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Morphological and Molecular Characterization of *Discocriconemella inarata*, an Endemic Nematode from North American Native Tallgrass Prairies

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Abstract: Discocriconemella inarata, a plant parasitic nematode species originally discovered in a virgin tallgrass prairie in northwest Iowa, was re-examined by molecular and morphological analyses of topotype material. This species has never been recorded in cultivated fields and could potentially serve as an indicator for high quality prairie habitats. DNA sequence from a conserved 3' portion of the 18S ribosomal gene exhibited an identical match between *D. inarata* topotype specimens and topotype specimens of *Mesocriconema xenoplax* from Fresno, California. Higher resolution sequence analyses using the internal transcribed spacer 1 (ITS1) and a portion of the mitochondrial gene cytochrome b (cytb) allowed discrimination of *D. inarata* apart from *M. xenoplax*. This pair of species formed a well-supported clade with other *Mesocriconema* species exclusive of tropical *Discocriconemella* species. Scanning electron microscopy confirmed the absence of submedian lobes on *D. inarata*, suggesting a secondary loss of this defining morphological characteristic for *Mesocriconema*. Observations and measurements of *D. inarata* juveniles were added for the first time. Surveys of other prairies within the Great Plains expanded the known distribution of this species.

Key words: Criconematidae *Discocriconemella inarata*, DNA taxonomy, endemic nematode, environmental indicator, native habitats, plant parasitic nematodes, ring nematodes, tallgrass prairie.

Discocriconemella inarata Hoffman, 1974, was originally described from Kalsow and Sheeder Prairies in northwest Iowa, two remnant, never cultivated tallgrass prairies of 64.7 and 10.1 hectares respectively. In Kalsow Prairie *D. inarata* was recorded as associated with unidentified native grasses and at Sheeder Prairie *Lathyrus venosus* Muhl., a rhizomatous perennial herb in Fabaceae, was listed as the plant host. The nematode species has never been reported from agricultural fields and its presence in remnant prairies makes it a potential below-ground indicator for undisturbed, native grasslands. Such remnant sites, formerly part of the largest tallgrass prairie on earth, are now rare due to the near total conversion in North America of the Central Tall Grasslands ecoregion to tilled cropland (Ricketts *et al.*, 1999; Savage, 2004). Today, only 12,140 ha of tallgrass prairie exist in Iowa, representing less than 0.1% of its original extent in the state (Dornbush, 2004; Samson and Knopf, 1994). Only 20 remnant prairie sites exist in southern Iowa and northern Missouri, none larger than 8 hectares in size (Ricketts *et al.*, 1999).

While conducting soil surveys to characterize nematode communities of native prairies in the Great Plains region of the U.S., we sampled both of the Iowa prairies known to contain *Discocriconemella inarata*. Specimens conforming to the original and only description of *D. inarata* were collected from Kalsow Prairie on four occasions between April 24, 2006 and September 26, 2007. To ensure accurate identification, combined molecular and morphological analyses were initiated. In addition to its status as a prairie endemic, *D. inarata* is the only *Discocriconemella* species found north of Mexico (Cid del Prado Vera & Loof, 1984; Nematode Geographical Distribution Committee of the Society of Nematologists, 1984). *Discocriconemella* De Grisse and Loof, 1965 includes 29 species according to Siddiqi (2000). Most of the species are found in tropical or subtropical climates, often in association with native forests, although notable exceptions, e.g. *D. addisababa* Abebe & Geraert, 1995; *D. mauritiensis* (Williams, 1960) De Grisse & Loof, 1965; and *D. uruguayensis* Vovlas & Lamberti, 1997, have been associated with grasses. There are no trees located on Kalsow Prairie and the climatic zone could be classified as temperate steppe (Bailey, 1996). The most recent review of the genus groups 22 species primarily on the basis of an expanded cephalic annule surrounding the labial plate that creates a disc-like appearance in lateral view (Vovlas, 1992). Although several genera in Criconematidae share this feature, the disc of *Discocriconemella* typically stands out due to the absence of an annule in close proximity to the disc. The region immediately posterior to the labial disc has been referred to as a collar, neck, or constricted region (Orton Williams, 1981; Raski & Luc, 1987) which accentuates the appearance of the disc. Other characteristics used to define the genus have changed since its creation, but they generally include the lack of differentiation between adult and juvenile cuticle ornamentation and a small curved body with smooth or crenate annules devoid of scales (Orton Williams, 1981; Siddiqi, 2000).

There are 18 recorded species of Criconematidae associated with remnant prairies and woodlands in Iowa (Hoffman, 1974a; 1974b), many with overlapping distributions and indistinct species boundaries. Juveniles, which lack the diagnostic characters associated with the female reproductive system, are often the predominant life stage in soil collections. Application of nematodes as environmental indicators requires explicit means of species discrimination. In this report we first establish

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the identity of topotype specimens as *Disocriconemella inarata* based on comparisons with the original line drawings, measurements and the holotype specimen. Then we use molecular and morphological data for species delimitation and suggest reconsideration of *D. inarata* as a member of the genus *Discocriconemella*.

Materials and Methods

Nematode collection sites: Sheeder (N41 41.386', W94 35.236') and Kalsow Prairies (N42 34.416', W94 33.614') are located in the central and northwest portion of Iowa at 378m elevation. Both sites are managed by the Iowa Department of Natural Resources as part of the state Prairie Reserve System. Other collection sites utilized for this study are listed in Table 1.

Nematode sampling and extraction: Soil cores were taken using Oakfield tubes to a depth of 25cm, either in a focal sampling effort targeting specific plant species or as bulked soil samples within a 40 x 40m grid. Nematodes were extracted from soil by suspension and flotation of the nematodes in water followed by sieving over a nested set of standard sieves (60 mesh to remove debris, then nematode collection on 400 mesh) and sugar centrifugation (Jenkins, 1964).

Nematode examination: For specimens used in DNA analyses, a living nematode was placed into a drop of water for temporary slide construction. Nematodes were observed by differential interference contrast microscopy on a Leica DMLB microscope, images recorded by a Leica DC300 video camera, and measurements obtained using an eyepiece micrometer at 1000x magnification. After nematode measurement the slide was carefully dismantled, the nematode was recovered using a fine insect pin pick, added to an 18 ul drop of sterile ddH_2O, and then smashed on a cover slip with a clear, sterile micropipette tip. Nematode residue is stored in PCR reaction tubes in a -20°C freezer until PCR amplification. Scanning electron microscopy was conducted on a Hitachi S-3000N scanning electron microscope. For SEM formalin fixed nematodes were passed through a graded series of ethanol dehydration, followed by critical point drying and gold coating prior to examination.

DNA analysis: Residue from each individual nematode served as a template for DNA amplifications (Powers & Harris, 1993). In a few cases, a single nematode produced high quality amplification for multiple primer sets used in this study. Small subunit (18S) rDNA, internal transcribed spacer 1 (ITS1), and mitochondrial cytochrome b (cytb) amplifications were performed in 50 µl reactions, each containing: 31.5 µl distilled water, 5 µl 10x PCR buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 1 µl dNTP mixture (2.5 mM each of dATP, dCTP, dGTP, and dTTP), additional 1.5 µl 25 mM $MgCl_2$, 1.0 µl of each primer (20 µM), 2.5 µl of JumpStart REDTaq polymerase (Sigma, St. Louis, MO; 1.0 u/µl), and 5 µl of DNA template. All PCR reactions were performed on a DNA Engine PTC-200 Peltier thermal cycler (MJ Research, Inc. Watertown, Massachusetts) with the following run parameters: one initial denaturation cycle at 95°C for 3 min, followed by 50 cycles at 95°C for 15 sec, 55 or 50°C for 15 sec, ramped increase at 0.5 °C per sec to 72°C for 1 min. A final elongation step was run at 72°C for 5 min. Negative controls (i.e., no template DNA) were included in each amplification series. The following primers were used in this study: 18S1.2a: 5'-CGATCAGATACCGCCCTAG-3' (forward) with 18Sr2b: 5'-TACAAAGGGCAGGGACGTAAT-3' (reverse) amplify approximately 592 nucleotides of the 3' region of the small ribosomal subunit, not including primers. Primer 18Sr2b (positions 1567 to 1547) is the reverse complement of primer rDNA2 from Vrain *et al.* (1992). Annealing temperature for this 18S primer set was 55°C.

ITS1 primer rDNA2:5'-TTGATTACGTCCCTGCCCTTT-3' is a modified version of the reverse complement of 18Sr2b (above) and is paired with rDNA1.58Sa: 5'-ACGAGCCGAGTGATCCACC-3' which is located within the 5.8S rDNA gene. This primer set produces a fragment of approximately 560 nucleotides, excluding primers, of which 176 nucleotides are the 3' end of 18S rDNA. Annealing temperature for this ITS1 primer set was 55°C.

Cytb primers CytB1F: 5'-KDAATTTTGGKAGWWTWYTRGG-3' (forward) and CytB1R: 5'-AGCACGYAAAATWSCRTAAGC-3' (reverse) are degenerate versions of primers 1F and 1R published in Nieberding et al., (2005). Excluding primers this set produces a 680 nucleotide product coding for the mitochondrial gene, cytochrome b. Annealing temperature for this cytb primer set was 50°C.

PCR products were purified and concentrated with Microcon-100 centrifugal filter units (Millipore Inc., Bedford, Massachusetts). Purified DNA was sent to Davis Sequencing Lab (Davis, California) and DNA Sequencing Lab (University of Arkansas for Medical Sciences) for direct sequencing in both directions. Amplification primers were used as sequencing primers. SSU sequences were edited and assembled using Sequence Navigator (Applied Biosystems, Foster City, California) and CodonCode Aligner (CodonCode Corp, Dedham, Massachusetts). DNA alignment was by MUSCLE 3.7 (Edgar, 2004). Maximum likelihood analysis was carried out by PHYML 3.0 (http://www.phylogeny.fr) using approximate likelihood-ratio tests for the estimation of branch support (Anisimova & Gascuel, 2006). Bayesian inference was implemented in the MrBayes 3.1.2 program (Huelsenbeck & Ronquist, 2001) using bootstrap assessment for support.

Vouchers and web presentation: Specimens examined during this project are maintained as digital vouchers at the Criconematina Barcode website (http://nematode.unl.edu/CriconematidProject.htm), amplified DNA and specimen residue is retained at -20°C in the University of Nebraska Nematology Laboratory, and permanent slides from other specimens in the series (when available) are formalin-fixed and mounted in glycerin

TABLE 1. List of individual specimens used in this study, their collection localities, and their amplification products.

Nematode ID	Species ID	Stage[a]	Locality	18S[b]	ITS1[b]	CYTB[b]
307	*Bakernema inaequale*	female	Pachaug State Forest, CT	HM116040	o	o
119005	*Criconema permistum*	female	Sheeder Prairie, IA	FJ489519	o	o
119007	*Criconemoides* sp.	female	Pammel Woods, IA	FJ489521	o	o
226064	*Crossonema fimbriatum*	female	Niobrara River bank, NE	AY911952	o	o
6	*Discocriconemella inarata*	female	Kalsow Prairie, IA	FJ489596	HM116058	o
7	*Discocriconemella inarata*	female	Kalsow Prairie, IA	o	HM116059	o
9	*Discocriconemella inarata*	female	Kalsow Prairie, IA	o	HM116060	o
11	*Discocriconemella inarata*	female	Kalsow Prairie, IA	HM116011	HM116063	o
125027	*Discocriconemella inarata*		Kalsow Prairie, IA	o	HM116051	HM116088
125028	*Discocriconemella inarata*		Kalsow Prairie, IA	o	HM116052	HM116089
125029	*Discocriconemella inarata*		Kalsow Prairie, IA	o	HM116053	HM116090
150022	*Discocriconemella inarata*	juvenile	Kalsow Prairie, IA	o	o	HM116091
150023	*Discocriconemella inarata*	female	Kalsow Prairie, IA	o	HM116054	o
150032	*Discocriconemella inarata*	female	Kalsow Prairie, IA	FJ489563	HM116055	HM116092
150033	*Discocriconemella inarata*	female	Kalsow Prairie, IA	o	HM116056	HM116093
223094	*Discocriconemella inarata*	female	9-Mile Prairie, NE	o	HM116069	o
223095	*Discocriconemella inarata*	juvenile	9-Mile Prairie, NE	o	HM116070	o
077063	*Discocriconemella inarata*	female	9-Mile Prairie, NE	o	o	HM116077
077042	*Discocriconemella inarata*	female	9-Mile Prairie, NE	o	o	HM116078
138012	*Discocriconemella* sp.	juvenile	Costa Rica[1], Plot 803	EU880007	o	o
132010	*Discocriconemella* sp.	juvenile	Costa Rica[1], Plot 801	EU879991	o	o
184027	*Discocriconemella* sp.	female	Costa Rica[2]	FJ489553	o	o
150034	*Discocriconemella inarata*	female	Kalsow Prairie, IA	o	o	HM116094
AY284622	*Hemicriconemoides pseudobrachyurus*		GenBank	AY284622	o	o
EU669914	*Hemicycliophora conida*		GenBank	EU669914	o	o
226063	*Lobocriconema thornei*	female	Homestead Prairie, NE	AY911948	o	o
1	*Lobocriconema thornei*	female	Plattsmouth, NE	FJ489593	o	o
AY284629	*Loofia thienemanni*		GenBank	AY284629	o	o
119006	*Mesocriconema curvatum*	juvenile	Sheeder Prairie, IA	o	o	HM116082
124088	*Mesocriconema curvatum*		Polk Co., NE	o	o	HM116083
18	*Mesocriconema curvatum*	female	Chase Co., NE	o	HM116061	o
19	*Mesocriconema curvatum*	female	Williams Prairie, IA	HM116007	HM116062	o
26	*Mesocriconema curvatum*		Brookings, SD	o	HM116064	o
155077	*Mesocriconema curvatum*	female	Konza Prairie, KS	o	o	HM116095
223086	*Mesocriconema curvatum*	female	Lincoln Country Club, NE	AY919190	o	o
223090	*Mesocriconema curvatum*	female	9-Mile Prairie, NE	o	HM116065	o
223091	*Mesocriconema curvatum*		Konza Prairie, NE	o	HM116066	o
223092	*Mesocriconema curvatum*		Lincoln Country Club, NE	o	HM116067	o
223093	*Mesocriconema curvatum*	female	Lincoln Country Club, NE	o	HM116068	o
075035	*Mesocriconema curvatum*	female	Konza Prairie, KS	o	o	HM116076
077057	*Mesocriconema curvatum*	female	9-Mile Prairie, NE	o	o	HM116079
199024	*Mesocriconema curvatum*	female	Reichelt Remnant Prairie, IA	o	o	HM116097
124090	*Mesocriconema rusticum*		Waldo Co., ME	o	o	HM116084
124091	*Mesocriconema rusticum*		Waldo Co., ME	o	o	HM116085
199022	*Mesocriconema rusticum*	female	Lamoille Co., VT	FJ489580	o	o
223085	*Mesocriconema rusticum*	female	Lincoln Country Club, NE	AY919188	o	o
4	*Mesocriconema xenoplax*	female	Plattsmouth, NE	HM116002	HM116057	o
223089	*Mesocriconema xenoplax*	female	UC-Davis, CA *Type locality*	AY919192	o	o
AY284627	*Mesocriconema xenoplax*		GenBank	AY284627	o	o
AY284626	*Mesocriconema xenoplax*		GenBank	AY284626	o	o
074051	*Mesocriconema xenoplax*	female	UC-Davis, CA	o	o	HM116080
223096	*Mesocriconema xenoplax*	juvenile	Fresno, CA	o	HM116071	o
223097	*Mesocriconema xenoplax*	juvenile	Fresno, CA	o	HM116072	o
223098	*Mesocriconema xenoplax*		UC-Davis, CA	o	HM116073	o
074052	*Mesocriconema xenoplax*	female	UC-Davis, CA	o	o	HM116081
184020	*Mesocriconema* sp.	female	Costa Rica[1]	o	o	HM116096
151049	*Nothocriconemoides* sp.	female	Costa Rica[1]	FJ489536	o	HM116086
151052	*Nothocriconemoides* sp.	female	Costa Rica[1]	o	o	HM116087
223099	*Ogma decalineatum*	female	9-Mile Prairie, NE	o	HM116074	o
223100	*Ogma decalineatum*	juvenile	9-Mile Prairie, NE	o	HM116075	o
226065	*Ogma decalineatum*	female	9-Mile Prairie, NE	AY919221	o	o
29	*Ogma octangulare*		Mt. Philo, VT	HM116029	o	o
EU669919	*Ogma menzeli*		GenBank	EU669919	o	o
AY284630	*Paratylenchus straeleni*		GenBank	AY284630	o	o
193072	*Xenocriconemella macrodora*	female	Grinnell, IA	FJ489556	o	o
15	*Xenocriconemella* sp.	female	Minneapolis, MN	FJ489599	o	o

Costa Rica[1] –La Selva Biological Research Station; Costa Rica[2] –Las Cruces Biological Experiment Station.

[a] blank = undetermined.

[b] GenBank numbers for sequence used in phylogenetic trees; (o) sequence not available for phylogenetic analysis.

on Cobb slides. All slides are maintained at the University of Nebraska Nematology Collection housed in the Department of Plant Pathology.

The holotype slides of *Discocriconemella inarata* Hoffman, 1974 and *Mescocriconema discus* (Thorne & Malek, 1968) Loof & De Grisse, 1989 were loaned by the USDA Nematology Laboratory.

Results and Discussion

Taxonomy: Three lines of morphological evidence were used to determine that specimens collected at the type locality were indeed *Discocriconemella inarata*. A combination of light and scanning microscopy images of topotype specimens compared to the original line drawings of Hoffman (1974a) are presented in Figure 1. Generally good concordance is observed between the line drawings and recently collected specimens. The symmetry of the cephalic annules seen in face views (Figure 1B) and the configuration of the projections on the anterior lip of the vulva (Figure 1C) are strikingly similar in both line drawings and SEM images. Most important from a diagnostic perspective is the conformation of the cephalic region which is discussed in more detail below. Side by side profiles of heads and tails of topotype specimens with the *D. inarata* holotype are displayed in Figure 2. Again, overall good concordance was observed between the holotype and topotype specimens. Measurements from paratypes and topotype specimens are compared in Table 2. Included in this table are measurements from a second population of *D. inarata* from Sheeder Prairie described by Hoffman (1974a) as well as an isolate that Hoffman (1974b) referred to as the short-stylet form of *Mesocriconema xenoplax* (Raski, 1952) De Grisse & Loof, 1965. All measurements are consistent with the contention that topotype specimens are *D. inarata*. Recorded for the first time are measurements of *D. inarata* juveniles. Morphometrically they are nearly identical to *M. curvatum* (Raski, 1952) Loof & De Grisse, 1989 juveniles, however the two species can be differentiated based on the presence of faint crenations on annules of juvenile *M. curvatum* and the absence of submedian lobes in *D. inarata*.

Orton Williams (1981) mentioned that several of the morphological features of *Discocriconemella inarata*, smooth annules, a sigmoid vagina and open vulva, "remove it from most of the known species of *Discocriconemella*". The sigmoid vagina, mentioned as a diagnostic characteristic in the original description of *D. inarata* (Figure 2C, D) is also observed in *D. addisababa* Abebe & Geraert, 1995, *D. degrisse* Loof & Sharma, 1980, and *D. perseae* Cid Del Prado Vera & Loof, 1984. Topotype specimens of *D. inarata* possess this trait although variation exists among individuals with respect to the degree of curvature at either end of the canal. In the observation of living specimens, vaginas became more curved with the characteristic

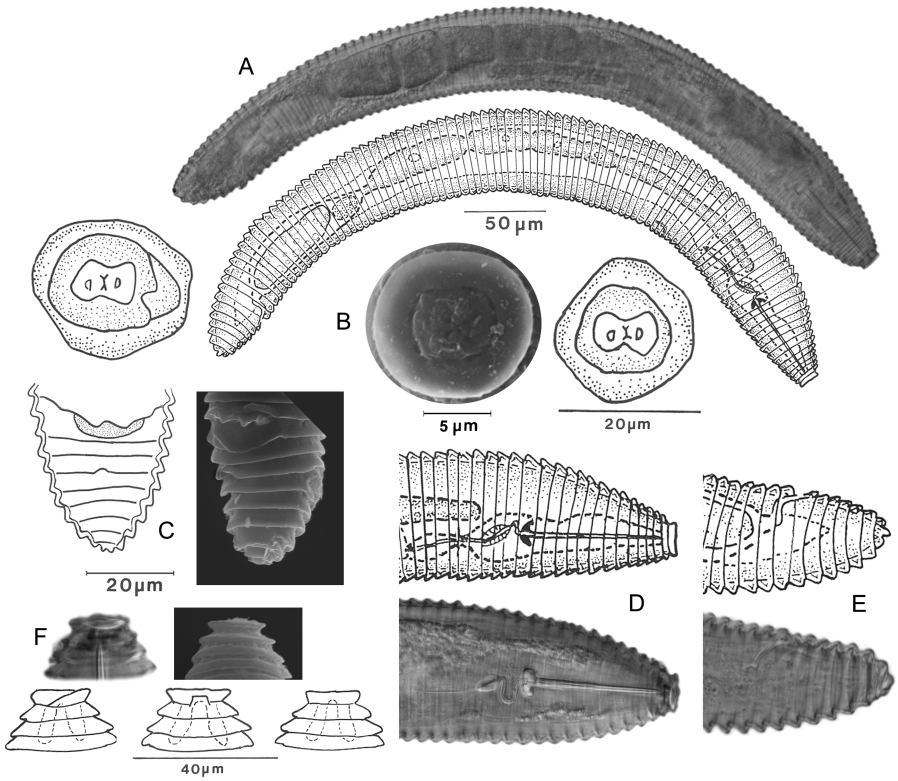

Fig. 1. A combination of light and scanning microscopy images of *Discocriconemella inarata* topotype specimens compared to line drawings of Hoffman (1974a). A. whole body of female. B. SEM face view of female. C. Female vulva and post vulval region. D. Female anterior profile. E. Female posterior and vagina. F. Light micrograph and SEM profiles of female cephalic region.

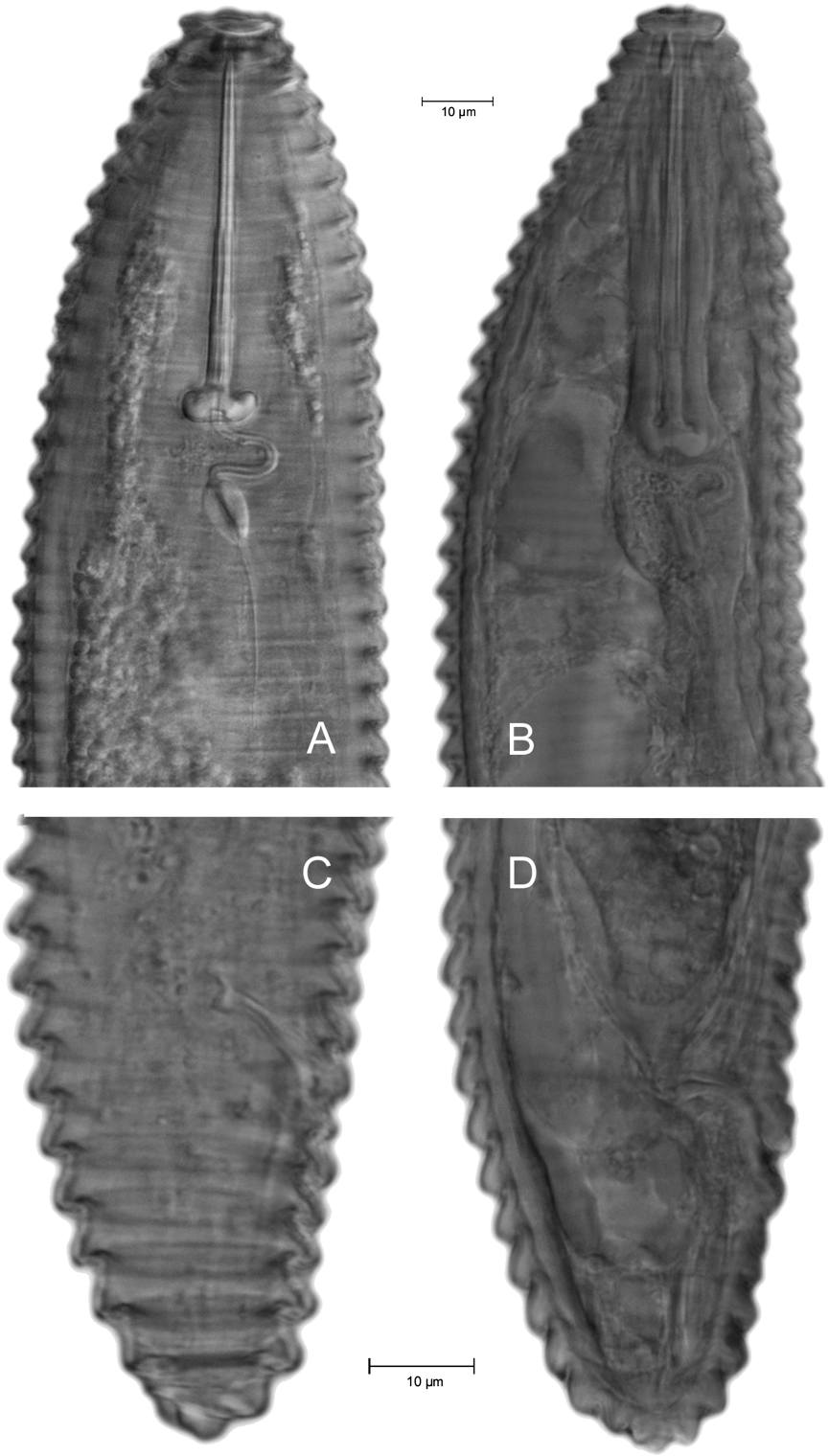

FIG. 2. Light micrograph comparison of *Discocriconemella inarata* holotype (2b, d) with topotype (2a, c) specimens from Kalsow Prairie, Iowa.

contraction of the posterior annules observed in criconematids. Both Orton Williams (1981) and Cid del PradoVera & Loof (1984) have remarked on the anomalous feature of the open vulva and the anterior bi-lobed or ornamented vulva lip as seen in *D. inarata* (Figures 1C and 3D). At least ten species of *Discocriconemella* are described as having an open vulva, although only *D. mineira* Vovlas, Ferraz & Santos, 1989, *D. morelensis* Cid Del Prado Vera & Loof, 1984, and *D. inarata* are described as possessing bilobed projections of the anterior vulval margin. *Discocriconemella* typically have smooth margins around the vulva lips (Figure 3C).

TABLE 2. Measurements of *Discocriconemella inarata*.

Discocriconemella inarata measurements (μm) and ratios	Holotype[a] ♀	Paratypes[a] ♀	Kalsow Prairie[b] ♀	Kalsow Prairie[b] juveniles	*Mesocriconema xenoplax* short stylet form[c] ♀	*Discocriconemella inarata*[a] Sheeder Prairie ♀	*Mesocriconema curvatum*[d] ♀
n		16	16	9	16	7	?
L	516	422 (354-486)	414.3± 68.6 (323-540)	373.3±43.9 (316-423)	464 (397-603)	427 (378-499)	303-452
V	93	92 (91-94)	91.9±1.2 (90-94)	na	93.8 (92.8-99.5)	93 (93-94)	90.8-96.3
R	104	93 (77-100)	96.1±3.6 (89-103)	97.2±5.1 (87-103)	99 (87-116)	90 (88-93)	50-88
Rv	9	9 (8-9)	7.5±0.6 (7-9)	na	8 (7-10)	8 (7-9)	6-10
Rex	-	26 (25-26)	25.5± 0.8 (24-27)	28.0	27 (24-29)	25	21-29
Lsty	57	55 (51-61)	57.4±2.9(53-62)	46.9±1.7 (45-49)	52 (48-56)	55 (52-58)	47-67
Leso	-	-	99.7±11.0 (80-125)	86+6.2 (75-95)	-	-	-
Lpv	-	-	32.7±4.9 (25-45)	-	-	-	-
mbw	-	-	49.1±6.9 (35-62)	45.5±6.0 (37-56)	-	-	-
vbw	-	-	33.6±5.2 (25-45)	-	-	-	-
a	9.9	9.1 (7.1-11.3)	8.6±1.9 (5.7-12.9)	8.6±1.3 (7.1-10.3)	-	10.0 (9.2-10.8)	8.5-12.9
b	4.7	4.2 (3.6-5.6)	4.2±0.7 (2.8-5.4)	4.3±0.4 (3.6-4.8)	-	4.2 (3.8-4.8)	3.2-4.5
Lpv/vbw	-	-	1.0±0.2 (0.7-1.3)	-	-	-	-
st % L	-	-	14.2±2.4 (10.2-18.5)	12.7±2.1 (10.6-15.3)	-	-	-

L = body length, V = ratio of length to vulva by body length, R = number body annules, Rv = number of body annules between vulva and body terminus, Rex = number of annules from anterior end to excretory pore, Lsty = stylet length, Leso = anterior end to base of esophagus, Lpv = length posterior to vulva, mbw = mid body width, vbw = vulva body width, a = length/mbw, b = length/Leso, st % L = stylet length x100/body length

[a] Hoffman 1974a.
[b] this study.
[c] Hoffman 1974b.
[d] Raski 1952.

Two characteristics best observed by SEM also indicate *D. inarata* is distinct when compared with the majority of species in *Discocriconemella*. First, the cephalic annule in *D. inarata* is not dramatically offset or separated from the first body annule. SEM profiles of *D. inarata* topotypes exhibit a smaller "collar" when compared to specimens conforming to *Discocriconemella limitanea* (Luc, 1959) De Grisse & Loof, 1965 from Costa Rica (Figure 3A, B). The *en face* view of *D. inarata*, exhibits a hexagonal labial plate surrounded by a circular cephalic annule with smooth edges (Figure 4A, C). Loof & De Grisse (1989) refer to a similar character state in *Xenocriconemella macrodora* (Taylor, 1936) De Grisse & Loof, 1965 (Figure 4E) in which a small cephalic annule is surrounded and enveloped by a much larger second annule. There is no evidence of submedian lobes or pseudo-lobes in *D. inarata*. Submedian lobes are a defining characteristic of *Mesocriconema* Andrássy, 1965 and generally can be observed in lateral view at 1000x by light microscopy. Submedian lobes are present in *M. curvatum* (Raski, 1952) Loof & De Grisse, 1989 and *M. xenoplax* (Raski, 1952) Loof & De Grisse, 1989, but their absence in *D. inarata* is not always easy to verify unless *en face* views are available. Three species of *Discocriconemella*, *D. degrissei* Loof & Sharma, 1980, *D. mineira* Vovlas, Ferraz, & dos Santos 1989, and *D. morelensis* Cid del Prado Vera & Loof, 1984 are reported to possess weakly developed or rudimentary submedian lobes (Vovlas, 1992).

DNA Sequence comparison: DNA comparison of the 3' barcode region of the 18S ribosomal gene revealed a perfect match, 635/635 identical nucleotides for *Discocriconemella inarata* and *Mesocriconema xenoplax* (Figure 5). This is in sharp contrast to the comparison between *D. inarata* and *D. limitanea* (Luc, 1959) De Grisse & Loof, 1965, the type species of the genus. When *D. inarata* is compared with specimens of *D. limitanea* from Costa Rican lowland rain forests, 614/635 to 618/635 nucleotides are shared across this 18S region. Two isolates from the type locality of *M. xenoplax*, one from culture at University of California, Davis (Table 1, Nematode ID#223089) and the other from a vineyard near Fresno, CA field collection (AY146454) were identical in this genetic marker to *D. inarata*. A third isolate of *M. xenoplax* from a hardwood forest near Plattsmouth, Nebraska (ID#4) also identically matched the 3'-18S barcode of *D. inarata*. *M. curvatum*, however, exhibited 5 fixed unique nucleotide polymorphisms in this marker when compared with *M. xenoplax* and *D. inarata*. All specimens identified as *Mesocriconema curvatum* based on morphology and the presence of medium sized submedian lobes had identical sequence for the 18S barcode. Representatives of this species were obtained from native prairies throughout the Great Plains including Kalsow Prairie, as well as agricultural fields and golf course turf grass within the region. A 50% majority rule Bayesian consensus tree of the 3'-18s region shows *D. inarata*, together with *M. xenoplax*, nested within larger *Mesocriconema* clades suggesting that the evolutionary affinities of *D. inarata* are more closely aligned with *Mesocriconema* than *Discocriconemella* (Figure 5).

Application of two other genetic markers provided a higher resolution analysis of taxonomic relationships among these species and provided support for the delimitation of *Discocriconemella inarata* (Figure 6A, B). The maximum likelihood analysis of ITS1 and cytochrome b exhibited strong likelihood-ratio support for *D. inarata*

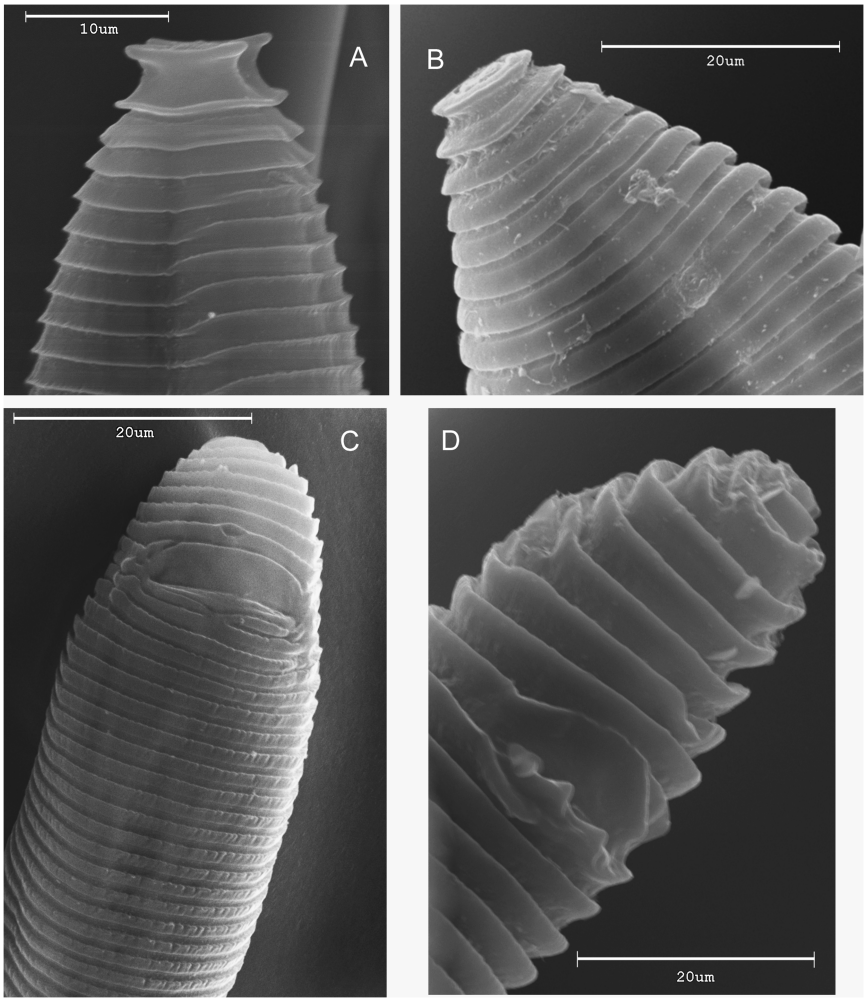

FIG. 3. Scanning electron micrograph images of *Discocriconemella limitanea* anterior profile (3a) and female ventral view of *D. limitanea* vulva (3c) compared to anterior profile (3b) and vulva (3d) of *D. inarata* from Kalsow Prairie.

as a monophyletic group, 0.96 and 1.0 respectively. For the ITS1 primer set (Figure 6A), three nucleotide substitutions were fixed and "pure" for *D. inarata* (present in all specimens and not found in the other *Mesocriconema* species in the dataset – see DeSalle *et al.*, 2005), and in the cytochrome b marker 21 pure characters were shared by all *D. inarata* specimens (Figure 6B). Two specimens from 9-mile Prairie in Lincoln, Nebraska were grouped together in the *D. inarata* clade by both sets of characters, expanding the observed distribution of this species.

Using the cytochrome b marker, moderate likelihood-ratio support existed for *Mesocriconema xenoplax* as sister species to *Discocriconemella inarata* (0.72) and for monophyly of *M. curvatum* (0.55). There were 17 fixed nucleotides in the cytb dataset that diagnostically supported the *M. xenoplax* topotype isolate as representing a unique evolutionary linage. The maximum likelihood tree for ITS1 displayed paraphyletic relationships for both *M. curvatum* and *M. xenoplax*. Specimens identified as *M. xenoplax* from Europe (AY284625, AY284626, AY284627) did not group with North America *M. xenoplax* specimens using the 18S primer set. The possibility of additional identifiable lineages existing within *M. xenoplax* and *M. curvatum* clades needs to be examined by sampling across the known ranges of both species.

Taxonomic conclusions: D. inarata does not appear to belong to *Discocriconemella*. DNA sequence of ribosomal DNA indicates that the species is distantly related to *Discocriconemella* and more closely related to *Mesocriconema xenoplax* and other species of *Mesocriconema* (Powers *et al.*, 2009). Orton Williams (1981) noted that the smooth body annules, sigmoid vagina, and open vulva of *D. inarata* distance it from other members of that genus. Cid del Prado Vera & Loof (1984) referred to its "*Criconemella* characteristics" of an open vulva with ornamented anterior lip and large-sized body. The close relationship between these species is problematic from a taxonomic perspective because *D. inarata* does not appear to have submedian lobes, the defining character of *Mesocriconema*. It may be possible that they are reduced in size and obscured by the large cephalic disc-like annule. However, face views of living specimens and *en face* observation of the original description show no evidence of submedian lobes (Hoffman, 1974a; Powers personal observation). Therefore we hypothesize that the submedian

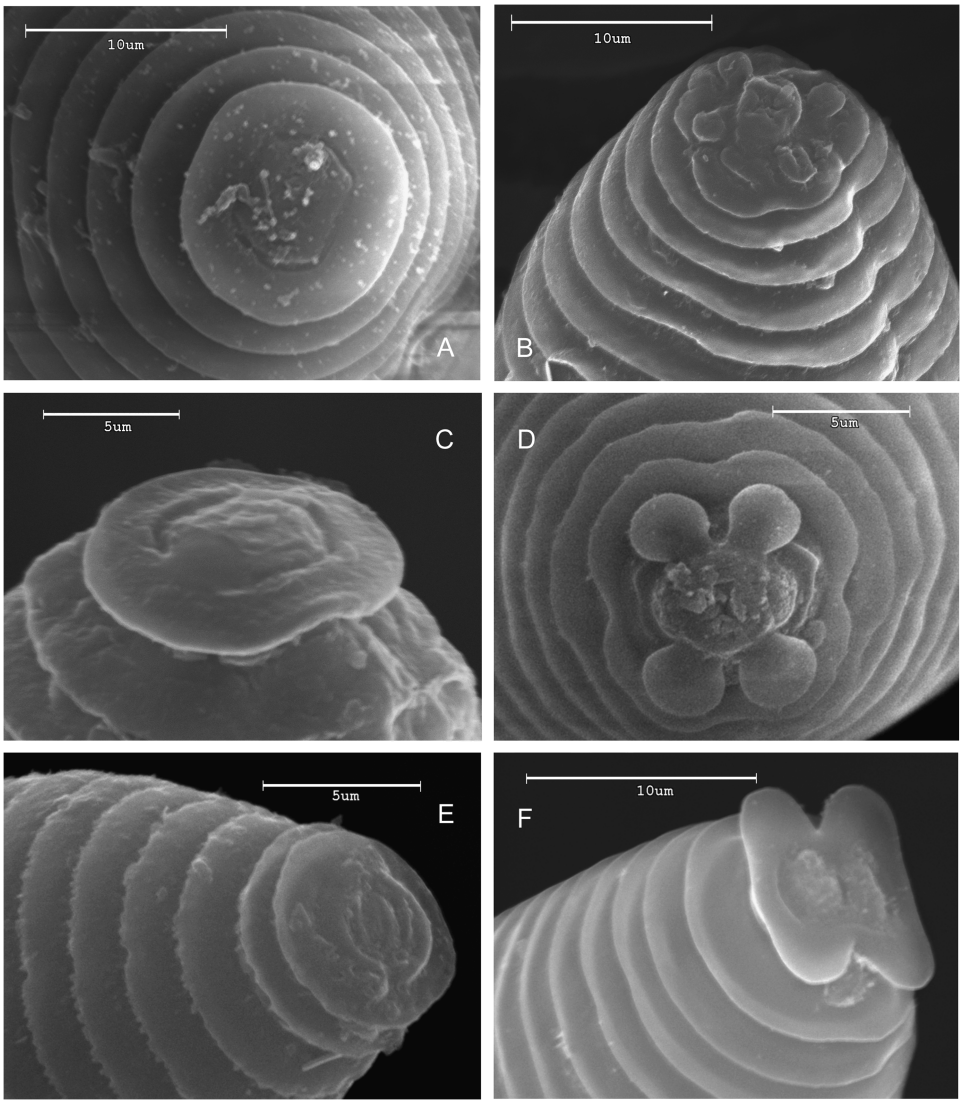

FIG. 4. Scanning electron micrograph images of *D. inarata* face view (4a, c) and face views of *Mesocriconema curvatum* (4b), *M. rusticum* (4d), *Xenocriconemella macrodora* (4e) and *Discocriconemella limitanea* (4f). Note hexagonal labial plate and lack of submedian lobes in *D. inarata*. Submedian lobes are conspicuous on *M. curvatum*, strongly developed on *M. rusticum*, and absent in *X. macrodora* and *D. limitanea*.

lobes of *D. inarata* were secondarily lost, and the placement of *D. inarata* into the genus *Discocriconemella* was primarily based on the homoplasy of a greatly enlarged first cephalic annule.

DNA sequence of ITS1 and cytochrome b clearly demonstrate that *Discocriconemella inarata* is distinct from *Mesocriconema xenoplax*, *M. curvatum*, and *M. rusticum* (Micoletzky, 1915) De Grisse & Loof, 1965, but part of a larger *Mesocriconema* clade. Ebsary (1982) considered *D. inarata* a synonym of *Criconemella discus* (Thorne & Malek, 1968) Luc and Raski, 1981 based on an apparent discrepancy between the original description of *C. discus* and the key provided for *Criconemoides* in that publication (Thorne & Malek, 1968). The discrepancy centers on the interpretation of Thorne's statement: "First labial annule forming 4 broad, flat lobes, definitely set off by a narrow annule." Thorne's key requires a choice of "lip regions without sublateral lobes" in order to proceed to *C. discus*. Ebsary (1982) wrote "Thorne & Malek (1968) stated that large submedian lobes were present in *Criconemoides discus* but their diagrams did not illustrate these structures." We have examined the holotype and paratype specimens of *C. discus* (slide Criconemoides 3, Thorne & Malek, 1968) and observed that the first cephalic annule is arranged in four parts and clearly dissimilar to the continuous cephalic disk that surrounds the labial plate in *D. inarata*. Also the four adult females on the slide had stylet lengths that averaged 68 um, outside the upper range of *D. inarata*. Soil samples from the type locality in South Dakota recovered *M. curvatum*, but no specimens that resembled *M. discus*. We believe *M. discus* should be considered a valid species, but efforts should be made to collect fresh material suitable for DNA analysis.

The original description of *D. inarata* reads as follows:

Only females were found. The ventrally curved body is widest in the anterior one-third. The head is comprised of a single offset disk-shaped

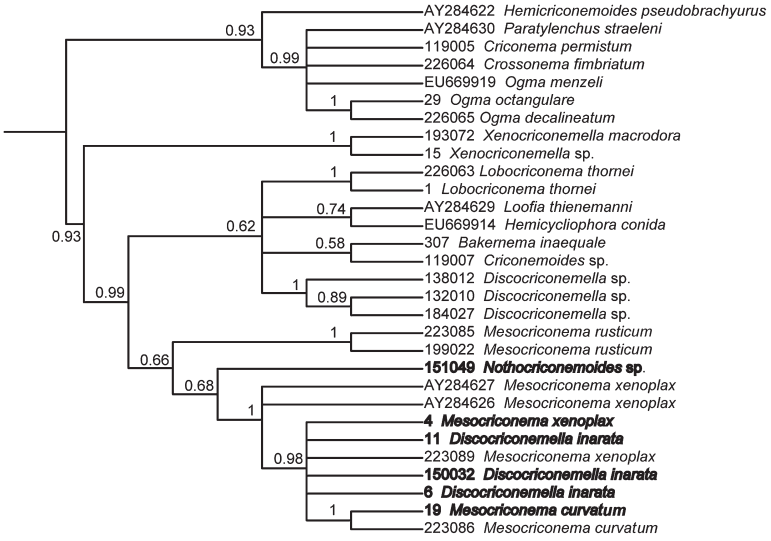

FIG. 5. Bayesian inference 50% majority rule consensus tree of 18S 3' region of criconematid specimens. Terminal nodes of branches are labeled with nematode ID numbers that correspond to information presented in Table 1. Species names are applied according to the combined molecular and morphological characters. Numbers at nodes are bootstrap support values.

anteriorly directed annule. The head annule may be complete or discontinuous. Sublateral lobes are absent. The body annules are retorse with smooth posterior edges and occasional anastomoses. A typical criconematoid esophagus is present. The tip of the single, outstretched ovary often extends anterior to the basal bulb. A spermatheca, usually containing sperm, is located in the anterior portion of the uterus. The anterior vulval lip is bilobed. The vagina is sigmoid and the vulva is open.

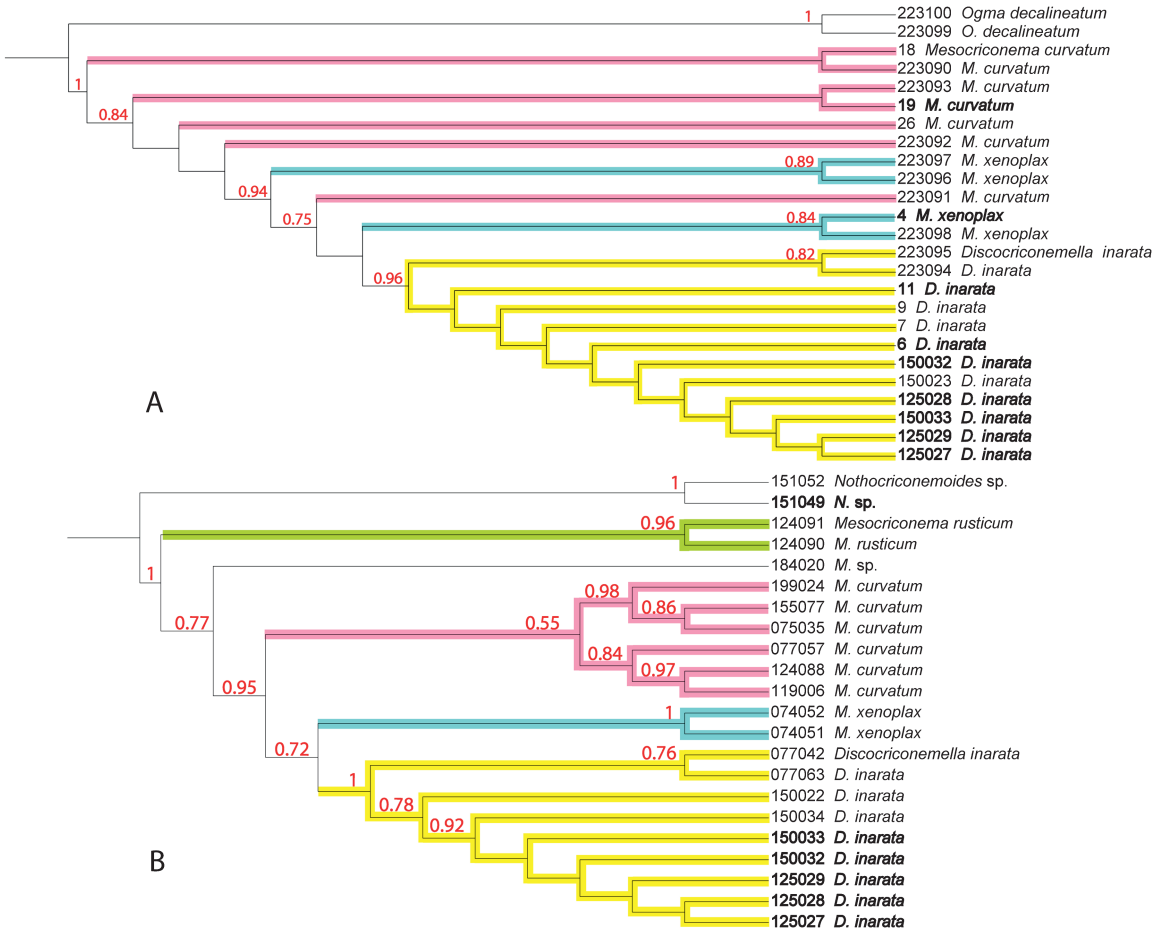

FIG. 6. Maximum likelihood tree of internal transcribed spacer 1 (ITS1) of the nuclear ribosomal gene region (A) and cytochrome-b of the mitochondrial genome (B) of criconematid specimens. Species names are applied according to the combined molecular and morphological characters. Numbers at nodes are approximate likelihood-ratio test support values.

The species description should be emended to include the following characteristics. Females are generally less than 0.5 mm in length, with a disc-like first cephalic annule surrounding a hexagonal labial plate that is flush with the disc surface. There are no apparent submedian lobes. The first cephalic annule is smaller in circumference than the second cephalic annule, and not separated by a distinct neck or collar. Annules of the adults are smooth, usually with a single anastomosis, but occasionally specimens were observed with as many as seven anastomoses. Annules of juveniles usually have crenate posterior edges, although later stages may possess crenations only on the posterior third of the body. The anterior vulval lip of the female has two posterior-directed pointed projections. While it is clear that *D. inarata* does not belong in the genus *Discocriconemella*, we feel it is prudent to conduct a more extensive taxonomic analysis of *Mesocriconema* species before recommending nomenclatural changes.

Ecology: The host associations of *Discocriconemella inarata* are not well established. Bulk soil sampling under the predominant native grasses, *Andropogon gerardii* Vitman and *Sporobolus heteroleptis* (Gray) Gray recovered a low frequency of *D. inarata* mixed with *Mesocriconema curvatum* and *M. rusticum*. The density of prairie plant roots and the diversity of plant species, 81 species recorded in a 1999 survey of Kalsow prairie (Dornbush, 2004), make it difficult to exclude the possibility that *D. inarata* is specifically associated with a minor component of the plant community. *Lathyrus venosus*, the legume recognized as a host for *D. inarata* from Sheeder Prairie by Hoffmann (1974a) has disappeared from Kalsow Prairie where in a 1949 survey of the plant community *L. venosus* was present in 5% of the $1m^2$ quadrats sampled (Moyer, 1953). Neither the legume nor the nematode was found in our repeated sampling of Sheeder Prairie. These results suggest that specific nematode-host associations may exist within native prairies and that soil nematode diversity may be linked to the diversity of the plant community.

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