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Description and Distribution of Three Criconematid Nematodes from Hangzhou, Zhejiang Province, China

Maria Munawar,[1]
Powers Thomas O.,[2]
Tian Zhongling,[1] Harris Timothy,[2]
Higgins Rebecca,[2] and
Zheng Jingwu[1,3]*

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

[2]Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

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

*E-mail: jwzheng@zju.edu.cn.

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Abstract

Populations of *Criconemoides parvus*, *Discocriconemella hengsungica*, and *Discocriconemella limitanea*, isolated in Hangzhou, China from the rhizosphere soil of woody perennials were characterized morphologically and molecularly. The morphometric data of the Chinese populations were compared with populations from other regions of the world. DNA barcoding with the mitochondrial COI gene confirmed conspecificity of Chinese and Costa Rican populations of *D. limitanea*. Phylogenetic assessment using a near full-length 18S ribosomal DNA sequence provided weak support for a grouping of *Criconemoides parvus* from China and *C. annulatus* from western North America. The phylogenetic position of *D. hengsungica* from China and an unknown species of *Discocriconemella* from Thailand relative to *D. limitanea* suggests that the genus *Discocriconemella* is not monophyletic. The study provides the first record of *D. hengsungica* in China and confirms the presence of *C. parvus* previously reported from China. Biogeographic implications of these nematode distributions are discussed.

Key words

Criconemoides parvus, Discocriconemella hengsungica, D. limitanea, DNA barcoding, Nematode morphology, Phylogeny, Scanning electron microscopy.

Species of genera *Criconemoides* (Taylor, 1936) and *Discocriconemella* (De Grisse & Loof, 1965) have global distributions (Geraert, 2010, Eskandari et al., 2010) and are known to be associated with agricultural crops, grasslands and woody perennials (Siddiqi, 2000). At present, the genus *Criconemoides* contains 42 valid species (Geraert, 2010) with only three species (*C. informis* (Micoletzky, 1922) Taylor, 1936; *C. parvus* Raski, 1952, and *C. zavadskii* (Taulaganov, 1941) Raski, 1958) reported from China. *Discocriconemella,* after the transfer of *D. inarata* Hoffman, 1974 to *Mesocriconema* (Powers et al., 2010; 2014) contains 27 valid species. Only *D. limitanea* (Luc, 1959) De Grisse and Loof, 1965 was formerly known to be reported from China (Yin et al., 1994; Ye et al., 1997; Zhang et al., 1997; Li et al., 2006).

During a routine nematological survey of Hangzhou city, Zhejiang province, China, large populations of three criconematids were recovered from the rhizosphere of woody perennials. Morphological studies revealed the identity of these nematodes as *C. parvus*, *D. hengsungica* (Choi & Geraert, 1975) and *D. limitanea*. Previously, *C. parvus* was reported from Shandong (Liu et al., 2004) and Liaoning provinces (Tan and Ye, 2009) in *Pisum sativum* and *Pinus* sp. rhizosphere, respectively. However, no morphological descriptions or photo documentations were presented in the Chinese literature to confirm the actual identity of *C. parvus*. Similarly, *D. limitanea* was reported from Guangzhou (Yin et al., 1994), Guangdong (Ye et al., 1997), Fujian (Zhang et al., 1997) and Yunan (Li et al., 2006) provinces, in the rhizosphere of fruits and *Rosaceae* plants. Most of the descriptions are in Chinese and without photo documentation or molecular data.

Discocriconemella hengsungica was originally described from Korea, and is the only record of its occurrence (Choi and Geraert, 1975), but there is no molecular information available for this species.

Thus, the objectives of the study were to: (1) establish the identity of these three species by morphological and molecular characterization, (2) integrate the morphometric characterization of Chinese populations of *D. limitanea* and *C. parvus* with measurements reported from different countries, (3) evaluate the phylogenetic and biogeographic relationships of these species within Criconematidae using 18S and COI DNA sequence.

Materials and methods

Nematode detection and morphological observations: Soil samples were collected from undisturbed natural locations in the Hangzhou Botanical Garden. Nematodes were extracted from soil using a modified Cobb sieving and flotation–centrifugation method (Jenkins, 1964). Nematodes were killed and fixed in hot 4% formaldehyde, infiltrated with glycerin following the method of Seinhorst (1959), and mounted on slides for observation and preservation. The measurements and light micrographs of nematodes were accomplished using an ocular micrometer and a Zeiss Stemi 2000-C compound microscope.

Nematodes were also examined using a Hitachi TM-1000 scanning electron microscope (SEM). 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 cocodylate 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. Specimens from Costa Rica were processed for SEM using the methods described in Powers et al. (2010).

Molecular analyses

DNA samples from China were prepared according to Zheng et al. (2003). Individual nematodes were transferred into an Eppendorf tube containing 16 µL ddH2O. Two microliters PCR buffer solution was added to each tube. Nematodes were crushed using a sterilized pipette tip, briefly spun and immediately frozen at –68°C for at least 30 min. The tubes were heated to 85°C for 2 min, briefly spun, followed by the addition of 2 µL proteinase K. The tubes were incubated at 56°C for 1 to 2 hrs, followed by 10 min at 95°C. After incubation, these tubes were cooled at 4°C and used for PCR (Zheng et al., 2003). Several sets of primers (synthesized by Invitrogen, Shanghai, China) were used in the PCR analyses to amplify the near full-length18S region of rDNA and COI region. Two sets of primers: the forward 18S39F (5′-AAA GAT TAA GCC ATG CAT G-3′) and the reverse 18S977R (5′-TTT ACG GTT AGA ACT AGG GCG G-3′), the forward 18S900F (5′-AAG ACG GAC TAC AGC GAA AG-3′) and the reverse 18S1713R (5′-TCA CCT ACA GCT ACC TTG TTA CG-3′) for amplification of the nearly full-length 18S rRNA (Olson et al., 2017). For the amplification of COI the primers used were COI-F5-(5′-AATWTWGGTGTTGGAACTTCTT-GAAC-3′) and COI-R9-(5′ CTTAAAACATAATGRAAAT-GWGCWACWACATAATAAGTATC-3) (Powers et al., 2014). The 25-µl PCR was performed using 2x-TsingKe Master Mix DNA polymerase (Beijing TsingKe Biotech Co., Ltd) according to the manufacturer's protocol in a BIOER-XP thermocycler. The thermal cycler program for 18S and COI was as follows: denaturation at 95°C for 5 min, followed by 40 cycles (18S) or 50 cycles (COI) of denaturation at 94°C for 30 s, annealing at 50°C (18S) or 48°C (COI) for 30 s, and extension at 72°C for 90 s. A final extension was performed at 72°C for 5 min as described by Powers et al. (2014) and Olson et al. (2017). PCR products were separated and visualized on 1% agarose gels and stained with ethidium bromide. PCR products of sufficiently high quality were sent for sequencing by Invitrogen (Shanghai, China).

Phylogenetic analysis

Phylogenetic trees were constructed by maximum likelihood (ML) in MEGA version 6. Sequences were edited using CodonCode Aligner version 4.2 (http://www.codoncode.com/) and aligned using Muscle within MEGA version 6 (Tamura et al., 2013). Gap opening penalty was set at –400 with a gap extension penalty of 0. The general time reversible model with Gamma-distributed rates plus invariant sites (GTR+G+I) was determined to be the best substitution model by Bayesian Information Criterion using the Best Fit Substitution Model tool in MEGA 6.0. The ML trees used the all sites option for gaps and 200 bootstrap replications to assess clade support. The 18S tree used all the taxa previously presented in Powers et al. (2017) plus the eight new sequences from China. The COI tree includes the same taxa as the 18S tree, adding 79 new COI sequences to GenBank, plus 11 new sequences from China. GenBank accession numbers and associated metadata are presented in supplementary Table 1.

Figure 1: Light photomicrographs of *Criconemoides parvus*: A: entire female; B, C: head region; D: mid-body (arrows showing anastomosis); E, F: pharyngeal region (arrows showing position of the excretory pore); G, posterior region showing the reproductive system; H: posterior region showing crenation on annuli; I-K: female tail (arrows showing position of vulva and anus; scale bars = A = 50 µm, all others 10 µm).

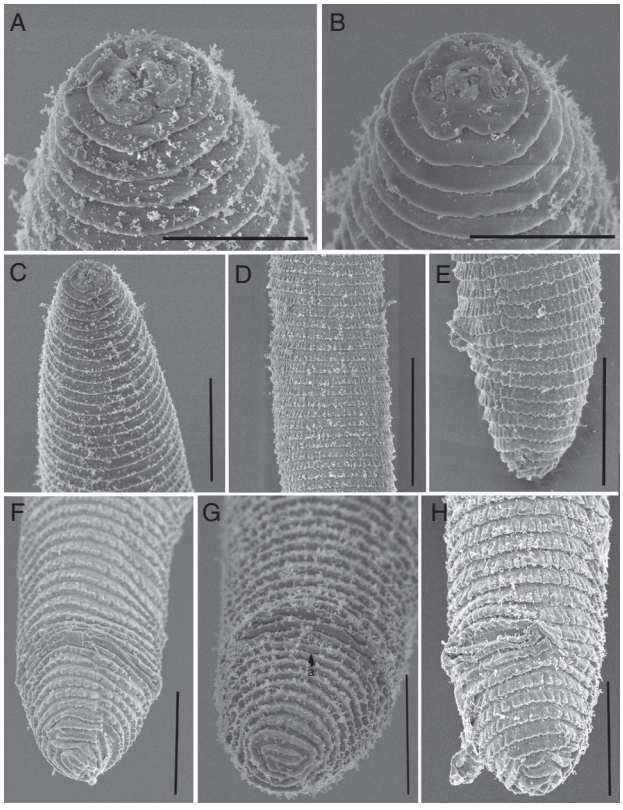


Figure 2: Scanning electron microscopy of *Criconemoides parvus*: A-C: lip region; D: mid-body showing crenations; E-H: posterior region of the female showing vulva and anus (arrows showing position of anus; scale bars = A, B = 5 µm; C, F, G, H = 10 µm; D = 20 µm).

Results

Systematics

Criconemoides parvus (Raski, 1952) (Figs. 1, 2; Table 1).

Description

Female: Body cylindrical, ventrally arcuate after heat relaxation. The cephalic region is flat, continuous with the body contour. *En face* view, an oral disc with slightly elevated lateral pseudolips, oral aperture slit-like, with submedian lobes absent. Surrounding and apparently fused with the oral disc is a single labial annulus with dorsal and ventral indentations. Body annuli retrorse with posterior margins finely crenate, more prominent on the posterior body, anastomoses common in the middle of the body. Stylet is short with rounded basal knobs, DGO indistinct, and oesophagus criconematoid. Excretory pore at the base of the oesophageal bulb. Gonad monodelphic, outstretched, spermatheca oblong, filled with rod-shaped sperm, vagina straight, vulva closed, anterior and posterior annuli around the vulva larger than the preceding body annuli; discontinuous annuli are more common near the region of the vulva. Tail conoid ending in a rounded terminus and the anus is indistinct.

Male: Not found.

Locality and habitat: The population was found in the rhizosphere of *Punica granatum* L. from Xixi wetland, Hangzhou, Zhejiang Province, China on

Table 1. Morphometric data and distribution of *Criconemoides parvus.* All measurements in µm.

Authors	This study	Raski (1952)[a]	Loof (1991)	Eskandari et al. (2010)	Mirghasemi et al. (2014)	Rashid et al. (1986)	Popovivi and Ciobanu (2000)	Liskova et al. (2004)
Origin	Chinese population	Berkeley, California, USA	Iran	Iran	Iran	Brazil	Romania	Slovak Republic
Host	Pomegranate	*Artemesia* sp.	*Populus* sp.	–	Tea	*Theobroma cacao*	–	*Robinia pseudoacacia*
n	15	–	33	23	8	29	1	1
L	270.4–324.5	259–295	240–330	252–313	260–346	210–270	299	280
a	12.8–15.0	11.7–14.5	16–Oct	8.8–13.5	11.3–14.4	12–Aug	12.4	11.6
b	3.7–4.2	3.0–3.4	3.2–4.2	3.2–4.1	3.1–4	3.0–3.6	3.9	3.3
c	20.7–32.1	–	21–55	21.7–45.4	47.9–65	18–47	33.2	23.3
V	93.6–95.6	92.5–95.9	94–97	93.6–96.7	95.9–96.5	91–94	95	94.3
VL/VB	0.7–1.2	–	0.6–0.9	0.6–0.9	0.7–0.80	0.4–1.1	0.7	0.7
Stylet	26.5–30.3	38–41	26–32	30–43	30.2–36.1	34.5–43	35	41
Stylet %L	8.7–10.6	–	11–Sep	10.4–15.9	–	14–18	12	14.6
R	140.0–168	142–156	142–172	144–167	148–160	124–141	173	178
Rex	43.0–48	46–49	41–53	45–53	–	39–52	50	–
RV	9.0–13.0	11–12	8–12	9–13	8–10	8–11	12	16
RVan	2.0–3	–	0–4	0–2	3–5	1–3	2	6
Ran	6.0–10	–	6–11	7–11	5–7	6–9	9	10
Tail length	9.1–15.6	–	–	6–14	–	6–11	–	–
Male	Unknown	Unknown	–	–	–	Known	–	–

[a]Original description.

May 5, 2017. The geographical location of the sampling site is 30°16′23″N; 120°3′33″E.

Differential diagnosis: Males were not described in the original description by Raski (1952). The type locality was near Winnemucca, Nevada in the mountains of western North America around the roots of *Artemisia* sp. Subsequent reports mention females with spermatheca filled with sperm but it was not until 34 years later that Rashid et al., (1986) described a male from an earlier Netherlands collection that included males, but did not describe them (De Grisse and Loof, 1965). Another character not mentioned in the original description is the presence of anastomoses. An Iranian population reported by Loof & Barooti (1991) and a Romanian population by Liskova et al. (2004) described anastomoses as either absent or occasional. Specimens of the Chinese population had numerous anastomoses. Most other morphological characters of the Chinese populations match the original description.

Morphometrically, the three Iranian populations described by Loof and Barooti, (1991) have bodies that are slightly longer than the original description (240-346 µm *vs.* 259-295 µm) and stylets that are shorter (26-32 µm *vs.* 38-41 µm). A Brazilian population reported by Rashid et al. (1986) had fewer body annuli (R = 124-141 *vs.* 142-156) as compared with the original description. Two New Zealand populations reported by Loof et al. (1997) and Wouts (2006) recorded slightly longer stylets (42-46 µm *vs.* 44-49 µm

vs. 38-41 µm, respectively) and relatively fewer body annuli (R = 126-169 *vs.* 128-147 *vs.* 142-156, respectively) as compared with the original description. The Romanian and Slovak Republic populations reported by Popovici and Ciobanu (2000) and Liskova et al. (2004) correspond well to the original description except for a higher number of body annuli (R = 173 *vs.* 178 vs 142-156, respectively). When compared with the original description, the Chinese population has a slightly longer body (270-324.5 µm *vs.* 259-295 µm) and a shorter stylet (26.5-30.3 µm *vs.* 38-41 µm).

Discocriconemella hengsungica (Choi and Geraert, 1975) (Figs. 3, 4; Table 2).

Description

Female: Body cylindrical, ventrally curved after heat relaxation. Labial region a disc-like appearance in profile. *En face* view, does not show a discrete oral disc, instead the stylet appears to be located centrally in an inner rectangular area surrounded by a continuous, broad labial annulus with deep ventral and dorsal indentations forming two pairs of dorsal and ventral lobes combined with distinct lateral bulges. The oral disc and amphid apertures are indistinct due to amphidal excretions in SEM images. The labial annulus is separated from the body annulus by a high neck or collar. Body annuli retrorse to angular, without anastomosis or interruptions. Stylet long and flexible with anchor-shaped knobs, DGO indistinct; oesophagus criconematoid. Excretory pore located near the middle of the oesophageal bulb. Gonad monodelphic, outstretched, some individuals with reflexed ovary, spermatheca rounded filled with spherical sperm, vagina straight, and vulva closed. Tail conoid broadly rounded, and terminal annuli displaced dorsally and the anus is indistinct.

Male: Not found.

Locality and habitat: The population was found in the rhizosphere of *Castanopsis sclerophylla* (Lindl.) Schott from a Botanical garden in Hangzhou, Zhejiang Province, China on March 28, 2017. The geographical location of the sampling site is ″30°15′17″N; 120°07′01″E.

Differential diagnosis: In the original description of *D. hengsungica* six specimens were studied. Only one female was observed with a few anastomoses. No anastomoses were observed on the Chinese specimens. The spermatheca was described as filled with sperm but no males were found. Similarly, the Chinese population had specimens with sperm-filled spermatheca, but no males were found. The original description lacks information on the morphology of the labial disc, position of excretory pore and anus, shape of vagina and vulva. Morphology of the Chinese population fits well with the characters included in the original description except for the complete absence of anastomoses. Morphometrically, the Chinese population is slightly longer (307-382 µm *vs.* 285-315 µm) with relatively longer stylets (100.3-113.5 µm *vs.* 104-108 µm) and less annuli from vulva to tail terminus (RV = 9-10 *vs.* 13-14). The slight morphometric differences could be attributed to fewer specimens studied in the original description and geographical variability.

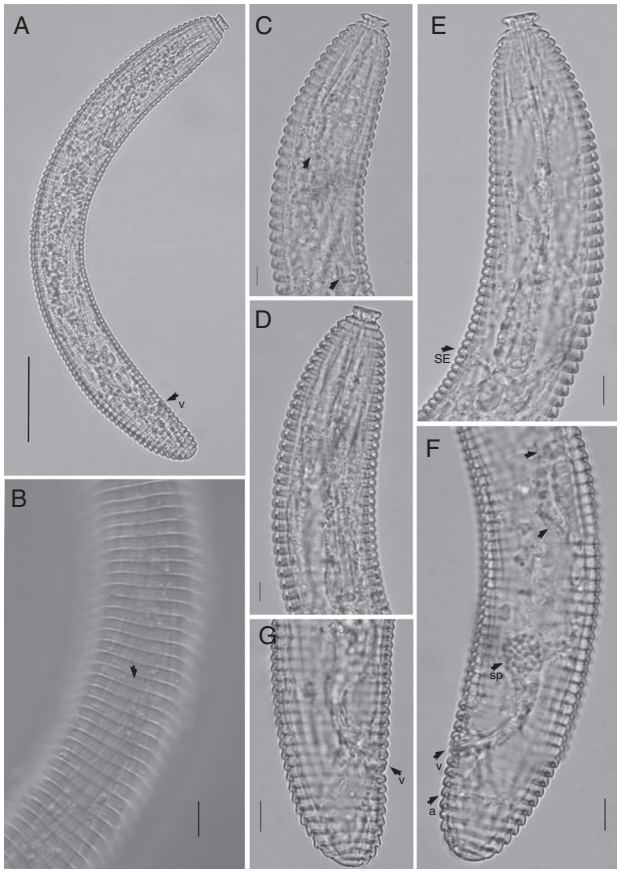


Figure 3: Light photomicrographs of *Discocriconemella hengsungica*: A: entire female; B: mid-body (arrow showing annuli without anastomosis); C-D: head region (arrows showing flexible stylet and basal knobs); E: pharyngeal region (arrow showing position of the excretory pore); F, posterior region showing the reproductive system (arrows showing reflexed ovary, spermatheca, position of vulva and anus); G: female tail ; scale bars = A = 50 µm, all others 10 µm).

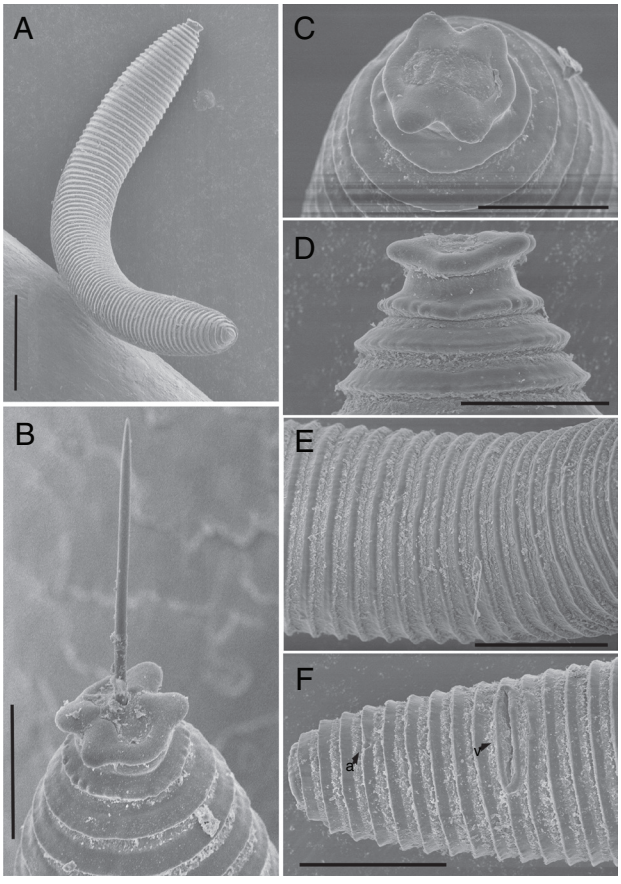


Figure 4: Scanning electron microscopy of *Discocriconemella hengsungica*: A: entire female; B-D: labial disc in different angles; E: mid-body annuli without anastomosis; F: posterior region of the female showing vulva and anus (arrows showing the position of anus; scale bars = A = 50 µm; B-D = 10 µm; E, F = 20 µm).

Table 2. Morphometric data of *Discocriconemella hengsungica*. All measurements in µm.

Authors	This study	Choi & Geraert, 1975[a]
Origin	China	Korea
Host	*Castanopsis sclerophylla*	*Zea mays*
n	15 females	5 females
L	333.1 ± 19.0(307.9–382.6)	285–315
a	9.4 ± 1.0(8.2–11.5)	8.2–9.8
b	2.5 ± 0.1(2.3–2.8)	2.5–2.6
c	19.6 ± 3.0(15.2–25.7)	–
c'	0.7 ± 0.1(0.5–0.8)	–
V	89.2 ± 0.8(87.8–90.4)	87–90
VL/VB	1.1 ± 0.1(1.0–1.3)	–
Stylet	107.4 ± 3.4(100.3–113.5)	104–108
Stylet % L	32.3 ± 1.7(28.6–34.6)	–
R	91.3 ± 2.2(88.0–94.0)	82–90
Rex	33.5 ± 1.6(30.0–36.0)	31
RV	9.8 ± 0.4(9.0–10.0)	13–14
RVan	5.2 ± 0.8(4.0–6.0)	7
Ran	4.7 ± 0.7(4.0–6.0)	7
Lip height	5.0 ± 0.5(4.0–5.7)	–
Pharynx	131.5 ± 3.3(126.4–137.5)	121
Max. body diam.	35.7 ± 2.7(30.1–39.3)	28
Vulva body diam.	31.9 ± 1.9(28.0–34.4)	–
Dis. from vulva to tail term.	35.9 ± 3.2(31.3–43.3)	–
Anal body diam.	26.5 ± 2.1(22.5–29.4)	–
Tail length	17.4 ± 2.8(12.0–21.8)	13

[a]Original description.

Discocriconemella limitanea (Figs. 5–8; Tables 3–4).

Description

Female: Body stout, ventrally arcuate after heat relaxation, lip region with disc-like appearance. *En face* view, a labial annulus with deep dorsal and ventral indentations, the oral opening appearing as a slit on a rounded oral disc flanked by two lateral amphidial apertures. The lateral edges of the labial annulus straight, lacking a central bulge. Body annuli retrorse, finely crenate edges, frequent anastomoses or discontinuous annuli that demarcate lateral lines. Stylet robust, anchor-shaped knobs, DGO indistinct. Oesophagus criconematoid. Excretory pore at the base of the oesophageal bulb. Gonad monodelphic, prodelphic, outstretched, spermatheca oblong

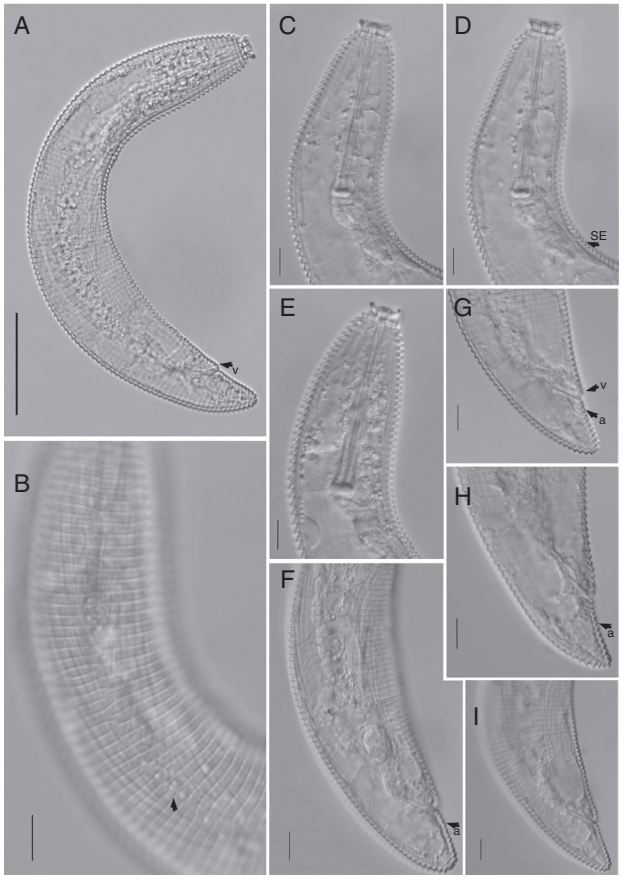


Figure 5: Light photomicrographs of *Discocriconemella limitanea*: A: entire female; B: mid-body (arrow showing anastomosis); C-E: pharyngeal region (arrow showing position of the excretory pore); F: posterior region showing the reproductive system; G-I, female tail (arrows showing position of vulva and anus; scale bars = A = 50 µm, all others 10 µm).

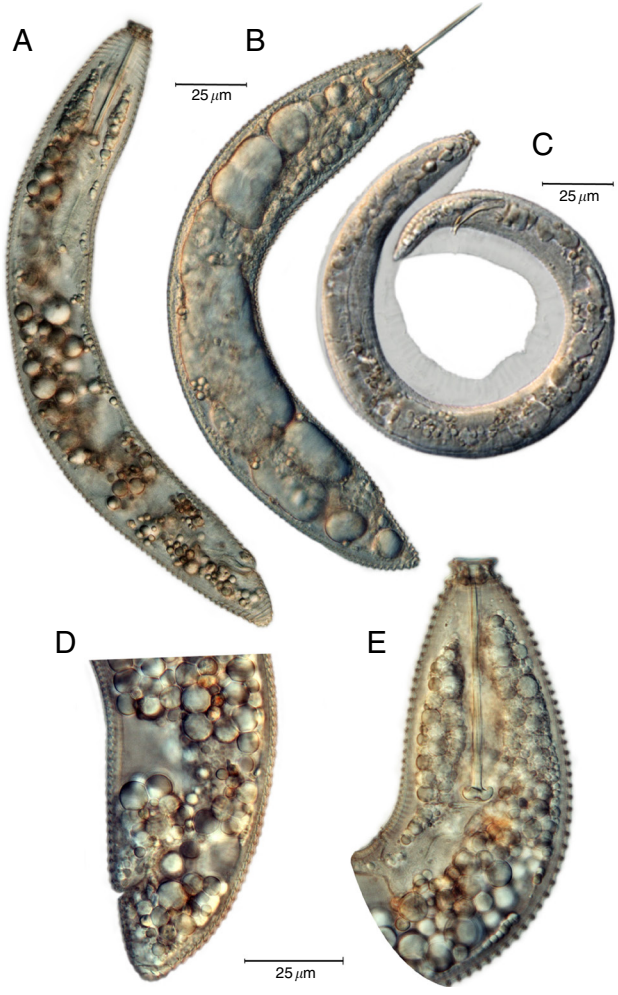


Figure 6: Light photomicrographs of *Discocriconemella limitanea* from La Selva, Costa Rica; A) entire female, PNID-184030; B) entire female, PNID-184026; C) entire male PNID-151041; D) female tail, PNID-184031; E) female anterior, PNID-184031.

rounded, filled with spherical sperm, vagina straight, vulva closed. Ventral post-vulval region straight, narrowing immediately posterior to the vulva, elongate-conoid. The terminal annulus is simple or lobed. Anus indistinct in light microscopy.

Male: Not found in Chinese population.

Locality and habitat: The population was found in the rhizosphere of *Magnolia grandiflora* Linn from a Botanical garden, Hangzhou, Zhejiang Province, China on April 13, 2017. The geographical position of the sampling site is ″30°15′09″N; 120°07′01″E.

Differential diagnosis: In the original description males were not described; however, most populations of *D. limitanea* are reported to have spermatheca filled with sperm. Several reports include the description of a male. Powers et al. (2011) listed a single male (GB #FJ489535) still within the cuticle of the previous molt (Fig. 6C). The specimen had a body length of 258 µm, spicule of 19 µm, and gubernaculum of 5 µm. In the female, the relatively abrupt constriction of the post-vulval body was not described or illustrated in the original description, but the populations from Malaysia (Sauer and Winoto, 1975), Brazil (Rashid et al., 1986), India (Rahaman and Ahmed, 1994) and Ecuador (Talavera and Hunt, 1997) reported the narrowing of the post-vulval body profile. The

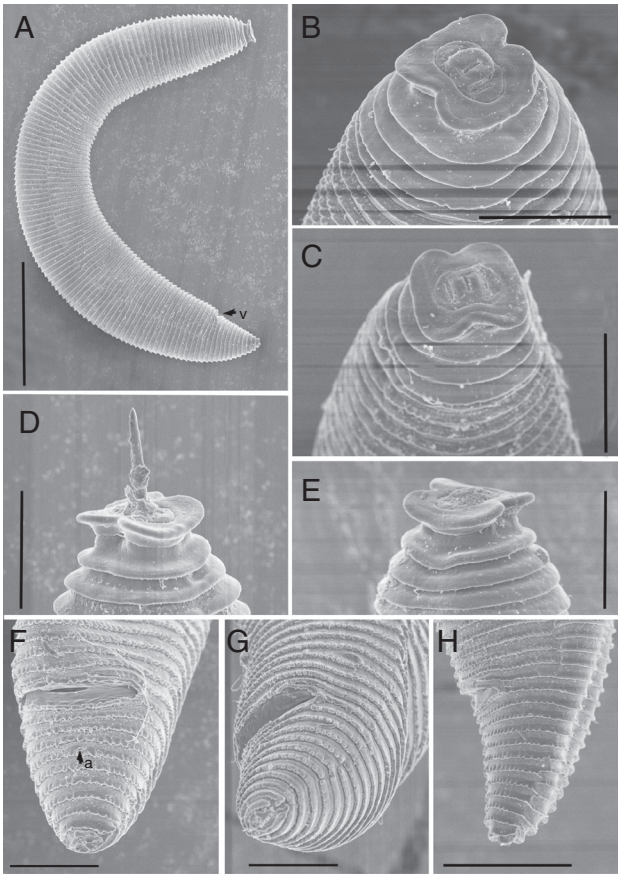


Figure 7: Scanning electron microscopy of *Discocriconemella limitanea*: A) entire female; B-E: oral disc in different angles; F-H: posterior region of the female showing vulva and anus (arrows showing position of the anus; scale bars = A = 50 µm; B-G = 10 µm; H = 20 µm).

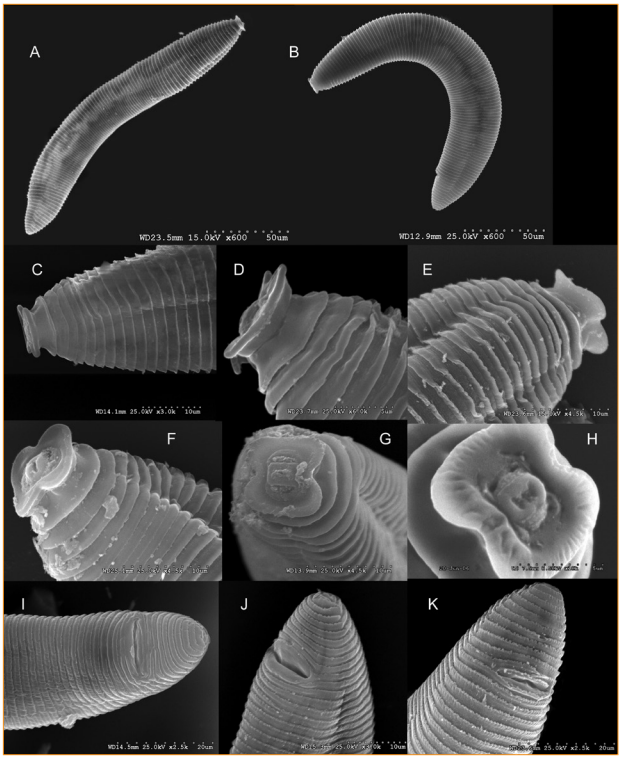


Figure 8: Scanning electron microscopy of *Discocriconemella limitanea* female from La Selva and Las Cruces, Costa Rica: A,B) entire female; C-H) head profiles in different angles; I-K) female posterior region showing vulva and anus.

South African population (Van den berg and Cadet, 1992) was reported to have distinct tooth-like projections on the margins of the ventral body annuli. The Brazilian population (Loof and Sharma, 1980) was reported to have a conspicuous break between the fourth and fifth annuli.

Morphometrically, the Congo population (Coomans, 1966) is slightly longer than the original description (260-280 µm *vs.* 207-228 µm). The Ivory Coast population (Luc, 1970) was reported to have the shortest body length (180 µm) and smallest stylet (38 µm) in the population compared with the original description. The Malaysian and Ecuadorean populations were reported to have larger V values (90-93 *vs.* 89.1-94.3 *vs.* 87-89, respectively) and smaller stylets (45-53 µm *vs.* 35-51 µm *vs.* 52-53 µm, respectively) in relation to the original description. The two Brazilian populations reported by Loof and Sharma (1980) and Rashid et al. (1986) are a mixture of small and large specimens. These two populations also differ from each other morphometrically; the notable difference of these two populations from the original description is the variable body length (167-306 µm *vs.* 190-280 µm *vs.* 207-228 µm, respectively) and stylet lengths (50-77 µm *vs.* 43-52 µm *vs.* 52-53 µm respectively). The South African, Indian and Venezuelan populations are morphologically close to the original description, except that the South African population has a longer body length (260-280 µm *vs.* 207-228 µm) while the body length of the Venezuelan population is shorter (191-280 µm *vs.* 207-228 µm), and the Indian population was reported to have smaller stylets (48-51 µm *vs.* 52-53 µm). The Chinese population in this study matches well with the original description except for a slightly longer body length (220-260 µm

Table 3. Morphometric data and distribution of *Discocriconemella limitanea*. All measurements in µm.

Authors	This study	Luc (1959)[a]	Coomans (1966)	Luc (1970)	Sauer and Winoto (1975)	Rashid et al. (1986)	Van den Berg and Cadet (1992)	Rahaman and Ahmed (1994)	Talavera and Hunt, 1997	Crozzoli and Lamberti (2003)	Syn. *(D. repleta)* Pinochet and Raski 1976	Syn. *(D. repleta)* Loof and Sharma (1980)	*Syn. (D. repleta)* Vovlas et al. 1990	
Origin	China	Costa Rica	French Guinea	Congo	Ivory Cost	Malaysia	Brazil	South Africa	India	Eucador	Venezuela	Brazil	Brazil	Peru
Host	*Magnolia grandiflora*	Forest soil	*Cinchona succirubra*	–	*Coffea* sp.	Forest Soil	*Theobroma cacao*	Forest Soil	*Bambusa* sp.	Forest Soil	Forest Soil	*Theobroma cacao*	–	Forest Soil
n	15	5	3	8	20	11	36	11	20	18	–	10	–	8
L	223.9–270.1	225–270	207–228	260–280	180–250	200–250	190–280	220–260	180–240	190–270	191–202	250–290	187–271	219–245
a	5.9–7.5	6.8–8.7	5.8–7.6	8–11	7–8	5–8	7.5–9.5	5.9–7.4	6.2–6.7	7.6–12	5.6–6.4	7–8	6.8–8.8	8.3–9.5
b	2.6–3.2	1.9–3.3	2.5–2.6	2.8–2.9	2.5–3	2.7–3.2	2.6–3.2	2.4–2.6	2.5–2.7	2.7–3.5	2.5–2.8	2.6–3.1	–	2.8–3.1
c	15.7–24.7	–	16	17–25	–	20–33	20.4–46	17.8–22.3	14–21	15.5–20.5	15–20	20–26	–	27–39
V	90.2–92.5	89–93	87–89	88–92	84–90	90–93	89–94	88–91	87–91	89.1–94.3	88–90	92–95	–	92–93
VL/VB	0.8–1.2		–	–	–	–	0.6–1.1	–	0.87–1.1	0.8–1.4	–		–	0.8–0.9
Stylet	53.1–59.6	45–57	52–53	53–55	38–50	45–53	43–52	52.5–59.2	48–51	35–51	48–54	59–66	43–56	44–47
Stylet %L	20.2–25.3	18–25.3	–	–	–	–	17–23	22.3–25.2	23.5–26.6	16.6–19.7	–	–	–	18–20
R	96.0–114	98–121	90–110	102–110	84–113	95–120	99–111	107–113	104–120	103–138	96–110	117–116	98–118	107–114
Rex	33.0–37	33–41	34	32–36	–	35–38	34–44	28–33	34–39	37–47	–	37–42	–	30–34
RV	11.0–13.0	10–11	11–12	12–15	–	11–15	8–14	12–15	14–16	9–12	12–14	10–11	9–13	11–13
RVan	4.0–5.0	–	4	4–6	–	4–5	2–7	5–7	6–7	2–3	4–5	3–4	0–4	
Ran	6.0–8.0	–	7	7–9	–	6–9	5–10	5–8	7–9	6–8	7–8	7–8	6–10	5–7
Tail	9.8–16.4	–	–	–	–	–	4.5–13	10.3–14	–	10.5–16.5	–	–	–	5.6–8.9
Male	Unknown	Known	–	–	–	Known	–	–	Unknown	–	–	Known	–	–

[a]Original description.

Table 4. Morphometric data and distribution of *Discocriconemella limitanea* in Chinese provinces. All measurements in µm.

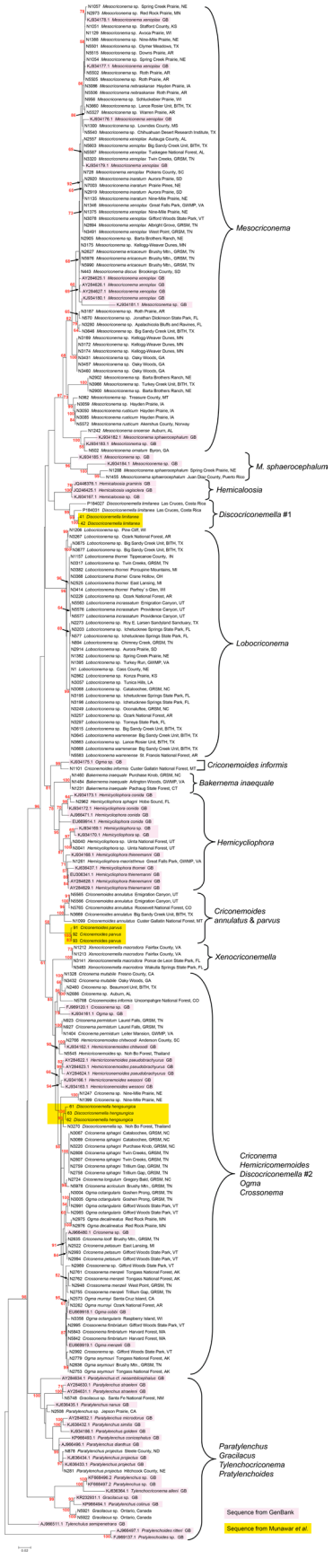
Authors	This study	Yin et al. (1994)	Ye et al. (1997)	Zhang et al. (1997)	Zhang et al. (1997)	Li et al. (2006)
Origin (Province, City)	Zhejiang Hangzhou	Guangzhou	Guangdong	Fujian, Xiamen	Fujian, Zhangpu	Yunan, Kunming
Host	Magnolia grandiflora	*Lychee*	–	Fruit trees	Fruit trees	Rosaceae plants
n	15	6	6	20	19	–
L	223.9–270.1	225–260	217–280	180–230	210–260	183–257
a	5.9–7.5	–	6.2–8.2	5.6–8.2	7–12	–
b	2.6–3.2	–	2.3–3.4	2.3–3.3	206–301	–
c	15.7–24.7	–	16.2–28.6	11–15	16–20	
V	90.2–92.5	89–91	89–91	90–93	87–91	–
VL/VB	0.8–1.2	–	094–1.06	–	–	–
Stylet	53.1–59.6	51–53	46–56	51–57	45–50	–
Stylet % L	20.2–25.3	–	–		–	–
R	96.0–114	110–122	90–98	92–104	94–110	96–103
Rex	33.0–37	–	30–34	30–37	30–36	34–37
RV	11.0–13.0	10–12	11–13	8–10	10–13	12–14
RVan	4.0–5.0	–	4–8	1–2	4–5	–
Ran	6.0–8.0	–	5–8	5–8	6–9	–
Tail	9.8–16.4	–	–	–	–	–
Male	Unknown	–	–	–	Known	–

vs. 207-228 µm) and longer stylet length (53-60 µm *vs.* 52-53 µm). Overall, the morphometrics are within the range of variation of the species according to the populations described by various authors.

Five additional populations of *D. limitanea* from China have been reported from Guangzhou, Guangdong, Fujian and Yunan provinces. Nematodes from all of these populations have overlapping morphometric ranges, fit well within with the original description and confirm to the species as described by multiple authors (Luc, 1970; Rashid et al., 1986; Rahaman and Ahmed, 1994; Talavera and Hunt, 1997).

Molecular profiles and phylogenetic status

Several key systematic features of criconematid nematodes are revealed by the 18S and COI phylogenetic trees. First, in the 18S tree (Fig. 9) which provides better resolution at the deeper nodes in the tree, there is strong bootstrap support (99%) for a clade that combines *Discocriconemella limitanea* from China with conspecific specimens from Costa Rica. This clade confirms the species identification and provides evidence of an amphi-Pacific disjunction, the first molecular data from a nematode to support this distribution pattern. Studies of many plant species suggest this is one of several intercontinental distribution patterns that link Asia and North America (Li and Wen, 2013; 2014; Fritsch et al., 2015). COI (Fig. 10) also supports this grouping at a lower support value (82%). Similarly, *Criconemoides parvus* groups with *C. annulatus* Cobb in Taylor, 1936 from western U.S. in the 18S tree, albeit at a relatively low support value



(58%). There are no molecular data of *C. parvus* from North America, although the type locality is in the western state of Nevada. The placement of *Discocriconemella hengsungica* and an unknown *Discocriconemella* specimen from Thailand, in both 18S and COI trees, provides strong evidence that the genus *Discocriconemella* is not a monophyletic group. *Discocriconemella hengsungica* is a member of a larger criconematid clade that predominantly includes nematodes that possess scales or projections on the cuticle in at least one life stage. *Xenocriconemella* (De Grisse and Loof, 1965) is also a member of this group which adds evidence that cuticle projections are not reliable taxonomic characters in establishing the genera (Powers et al., 2017).

Overall, the addition of these species from China to a reference dataset of criconematid nematodes provides insight into the biogeography of nematodes in general. It is likely that additional collections of plant parasitic nematodes from Asia will also contribute to fundamental questions of angiosperm biogeography (Raven and Axelrod, 1974; Fritsch et al., 2015; Wen et al., 2016).

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Figure 9: Maximum likelihood tree of 232 18S criconematid sequences. Substitution model GTR+G+I and 200 bootstrap replications. Each specimen is identified by a Nematode Identification number or GenBank Accession number (for taxa not sequenced by the authors), species name, and location as supplied by the author. Brackets are provided to indicate genera or specific species. Bootstrap values over 50 are applied by nodes in red. Specimens from China are highlighted in yellow.

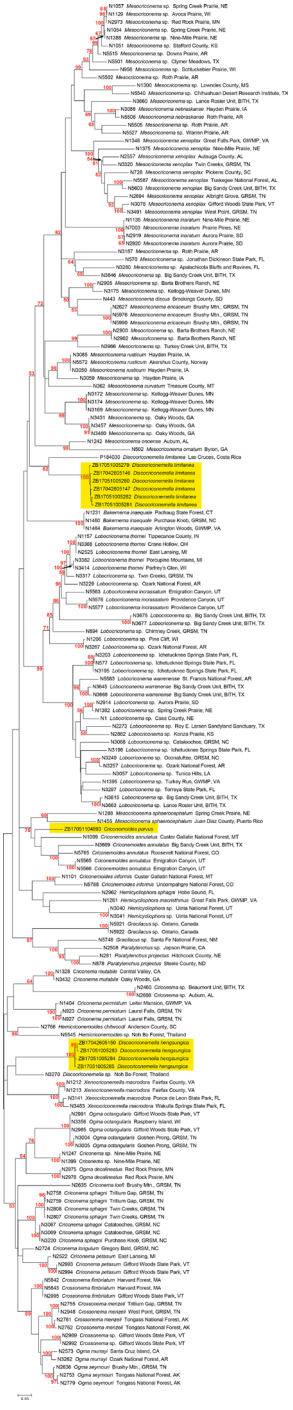

Figure 10: Maximum likelihood COI tree of 175 criconematid sequences. Substitution model GTR+G+I and 200 bootstrap replications. Each specimen is identified by a Nematode Identification number, species name and location. Bootstrap values of more than 50 are applied by nodes in red. Specimens from China are highlighted in yellow.

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Table AI. Table of location data and GenBank accession numbers for specimens appearing on the COI maximum likelihood tree, in tree order.

NID	Species	Stage	Locality**	Ecoregion	GenBank Accession #
1057	*Mesocriconema* sp.	J	Spring Creek Prairie, NE	Central Tall Grasslands	KJ788024
1129	*Mesocriconema* sp.	F	Avoca Prairie and Savanna State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	KJ788031
2973	*Mesocriconema* sp.	F	Red Rock Prairie Preserve, MN	Central Tall Grasslands	KY574752
1054	*Mesocriconema* sp.	F	Spring Creek Prairie, NE	Central Tall Grasslands	KJ788021
1388	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE	Central Tall Grasslands	KJ788053

1051	*Mesocriconema* sp.	F	Stafford County, KS	Central and Southern Mixed Grasslands	KJ788019
5515	*Mesocriconema* sp.	F	Downs Prairie Natural Area, AR	Mississippi Lowland Forests	KY574764
5501	*Mesocriconema* sp.	J	Clymer Meadows, TX	Texas Blackland Prairies	KY574813
956	*Mesocriconema* sp.	F	Schluckebier Prairie State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	KJ788015
5502	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	KY574731
1300	*Mesocriconema* sp.	F	Lowndes County, MS	Southeastern Mixed Forests	KJ787926
5540	*Mesocriconema* sp.	J	Chihuahuan Desert Research Institute, TX	Chihuahan Desert	MF770954
3660	*Mesocriconema* sp.	F	Lance Rosier Unit, BITH, TX	Piney Woods Forests	KY574795
3086	*Mesocriconema nebraskense*	F	Hayden Prairie Preserve, IA	Central Tall Grasslands	KY574679
5506	*Mesocriconema nebraskense*	J	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	KY574695
5505	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	KY574724
5527	*Mesocriconema* sp.	F	Warren Prairie Natural Area, AR	Piney Woods Forests	KY574807
1346	*Mesocriconema xenoplax*	F	Great Falls Park, GWMP, VA	Southeastern Mixed Forests	KY574831
1375	*Mesocriconema xenoplax*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	KJ787916
2557	*Mesocriconema xenoplax*	F	Autauga County, AL	Southeastern Mixed Forests	KY574633
3320	*Mesocriconema xenoplax*	J	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge Forests	KY574832
728	*Mesocriconema xenoplax*	F	Pickens County, SC	Southeastern Mixed Forests	KJ787873
5587	*Mesocriconema xenoplax*	F	Tuskegee National Forest, AL	Southeastern Mixed Forests	KY574626
5603	*Mesocriconema xenoplax*	J	Big Sandy Creek Unit, BITH, TX	Piney Woods Forests	MF770959
2694	*Mesocriconema xenoplax*	F	Albright Grove, GRSM, TN	Appalachian-Blue Ridge Forests	MF770909

3078	*Mesocriconema xenoplax*	F	Gifford Woods State Park, VT	New England Acadian Forests	KY574639
3491	*Mesocriconema xenoplax*	F	West Point, GRSM, TN	Appalachian-Blue Ridge Forests	MF770951
1135	*Mesocriconema inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	KJ787935
7003	*Mesocriconema inaratum*	F	Prairie Pines, NE	Central Tall Grasslands	MF770967
2919	*Mesocriconema inaratum*	F	Aurora Prairie Preserve, SD	Central Tall Grasslands	KY574657
2920	*Mesocriconema inaratum*	J	Aurora Prairie Preserve, SD	Central Tall Grasslands	MF770921
3187	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	KY574833
570	*Mesocriconema* sp.	J	Jonathan Dickinson State Park, FL	Florida Sand Pine Scrub	KJ788061
3280	*Mesocriconema* sp.	F	Apalachicola Bluffs and Ravines Preserve, FL	Southeastern Conifer Forests	KY574834
3646	*Mesocriconema* sp.	F	Neches Bottom and Jack Gore Unit, BITH, TX	Piney Woods Forests	KY574817
2905	*Mesocriconema* sp.	F	Barta Brothers Ranch, NE	Nebraska Sandhills Mixed Grasslands	KY574825
3175	*Mesocriconema* sp.	F	Kellogg-Weaver Dunes SNA, MN	Central Tall Grasslands	KY574826
443	*Mesocriconema discus*	J	Brookings County, SD	Central Tall Grasslands	KJ787868
2627	*Mesocriconema ericaceum*	F	Brushy Mtn., GRSM, TN	Appalachian-Blue Ridge Forests	KX290522
5976	*Mesocriconema ericaceum*	J	Brushy Mtn., GRSM, TN	Appalachian-Blue Ridge Forests	KX290542
5990	*Mesocriconema ericaceum*	F	Brushy Mtn., GRSM, TN	Appalachian-Blue Ridge Forests	KX290548
2900	*Mesocriconema* sp.	F	Barta Brothers Ranch, NE	Nebraska Sandhills Mixed Grasslands	MF770919
2902	*Mesocriconema* sp.	F	Barta Brothers Ranch, NE	Nebraska Sandhills Mixed Grasslands	MF770920
3966	*Mesocriconema* sp.	F	Turkey Creek Unit, BITH, TX	Piney Woods Forests	MF770953

3085	*Mesocriconema rusticum*	F	Hayden Prairie Preserve, IA	Central Tall Grasslands	MF770940
5572	*Mesocriconema rusticum*	F	Akershus County, Norway	Scandinavian and Russian Taiga	KY574621
3050	*Mesocriconema rusticum*	F	Hayden Prairie Preserve, IA	Central Tall Grasslands	MF770936
3059	*Mesocriconema* sp.	J	Hayden Prairie Preserve, IA	Central Tall Grasslands	MF770937
362	*Mesocriconema curvatum*	F	Treasure Co, MT	Northern Short Grasslands	KJ787847
3172	*Mesocriconema* sp.	J	Kellogg-Weaver Dunes SNA, MN	Central Tall Grasslands	MF770942
3174	*Mesocriconema* sp.	F	Kellogg-Weaver Dunes SNA, MN	Central Tall Grasslands	MF770943
3169	*Mesocriconema* sp.	F	Kellogg-Weaver Dunes SNA, MN	Central Tall Grasslands	KY574821
3431	*Mesocriconema* sp.	F	Oaky Woods Wildlife Management Area, GA	Southeastern Mixed Forests	KY574822
3457	*Mesocriconema* sp.	F	Oaky Woods Wildlife Management Area, GA	Southeastern Mixed Forests	KY574823
3460	*Mesocriconema* sp.	F	Oaky Woods Wildlife Management Area, GA	Southeastern Mixed Forests	MF770949
1242	*Mesocriconema onoense*	F	Auburn University, Auburn, AL	Southeastern Mixed Forests	KJ787834
502	*Mesocriconema ornatum*	F	USDA Southeastern Fruit and Nut Tree Reseach Station, GA	Southeastern Mixed Forests	KJ787824
P184030	*Discocriconemella limitanea*	F	Las Cruces Biological Station, Costa Rica	Isthmian-Pacific Moist forests	KJ788069
ZB17051005279	*Discocriconemella limitanea*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770975
ZB17042605146	*Discocriconemella limitanea*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770973
ZB17051005280	*Discocriconemella limitanea*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770976
ZB17042605147	*Discocriconemella limitanea*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770974

ZB17051005282	*Discocriconemella limitanea*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770978
ZB17051005281	*Discocriconemella limitanea*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770977
1231	*Bakernema inaequale*	F	Pauchaug State Forest, WI	Northeastern Coastal Forests	MF770896
1460	*Bakernema inaequale*	F	Purchase Knob, GRSM, NC	Appalachian-Blue Ridge Forests	MF770902
1484	*Bakernema inaequale*	F	Arlington Woods, GWMP, VA	Southeastern Mixed Forests	MF770903
1157	*Lobocriconema thornei*	F	Tippecanoe County, IN	Central Forest-Grassland Transition Zone	KU236522
3368	*Lobocriconema thornei*	F	Crane Hollow Preserve, OH	Appalachian Mixed Mesophytic Forests	KU236539
2525	*Lobocriconema thornei*	F	Michigan State University, East Lansing, MI	Southern Great Lakes Forests	KU236534
3382	*Lobocriconema thornei*	F	Porcupine Mountains Wilderness State Park, MI	Western Great Lakes Forest	KU236626
3414	*Lobocriconema thornei*	F	Parfrey's Glen State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	KU236627
3317	*Lobocriconema* sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge Forests	KU236521
3229	*Lobocriconema* sp.	F	Ozark National Forest, AR	Central US Hardwood Forests	KU236631
5563	*Lobocriconema incrassatum*	F	Emigration Canyon, UT	Wasatch and Uinta Montane Forests	KU236508
5576	*Lobocriconema incrassatum*	J	Providence Canyon, Cache County, UT	Wasatch and Uinta Montane Forests	KU236620
5577	*Lobocriconema incrassatum*	F	Providence Canyon, Cache County, UT	Wasatch and Uinta Montane Forests	KU236621

3675	*Lobocriconema* sp.	F	Canyonlands, BITH, TX	Piney Woods Forests	KU236623
3677	*Lobocriconema* sp.	F	Canyonlands, BITH, TX	Piney Woods Forests	KU236624
894	*Lobocriconema* sp.	F	Chimney Creek, GRSM, TN	Appalachian-Blue Ridge Forests	KU236496
1206	*Lobocriconema* sp.	F	Pine Cliff State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	KU236491
3267	*Lobocriconema* sp.	F	Ozark National Forest, AR	Central US Hardwood Forests	KU236495
3203	*Lobocriconema* sp.	F	Ichetucknee Springs State Park, FL	Southeastern Conifer Forests	KU236554
577	*Lobocriconema* sp.	F	Ichetucknee Springs State Park, FL	Southeastern Conifer Forests	KU236548
3195	*Lobocriconema* sp.	F	Ichetucknee Springs State Park, FL	Southeastern Conifer Forests	KU236552
5583	*Lobocriconema warrenense*	F	St. Francis National Forest, AR	Mississippi Lowland Forests	MF770958
3645	*Lobocriconema warrenense*	F	Big Sandy Creek Unit, BITH, TX	Piney Woods Forests	KU236546
3668	*Lobocriconema warrenense*	F	Big Sandy Creek Unit, BITH, TX	Piney Woods Forests	KU236547
2914	*Lobocriconema* sp.	F	Aurora Prairie Preserve, SD	Central Tall Grasslands	KU236570
1382	*Lobocriconema* sp.	J	Spring Creek Prairie, NE	Central Tall Grasslands	KU236568
1	*Lobocriconema* sp.	F	Timmas Farm State Ecological Preserve, NE	Central Tall Grasslands	KU236629
2273	*Lobocriconema* sp.	F	Roy E. Larsen Sandyland Sanctuary, TX	Piney Woods Forests	KU236555
2862	*Lobocriconema* sp.	F	Konza Prairie Biological Station, KS	Flint Hills Tall Grasslands	KU236557
3068	*Lobocriconema* sp.	F	Cataloochee, GRSM, NC	Appalachian-Blue Ridge Forests	KU236601
3196	*Lobocriconema* sp.	F	Ichetucknee Springs State Park, FL	Southeastern Conifer Forests	KU236613
3249	*Lobocriconema* sp.	F	Oconaluftee, GRSM, NC	Appalachian-Blue Ridge Forests	KU236571
3257	*Lobocriconema* sp.	F	Ozark National Forest, AR	Central US Hardwood Forests	KU236572
3057	*Lobocriconema* sp.	F	Tunica Hills State Wildlife Refuge, LA	Mississippi Lowland Forests	KU236597

1395	*Lobocriconema* sp.	F	Leiter Manxion & Turkey Run, GWMP, VA	Southeastern Mixed Forests	KU236630
3297	*Lobocriconema* sp.	F	Torreya State Park, FL	Southeastern Conifer Forests	KU236608
3615	*Lobocriconema* sp.	F	Big Sandy Creek Unit, BITH, TX	Piney Woods Forests	KU236588
3663	*Lobocriconema* sp.	F	Lance Rosier Unit, BITH, TX	Piney Woods Forests	KU236590
1288	*Mesocriconema sphaerocephalum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	MF770898
1455	*Mesocriconema sphaerocephalum*	F	Juan Diaz County, Puerto Rico	Puerto Rican Moist Forests	MF770901
ZB17051104893	*Criconemoides parvus*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770968
1099	*Criconemoides annulatus*	F	Custer Gallatin National Forest, MT	South Central Rockies Forests	MF77089
3669	*Criconemoides annulatus*	F	Canyonlands Unit, BITH, TX	Piney Woods Forests	MF770952
5765	*Criconemoides annulatus*	F	Roosevelt National Forest, CO	Colorado Rockies Forests	MF770961
5565	*Criconemoides annulatus*	F	Emigration Canyon, UT	Wasatch and Uinta Montane Forests	MF770956
5566	*Criconemoides annulatus*	F	Emigration Canyon, UT	Wasatch and Uinta Montane Forests	MF770957
1101	*Criconemoides informis*	F	Gallatin National Forest, MT	South Central Rockies Forests	KJ787842
5788	*Criconemoides informis*	J	Uncompahgre National Forest, CO	Colorado Rockies Forests	MF770962
2962	*Hemicycliophora* cf. *sphagni*	F	Hobe Sound National Wildlife Refuge, FL	Florida Sand Pine Scrub	MF770923
1261	*Hemicycliophora* cf. *macristhmus*	F	Great Falls Park, GWMP, VA	Southeastern Mixed Forests	KJ788066
3040	*Hemicycliophora* sp.	J	Uinta-Wasatch-Cache National Forest, UT	Wasatch and Uinta Montane Forests	MF770934
3041	*Hemicycliophora* sp.	J	Uinta-Wasatch-Cache National Forest, UT	Wasatch and Uinta Montane Forests	MF770935
5921	*Gracilacus wuae*	J	Ontario, Canada	Eastern Great Lakes lowland forests	MF770965

5922	*Gracilacus wuae*	J	Ontario, Canada	Eastern Great Lakes lowland forests	MF770966
5748	*Gracilacus* sp.	F	Santa Fe National Forest, NM	Colorado Rockies Forests	MF770960
2508	*Paratylenchus* sp.	F	Jepson Prairie Preserve, CA	California Central Valley Grasslands	MF770905
281	*Paratylenchus projectus*	F	Hitchcock County, NE	Central and Southern Mixed Grasslands	MF770889
878	*Paratylenchus projectus*	F	Steele County, ND	Northern Mixed Grasslands	MF770890
1328	*Criconema mutabile*	F	Fresno County, CA	California Central Valley Grasslands	KU236637
3432	*Criconema mutabile*	F	Oaky Woods Wildlife Management Area, GA	Southeastern Mixed Forests	MF770948
2460	*Criconema* sp.	F	Beaumont Unit, BITH, TX	Piney Woods Forests	MF770904
2686	*Criconema* sp.	F	Auburn University, Auburn, AL	Southeastern Mixed Forests	MF770908
1404	*Criconema permistum*	F	Leiter Mansion & Turkey Run, GWMP, VA	Southeastern Mixed Forests	MF770900
923	*Criconema permistum*	F	Laurel Falls Trail, GRSM, TN	Appalachian-Blue Ridge Forests	MF770891
927	*Criconema permistum*	F	Laurel Falls Trail, GRSM, TN	Appalachian-Blue Ridge Forests	MF770892
2766	*Hemicriconemoides chitwoodi*	F	Anderson County, SC	Southeastern Mixed Forests	MF770916
5545	*Hemicriconemoides* sp.	F	Noh Bo Forest, Thailand	Kayah-Karen Montane Rain Forests	MF770955
ZB17042605150	*Discocriconemella hengsungica*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770969
ZB17051005283	*Discocriconemella hengsungica*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770970
ZB17051005284	*Discocriconemella hengsungica*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770971

ZB17051005285	*Discocriconemella hengsungica*	F	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF770972
3270	*Discocriconemella* sp.	F	Noh Bo Forest, Thailand	Kayah-Karen Montane Rain Forests	MF770946
1212	*Xenocriconemella macrodora*	F	Accotink Watershed, VA	Southeastern Mixed Forests	MF770894
1213	*Xenocriconemella macrodora*	F	Accotink Watershed, VA	Southeastern Mixed Forests	MF770895
3141	*Xenocriconemella macrodora*	F	Ponce de Leon State Park, FL	Southeastern Conifer Forests	MF770941
3483	*Xenocriconemella macrodora*	F	Wakulla Springs State Park, FL	Southeastern Conifer Forests	MF770950
2991	*Ogma octangularis*	F	Gifford Woods State Park, VT	New England Acadian Forests	MF770928
3356	*Ogma octangularis*	F	Raspberry Island, APIS, WI	Western Great Lakes Forest	MF770947
2985	*Ogma octangularis*	F	Gifford Woods State Park, VT	New England Acadian Forests	MF770926
3004	*Ogma octangularis*	F	Goshen Prong, GRSM, TN	Appalachian-Blue Ridge Forests	MF770932
3005	*Ogma octangularis*	F	Goshen Prong, GRSM, TN	Appalachian-Blue Ridge Forests	MF770933
1247	*Criconema* sp.	F	Nine-Mile Prairie, NE	Central Tall Grasslands	MF770897
1399	*Criconema* sp.	F	Nine-Mile Prairie, NE	Central Tall Grasslands	MF770899
2975	*Ogma decalineatus*	F	Red Rock Prairie Preserve, MN	Central Tall Grasslands	MF770924
2976	*Ogma decalineatus*	F	Red Rock Prairie Preserve, MN	Central Tall Grasslands	MF770925
2635	*Criconema loofi*	F	Brushy Mtn., GRSM, TN	Appalachian-Blue Ridge Forests	KX290563
2758	*Criconema sphagni*	F	Trillium Gap, GRSM, TN	Appalachian-Blue Ridge Forests	MF770912
2759	*Criconema sphagni*	F	Trillium Gap, GRSM, TN	Appalachian-Blue Ridge Forests	MF770913
2808	*Criconema sphagni*	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge Forests	MF770918

2807	*Criconema sphagni*	J	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge Forests	MF770917
3067	*Criconema sphagni*	F	Cataloochee, GRSM, NC	Appalachian-Blue Ridge Forests	MF770938
3069	*Criconema sphagni*	F	Cataloochee, GRSM, NC	Appalachian-Blue Ridge Forests	MF770939
3220	*Criconema sphagni*	F	Purchase Knob, GRSM, NC	Appalachian-Blue Ridge Forests	MF770944
2724	*Criconema longulum*	J	Gregory Bald, GRSM, NC	Appalachian-Blue Ridge Forests	MF770910
2522	*Criconema petasum*	F	Michigan State University, East Lansing, MI	Southern Great Lakes Forests	MF770906
2993	*Criconema petasum*	F	Gifford Woods State Park, VT	New England Acadian Forests	KU236641
2994	*Criconema petasum*	F	Gifford Woods State Park, VT	New England Acadian Forests	MF770930
5842	*Crossonema fimbriatum*	F	Simes Tract, Harvard Forest LTER, MA	New England Acadian Forests	MF770963
5843	*Crossonema fimbriatum*	F	Simes Tract, Harvard Forest LTER, MA	New England Acadian Forests	MF770964
2995	*Crossonema fimbriatum*	F	Gifford Woods State Park, VT	New England Acadian Forests	MF770931
2755	*Crossonema menzeli*	F	Trillium Gap, GRSM, TN	Appalachian-Blue Ridge Forests	MF770911
2948	*Crossonema menzeli*	F	West Point, GRSM, TN	Appalachian-Blue Ridge Forests	MF770922
2761	*Crossonema menzeli*	F	Tongass National Forest, AK	Northern Pacific Coastal Forests	MF770914
2762	*Crossonema menzeli*	F	Tongass National Forest, AK	Northern Pacific Coastal Forests	MF770915
2989	*Crossonema* sp.	F	Gifford Woods State Park, VT	New England Acadian Forests	MF770927
2992	*Crossonema* sp.	F	Gifford Woods State Park, VT	New England Acadian Forests	MF770929

2573	*Ogma murrayi*	F	Santa Cruz Island Reserve, CA	California Coastal Sage and Chaparral	MF770907
3262	*Ogma murrayi*	F	Ozark National Forest, AR	Central US Hardwood Forests	MF770945
2636	*Ogma seymouri*	F	Brushy Mtn., GRSM, TN	Appalachian-Blue Ridge Forests	KX290587
2753	*Ogma seymouri*	F	Tongass National Forest, AK	Northern Pacific Coastal Forests	KX290594
2779	*Ogma seymouri*	F	Tongass National Forest, AK	Northern Pacific Coastal Forests	KX290599

Table AII. Table of location data and GenBank accession numbers for new *Discocriconemella limitanea, Criconemoides parvus* and *Discocriconemella hengsungica* specimens appearing on the 18S maximum likelihood tree, in tree order.

ZB17052504993	*Discocriconemella limitanea*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795592
ZB17052504991	*Discocriconemella limitanea*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795591
ZB17052504983	*Criconemoides parvus*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795587
ZB17052504981	*Criconemoides parvus*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795586
ZB17052504979	*Criconemoides parvus*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795585
ZB17052504987	*Discocriconemella hengsungica*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795589
ZB17052504985	*Discocriconemella hengsungica*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795588
ZB17052504989	*Discocriconemella hengsungica*	Hangzhou, Zhejiang Province, China	Eastern coast of China, flooded grasslands and savannas	MF795590