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 Jingwu1,3*
1Institute of Biotechnology, College of Agriculture & Biotechnology, Hangzhou 310058, Zhejiang, P. R. China.
2Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.
3Ministry of Agriculture Key Lab of Molecular Biology of Crop Pathogens and Insects, Hangzhou 310058, P. R. China.
*E-mail: jwzheng@zju.edu.cn.
This article was edited by Zafar Handoo.
Received for publication August 15, 2017.

Abstract

Populations of Criconemoides parvus, Discocriconemella hengsungi-ca, 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. hengsungi-ca 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. hengsungi-ca 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 hengsungi-ca, 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. hengsungi-ca (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 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. 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-length 18S 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 AGC GTT AGA ACT AGG GCG G-3′), the forward 18S900F (5′-AAG AGC 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′-AATWTGGGTGTTGAACCTTCTTT-3′ and COI-R9-5′ CTATAACATAATGAAATGGWGCWACCATATAAGTATC-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.
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
Three criconematids from China

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).

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

| Authors                  | This study | Raski (1952) | 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 | Artemisia sp. | Populus sp. | – | Tea | Theobroma cacao | – | Robinia pseudoacacia |
| n                        | 15         | 33           | 23          | 8           | 29         | 1       | 1           | 280      |
| 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        |          |
| R                        | 26.5–30.3  | 38–41       | 36–32      | 30–43      | 30.2–36.1  | 34.5–43 | 35         | 41       |
| 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   | –          | –        |

\^Original description.
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; 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 retrose 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.
Three criconematids from China

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).

*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.

| Table 2. Morphometric data of *Discocriconemella hengsungica*. All measurements in µm. |
|---|---|---|
| Authors | This study | Choi & Geraert, 1975a |
| 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 |

*aOriginal description.*
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
Three criconematids from China

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 Ecuadorian 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 vs. 207-228 μm).
Table 3. Morphometric data and distribution of *Discocriconemella limitanea*. All measurements in µm.

| Authors                  | This study | Luc (1959)* | 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) | Syn. (D. repleta) | Syn. (D. repleta) |
|--------------------------|------------|-------------|----------------|------------|--------------------------|----------------------|-------------------------------|--------------------------|------------------------|---------------------------|----------------|----------------|----------------|
| Origin                   | China      | Costa Rica  | French Guinea  | Congo      | Malaysia                 | Brazil               | South Africa                  | India                     | Eucador                | Theobroma  | Brazil       | Peru      |
| n                        | 15         | 5           | 3              | 8          | 20                       | 11                   | 36                            | 11                       | 10                     | 20                       | 18              | 11            | 5         |
| L                        | 223.9–270.1| 225–270     | 207–228        | 260–280    | 180–250                  | 200–250              | 190–280                      | 220–260                  | 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   |
| 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                      | –                        | 0.8–1.1               | 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     |
| 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    |
| Rex                      | 33.0–37    | 33–41       | 34              | 32–36      | –                        | 35–38                | 34–44                        | 28–33                    | 34–39                  | 37–47                     | –               | 37–42         | –         |
| 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      |
| 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             | 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                    | –               | –             | –         |

*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        | 53.1–69.6   | 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          | 4.0–6.0     | –               | 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.
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).

**Acknowledgments**

This research was supported by the National Natural Science Foundation of China (Project no. 31371921) and by U.S. National Science Foundation Proposal DEB-1145440. The authors acknowledge Dr. N. H. Rong and X. Li, for providing assistance in the preparation of SEM and R. Cai and N. Qu for helping with the samplings.

---

**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.
Three criconematids from China

References

Choi, Y.E., and Geraert, E. 1975. Criconematids from Korea with the description of eight new species (Nematoda: Tylenchida). *Nematologica* 21(1): 35–52.

Coomans, A. 1966. Some nematodes from Congo. *Revue de Zoologie et Botanique Africane* 74: 287–312.

Crozzoli, R., and Lamberti, F. 2003. Species of *Criconemoides* Taylor, 1936, *Discocriconemella* De Grisse & Loof, 1965 and *Hemicriconemoides* Chitwood & Birchfield, 1957 occurring in Venezuela, with description of *Criconemoides tiaraensis* sp. n. (Nematoda: Criconematidae). *Russian Journal of Nematology* 11: 67–79.

De Grisse, A.T., and Loof, P.A.A. 1965. Revision of the genus *Criconemoides* (Nematoda). *Overdruk uit de Mededelingen van Landbouwhoeschool en de Opzoekingsstations van de Staat Gent* 30: 577–603.

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

Eskandari, A., Karegar, A., Pourjan, E., Van den Berg, E., and Tiedt, L.R. 2010. Additional data on some poorly known species of *Criconemoides* Taylor, 1936 (Nematoda: Criconematidae). *Nematology* 4: 505–18.

Fritsch, P.W., Manchester, S.R., Stone, R.D., Cruz, B.C., and Almeda, F. 2015. Northern Hemisphere origins of the amphi-Pacific tropical plant family Symplocaceae. *Journal of Biogeography* 42: 891–901.

Geraert, E. 2010. *Criconematidae of the world-identification of the family Criconematidae* (Nematoda) Gent, Academia Press, New York.

Hoffmann, J.K. 1974. Morphological variation in species of *Bakernema*, *Criconema*, and *Criconemoides* (Criconematidae: Nematoda). *Iowa State Journal of Research* 49: 137–53.

Jenkins, W.R. 1964. A rapid centrifugal-flotation method for separating nematodes from soil. *Plant Disease Reporter* 48: 692.

Joyce, S., Reid, A., Driver, F., and Curran, J. 1994. Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes., in Burnell, A.M., Ehlers, R.U., and 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. 178–87.

Li, R., and Wen, J. 2013. Phylogeny and biogeography of *Dendropanax* (Araliaceae), an amphi-Pacific
disjunct genus between tropical/subtropical Asia and the Neotropics. Systematic Botany 38: 536–51.

Li, R., and Wen, J. 2014. Phylogeny and biogeography of Asian Schefflera (Araliaceae) based on nuclear and plastid DNA sequences data. Journal of Systematics and Evolution 52: 431–49.

Li, W.F., Yang, Y.L., Li, S.K., and Hu, X.Q. 2006. Preliminary investigation on parasitic nematode species of flowers and plants in Rosaceae. Southwest China Journal of Agricultural Sciences 19: 906–11.

Liskova, M., Vovlas, N., and Sasanelli, N. 2004. Criconematidae (Nematoda) in the Slovak Republic. Helminthologica 3: 161–70.

Liu, W., Zhao, H., Feng, G., Duan, F., and Ni, X. 2004. Species of nematodes parasitizing on vegetables in Shandong Province, Proceedings of the annual meeting of Chinese society for Plant Pathology, pp. 297–300.

Loof, P.A.A., and Barooti, S. 1991. New records of species of Criconematidae from Iran with the description of Criconemoides decipiens sp. n. (Nematoda: Tylenchida). Nematologica Mediterranea 19: 83–95.

Loof, P.A.A., Wouts, W.M., and Yeates, G.W. 1997. Criconematidae (Nematoda: Tylenchida) from the New Zealand region: Genera Mesocriconema, Discocriconemella, and Hemicriconemoides. New Zealand Journal of Zoology 2: 123–51.

Loof, P.A.A., and Sharma, R.D. 1980. Discocriconemella species from Bahia State, Brazil (Nematoda: Criconematidae). Mededelingen van de Fakulteit Landbouwwetenschappen Rijksuniversiteit Gent 45: 795–806.

Luc, M. 1959. Nouveaux Criconematidae de la zone intertropicale (Nematoda: Tylenchida). Nematologica 4: 16–22.

Luc, M. 1970. Contribution a l’ ‘etude du genre’ Criconemoides Taylor, 1936 (Nematoda: Criconematidae). Cahiers O.R.S.T.O.M., Serie Biologie 11: 69–131.

Micoletzky, H. 1922. Die freilebenden Erd-Nematoden mitbesonderer Berücksichtigung der Steiermark und der Bukowina, zugleich mit einer Revision sämtlicher nicht mariner, freilebender Nematoden in Form von Genus-Beschreibungen und Bestimmungsschlüsseln. Archiv für Naturgeschichte A87: 1–650.

Olson, M., Harris, T., Higgins, R., Mullin, P., Powers, K., Olson, S., and 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.

Pinochet, J., and Raski, D.J. 1976. Discocriconemella repleta n.sp., and the male of Criconemoides insitusatus Hoffmann, 1974 (Criconematidae: Nematoda). Journal of Nematology 8: 327–330.

Popovic, J., and Ciobanu, M. 2000. New morphometric data and geographical distribution of criconematids species (Nematoda: Criconematidae) in Romania. Studia, Universitatis Babes-Bolyai. Biologia 45: 39–55.

Powers, T.O., Bernard, E.C., Harris, T., Higgins, R., Olson, M., Lodema, M., Mullin, P., Sutton, L., and Powers, K.S. 2014. COI haplotype groups in Mesocriconema (Nematoda: Criconematidae) and their morphospecies associations. Zootaxa 3827(2): 101–46.

Powers, T.O., Bernard, E.C., Harris, T., Higgins, R., Olson, M., Lodema, M., Matczyszyn, J., Mullin, P., Sutton, L., and Powers, K.S. 2016. Species discovery and diversity in Lobocriconema (Criconematidae: Nematoda) and related plant-parasitic nematodes from North American ecoregions. Zootaxa 3: 301–44.

Powers, T., Harris, T., Higgins, R., Mullin, P., and Powers, K. 2017. An 18S rDNA Perspective on the Classification of Criconematoidea. Journal of Nematology 49: 236–244.

Powers, T., Harris, T., Higgins, R., Mullin, P., Sutton, L., and Powers, K. 2011. MOTUs, morphology, and biodiversity estimation: a case study using nematodes of the suborder Criconematina and a conserved 18S DNA barcode. Journal of Nematology 43(1): 35.

Powers, T.O., Harris, T., Higgins, R., Sutton, L., and 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.

Rahaman, P.F., and Ahmed, W. 1994. Observations on Discocriconemella limita with comments on D. repleta and D. barberi (Nematoda: Criconematidae). Afro-Asian Journal of Nematology 4: 201–9.

Rashid, F., Geraert, E., and Sharma, R.D. 1986. Criconematidae (Nematoda) from Brazil. Nematologica 32: 374–97.

Raski, D.J. 1952. On the morphology of Criconemoides Taylor, 1936 with description of six new species (Nematoda: Criconematidae). Proceedings of the Helminthological Society of Washington 19: 85–99.

Raski, D.J. 1958. Nomenclatorial note on the genus Criconemoides (Nematoda: Criconematidae) with a key to the species. Proceedings of the Helminthological Society of Washington 25: 139–42.

Raven, P.H., and Axelrod, D.I. 1974. Angiosperm biogeography and past continental movements. Annals of the Missouri Botanical Garden 61(3): 539–673.

Sauer, M.R., and Winoto, R. 1975. Species of Discocriconemella from Malaysia. Nematologica 21: 333–40.

Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 67–9.
Three criconematids from China

Siddiqi, M.R. 2000. *Tylenchida parasites of plants and insects*, 2nd ed., Commonwealth Agricultural Bureaux, Wallingford, UK.

Talavera, M., and Hunt, D.J. 1997. Observations on species of *Discocriconemella* De Grisse & Loof, 1965 and *Macroposthonia* de Man, 1880 (Nematoda: Tylenchida: Criconematidae) from Ecuador, with the proposal of *M. napoensis* n. sp. and *M. planiloba* n. sp. *Systematic parasitology* 36: 133–42.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–9.

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 |

Vovlas, N., Ciancio, A., and Carbonell-Torres, E. 1990. Criconematids from Peru with description of *Ogma andense* sp. n. *Nematology Mediterranea* 18: 243–252.

Wen, J., Nie, Z.L., and Ickert-Bond, S.M. 2016. Intercontinental disjunctions between eastern Asia and western North America in vascular plants highlight the biogeographic importance of the Bering land bridge from late Cretaceous to Neogene. *Journal of Systematics and Evolution* 54: 469–490.

Wouts, W.M. 2006. *Criconematina* (Nematoda: Tylenchida). *Fauna of New Zealand* 55: 1–228.

Ye, W., Lin, W., and Cai, W. 1997. Some criconematids from China. International. *International Journal of Nematology* 7: 137–41.

Yin, Y., Xuebao, G., and Feng, Z. 1994. Three new records of plant nematodes in China. *Journal of south China Agriculture University* 2: 23–5.

Zhang, S., Guokun, L., and Cai, X. 1997. Notes of species of *Criconematidae* in Fujian, China. *Journal of Fujian Agriculture University* 4: 427–31.

Zheng, J., Subbotin, S.A., He, S., Gu, J., and Moens, M. 2003. Molecular characterization 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.

Tan, J.J., and Ye, J.R. 2009. List of nematodes in Pine in China. *Forest Pest and Disease* 5: 24–35.

Taulaganov, A. 1941. Description of a new species of nematodes of the genus *Hoplolaimus*. *Trudy Uzbekskoi gosudarskoi Universiteta H.S. 16, Biologiya* 11: 21–2.

Taylor, 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., and Cadet, P. 1992. On five plant parasitic tylenchs from Martinique (Nemata). *Fundamental and Applied Nematology* 15: 431–42.
|   | Species          | Gender | Location                  | Ecoregion                        | Accession |
|---|------------------|--------|---------------------------|----------------------------------|-----------|
|1051| Mesocriconema sp. | F      | Stafford County, KS       | Central and Southern Mixed       | KJ788019  |
||                  |        |                           | Grasslands                       |           |
|5515| Mesocriconema sp. | F      | Downs Prairie Natural     | Mississippi Lowland Forests      | KY574764  |
||                  |        | Area, AR                  |                                  |           |
|5501| Mesocriconema sp. | J      | Clymer Meadows, TX        | Texas Blackland Prairies         | KY574813  |
|956 | Mesocriconema sp. | F      | Schluckebier Prairie      | Upper Midwest Forest-Savanna     | KJ788015  |
||                  |        | State Natural Area, WI    | Transition Zone                  |           |
|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 | Chihuahuan 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  |
| Record | Genus and Species       | Sex | Location 1                           | Location 2       | Location 3 | Journal Entry |
|--------|-------------------------|-----|-------------------------------------|------------------|------------|--------------|
| 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* ericaeum | F   | Brushy Mtn., GRSM, TN               | Appalachian-Blue Ridge Forests | KX290522 |
| 5976   | *Mesocriconema* ericaeum | J   | Brushy Mtn., GRSM, TN               | Appalachian-Blue Ridge Forests | KX290542 |
| 5990   | *Mesocriconema* ericaeum | 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 |
| Accession | Species, Subspecies | Location | Map Location | GenBank Accession |
|-----------|---------------------|----------|--------------|------------------|
| 3085      | *Mesocriconema* *rusticum* | Hayden Prairie Preserve, IA | Central Tall Grasslands | MF770940 |
| 5572      | *Mesocriconema* *rusticum* | Akershus County, Norway | Scandinavian and Russian Taiga | KY574621 |
| 3050      | *Mesocriconema* *rusticum* | Hayden Prairie Preserve, IA | Central Tall Grasslands | MF770936 |
| 3059      | *Mesocriconema* sp. | Hayden Prairie Preserve, IA | Central Tall Grasslands | MF770937 |
| 362       | *Mesocriconema* *curvatum* | Treasure Co, MT | Northern Short Grasslands | KJ787847 |
| 3172      | *Mesocriconema* sp. | Kellogg-Weaver Dunes SNA, MN | Central Tall Grasslands | MF770942 |
| 3174      | *Mesocriconema* sp. | Kellogg-Weaver Dunes SNA, MN | Central Tall Grasslands | MF770943 |
| 3169      | *Mesocriconema* sp. | Kellogg-Weaver Dunes SNA, MN | Central Tall Grasslands | KY574821 |
| 3431      | *Mesocriconema* sp. | Oaky Woods Wildlife Management Area, GA | Southeastern Mixed Forests | KY574822 |
| 3457      | *Mesocriconema* sp. | Oaky Woods Wildlife Management Area, GA | Southeastern Mixed Forests | KY574823 |
| 3460      | *Mesocriconema* sp. | Oaky Woods Wildlife Management Area, GA | Southeastern Mixed Forests | MF770949 |
| 1242      | *Mesocriconema* *onoense* | Auburn University, Auburn, AL | Southeastern Mixed Forests | KJ787834 |
| 502       | *Mesocriconema* *ornatum* | USDA Southeastern Fruit and Nut Tree Research Station, GA | Southeastern Mixed Forests | KJ787824 |
| P184030   | *Discocronemella* *limitanea* | Las Cruces Biological Station, Costa Rica | Isthmian-Pacific Moist forests | KJ788069 |
| ZB17051005279 | *Discocronemella* *limitanea* | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770975 |
| ZB17042605146 | *Discocronemella* *limitanea* | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770973 |
| ZB17051005280 | *Discocronemella* *limitanea* | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770976 |
| ZB17042605147 | *Discocronemella* *limitanea* | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770974 |
| Accession | Species                        | Location                                      | Geographical Features                                      | Accession Numbers |
|-----------|--------------------------------|-----------------------------------------------|-------------------------------------------------------------|-------------------|
| 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          |
| Record | Lobocriconema Species | Sex | Location | Forest Type |
|--------|----------------------|-----|----------|-------------|
| 3675   | Lobocriconema sp.    | F   | Canyonlands, BITH, TX | Piney Woods Forests |
| 3677   | Lobocriconema sp.    | F   | Canyonlands, BITH, TX | Piney Woods Forests |
| 894    | Lobocriconema sp.    | F   | Chimney Creek, GRSM, TN | Appalachian-Blue Ridge Forests |
| 1206   | Lobocriconema sp.    | F   | Pine Cliff State Natural Area, WI | Upper Midwest Forest-Savanna Transition Zone |
| 3267   | Lobocriconema sp.    | F   | Ozark National Forest, AR | Central US Hardwood Forests |
| 3203   | Lobocriconema sp.    | F   | Ichetucknee Springs State Park, FL | Southeastern Conifer Forests |
| 577    | Lobocriconema sp.    | F   | Ichetucknee Springs State Park, FL | Southeastern Conifer Forests |
| 3195   | Lobocriconema sp.    | F   | Ichetucknee Springs State Park, FL | Southeastern Conifer Forests |
| 5583   | Lobocriconema sp.    | F   | St. Francis National Forest, AR | Mississippi Lowland Forests |
| 3645   | Lobocriconema sp.    | F   | Big Sandy Creek Unit, BITH, TX | Piney Woods Forests |
| 3668   | Lobocriconema sp.    | F   | Big Sandy Creek Unit, BITH, TX | Piney Woods Forests |
| 2914   | Lobocriconema sp.    | F   | Aurora Prairie Preserve, SD | Central Tall Grasslands |
| 1382   | Lobocriconema sp.    | J   | Spring Creek Prairie, NE | Central Tall Grasslands |
| 1      | Lobocriconema sp.    | F   | Timmas Farm State Ecological Preserve, NE | Central Tall Grasslands |
| 2273   | Lobocriconema sp.    | F   | Roy E. Larsen Sandylan Sanctuary, TX | Piney Woods Forests |
| 2862   | Lobocriconema sp.    | F   | Konza Prairie Biological Station, KS | Flint Hills Tall Grasslands |
| 3068   | Lobocriconema sp.    | F   | Cataloochee, GRSM, NC | Appalachian-Blue Ridge Forests |
| 3196   | Lobocriconema sp.    | F   | Ichetucknee Springs State Park, FL | Southeastern Conifer Forests |
| 3249   | Lobocriconema sp.    | F   | Oconaluftee, GRSM, NC | Appalachian-Blue Ridge Forests |
| 3257   | Lobocriconema sp.    | F   | Ozark National Forest, AR | Central US Hardwood Forests |
| 3057   | Lobocriconema sp.    | F   | Tunica Hills State Wildlife Refuge, LA | Mississippi Lowland Forests |
| ID     | Species                      | Location                        | Geographic Region          | Accession Number |
|--------|------------------------------|---------------------------------|-----------------------------|------------------|
| 1395   | *Lobocriconema* sp.          | Leiter Manxion & Turkey Run, GWMP, VA | Southeastern Mixed Forests | KU236630         |
| 3297   | *Lobocriconema* sp.          | Torreya State Park, FL          | Southeastern Conifer Forests | KU236608         |
| 3615   | *Lobocriconema* sp.          | Big Sandy Creek Unit, BITH, TX  | Pinney Woods Forests        | KU236588         |
| 3663   | *Lobocriconema* sp.          | Lance Rosier Unit, BITH, TX     | Pinney Woods Forests        | KU236590         |
| 1288   | *Mesocriconema sphaerocephalum* | Spring Creek Prairie, NE | Central Tall Grasslands     | MF770898         |
| 1455   | *Mesocriconema sphaerocephalum* | Juan Diaz County, Puerto Rico | Puerto Rican Moist Forests  | MF770901         |
| ZB17051104893 | *Criconemoides parvus* | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770968 |
| 1099   | *Criconemoides annulatus*    | Custer Gallatin National Forest, MT | South Central Rockies Forests | MF77089         |
| 3669   | *Criconemoides annulatus*    | Canyonlands Unit, BITH, TX      | Pinney Woods Forests        | MF770952         |
| 5765   | *Criconemoides annulatus*    | Roosevelt National Forest, CO   | Colorado Rockies Forests    | MF770961         |
| 5565   | *Criconemoides annulatus*    | Emigration Canyon, UT           | Wasatch and Uinta Montane Forests | MF770956 |
| 5566   | *Criconemoides annulatus*    | Emigration Canyon, UT           | Wasatch and Uinta Montane Forests | MF770957 |
| 1101   | *Criconemoides informis*     | Gallatin National Forest, MT    | South Central Rockies Forests | KJ787842         |
| 5788   | *Criconemoides informis*     | Uncomphagre National Forest, CO | Colorado Rockies Forests    | MF770962         |
| 2962   | *Hemicycliophora cf. sphagni* | Hobe Sound National Wildlife Refuge, FL | Florida Sand Pine Scrub     | MF770923         |
| 1261   | *Hemicycliophora cf. macristhmus* | Great Falls Park, GWMP, VA | Southeastern Mixed Forests | KJ788066         |
| 3040   | *Hemicycliophora* sp.       | Uinta-Wasatch-Cache National Forest, UT | Wasatch and Uinta Montane Forests | MF770934 |
| 3041   | *Hemicycliophora* sp.       | Uinta-Wasatch-Cache National Forest, UT | Wasatch and Uinta Montane Forests | MF770935 |
| 5921   | *Gracilacus wuae*            | Ontario, Canada                 | Eastern Great Lakes lowland forests | MF770965 |
| ID   | Species                          | Location                        | Habitat                          | Accession |
|------|----------------------------------|----------------------------------|----------------------------------|-----------|
| 5922 | *Gracilacus wuae*                 | Ontario, Canada                  | Eastern Great Lakes lowland forests | MF770966  |
| 5748 | *Gracilacus* sp.                 | Santa Fe National Forest, NM     | Colorado Rockies Forests          | MF770960  |
| 2508 | *Paratylanchus* sp.              | Jepson Prairie Preserve, CA      | California Central Valley Grasslands | MF770905  |
| 281  | *Paratylanchus projectus*        | Hitchcock County, NE             | Central and Southern Mixed Grasslands | MF770889  |
| 878  | *Paratylanchus projectus*        | Steele County, ND                | Northern Mixed Grasslands         | MF770890  |
| 1328 | *Criconema mutabile*             | Fresno County, CA                | California Central Valley Grasslands | KU236637  |
| 3432 | *Criconema mutabile*             | Oaky Woods Wildlife Management Area, GA | Southeastern Mixed Forests       | MF770948  |
| 2460 | *Criconema* sp.                  | Beaumont Unit, BITH, TX          | Piney Woods Forests               | MF770904  |
| 2686 | *Criconema* sp.                  | Auburn University, Auburn, AL    | Southeastern Mixed Forests        | MF770908  |
| 1404 | *Criconema permistum*            | Leiter Mansion & Turkey Run, GWMP, VA | Southeastern Mixed Forests       | MF770900  |
| 923  | *Criconema permistum*            | Laurel Falls Trail, GRSM, TN     | Appalachian-Blue Ridge Forests    | MF770891  |
| 927  | *Criconema permistum*            | Laurel Falls Trail, GRSM, TN     | Appalachian-Blue Ridge Forests    | MF770892  |
| 2766 | *Hemicriconemoides chitwoodi*    | Anderson County, SC              | Southeastern Mixed Forests        | MF770916  |
| 5545 | *Hemicriconemoides* sp.          | Noh Bo Forest, Thailand          | Kayah-Karen Montane Rain Forests  | MF770955  |
| ZB17042605150 | *Discocriconemella* hensungica | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770969  |
| ZB17051005283 | *Discocriconemella* hensungica | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770970  |
| ZB17051005284 | *Discocriconemella* hensungica | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas | MF770971  |
| Accession ID | Species and Synonym | Gender | Location and Details | Geographic and Ecological Details |
|--------------|---------------------|--------|----------------------|----------------------------------|
| ZB17051005285 | Discocriconemella hengsungica | F | Hangzhou, Zhejiang Province, China | Eastern coast of China, flooded grasslands and savannas |
| 3270         | Discocriconemella sp. | F | Noh Bo Forest, Thailand | Kayah-Karen Montane Rain Forests |
| 1212         | Xenocriconemella macrodora | F | Accotink Watershed, VA | Southeastern Mixed Forests |
| 1213         | Xenocriconemella macrodora | F | Accotink Watershed, VA | Southeastern Mixed Forests |
| 3141         | Xenocriconemella macrodora | F | Ponce de Leon State Park, FL | Southeastern Conifer Forests |
| 3483         | Xenocriconemella macrodora | F | Wakulla Springs State Park, FL | Southeastern Conifer Forests |
| 2991         | Ogma octangularis | F | Gifford Woods State Park, VT | New England Acadian Forests |
| 3356         | Ogma octangularis | F | Raspberry Island, APIS, WI | Western Great Lakes Forests |
| 2985         | Ogma octangularis | F | Gifford Woods State Park, VT | New England Acadian Forests |
| 3004         | Ogma octangularis | F | Goshen Prong, GRSM, TN | Appalachian-Blue Ridge Forests |
| 3005         | Ogma octangularis | F | Goshen Prong, GRSM, TN | Appalachian-Blue Ridge Forests |
| 1247         | Criconema sp. | F | Nine-Mile Prairie, NE | Central Tall Grasslands |
| 1399         | Criconema sp. | F | Nine-Mile Prairie, NE | Central Tall Grasslands |
| 2975         | Ogma decalineatus | F | Red Rock Prairie Preserve, MN | Central Tall Grasslands |
| 2976         | Ogma decalineatus | F | Red Rock Prairie Preserve, MN | Central Tall Grasslands |
| 2635         | Criconema loofi | F | Brushy Mtn., GRSM, TN | Appalachian-Blue Ridge Forests |
| 2758         | Criconema sphagni | F | Trillium Gap, GRSM, TN | Appalachian-Blue Ridge Forests |
| 2759         | Criconema sphagni | F | Trillium Gap, GRSM, TN | Appalachian-Blue Ridge Forests |
| 2808         | Criconema sphagni | F | Twin Creeks, GRSM, TN | Appalachian-Blue Ridge Forests |
| #   | Species                  | Sex | Location                        | Region                      | Accession  |
|-----|--------------------------|-----|---------------------------------|-----------------------------|------------|
| 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   |
Three criconematids from China

| Location ID | Taxon                  | Gender | Location Name, Province, Region | Location Description                  | GenBank Accession Number |
|-------------|------------------------|--------|---------------------------------|---------------------------------------|--------------------------|
| 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.

| Location ID | Taxon                  | Location Name, Province, Region | Location Description                  | GenBank Accession Number |
|-------------|------------------------|---------------------------------|---------------------------------------|--------------------------|
| 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                 |