vulva); B: Cuticular annuli at mid-body; C: Lip region with cuticle; D-F: Lip region; G: Pharyngeal region with arrows showing position of base of pharyngeal bulb (ph.b) and excretory pore (exp); H-K: Tail region with arrows showing position of vulva (v) and anus (a). (Scale bars: A = 20 μm; B-K = 10 μm.)

Fig. 2. Scanning Electron Micrographs of female *Hemicriconemoides chitwoodi* Esser, 1960. A: Entire body (v = vulva); B-D: Lip regions; E: Cuticular annuli at mid-body; F-H: Tail region with arrows showing position of vulva (v) and anus (a). (Scale bars: A = 100 μm; B, D = 10 μm; C = 5 μm; E, H = 30 μm; F, G = 20 μm.)

Fig. 3. Light photomicrographs of female *Hemicriconemoides fujianensis* Zhang, 1998, Anhui Province population. A: Entire body (v = vulva); B: Entire body cuticle pulled over lip region (v = vulva); C, D: Lip region; E: Lip region with cuticle; F: Pharyngeal region with arrows showing position of base of pharyngeal bulb (ph.b) and excretory pore (exp); G: Cuticular annuli at mid-body; H: Posterior region showing entire gonad showing position of vulva (v); I, J: Tail region with arrows showing position of vulva (v) and anus (a). (Scale bars: A, B = 20 μm; C-J = 10 μm.)

Fig. 4. Scanning Electron Micrographs of female *Hemicriconemoides fujianensis* Zhang, 1998, Anhui Province population. A: Entire body (v = vulva); B-D: Lip regions; E: Cuticular annuli at mid-body; F-H: Tail region with arrows showing position of vulva (v) and anus (a), the lower arrow in H showing annuli breaking at tail terminus. (Scale bars: A = 100 μm; B, D = 10 μm; C = 5 μm; E = 50 μm; F-H = 20 μm.)

Fig. 5. Light photomicrographs of female *Hemicriconemoides fujianensis* Zhang, 1998, Zhejiang Province population. A: Entire body (v = vulva); B: Cuticular annuli at mid-body; C: Lip region with cuticle; D, E: Lip region; F: Pharyngeal region with arrows showing position of base of pharyngeal bulb (ph.b) and excretory pore (exp); G-J: Tail region with arrows showing position

of vulva (v) and anus (a). (Scale bars: A = 20 μm; B-J = 10 μm.)

Fig. 6. Scanning Electron Micrographs of female *Hemicriconemoides fujianensis* Zhang, 1998, Zhejiang Province population. A: Entire body (v = vulva); B-D: Lip region; E: Cuticular annuli at mid-body; F-H: Tail region with arrows showing position of vulva (v) and anus (a). (Scale bars: A = 100 μm; B, D = 10 μm; C = 5 μm; E = 40 μm; F, G = 20 μm; H = 30 μm.)

Fig. 7. Light photomicrographs of female *Hemicriconemoides parasinensis* Chen & Liu, 2003. A: Entire body (v = vulva); B: Entire body cuticle pulled over lip region (v = vulva); C: Lip region with cuticle; D: Lip region; E, F: Pharyngeal region with arrows showing position of base of pharyngeal bulb (ph.b) and excretory pore (exp); G: Posterior region showing entire gonad; H: Cuticular annuli; I-K: Tail region with arrows showing position of vulva (v) and anus (a). (Scale bars: A, B = 20 μm; C-K = 10 μm.)

Fig. 8. Scanning Electron Micrographs of female *Hemicriconemoides parasinensis* Chen & Liu, 2003. A: Entire body cuticle pulled over lip region (v = vulva); B: Entire body (v = vulva); C, D: Lip region; E, F: Tail region with arrows showing position of vulva (v) and anus (a); G: Tail region with arrow showing position of vulva (v); H: Cuticular annuli at mid-body. (Scale bars: A, B = 100 μm; C, D = 10 μm; E-H = 20 μm.)

Fig. 9. Light photomicrographs of female *Criconemoides myungsugae* Choi & Geraert, 1975. A: Entire body (v = vulva); B: Cuticular annuli showing anastomoses (arrows); C-E: Lip region; F: Pharyngeal region with arrows showing position of base of pharyngeal bulb (phar.bulb) and excretory pore (exp); G, I: Tail region with arrows showing position of anus (a); H: Tail region with arrows showing position vulva (v) and of anus (a). (Scale bars: A = 20 μm; B-I = 10 μm.)

Fig. 10. Scanning Electron Micrographs of female *Criconemoides myungsugae* Choi & Geraert, 1975. A, B: Lip region; C: Cuticular annuli at mid-body; D-F: Tail region showing position of vulva and anus. (Scale bars: A, B = 10 μm; C-F = 20 μm.)

Supplementary figures

Supplementary Fig. S1. Phylogenetic relationships within populations and species of *Hemicriconemoides* as inferred from Bayesian analysis using the D2-D3 of 28S rRNA gene sequence dataset with the GTR + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold. * Identified as *H. strictathecatus* by Subbotin *et al.* (2005) and Van den Berg *et al.* (2014); ** identified as *H. strictathecatus* by Van den Berg *et al.* (2014).

Supplementary Fig. S2. Phylogenetic relationships within populations and species of *Hemicriconemoides* as inferred from Bayesian analysis using the ITS rRNA gene sequence dataset with the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold. * Identified as *H. litchi* by Chen *et al.* (2011) and *H. strictathecatus* by Van den Berg *et al.* (2014); ** identified as *H. strictathecatus* by Van den Berg *et al.* (2014); *** identified as *H. mangiferae* by Chen *et al.* (2011); **** identified as *H. californianus* by Chen *et al.* (2011).

Supplementary Fig. S3. Phylogenetic relationships within populations and species of *Hemicriconemoides* as inferred from Bayesian analysis using the partial 18S rRNA gene sequence dataset with the GTR + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

Supplementary Fig. S4. Phylogenetic relationships within populations and species of *Hemicriconemoides* as inferred from Bayesian analysis using the partial *cox*I gene with the GTR + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

Supplementary Fig. S5. Phylogenetic relationships within populations and species of *Criconemoides* as inferred from Bayesian analysis using the partial 28S gene sequence dataset with the GTR + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

Supplementary Fig. S6. Phylogenetic relationships within populations and species of *Criconemoides* as inferred from Bayesian analysis using the ITS gene sequence dataset with the GTR + G. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

Supplementary Fig. S7. Phylogenetic relationships within populations and species of *Criconemoides* as inferred from Bayesian analysis using *cox*I gene sequence dataset with the GTR + I + G model. Posterior probability more than 70% is given for appropriate clades. Newly obtained sequences are indicated in bold.

Table 1. Morphometrics of female *Hemicriconemoides chitwoodi* Esser, 1960. All measurements are in μm and in the form: mean ± s.d. (range).

Character	This study Zhejiang	Esser, 1960 (Type pop.)	Wang, 1993 Shaanxi	Lin *et al*., 2014 Yunnan	Van den Berg *et al*., 2014 Topotype, USA
n	15	13	19	27	7
L	525 ± 36.8 (456-580)	540 (480-590)	460 (390-520)	507 (441-574)	496 (435-537)
a	17.2 ± 1.2 (15.5-19.4)	15.5 (13.4-17.0)	16.3 (13.5-19.1)	16.9 (11.9-20.5)	13.6 (11.9-16.2)
b	4.2 ± 0.2 (3.9-4.7)	3.8 (3.5-4.1)	3.8 (3.3-4.2)	3.1 (3.1-4.3)	3.7 (3.5-3.8)
c	18.0 ± 1.6 (14.2-20.2)	15.7 (11.2-21.7)	18.6 (15.4-21.8)	17.9 (15.2-22.1)	17.2 (15.2-19.1)
c'	1.6 ± 0.2 (1.4-1.9)	–	1.6 (1.3-1.8)	1.5 (1.1-1.9)	1.3 (1.1-1.4)
V	90.7 ± 0.6 (89.6-91.6)	90.1 (88.1-91.0)	91 (90-92)	90.6 (89.7-91.4)	90 (84-92)
VL/VB	2.1 ± 0.2 (1.9-2.5)	(1.9-2.6)	–	–	1.6 (1.3-1.9)
R	118 ± 3.4 (110-122)	124 (116-133)	–	124 (114-141)	122 (118-125)
Rex	36 ± 1.8 (33-39)	(33-36)	–	33 (30-36)	35 (33-37)
RV	12.3 ± 0.6 (11.0-13.0)	(12-16)	–	13 (10-15)	14 (13-16)
RVan	4.9 ± 0.3 (4.0-5.0)	(3-6)	–	4.6 (4-6)	3 (3-4)
Ran	7.4 ± 0.6 (6.0-8.0)	(8-11)	–	8.3 (6-11)	10 (9-11)
Lip height	12.9 ± 1.2 (11.3-15.5)	–	–	–	–
Lip diam.	5.4 ± 0.4 (4.7-6.1)	–	–	–	–
Stylet	82 ± 2.3 (79-86)	91 (85-95)	80 (77-84)	80 (77-84)	88 (81-96)
Stylet %	15.8 ± 1.0 (14.1-17.6)	–	–	–	17.8 (16.5-20.1)
Pharynx length	124 ± 5.0 (116-131)	–	–	–	134 (122.5-144)
Max. body diam.	30.7 ± 2.2 (27.0-34.7)	–	–	30.5 (24.5-38.5)	37.0 (33.0-42.5)
Vulval body diam.	22.9 ± 1.4 (20.1-25.7)	–	–	–	30.5 (25.5-36.0)
Vulva to tail tip	48.7 ± 4.8 (38.1-57.6)	–	–	–	–
Anal body diam.	17.9 ± 1.3 (15.9-20.3)	–	–	–	24.5 (18.5-35.0)
Tail length	29.4 ± 3.3 (22.7-34.8)	–	–	28.6 (22.7-35)	29.0 (25.0-31.5)

*VL/LB= Distance from terminus to vulva divided by body width at vulva

Table 2. Morphometrics of female *Hemicriconemoides fujianensis* Zhang, 1998. All measurements are in μm and in the form: mean ± s.d. (range).

Character	This study Anhui	This study Zhejiang	Zhang, 1998 Fujian (Type pop.)
n	15	15	25
L	545 ± 28.9 (465-583)	508 ± 16.1 (487-540)	470 (380-510)
a	15.3 ± 0.8 (13.6-16.4)	13.8 ± 0.8 (12.7-15.3)	17 (13-32)
b	4.9 ± 0.2 (4.5-5.5)	4.8 ± 0.2 (4.6-5.1)	4.5 (3.8-5.4)
c	26.1 ± 3.7 (21.3-36.6)	30.2 ± 3.0 (25.7-34.4)	19.5 (16.9-24)
c'	1.0 ± 0.1 (0.8-1.2)	0.8 ± 0.1 (0.7-1.0)	1.3 (1.1-1.6)
V	91.3 ± 0.5 (90.2-92.4)	91.8 ± 0.5 (90.9-93.1)	92 (90-94)
VL/VB	1.7 ± 0.1 (1.6-1.9)	1.5 ± 0.1 (1.3-1.6)	1.5 (1.2-1.8)
R	103 ± 2.1 (100-107)	97 ± 3.1 (92-103)	124 (118-130)
Rex	29.5 ± 1.5 (28.0-32.0)	27.0 ± 1.1 (25.0-29.0)	32 (30-33)
RV	10.2 ± 0.6 (9.0-11.0)	9.1 ± 0.7 (8.0-10.0)	11 (9-12)
RVan	5.0 ± 0.0 (5.0-5.0)	4.4 ± 0.5 (4.0-5.0)	2-4
Ran	5.2 ± 0.6 (4.0-6.0)	4.7 ± 0.5 (4.0-5.0)	6-8
Lip height	6.0 ± 0.6 (5.0-6.9)	6.0 ± 0.6 (5.2-6.8)	–
Lip diam.	14.1 ± 0.8 (12.3-15.3)	14.9 ± 0.9 (12.3-16.3)	–
Stylet	62 ± 1.8 (60-65)	59 ± 2.0 (56-62)	66 (63-70)
Stylet %	11.4 ± 0.6 (10.8-12.9)	11.7 ± 0.5 (10.9-12.4)	–
Pharynx length	111 ± 4.9 (102-121)	106 ± 2.7 (100-112)	–
Max. body diam.	35.5 ± 1.3 (32.9-37.9)	36.9 ± 1.8 (34.6-40.1)	–
Vulval body diam.	27.3 ± 1.2 (24.8-29.1)	28.3 ± 1.1 (26.4-30.5)	–
Vulva to tail tip	47.2 ± 3.2 (40.2-51.6)	41.6 ± 2.7 (35.4-46.8)	–
Anal body diam.	20.7 ± 1.4 (18.0-22.2)	20.2 ± 1.1 (18.1-21.9)	–
Tail length	21.1 ± 2.4 (15.2-24.5)	17.0 ± 1.8 (14.7-20.3)	–

*VL/LB= Distance from terminus to vulva divided by body width at vulva

Table 3. Morphometrics of female *Hemicriconemoides parasinensis* Chen & Liu, 2003. All measurements are in μm and in the form: mean ± s.d. (range).

Character	This study	Chen & Liu, 2003	Chen & Liu, 2003	Chen *et al*., 2008
		Liaoning	Henan	
	Zhejiang	(Type pop.)	(Type pop.)	Taiwan
n	15	20	21	*
L	469 ± 30.3 (400-501)	405 (354-448)	482 (426-516)	(340-480)
a	14.0 ± 1.1 (12.2-15.6)	12.7 (10.7-13.8)	14 (12.1-15.4)	(11.5-15.2)
b	3.7 ± 0.3 (3.3-4.5)	3.3 (2.9-3.5)	3.8 (3.4-4.2)	(3.2-3.8)
c	20.7 ± 2.9 (16.3-27.5)	23.4 (19.3-32.8)	22.6 (15.4-32.3)	(17.5-33.6)
c'	1.2 ± 0.1 (1.0-1.4)	0.8 (0.6-1.0)	1.0 (0.7-1.3)	(0.6-1.3)
V	91.4 ± 0.8 (90.0-92.7)	92.4 (91.0-93.9)	91.7 (90.4-93.0)	(89.6-93.4)
VL/VB	1.7 ± 0.2 (1.4-2.1)	–	–	(1.0-1.7)
R	107 ± 3.4 (101-113)	101 (96-110)	105 (97-112)	(99-113)
Rex	33.9 ± 2.4 (30.0-36.0)	31 (29-33)	31 (29-33)	(28-33)
RV	10.5 ± 0.8 (9.0-12.0)	9 (7-10)	10 (9-12)	(8-12)
RVan	4.3 ± 0.6 (3.0-5.0)	–	–	–
Ran	6.2 ± 0.4 (6.0-7.0)	5 (4-6)	6 (4-7)	(4-8)
Lip height	5.2 ± 0.3 (4.8-5.6)	–	–	–
Lip diam.	11.1 ± 1.0 (9.3-12.5)	–	–	–
Stylet	87 ± 2.7 (82-90)	84 (76-86)	87 (80-92)	(73.7-92)
Stylet %	18.6 ± 1.3 (16.6-22.0)	–	–	–
Pharynx length	125 ± 5.9 (112-134)	–	–	–
Max. body diam.	33.7 ± 2.9 (28.6-40.6)	–	–	–
Vulval body diam.	23.6 ± 1.4 (20.9-26.7)	25.1 (20-30)	27.7 (24-30)	(25-40)
Vulva to anus	17.4 ± 2.9 (13.2-24.3)	–	–	–
Vulva to tail tip	40.3 ± 3.8 (35.7-45.9)	–	–	–
Anal body diam.	19.0 ± 1.3 (17.1-20.7)	19.4 (14-22)	22.8 (20-26)	(14-22)
Tail length	22.9 ± 2.4 (18.2-26.7)	16.4 (10-22)	22 (16-32)	(11-25)

*VL/LB= Distance from terminus to vulva divided by body width at vulva

*consolidated data from four populations from Taiwan.

Table 4. Morphometrics of female *Criconemoides myungsugae* Choi & Geraert, 1975. All measurements are in μm and in the form: mean ± s.d. (range).

Character	This study	Choi & Geraert, 1975	Choi *et al*., 2000	Eskandari *et al*., 2010
	Zhejiang	(Type pop.)	Korea	Iran
n	15	9	13	18
L	527 ± 35.5 (459-574)	445-500	450 (410-490)	546 (455-630)
a	12.0 ± 1.3 (9.7-14.5)	10-12	10.9 (10.1-11.4)	11 (9.4-13.3)
b	4.6 ± 0.3 (4.2-5.1)	3.7-4	3.7 (3.6-4.0)	4.4 (3.8-4.8)
c	31.7 ± 5.6 (23.4-40.0)	20-24	23 (21-26)	22.2 (16.8-27.9)
c'	0.7 ± 0.1 (0.5-1.0)	–	–	–
V	93.2 ± 0.6 (92.0-94.1)	92	91-92	92.2 (91.1-93.7)
VL/VB	1.2 ± 0.1 (1.0-1.4)	–	1.1-1.2	1.2 (0.9-1.3)
R	111 ± 2.9 (104-115)	122-127	122 (119-125)	119 (111-127)
Rex	30.0 ± 1.2 (27.0-32.0)	29-34	31 (29-34)	31 (30-34)
RV	9.0 ± 2.6 (8.0-10.0)	10-12	12.8 (11-14)	11 (9-12)
RVan	4.6 ± 0.5 (4.0-5.0)	6	5 (4-7)	2 (1-3)
Ran	4.4 ± 0.5 (4.0-5.0)	5-8	6.8 (5-8)	7 (6-9)
Stylet	65 ± 2.9 (59-69)	66-68	68 (66-72)	64 (62-71)
Stylet %	12.3 ± 0.7 (10.9-13.9)	–	–	11.8 (10.6-14.5)
Pharynx length	114 ± 4.6 (107-121)	–	–	125 (112-137)
Max. body diam.	44.5 ± 5.3 (35.2-50.8)	–	–	50 (41-59)
Vulval body diam.	29.9 ± 2.0 (26.4-32.5)	–	–	–
Vulva to tail tip	36.0 ± 3.6 (29.3-41.4)	–	–	–
Anal body diam.	23.3 ± 2.0 (20.0-27.0)	–	–	–
Tail length	17.2 ± 3.6 (12.0-23.0)	–	–	25 (20-31)

*VL/LB= Distance from terminus to vulva divided by body width at vulva