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Species Delimitation and Description of *Mesocriconema nebraskense* n. sp. (Nematoda: Criconematidae), a Morphologically Cryptic, Parthenogenetic Species from North American Grasslands

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Abstract: Nematode surveys of North American grasslands conducted from 2010 to 2015 frequently recovered a species of criconematid nematode morphologically resembling *Mesocriconema curvatum*. These specimens were recovered from remnant native prairies in the central tallgrass ecoregion of North America, and not from surrounding agroecosystems. Historical records indicate that *M. curvatum* is a cosmopolitan species feeding on a wide range of agronomic and native plants. DNA barcoding indicates North American grasslands contain at least 10 phylogenetically distinct lineages of *Mesocriconema* that resemble, but are not, *M. curvatum*. Analysis of the two most common lineages reveals two distinctly different population structures. The variation in population structure suggests unique evolutionary histories associated with their diversification. These two major lineages share a sympatric distribution and their slight morphological differences contrast with a high level of genetic separation. Based on their genetic divergence, fixed diagnostic nucleotides, population structure, species delimitation metrics, and a sympatric distribution, we believe that one of these distinct lineages warrants formal nomenclatural recognition. Herein, we provide formal recognition for *Mesocriconema nebraskense* n. sp. and discuss its relationship to other *Mesocriconema* lineages discovered in native North American grasslands.

Key words: biogeography, cryptic species, nematode distribution, network analysis, plant-parasitic nematodes, tallgrass prairies, taxonomy, phylogeny.

During the course of soil surveys conducted to assess nematode biodiversity in native grasslands of North America, a single criconematid morphospecies was found to be widespread and abundant. Using dichotomous and synoptic keys (Hoffmann, 1974; Brzeski, 2002; Geraert, 2010), this morphospecies was identified as *Mesocriconema curvatum* (Raski, 1952) Loof and De Grisse, 1989. This plant-parasitic taxon has previously been recognized as a component of the nematode community in central tallgrass prairies (Norton and Ponchillia, 1968; Schmitt and Norton, 1972; Norton, 1978; Powers et al., 2010). Historically, it was recorded as associated with a broad range of hosts from monocots to dicots, annual herbaceous plants to conifer and hardwood trees, and in agroecosystems as well as native plant communities. Geographically, *M. curvatum* has been reported from six continents in both tropical and temperate climates. Judging from these reports, either this species is an extreme generalist, adapted to a multitude of environments and plant hosts, or *M. curvatum* as recorded in the literature is actually a composite of morphologically similar but genetically distinct lineages, possibly cryptic species (Fontaneto et al., 2008; Nadler and De León, 2011; Ristau et al., 2013; Palomares-Rius et al., 2014).

In a previous taxonomic analysis of species of *Mesocriconema* Andrássy, 1965, from North America, molecular and morphological analyses were used to differentiate formally described members of the genus as well as lineages lacking a formal description (Powers et al., 2014). With few exceptions, the specimens collected from native grasslands were determined to be phylogenetically distinct from specimens found in agroecosystems, with the agricultural group of specimens judged to most closely conform to the original description of *M. curvatum* (Powers et al., 2014). The question remained, however, as to the taxonomic status of the grassland specimens. In this study, we use a species concept that recognizes species as separately evolving metapopulation lineages (De Queiroz, 2007) and apply species delimitation methods that incorporate character state and genetic distance analyses (DeSalle et al., 2005; Pons et al., 2006; Wiens, 2007; Puillandre et al., 2012). The lineages discovered during these analyses appear to represent members of a group of morphologically similar, parthenogenetic species endemic to North American grasslands. Two of the lineages were geographically widespread across the central grasslands and were usually abundant when found in remnant prairies. In an earlier study, these two lineages were referred to as haplotype group (hg) 18 and hg 24 (Powers et al., 2014). In the current study, the number of grassland collection sites with positive finds has been expanded to 35, and the number of specimens studied of hg 18 and hg 24 has been increased to 98 and 100 specimens, respectively. Negative collection sites helped define the geographic limits of these groups. A statistical parsimony network analysis provided information about the population structure and haplotype relationships for both lineages. In this study, hg 18 is deemed deserving of a unique, formal species

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description based on phylogenetic clade support, reciprocal monophyly, genetic distance metrics, species delimitation analyses, and discriminant function analyses (DFA) that provide evidence of a degree of morphological distinction. Haplotype group 24, while exhibiting some of the same characteristics suggesting lineage distinction, is not as readily delineated and requires additional study before formal nomenclatural recognition.

Materials and Methods

Nematode collection: Nematodes were obtained from soil samples collected using a standardized collection procedure to facilitate consistent and optimal recovery between sites (Neher et al., 1995). Soil cores were taken randomly within a 40 × 40 m grid using Oakfield tubes with a 3-cm diameter at a maximum depth of 30 cm. Samples consisted of approximately 1,000 cc of bulked soil from a single grid and were stored at 8°C until nematodes from a 200-ml subsample were isolated using a modified flotation-sieving and centrifugation method (Jenkins, 1964).

Samples included in this study were collected from 35 sites recorded in Table 1 and Fig. 1. The collection sites represent 13 ecoregions as designated by the World Wildlife Fund (Olson et al., 2001). Twenty-eight of these sites were native prairie remnants managed to maintain native plant diversity. The most intensively sampled ecoregion was the central tallgrass prairie with 14 sites. Other grassland ecoregions were the Flint Hills tall grasslands, Texas Blackland prairies, and central and southern mixed grasslands. Two of the study sites, Nine-Mile Prairie and Spring Creek Prairie of the central tallgrass ecoregion, were sampled intensively over multiple years and seasons during the survey. Several other sampling sites were not officially designated as grasslands; nonetheless, they had a substantial grass component. These included grassy balds of the Smoky Mountains (Jenkins, 2007), southern longleaf pine–wiregrass communities, dunegrass communities along the Atlantic Coast (Noss, 2013), and northern oak savannas in Wisconsin and Minnesota (Hoffman, 2002).

Datasets: Six datasets are discussed in this study:

1) A 608-specimen dataset of COI DNA sequences of all *Mesocriconema* collected in North America.
2) A 191-specimen dataset of COI DNA sequences reduced to unique sequences by removing all redundant sequences from the 608 dataset. This dataset was used in analyses to generate Fig. 2.
3) A 74-specimen dataset of unique COI DNA sequences from hg 18 to 27 (Fig. 3).
4) A 256-specimen dataset of COI DNA sequences that include all of the specimens that formed hg 18 to 27. This dataset was used in network analyses, population analyses, and geographic distribution.
5) A 36-specimen dataset of internal transcribed spacer 1 (ITS1) DNA representing members of hg 18 to 27 as determined by COI DNA analysis (Fig. 4).
6) A 161-specimen dataset of all female specimens in hg 18, 19, 24, and 25. This dataset was used in morphological analyses including the DFA (Fig. 5).

Microscopic analysis and documentation: Nematodes isolated from soil were first examined using a dissecting stereomicroscope, and specimens recognized as belonging to Criconematina were handpicked for compound light microscope analysis. Individual nematodes were mounted on glass slides, measured, and digitally photographed using a Leica DMLB light microscope with differential interference contrast optics and a Leica DC300 video camera. A set of 16 standard measurements were taken on individual specimens allowing for the combined retention of morphological characters and molecular data. Both adult females and juveniles were subjected to morphological and molecular analyses, but only adult females were included in morphological comparisons for species delimitation. No male *Mesocriconema* specimens were encountered in this study. Nematodes were prepared for scanning electron microscopy (SEM) by fixation in 4% formalin followed by dehydration in a graded series of alcohol to 100% ethyl alcohol, critical point drying, mounting on SEM specimen stubs, and coating with gold. Images were obtained on a Hitachi S-3000N scanning electron microscope. Microscopic images of all specimens were stored in an in-house database in the Department of Plant Pathology at University of Nebraska-Lincoln, and images of specimens with DNA barcodes have been deposited in the Barcode of Life Database (http://v4.boldsystems.org/).

Polymerase chain reaction amplification: Following documentation, nematodes were removed from slides and crushed in 18 µl of sterile deionized distilled water using the tip of a micropipette and transferred to a polymerase chain reaction (PCR) microfuge tube. DNA was amplified by PCR and sequenced as described by Powers et al. (2014). The COI primer sequences COI-F5 (5′-AATWTWGGTGTTG GAACTTCTTGAAC-3′) and COI-R9 (5′-CTTAAAA CATAATGRAAATGWGCWACWACATAATAAGTATC-3′) resulted in an approximately 790-base pair (bp) amplification product. Following removal of primer sequences, 721 bp of sequence were used in genetic analyses. Near complete 18s ribosomal DNA sequence was obtained for representative *Mesocriconema* specimens using the following two primers sets: 18s39F (5′-AAAGATTAAGCCATGCATG-3′) and 18s977R (5′-TTTACGGTTAGAACTAGGGCGG-3′) produce a 0.97-kb amplification product which is reduced to 951 bp when primers are trimmed off. The second set, 18s900F (5′-AAGACGGACTACAGCGAAAG-3′) and 18s1713R (5′-TCACCTACAGCTACCTTGTTACG-3′)

TABLE 1. Specimen location data for *Mesocriconema nebraskense* n. sp. and other *Mesocriconema* spp.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
18	600	*M. nebraskense*	F	Aurora Prairie, SD[b]	Central Tall Grasslands	COI	KJ787959
18	825	*M. nebraskense*	F	Sheeder Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787960
18	827	*M. nebraskense*	J	Sheeder Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787961
18	828	*M. nebraskense*	F	Sheeder Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787962
18	829	*M. nebraskense*	F	Sheeder Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787963
18	832	*M. nebraskense*	F	Kalsow Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787964
18	833	*M. nebraskense*	F	Kalsow Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787965
18	835	*M. nebraskense*	J	Kalsow Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787966
18	836	*M. nebraskense*	F	Kalsow Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787967
18	842	*M. nebraskense*	F	Doolittle Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787968
18	843	*M. nebraskense*	J	Doolittle Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787969
18	845	*M. nebraskense*	F	Doolittle Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787970
18	884	*M. nebraskense*	F	Doolittle Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ787971
						ITS1	KY574859
18	888	*M. nebraskense*	F	Reichelt Unit, Rock Creek State Park, IA[b]	Central Tall Grasslands	COI	KJ787972
18	1050	*M. nebraskense*	J	Stafford County, KS[d]	Central and Southern Mixed Grasslands	COI	KJ787973
18	1074	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787974
						ITS1	KY574860
18	1091	*M. nebraskense*	J	Stafford County, KS[d]	Central and Southern Mixed Grasslands	COI	KJ787975
18	1092	*M. nebraskense*	F	Stafford County, KS[d]	Central and Southern Mixed Grasslands	COI	KJ787976
18	1093	*M. nebraskense*	J	Stafford County, KS[d]	Central and Southern Mixed Grasslands	COI	KJ787977
18	1094	*M. nebraskense*	F	Stafford County, KS[d]	Central and Southern Mixed Grasslands	COI	KJ787978
18	1112	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787979
18	1246	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787980
18	1265	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787981
18	1313	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787982
18	1315	*M. nebraskense*	J	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787983
18	1318	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ787984
18	1343	*M. nebraskense*	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ787985
18	1352	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ787986
18	1374	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787987
18	1378	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787988
18	1379	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787989
18	1380	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787990
18	1386	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787991
18	1405	*M. nebraskense*	J	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574661
18	1407	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574662
18	1411	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574663
18	1413	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787992
18	1415	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787993
18	1429	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787994
18	1430	*M. nebraskense*	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787995
18	1433	*M. nebraskense*	J	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787996
18	1434	*M. nebraskense*	J	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ787997

(*Continued*)

TABLE 1. Continued.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
18	2815	*M. nebraskense*	F	Nine-Mile Prairie Adjoining Land, NE[e]	Central Tall Grasslands	COI	KY574664
18	2816	*M. nebraskense*	F	Nine-Mile Prairie Adjoining Land, NE[e]	Central Tall Grasslands	COI	KY574665
18	2817	*M. nebraskense*	F	Nine-Mile Prairie Adjoining Land, NE[e]	Central Tall Grasslands	COI	KY574666
18	2822	*M. nebraskense*	F	Nine-Mile Prairie Adjoining Land, NE[e]	Central Tall Grasslands	COI	KY574667
18	2832	*M. nebraskense*	F	Nine-Mile Prairie, NE[c]	Central Tall Grasslands	COI	KY574668
18	2865	*M. nebraskense*	F	Konza Prairie Biological Station, KS[b]	Flint Hills Tall Grasslands	COI	KY574669
18	2885	*M. nebraskense*	F	Konza Prairie Biological Station, KS[b]	Flint Hills Tall Grasslands	COI	KY574670
18	2886	*M. nebraskense*	J	Konza Prairie Biological Station, KS[b]	Flint Hills Tall Grasslands	COI	KY574671
18	2895	*M. nebraskense*	J	Konza Prairie Biological Station, KS[b]	Flint Hills Tall Grasslands	COI	KY574672
18	2927	*M. nebraskense*	J	Aurora Prairie, SD[b]	Central Tall Grasslands	COI	KY574673
18	2928	*M. nebraskense*	F	Aurora Prairie, SD[b]	Central Tall Grasslands	COI	KY574674
18	2997	*M. nebraskense*	F	Cades Cove, Great Smoky Mountains National Park, TN[d]	Appalachian-Blue Ridge Forests	COI	KY574675
18	3000	*M. nebraskense*	F	Cades Cove, Great Smoky Mountains National Park, TN[d]	Appalachian-Blue Ridge Forests	COI	KY574676
						ITS1	KY574861
18	3031	*M. nebraskense*	F	Red Rock Prairie Preserve, MN[b]	Flint Hills Tall Grasslands	COI	KY574677
						ITS1	KY574862
18	3080	*M. nebraskense*	F	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574678
18	3086	*M. nebraskense*	F	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574679
						18S	KY574844
18	3101	*M. nebraskense*	F	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574680
						ITS1	KY574863
18	3111	*M. nebraskense*	F	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574681
18	3139	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574682
18	3144	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574683
						ITS1	KY574864
18	3145	*M. nebraskense*	J	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574684
18	3146	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574685
18	3155	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574686
18	3156	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574687
18	3159	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574688
18	3162	*M. nebraskense*	J	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574689
18	3165	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574690
						ITS1	KY574865
18	3166	*M. nebraskense*	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574691
18	3230	*M. nebraskense*	F	Midewin National Tallgrass Prairie, IL[c]	Central Forest-Grassland Transition Zone	COI	KY574692
18	3231	*M. nebraskense*	F	Midewin National Tallgrass Prairie, IL[c]	Central Forest-Grassland Transition Zone	COI	KY574693
18	3236	*M. nebraskense*	F	Midewin National Tallgrass Prairie, IL[c]	Central Forest-Grassland Transition Zone	COI	KY574694
18	5506	*M. nebraskense*	J	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574695
						18S	KY574845
18	5944	*M. nebraskense*	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574696
18	5948	*M. nebraskense*	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574697
18	5950	*M. nebraskense*	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574698
18	5955	*M. nebraskense*	F	Homestead National Monument, NE[b]	Central Tall Grasslands	COI	KY574699

(*Continued*)

TABLE 1. Continued.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
18	5957	*M. nebraskense*	F	Homestead National Monument, NE[b]	Central Tall Grasslands	COI	KY574700
18	7006	*M. nebraskense*	F	Prairie Pines, NE[b]	Central Tall Grasslands	COI	KY574701
18	7013	*M. nebraskense*	F	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574702
18	7014	*M. nebraskense*	F	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574703
18	7016	*M. nebraskense*	F	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574704
18	7017	*M. nebraskense*	J	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574705
18	7019	*M. nebraskense*	F	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574706
18	7028	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574707
18	7030	*M. nebraskense*	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574708
18	7035	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574709
18	7036	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574710
18	7037	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574711
18	7038	*M. nebraskense*	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574712
18	7039	*M. nebraskense*	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574713
18	7041	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574714
18	7042	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574715
18	7043	*M. nebraskense*	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574716
18	7044	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574717
18	7045	*M. nebraskense*	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574718
18	P77025	*M. nebraskense*	F	Konza Prairie Biological Station, KS[b]	Flint Hills Tall Grasslands	COI	KJ787998
19	919	*Mesocriconema* sp.	J	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ787999
19	920	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788000
19	943	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788001
19	1168	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788002
19	1169	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788003
19	1170	*Mesocriconema* sp.	J	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788004
19	2996	*Mesocriconema* sp.	F	Cades Cove, Great Smoky Mountains National Park, TN[d]	Appalachian-Blue Ridge Forests	COI	KY574719
						ITS1	KY574866
19	2999	*Mesocriconema* sp.	F	Cades Cove, Great Smoky Mountains National Park, TN[d]	Appalachian-Blue Ridge Forests	COI	KY574720
19	3190	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574721
19	3191	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574722
19	5504	*Mesocriconema* sp.	J	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574723
						ITS1	KY574867
19	5505	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574724
						18S	KY574846
19	5514	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574725
						ITS1	KY574868
19	5519	*Mesocriconema* sp.	F	Downs Prairie, AR[b]	Mississippi Lowland forests	COI	KY574726
						ITS1	KY574869
19	5525	*Mesocriconema* sp.	J	Downs Prairie, AR[b]	Mississippi Lowland forests	COI	KY574727
						ITS1	KY574870
20	1270	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788005
20	1278	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788006

(*Continued*)

TABLE 1. Continued.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
20	1303	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788007
						ITS1	KY574871
20	1351	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788008
20	2517	*Mesocriconema* sp.	F	Attwater Prairie Chicken National Wildlife Refuge, TX[b]	Western Gulf Coastal Grasslands	COI	KY574728
						ITS1	KY574872
21	918	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788009
21	942	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788010
						ITS1	KY574873
22	321	*Mesocriconema* sp.	F	Baltimore County, MD[b]	Southeastern Mixed Forests	COI	KJ788011
						ITS1	KY574874
22	363	*Mesocriconema* sp.	F	Baltimore County, MD[b]	Southeastern Mixed Forests	COI	KJ788012
						ITS1	KY574875
22	1167	*Mesocriconema* sp.	J	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788013
22	2518	*Mesocriconema* sp.	F	Attwater Prairie Chicken National Wildlife Refuge, TX[b]	Western Gulf Coastal Grasslands	COI	KY574729
22	2520	*Mesocriconema* sp.	J	Attwater Prairie Chicken National Wildlife Refuge, TX[b]	Western Gulf Coastal Grasslands	COI	KY574730
						ITS1	KY574876
22	5502	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574731
						18S	KY574847
23	609	*Mesocriconema* sp.	F	Avoca Prairie and Savanna State Natural Area, Iowa County, WI[b]	Upper Midwest Forest-Savanna Transition Zone	COI	KJ788014
23	956	*Mesocriconema* sp.	F	Schluckebier Prairie State Natural Area, WI[c]	Upper Midwest Forest-Savanna Transition Zone	COI	KJ788015
						18S	KY574848
23	3061	*Mesocriconema* sp.	F	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574732
						ITS1	KY574877
23	3100	*Mesocriconema* sp.	J	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574733
24	608	*Mesocriconema* sp.	F	Meade County, SD[e]	Northern Short Grasslands	COI	KJ788016
24	741	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788017
24	855	*Mesocriconema* sp.	J	Doolittle Prairie State Preserve, IA[b]	Central Tall Grasslands	COI	KJ788018
24	1051	*Mesocriconema* sp.	F	Stafford County, KS[d]	Central and Southern Mixed Grasslands	COI	KJ788019
						18S	KY574849
24	1053	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788020
24	1054	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788021
						18S	KY574850
24	1055	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788022
24	1056	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788023
24	1057	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788024
						18S	KY574851
24	1058	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788025
24	1064	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788026
24	1066	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788027
24	1075	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788028
24	1109	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788029

(*Continued*)

TABLE 1. Continued.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
24	1127	*Mesocriconema* sp.	F	Avoca Prairie and Savanna State Natural Area, Iowa County, WI[b]	Upper Midwest Forest-Savanna Transition Zone	COI	KJ788030
24	1129	*Mesocriconema* sp.	F	Avoca Prairie and Savanna State Natural Area, Iowa County, WI[b]	Upper Midwest Forest-Savanna Transition Zone	COI	KJ788031
						18S	KY574852
24	1142	*Mesocriconema* sp.	F	Avoca Prairie and Savanna State Natural Area, Iowa County, WI[b]	Upper Midwest Forest-Savanna Transition Zone	COI	KJ788032
24	1166	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KJ788033
24	1238	*Mesocriconema* sp.	F	Hitchcock County, NE[b]	Central and Southern Mixed Grasslands	COI	KJ788034
24	1239	*Mesocriconema* sp.	F	Hitchcock County, NE[b]	Central and Southern Mixed Grasslands	COI	KJ788035
24	1240	*Mesocriconema* sp.	J	Hitchcock County, NE[b]	Central and Southern Mixed Grasslands	COI	KJ788036
24	1245	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788037
24	1264	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788038
24	1271	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788039
24	1277	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788040
24	1286	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788041
24	1287	*Mesocriconema* sp.	J	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788042
24	1289	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788043
24	1307	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574734
24	1308	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574735
24	1314	*Mesocriconema* sp.	J	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788044
24	1317	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788045
24	1342	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788046
24	1359	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788047
24	1360	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788048
24	1370	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788049
24	1371	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788050
24	1383	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788051
24	1384	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788052
24	1388	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788053
						18S	KY574853
24	1389	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[c]	Central Tall Grasslands	COI	KJ788054
24	1390	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[c]	Central Tall Grasslands	COI	KJ788055
24	1391	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[c]	Central Tall Grasslands	COI	KJ788056
24	1393	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[c]	Central Tall Grasslands	COI	KJ788057
24	1394	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[c]	Central Tall Grasslands	COI	KJ788058
24	1400	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574736
24	1401	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574737
24	1409	*Mesocriconema* sp.	J	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KY574738
24	1427	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KJ788059
24	1428	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[b]	Central Tall Grasslands	COI	KJ788060
24	2506	*Mesocriconema* sp.	J	Four Canyon Preserve, OK[c]	Central and Southern Mixed Grasslands	COI	KY574739
24	2646	*Mesocriconema* sp.	F	Lincoln County, NE[e]	Central Tall Grasslands	COI	KY574740
24	2671	*Mesocriconema* sp.	F	Lincoln County, NE[e]	Central Tall Grasslands	COI	KY574741
24	2672	*Mesocriconema* sp.	F	Lincoln County, NE[e]	Central Tall Grasslands	COI	KY574742

(*Continued*)

TABLE 1. Continued.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
24	2673	*Mesocriconema* sp.	F	Lincoln County, NE[e]	Central Tall Grasslands	COI	KY574743
24	2811	*Mesocriconema* sp.	J	Nine-Mile Prairie Adjoining Land, NE[d]	Central Tall Grasslands	COI	KY574744
24	2813	*Mesocriconema* sp.	F	Nine-Mile Prairie Adjoining Land, NE[d]	Central Tall Grasslands	COI	KY574745
24	2814	*Mesocriconema* sp.	J	Nine-Mile Prairie Adjoining Land, NE[d]	Central Tall Grasslands	COI	KY574746
						ITS1	KY574878
24	2845	*Mesocriconema* sp.	J	Nine-Mile Prairie Adjoining Land, NE[d]	Central Tall Grasslands	COI	KY574747
24	2847	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE[c]	Central Tall Grasslands	COI	KY574748
24	2939	*Mesocriconema* sp.	F	Plover Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574749
24	2940	*Mesocriconema* sp.	F	Plover Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574750
24	2941	*Mesocriconema* sp.	F	Plover Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574751
24	2973	*Mesocriconema* sp.	F	Red Rock Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574752
						18S	KY574854
24	2983	*Mesocriconema* sp.	F	Red Rock Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574753
24	2984	*Mesocriconema* sp.	J	Red Rock Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574754
24	3027	*Mesocriconema* sp.	F	Red Rock Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574755
24	3028	*Mesocriconema* sp.	J	Red Rock Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574756
24	3030	*Mesocriconema* sp.	F	Red Rock Prairie Preserve, MN[b]	Central Tall Grasslands	COI	KY574757
24	3082	*Mesocriconema* sp.	J	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574758
24	3114	*Mesocriconema* sp.	F	Hayden Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574759
24	3143	*Mesocriconema* sp.	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574760
24	3147	*Mesocriconema* sp.	F	Cayler Prairie Preserve, IA[b]	Central Tall Grasslands	COI	KY574761
						ITS1	KY574879
24	3186	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574762
24	3188	*Mesocriconema* sp.	F	Roth Prairie Natural Area, AR[c]	Mississippi Lowland Forests	COI	KY574763
						ITS1	KY574880
24	5515	*Mesocriconema* sp.	F	Downs Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574764
						18S	KY574855
24	5516	*Mesocriconema* sp.	F	Downs Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574765
						ITS1	KY574881
24	5517	*Mesocriconema* sp.	J	Downs Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574766
						ITS1	KY574882
24	5518	*Mesocriconema* sp.	F	Downs Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574767
24	5520	*Mesocriconema* sp.	J	Downs Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574768
						ITS1	KY574883
24	5526	*Mesocriconema* sp.	J	Downs Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574769
						ITS1	KY574884
24	5933	*Mesocriconema* sp.	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574770
24	5937	*Mesocriconema* sp.	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574771
24	5938	*Mesocriconema* sp.	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574772
24	5939	*Mesocriconema* sp.	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574773
24	5940	*Mesocriconema* sp.	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574774
24	5947	*Mesocriconema* sp.	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574775
24	5949	*Mesocriconema* sp.	F	Homestead National Monument, NE[c]	Central Tall Grasslands	COI	KY574776
24	5951	*Mesocriconema* sp.	F	Homestead National Monument, NE[b]	Central Tall Grasslands	COI	KY574777

(*Continued*)

TABLE 1. Continued.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
24	5952	*Mesocriconema* sp.	F	Homestead National Monument, NE[b]	Central Tall Grasslands	COI	KY574778
24	5954	*Mesocriconema* sp.	F	Homestead National Monument, NE[b]	Central Tall Grasslands	COI	KY574779
24	5956	*Mesocriconema* sp.	F	Homestead National Monument, NE[b]	Central Tall Grasslands	COI	KY574780
24	5984	*Mesocriconema* sp.	F	Prairie Pines, NE[b]	Central Tall Grasslands	COI	KY574781
24	5996	*Mesocriconema* sp.	F	Prairie Pines, NE[b]	Central Tall Grasslands	COI	KY574782
24	7004	*Mesocriconema* sp.	F	Prairie Pines, NE[b]	Central Tall Grasslands	COI	KY574783
24	7020	*Mesocriconema* sp.	J	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574784
24	7021	*Mesocriconema* sp.	J	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574785
24	7022	*Mesocriconema* sp.	J	Prairie Pines, NE[c]	Central Tall Grasslands	COI	KY574786
24	7029	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574787
24	7031	*Mesocriconema* sp.	J	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574788
24	7040	*Mesocriconema* sp.	F	Spring Creek Prairie, NE[b]	Central Tall Grasslands	COI	KY574789
25	1495	*Mesocriconema* sp.	F	Big Sandy Creek Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574790
25	2337	*Mesocriconema* sp.	F	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574791
25	2707	*Mesocriconema* sp.	F	Appling County, GA[e]	Southeastern Conifer Forests	COI	KY574792
25	3622	*Mesocriconema* sp.	F	Turkey Creek Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574793
25	3657	*Mesocriconema* sp.	F	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI ITS1	KY574794 KY574885
25	3660	*Mesocriconema* sp.	F	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI 18S	KY574795 KY574856
25	3662	*Mesocriconema* sp.	F	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574796
25	3664	*Mesocriconema* sp.	F	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574797
25	3665	*Mesocriconema* sp.	J	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574798
25	4003	*Mesocriconema* sp.	F	Turkey Creek Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574799
25	5624	*Mesocriconema* sp.	J	Turkey Creek Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574800
25	5675	*Mesocriconema* sp.	J	Big Sandy Creek Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574801
25	5692	*Mesocriconema* sp.	J	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574802
25	5693	*Mesocriconema* sp.	J	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574803
25	5695	*Mesocriconema* sp.	F	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574804
25	5698	*Mesocriconema* sp.	F	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574805

(*Continued*)

TABLE 1. Continued.

Haplotype[a]	NID	Species	Stage	Locality	Ecoregion	Marker	GenBank accession number
25	5699	*Mesocriconema* sp.	J	Lance Rosier Unit, Big Thicket National Preserve, TX[c]	Piney Woods Forests	COI	KY574806
26	5527	*Mesocriconema* sp.	F	Warren Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574807
						18S	KY574857
26	5528	*Mesocriconema* sp.	F	Warren Prairie, AR[b]	Mississippi Lowland Forests	COI	KY574808
						ITS1	KY574886
27	2501	*Mesocriconema* sp.	J	Clymer Meadows, TX[b]	Texas Blackland Prairies	COI	KY574809
27	2511	*Mesocriconema* sp.	J	Clymer Meadows, TX[d]	Texas Blackland Prairies	COI	KY574810
27	3495	*Mesocriconema* sp.	J	Wichita Mountains National Wildlife Refuge, OK[b]	Central and Southern Mixed Grasslands	COI	KY574811
27	3496	*Mesocriconema* sp.	J	Wichita Mountains National Wildlife Refuge, OK[b]	Central and Southern Mixed Grasslands	COI	KY574812
27	5501	*Mesocriconema* sp.	J	Clymer Meadows, TX[b]	Texas Blackland Prairies	COI	KY574813
						18S	KY574858
S	2284	*Mesocriconema* sp.	J	Roy E. Larsen Sandylands, TX[b]	Piney Woods Forests	COI	KY574827

ITS1 = internal transcribed spacer 1; NID = nematode identification number.
[a] Number = group number, S = singleton.
[b] Remnant site.
[c] Restoration site.
[d] Reconstruction site.
[e] Agricultural site.

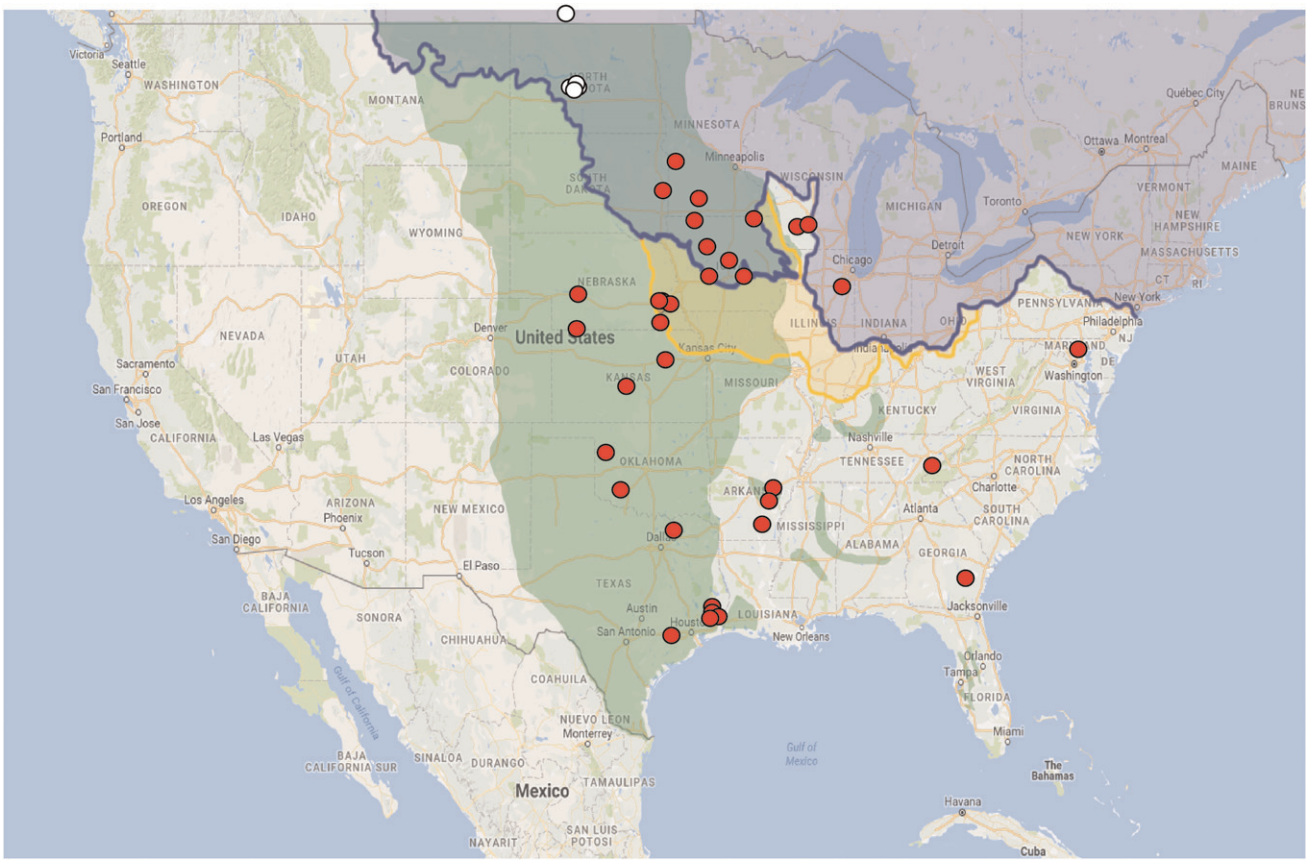

FIG. 1. Map of collection sites for specimens in haplotype groups 18 to 27. Red dots represent sampling sites positive for grassland specimens of *Mesocriconema*, white dots indicate grassland sites where no *Mesocriconema* were recovered from soil samples. Blue shaded area is the known limit of the Wisconsin glacial ice sheet, yellow shading indicates the furthest extent of glaciers during the Pleistocene ice ages. The historical range of the central grassland biome in the United States is shaded in dark green.

produce a 0.85-kb amplification product: 818 bp when the primers are trimmed off. Together the sets usually produce a final near-complete 18S product of 1,706 bp with a 63 bp overlap between sets. ITS1 sequence was generated from select specimens using ITS1 primer sequences rDNA2 (5′-TTGATTACGTCCCTGCCCTTT-3′) and rDNA1.58Sa (5′-ACGAGCCGAGTGATCCACC-3′).

Phylogenetic analysis: Phylogenies presented in this study were inferred using programs in Geneious R8 (Kearse et al., 2012) and MEGA 6 (Tamura et al., 2013). A reference maximum likelihood tree has previously been published for the genus *Mesocriconema* (Powers et al., 2014) and an updated version of that tree is presented in Fig. 2. This reference tree used COI sequence from the 608 *Mesocriconema* specimen dataset that was reduced by MacClade's Redundant Taxa Tool (Maddison and Maddison, 2000) to 191 unique haplotypes. Previously described haplotype groups and haplotype groups corresponding to Linnaean species are identified as colored blocks on the major branches of the tree. Five named morphospecies include specimens obtained from topotype localities: *Mesocriconema discus* (Thorne and Malek, 1968) Loof and De Grisse, 1989, *Mesocriconema ericaceum* Powers et al. (2016), *Mesocriconema inaratum* (Hoffmann, 1974) Powers et al. (2014), *Mesocriconema ornatum* (Raski, 1958) Loof and De Grisse, 1989, and *Mesocriconema xenoplax* (Raski, 1952) Loof and De Grisse, 1989. *Mesocriconema nebraskense* n. sp. and other closely related *Mesocriconema* haplotype groups collected from North American grasslands are uncolored on the maximum likelihood tree and are identified by boxed haplotype group numbers. A reduced 74 specimen dataset of unique haplotypes from hg 18 to 27 (Fig. 3) was also analyzed by neighbor-joining and Bayesian methods, which recognized identical haplotype groups based on branch topology and node support. It is this dataset, rooted by *M. inaratum*, that was subjected to the species delimitation methods discussed below.

Delimitation methods: Limits of candidate species were explored using the following methods: Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012), the Species Delimitation Plugin (Masters et al., 2011), implemented through Geneious R8 (Kearse et al., 2012) and statistical parsimony networks as implemented in the software program TCS (Clement et al., 2000; French et al., 2013), and DFA applied to a set of morphological characteristics of female specimens.

Automatic Barcode Gap Discovery (ABGD): ABGD is a delimitation method that groups DNA sequences into

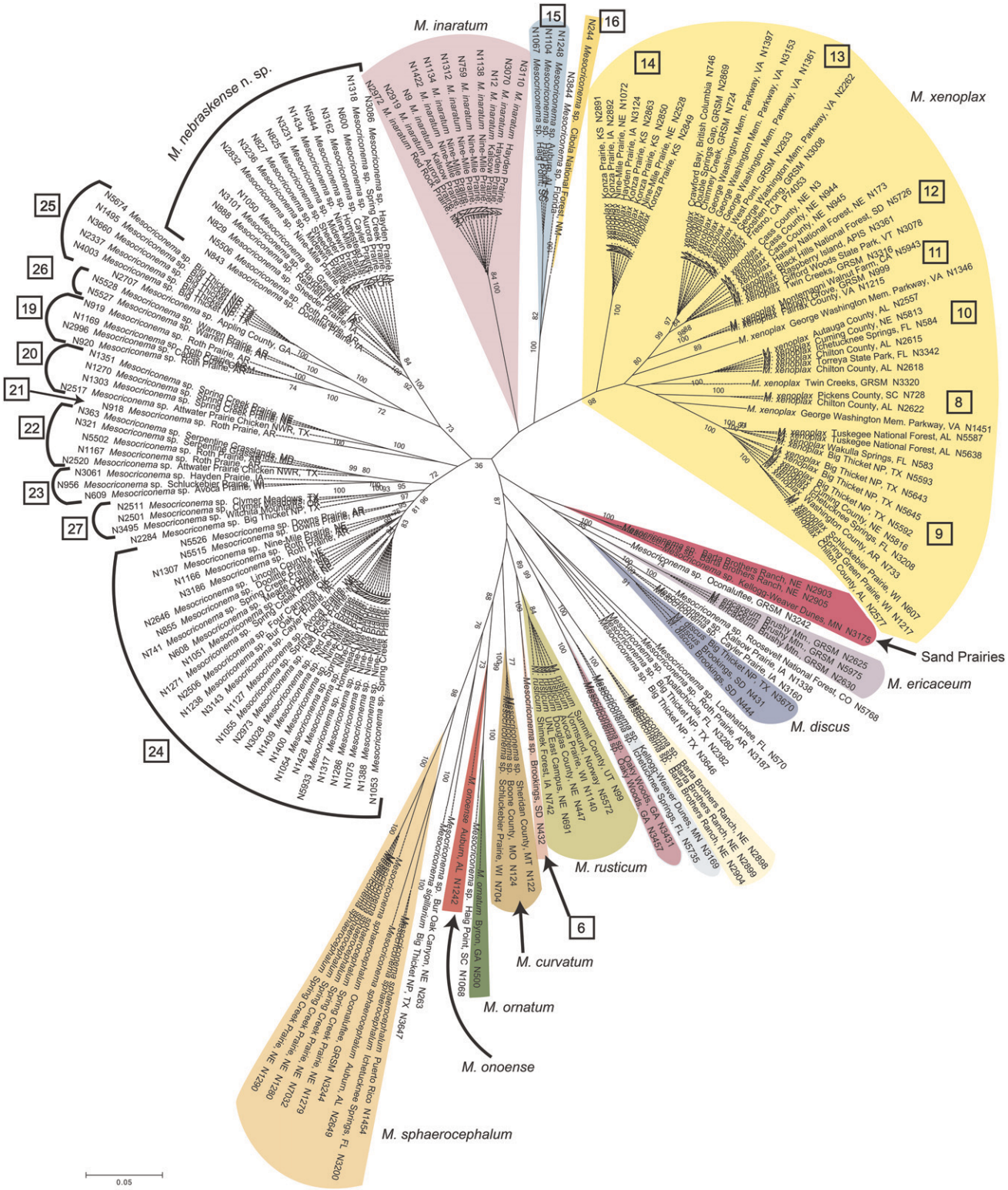

FIG. 2. Maximum likelihood tree of 191unique COI DNA sequences representing a set of 608 *Mesocriconema* specimens. Each terminal node includes a nematode identification number, taxon name, and location information. Haplotype groups have been boxed and given a group number. Haplotype groups that correspond to Linnaean names are identified based on analyses in Powers et al. (2014, 2016). *M. nebraskense* n. sp. and haplotype groups 19 to 27 are unshaded.

candidate species without a priori species hypotheses and operates under the assumption that a "gap" exists between intra- and interspecific diversity in the distribution of pairwise differences for any set of sequences. A barcode gap is statistically inferred and recursively partitions sequence data into groups of candidate species (Puillandre et al., 2012). The reduced grassland dataset was analyzed on the ABGD web-server

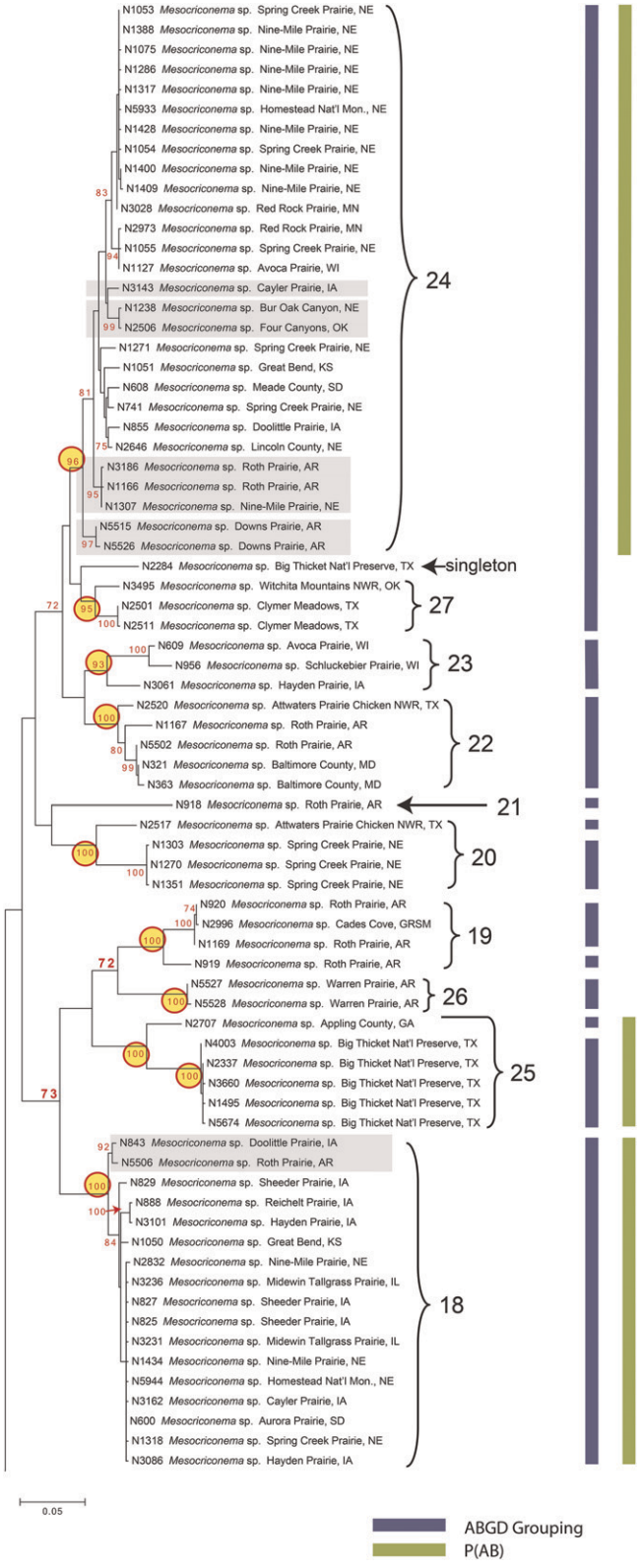

FIG. 3. Maximum likelihood tree of 74 unique COI sequences representing COI haplotype groups 18 to 27. Blue bars designate groupings recognized by Automatic Barcode Gap Discovery (ABGD and green bars indicate Rosenberg's test for reciprocal monophyly P (AB) groups. Taxa with gray shading represent subgroups that remain disconnected from haplotype groups 18 and 24 at the 95% connection limit.

(http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) using default values (Pmin = 0.001 and Pmax = 0.1) of prior maximum divergence of intraspecific diversity (i.e., species divergence) and the Kimura (K80) distance model, which accounts for the more frequent nature of transitional substitutions in protein coding sequences.

Species Delimitation Plugin (SDP): One metric in the species delimitation plugin available for the Geneious R8 software package was used to assess a priori species hypotheses supported in phylogenetic reconstructions. Highly supported nodes corresponding to candidate species were selected on phylogenetic trees within the plugin. Rosenberg's test for reciprocal monophyly (P(AB)) tests for clade distinctiveness under the null hypothesis that monophyly is an outcome of random branching events. Rodrigo's test (P(RD)), which assesses the probability that the degree of distinctiveness is due to random coalescent processes was not used because as stated, "for technical reasons, the probability cannot be computed when there are more than 40 taxa in the clade (Masters et al., 2011).

Haplotype networks: Haplotype networks are used to examine and visualize possible relationships and alternative evolutionary trajectories between DNA sequences of closely related specimens that may be masked in simple bifurcating trees. Networks can additionally be used to evaluate potential species boundaries based on empirical analyses that suggest intraspecific sequence divergence of less than 5% in COI often indicates species membership and corresponds with Linnaean names (Pons et al., 2006; Hart and Sunday, 2007; Chen et al., 2010).

The networks presented here were produced in the software package PopART (French et al., 2013) using algorithms developed for TCS (Clement et al., 2000). TCS calculates the maximum number of mutational steps that constitutes a parsimonious connection between two haplotypes, while conforming to a chosen connection limit (often 95%, although other limits could be evaluated) following statistical parsimony algorithms developed by Templeton et al. (1992). Haplotypes separated by more mutational steps than allowed by the designated connection limit remain disconnected. In a network visualization, unique haplotypes are represented as circles connected by hash marks indicating base pair changes between haplotypes. The size of the circle is proportional to the number of individuals conforming to that haplotype. Haplotype networks of groups 18 and 24 are presented in Figs. 6 and 7.

Population analyses: Basic nucleotide sequence population statistics including nucleotide and haplotype diversities and both Fu's Fs and Tajima's D neutrality tests were calculated in the software program DnaSP (Librado and Rozas, 2009). These statistics were calculated on the 10 haplotype groups supported in phylogenetic reconstructions on the 256 specimen grassland dataset.

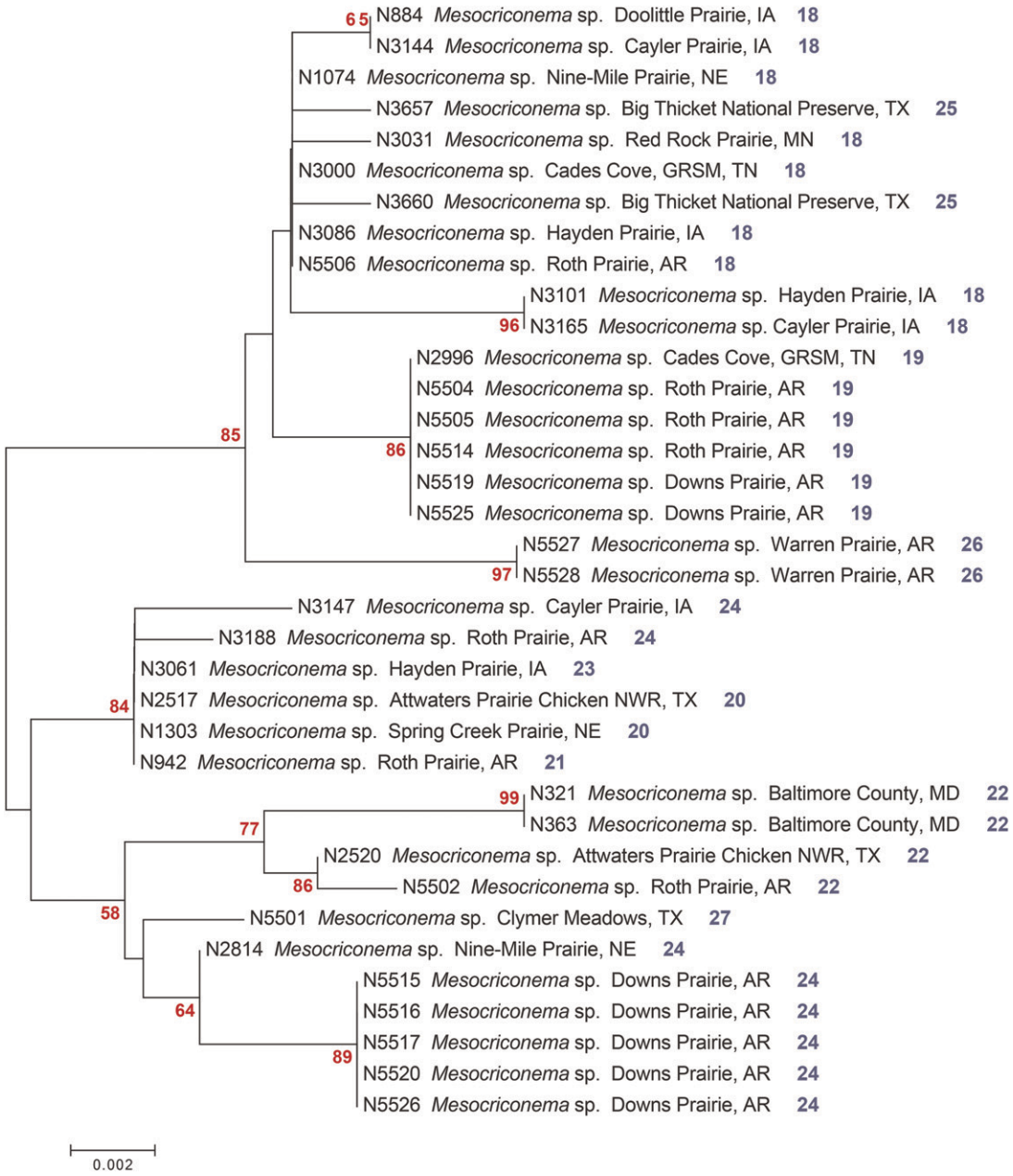

FIG. 4. ITS1 neighbor-joining tree of 36 unique *Mesocriconema* sequences. Terminal nodes include nematode identification number, taxon name, and location information. Numbers following the label correspond to COI haplotype group membership.

Neutrality tests determine whether patterns of genetic diversity within populations deviate from the expectation of a neutral model of evolution and a constant population size. Tajima's D (Tajima, 1989) is a neutrality test in which the D statistic tests the null hypothesis that all mutations are selectively neutral. Tajima's D is based on the principle that the neutral model estimates a correlation between the number of segregating sites and the average number of nucleotide differences. Fu's Fs (Fu and Li, 1993) also tests patterns of genetic variation in nucleotide data using the null hypothesis that the observed variation is a result of neutral selection. Unlike Tajima's D, Fu's Fs is based on branch length and coalescent theory, which states that all genes or alleles observed in a population are inherited by all members of the population from a common ancestor and assumes that genes do not undergo recombination and that genetic drift occurs under a stochastic model. Rejection of the null hypothesis indicates that observed diversity may be the result of selection or population subdivisions (Hartl et al., 1997; Ramírez-Soriano et al., 2008; Nei and Kumar, 2011).

Morphology and discriminant function analysis: Sixteen morphological characters were evaluated from 161 female *Mesocriconema* specimens by DFA. Discriminant function analyses are statistical procedures used to determine which variables in a data set potentially

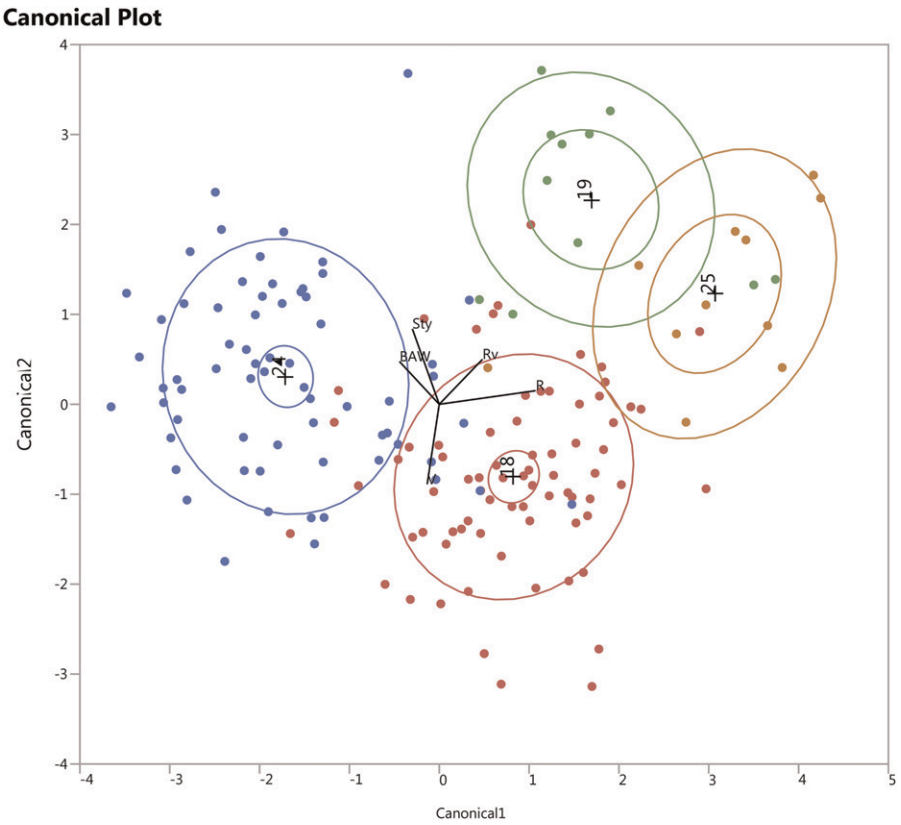

FIG. 5. Canonical plot of discriminant function analysis run on morphometrics from 161 females of *Mesocriconema* representing haplotype groups 18, 19, 24, and 25. Missing data points in the dataset were replaced with group means. Inner ellipse is the 95% confidence interval representing the true mean of each haplotype group. The normal (outer) ellipse region is estimated to contain 50% of the population for each group.

discriminate between two or more naturally occurring groups. DFA can be used to explore the effectiveness of a group of variables in predicting group membership. Used when groups are known a priori, DFA predicts categorical dependent variables, referred to as "grouping variables," based on one or more continuous independent variables, or "predictor variables" (Friedman, 1989; Hardle and Simar, 2007). A stepwise discriminant analysis was employed such that the first variable provides the greatest separation between groups. Successive variables were selected based on their ability to separate the known groups. Independent variables included in the analysis were assumed to be pairwise independent based on a multivariate normal distribution. Regularized, quadratic, and linear discriminant analyses were tested using proportional and equal prior probabilities for group membership. Juveniles and haplotype groups with less than six individuals were excluded from the analysis, leaving hg 18, 19, 24, and 25.

RESULTS

The location of 35 sampling sites positive for grassland specimens of *Mesocriconema*, as well as prairie sites in North Dakota and Canada where no *Mesocriconema* were recovered from soil samples are displayed in Fig. 1. Collection data for the full grassland dataset, which includes all 256 specimens in hg 18 to 27, is presented in Table 1. The dataset includes 182 adult females, 74 juveniles, and no males. The genetic relationship of the same grassland dataset reduced to unique sequences and compared to unique sequences in *Mesocriconema* hg 1 to 17 is depicted in the maximum likelihood phylogenetic tree shown in Fig. 2. As a distinct clade, the node representing hg 18 to 27 collectively did not have strong support with COI nucleotide or amino acid sequence data (not shown). However, in a near full-length 18S rDNA phylogenetic treatment of *Mesocriconema* specimens, a bootstrap support value of 95 provides strong support for the deeper node defining the clade (Powers et al., unpubl. data). Each of the individual haplotype groups are strongly supported in the maximum likelihood tree with bootstrap support values ranging from 93 to 100 (Fig. 3). Haplotype group membership was consistent across maximum likelihood, Bayesian, and neighbor-joining methods. Tree topology varied slightly within haplotype groups and at the deepest nodes in the trees. The deeper nodes in the COI tree, those that depict relationships among haplotype groups, generally have less support. Haplotype group

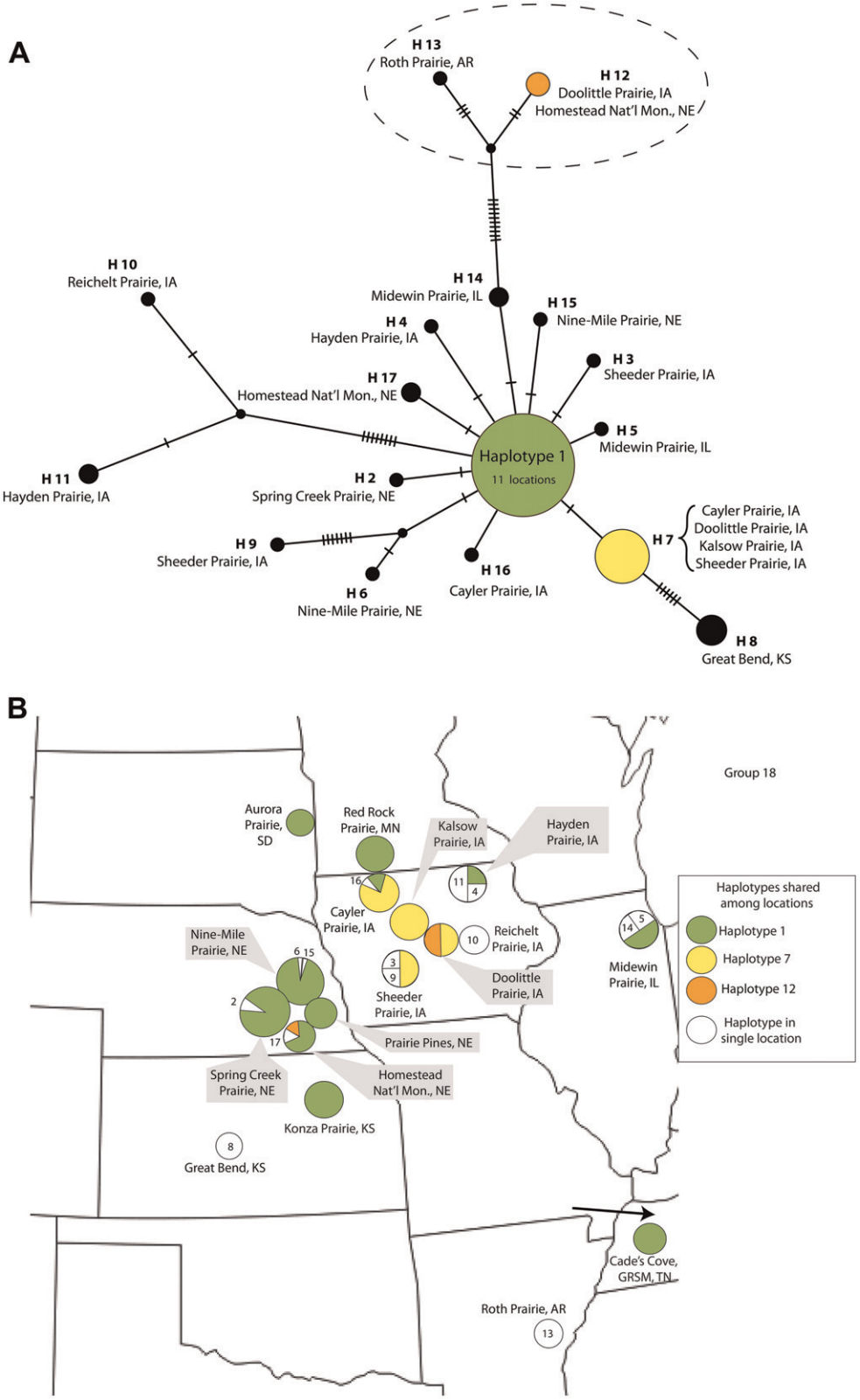

FIG. 6. Haplotype network and distribution map of *Mesocriconema nebraskense* n. sp., haplotype group 18. A. TCS haplotype network in which unique haplotype groups are represented as circles connected by hash marks indicating base pair changes between haplotypes. The size of the circle is proportional to the number of individuals conforming to that haplotype. Colored circles indicate haplotypes collected from multiple locations. Haplotypes enclosed by dashed lines correspond to groups that were disconnected at the 95% connection limit. B. Haplotype distribution map for *Mesocriconema nebraskense* n. sp. Haplotypes without colors indicate haplotypes found only at a single location.

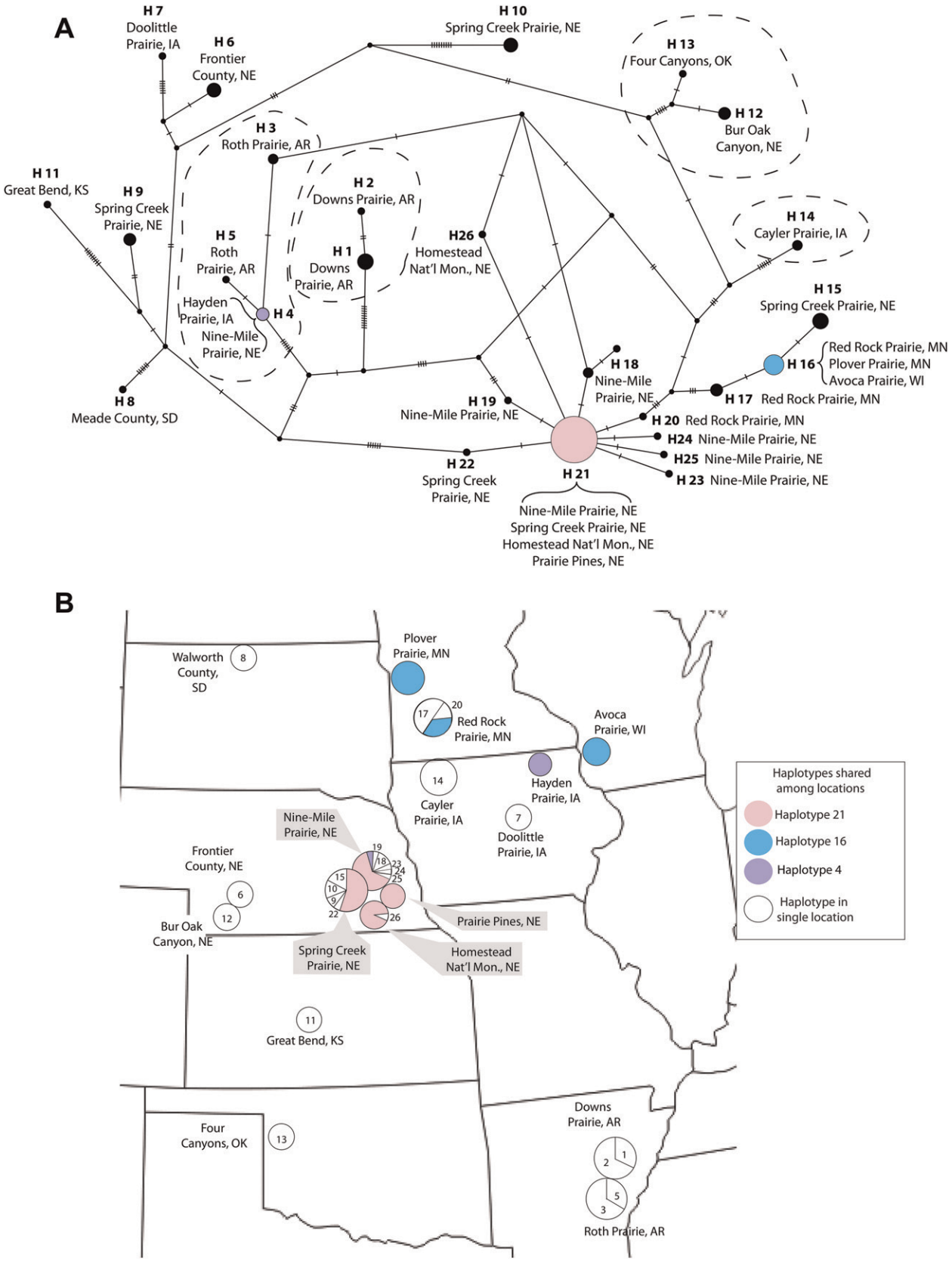

FIG. 7. Haplotype network and distribution map of haplotype group 24. A. TCS haplotype network in which unique haplotype groups are represented as circles connected by hash marks indicating base pair changes between haplotypes. The size of the circle is proportional to the number of individuals conforming to that haplotype. Colored circles indicate haplotypes collected from multiple locations. Haplotypes enclosed by dashed lines correspond to groups that were disconnected at the 95% connection limit. B. Haplotype distribution map for haplotype group 24. Haplotypes without colors indicate haplotypes found only at a single location.

18 is consistently recognized as a sister group to a clade containing hg 19, 25, and 26. This sister clade of hg 18 consists exclusively of specimens collected in the southern United States.

A summary of species delimitation analyses applied to the grassland dataset and superimposed on the maximum likelihood tree is presented in Fig. 3. ABGD partitions the dataset into groups congruent with the well-supported nodes in the phylogenetic trees, subdivides hg 19, 25, and 20, and merges group 27 with group 24. The major lineages, groups 18 and 24, are identified as distinct by P(AB) (Table 2). In the TCS network applying the 95% connection limit, four disconnected subgroups in group 24 and a single disconnected subgroup in group 18 were identified (Fig. 3). Lowering the connection limit to 93%, which permits an additional two substitutions in the comparisons, joins the subgroup in group 18. Two disconnected subgroups remain in group 24 at the 93% connection limit.

The pairwise mean genetic distance (p-distance) in COI sequences between all 256 specimens constituting hg 18 to 27 is presented in Table 3. For group 18, the closest mean distance is to groups 24 and 26, both at 10.6% difference. This mean distance to the closest haplotype group is comparable to the mean distances observed between other described species of *Mesocriconema* (Powers et al., 2014). Among all comparisons, the closest pairwise mean value is 5.7% between groups 24 and 27. Within haplotype group, mean distance between sequence pairs ranges from 0.0% in group 21 to 4.5% in group 23 (Table 2). Haplotype groups 18 and 24 have within-group mean distances of 0.4% and 1.4%, respectively.

An ITS1 neighbor-joining K2P tree of 36 representative specimens from hg 18 to 27 is shown in Fig. 4. The ITS1 alignment had 39 variable nucleotide sites and 15 autapomorphic sites out of 565 total nucleotides. Four gaps were inserted into the alignment. By comparison, the COI alignment features 267 variable nucleotide sites, with 33 autapomorphic sites and no gaps in the 721 nucleotide sequence. The relatively high percentage of autapomorphic characters and the possibility of individuals heteroplasmic for ITS1 reduce the confidence of haplotype associations depicted by this tree. Nonetheless, several taxa (e.g., groups 19, 22, and 26) appear to cluster according to COI haplotype group designation.

Basic statistics for 16 standard morphological measurements on females of hg 18, 19, 24, and 25 are presented in Table 4. Notable are the significantly larger values for R, Rv, and Rex in group 25 compared to the other haplotype groups. For hg 18 and 24, two groups that are co-distributed in native remnant prairies and the most frequently observed haplotypes in remnant prairies, mean values for R, stylet length (STY), body annulus width (BAW), and Rex aid in their discrimination. An indication of their diverging morphology is revealed by a discriminate function analysis of hg 18, 19, 24, and 25 (Fig. 5). A regularized ($\lambda = 0.1$, $\gamma = 0.9$) method with proportional priors of DFA was determined to perform best, classifying the 161 female nematode specimens into the correct haplotype groups, 84.50% of the time using the morphological variables in a given algorithm. The stepwise selection procedure suggested that a subset of five morphological variables, R, STY, V, BAW, and Rv best explain group membership. The derived canonicals quantify relationships between grouping and predictor variables and are illustrated on the canonical score plot presented in Fig. 5. Canonical 1 accounts for 72.5% of the between-group variation and Canonical 2 for 24.4% of the between-group variation, together explaining 96.9% of variation in haplotype group membership. Group means of the canonicals are plotted within circles representing 95% confidence intervals. Individual specimens are displayed as points, whereas the vectors point in the direction which that variable accounts for the greatest separation between groups.

TABLE 2. Haplotype group 18 to 27 summary statistics and neutrality tests.

Haplotype group	*n*	Number of mutations	Polymorphic mites	Number of haplotypes	p-distance within group	Haplotype diversity	Nucleotide diversity	Average number of differences	Fu's Fs	Tajima's D	Rosenberg's P(AB)
18	98	39	38	17	0.004	0.624	0.004	2.849	−3.857	−1.948**	2E-25***
19	15	34	34	4	0.023	0.619	0.024	17.048	12.206*	2.670**	9.10E-04
20	5	49	49	4	0.027	0.9	0.027	19.6	2.736	−1.257	0.02
21	2	0	0	1	0	0	0	0	n.d.	n.d.	0.02
22	6	37	36	5	0.023	0.933	0.023	16.733	1.675	0.208	0.00198
23	4	53	52	3	0.045	0.833	0.045	32.5	4.717	1.294	0.00198
24	101	66	63	27	0.014	0.806	0.014	10.221	−0.706	−0.635	7.00E-11***
25	17	50	49	6	0.029	0.721	0.030	21.096	10.211*	1.779**	7.70E-08***
26	2	2	2	2	0.003	1	0.003	2	0.693	n.d.	9.10E-04
27	5	26	26	3	0.022	0.800	0.022	15.6	4.879	1.863	0.07

* Indicates significant values for Fu's Fs test statistic.
** Indicates significant values for Tajima's D test statistic.
*** Indicates significance for Rosenberg's P(AB).

TABLE 3. Estimates of mean evolutionary divergence over sequence pairs between *Mesocriconema* haplotype groups 18 to 27. The analysis involved 255 nucleotide sequences, with a total of 721 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

	Group 18	Group 19	Group 20	Group 21	Group 22	Group 23	Group 24	Group 25	Group 26
Group 18									
Group 19	0.108								
Group 20	0.121	0.146							
Group 21	0.147	0.161	0.14						
Group 22	0.114	0.124	0.108	0.138					
Group 23	0.118	0.132	0.125	0.129	0.082				
Group 24	0.106	0.127	0.112	0.124	0.078	0.08			
Group 25	0.12	0.118	0.132	0.147	0.124	0.134	0.122		
Group 26	0.106	0.1	0.146	0.161	0.119	0.134	0.125	0.121	
Group 27	0.113	0.131	0.113	0.118	0.085	0.08	0.057	0.131	0.131

A TCS network and corresponding geographic map of haplotype distribution in group 18 is shown in Fig. 6A and B. Both network and map feature a single predominant haplotype (1) located at 11 sampling sites. Two other haplotypes were found at more than a single location. Haplotype 7 was confined to remnant prairies in central Iowa, whereas haplotype 12 was found in Homestead National Monument in Nebraska and Doolittle Prairie, a pothole prairie just north of Ames, Iowa. The network of hg 18 displays a star-like pattern (Avise, 2000; Nieberding et al., 2005; Walker et al., 2009) with nine haplotypes connected by one to two mutational steps from the most common haplotype. Star-like networks are characteristic of organisms with a relatively recent distribution and diversification. By contrast, the network and map of hg 24 show a more complex pattern of haplotype distribution and diversity (Fig. 7A and B). Only three of the 26 different haplotypes are found at more than a single location, with haplotype 21 localized to prairies in eastern Nebraska and haplotype 16 found in prairies in southern Minnesota and Wisconsin. Haplotype 4 was found in Nine-Mile Prairie, outside Lincoln, Nebraska, and Hayden Prairie in northeast Iowa. Although both hg 18

TABLE 4. Summary statistics of morphometric measurements of females diagnostic for *Mesocriconema* cf. sp. *curvatum* and sorted by their respective COI haplotype groups 18 (*Mesocriconema nebraskense* n. sp.), 19, 24, and 25.

Haplotype group	Length (L)					Number of body annuli (R)					Number of annuli from vulva to tail terminus (Rv)					Number of annuli anterior to excretory pore (Rex)				
	N	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum
18	74	507.9	44.5	393	606	74	102.9	6.1	84	113	74	7.8	1.0	6	11	74	28.0	1.5	24	31
19	11	538.3	52.7	470	648	11	107.7	6.1	99	120	11	9.5	0.7	9	11	11	27.8	1.7	25	31
24	67	551.0	60.1	405	675	67	90.8	5.8	80	104	67	7.0	0.9	5	9	67	24.6	2.0	20	32
25	11	583.5	40.5	530	669	11	119.5	9.4	109	143	11	9.9	1.4	7	12	11	31.5	2.8	24	34

Haplotype group	ESO					Stylet length					Stylet knob width					MBW				
	N	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum
18	74	117.2	10.2	73	135	74	52.6	2.7	45	59	*74*	10.0	0.7	9	11	74	40.5	4.0	32	50
19	11	124.2	6.6	113	135	11	57.3	3.1	53	62	11	10.1	0.5	9	11	11	42.0	3.7	36	48
24	67	128.6	9.5	105	170	67	56.8	3.4	48	66	67	10.6	0.9	9	13	67	45.0	5.0	32	58
25	11	124.5	6.5	112.5	135	11	54.4	2.9	52	62	11	9.7	1.0	7	11	11	40.1	2.9	34	45

Haplotype group	VUL					V					VBW					Body annulus width				
	N	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum
18	71	473.1	42.6	365	563	74	93.3	0.9	90.6	96	74	32.1	2.8	25	39	74	5.2	0.5	4.5	6.4
19	9	491.3	55.0	423	596	11	91.4	0.8	89.9	92.5	11	33.1	3.7	28	39	11	5.6	0.7	4.5	7
24	65	516.3	53.5	388	623	67	93.1	1.0	90.8	95.7	67	35.0	3.1	29	47	67	6.3	0.7	4.4	7.8
25	11	540.4	39.7	479	622	11	92.0	1.8	88	94	11	33.5	2.7	30	38	11	5.5	0.4	5	6.2

Haplotype group	(L − VUL)/VBW					a (L/MBW)					b (L/ESO)					Number of anastomoses				
	N	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum	*N*	Mean	SD	Minimum	Maximum
18	74	1.1	0.2	0.7	1.58	71	12.7	1.5	9.2	15.3	70	4.4	0.5	3.6	7.2	61	0.8	1.0	0	4
19	11	1.4	0.2	1.11	1.58	10	12.8	1.5	11.2	16	10	4.3	0.4	3.9	5.1	11	1.4	1.7	0	5
24	67	1.1	0.2	0.7	1.6	66	12.4	1.5	8.8	15.5	64	4.3	0.4	3.5	5.2	56	1.2	1.4	0	6
25	11	1.4	0.3	1	2.17	11	14.6	0.9	13.3	16.1	11	4.7	0.3	4.1	5.2	11	2.5	1.7	0	6

ESO = esophagus; MBW = median body width; VUL = vulval position from anterior; V = vulval position as a percentage of body length; VBW = vulval body width.

and 24 share a similar geographic distribution, the large number of mutational steps between haplotypes in group 24 suggests diversification occurring across a greater period of time.

Description

Mesocriconema nebraskense n. sp.
(Fig. 8, images of Holotype; Fig. 9, images of other specimens)

Measurements: See Table 4.

Holotype Specimen Measurements (nematode identification number [NID] 7028) from Spring Creek Prairie, Nebraska.

First lip annulus diameter = 15 μm, stylet length = 53 μm, knob width = 11 μm, knob height = 5 μm, dorsal esophageal gland orafice = 7 μm, anterior end to base of pharynx = 125 μm, anterior end to vulva = 507 μm, body length = 545 μm, mid-body width = 42 μm, body width at vulva = 35 μm, BAW = 5.0 μm, R = 111, Rv = 8, Ra = 6, Rex = 31, no anastomoses, anterior annulus to vulva with two pointed projections.

Females: Female body slightly curved ventrally when relaxed by heat, assuming an open arc-like shape (Fig. 8A). Annuli margins are smooth across the entire body, without any hint of crenation (Fig. 8E). Number of body annuli averages 103.6 (SD ± 6.3). Average width of annuli at mid-body is 5.2 μm. There is typically a single anastomosis on the body with four the maximum number of anastomoses observed from 73 specimens. The labial region is characterized by a rectangular oral disc with rounded edges. The slit-like amphid apertures are located laterally on the disc. Characteristically variable labial plates surround the oral disc. In size, the labial plates may be smaller than the submedian lobes or on some specimens appear to be fused with a fragmented labial annulus. More consistent in shape are four submedian lobes that often project above the plane of the cephalic contour when viewed laterally (Fig. 9A–E). The submedian lobes in SEM most often resemble a tongue with a central, longitudinal crease (Fig. 9M). The submedian lobes are seldom fused with labial plates. Subtending the labial plates and submedian lobes is the first complete body annulus, which averages 15 μm in diameter and often includes a single lateral notch on its margin. Second body annulus is usually 2 to 4 μm wider in profile than first annulus. Stylet averaging 52.6 (SD ± 2.7) μm in length, with robust stylet knobs that possess moderate anterior projections (Fig. 8B). The excretory pore location is generally on the 28th annulus from the anterior end, most often 2 to 6 annuli behind the posterior base of the pharyngeal bulb. Reproductive system terminating in a straight vagina, occasionally with posterior portion of cuticle-lined canal orientated parallel to body axis for length of a single annulus (Figs. 8C, 9H–K). Anterior to the vulva is a pair of pointed projections that may extend half the length of an annulus. On occasion, the projections are low and rounded. The postvulval region of the body tapers gradually, ending in a rounded, symmetrical terminus. SEM reveals the anal opening is located 2 to 3 annuli posterior to the vulva.

Male: No males of this species have been observed.

Juvenile: Body shape is similar to that of female. Annuli with crenate margins that may only be expressed on the posterior third of the body or extend across entire body. Total number of annuli approximately equal to that of adult females, but annulus width average 3.5 versus 5.2 μm for the adult. Body and STY for juveniles (n = 20) range from 280 to 443 μm and 37 to 48 μm, respectively. Anastomoses are less common on juvenile specimens compared to adults, lacking in more than 50% of specimens.

Differential diagnosis: Mesocriconema nebraskense n. sp. is morphologically very similar to *Mesocriconema* hg 24. Slight differences exist in mean values of the number of body annuli (*M. nebraskense* = 103.6 [±6.3] versus *Mesocriconema* hg 24 = 90.6 [±6.0]), location of the excretory pore in body annules (Rex) from the anterior end (*M. nebraskense* = 27.9 [±1.7] versus *Mesocriconema* hg 24 = 24.6 [±2.2]), BAW (*M. nebraskense* = 5.2 μm [±0.5] versus *Mesocriconema* hg 24 = 6.3 μm [±0.7]), and STY (*M. nebraskense* = 52.6 μm [±2.7] versus *Mesocriconem*a hg 24 = 56.9 μm [±3.5]). Two other prominent hg, hg 19 and 25, are more commonly observed in the southern grasslands of Texas and Arkansas. Haplotype group 19 is characterized by a more pointed tail (Powers et al., 2014) and a vulva positioned more anteriorly, and hg 25 has more body annuli (mean 119.5 [±9.4]) and an excretory pore positioned a greater distance from the anterior end (mean annuli number 31.5 [±2.8]). These haplotype groups are differentiated from *M. curvatum* which is smaller in body length (mean 396 ± 14 μm), total body annuli (mean R = 79 ± 4), and annuli number from anterior end to the excretory pore (mean Rex = 22 ± 1) (Powers et al., 2014).

Molecular diagnostic traits for M. nebraskense *n. sp.:* The COI marker sequence and alignment have been presented in Powers et al. (2016). Diagnostic nucleotides are designated by their position in the alignment, and the character state at that position (A,C,G,T). Sequence comparisons in this alignment include all 256 specimens in the grassland dataset that includes COI hg 18 to 27. The following diagnostic nucleotides are considered "pure" in that they are fixed for all specimens in the group and not found in other specimens in the dataset (DeSalle et al., 2005): markers COI-197, C; 199, T; 245, C; 247, T; 268, A; 605, C; 624, A.

Type locality and habitat: Holotype specimen (NID 7028) was collected from Spring Creek Prairie, Nebraska, by Kris and Tom Powers; latitude (decimal degrees) 40.6920460, longitude (decimal degrees) 96.8532120. Spring Creek Prairie is a remnant tallgrass prairie, managed by the Audubon Society, and

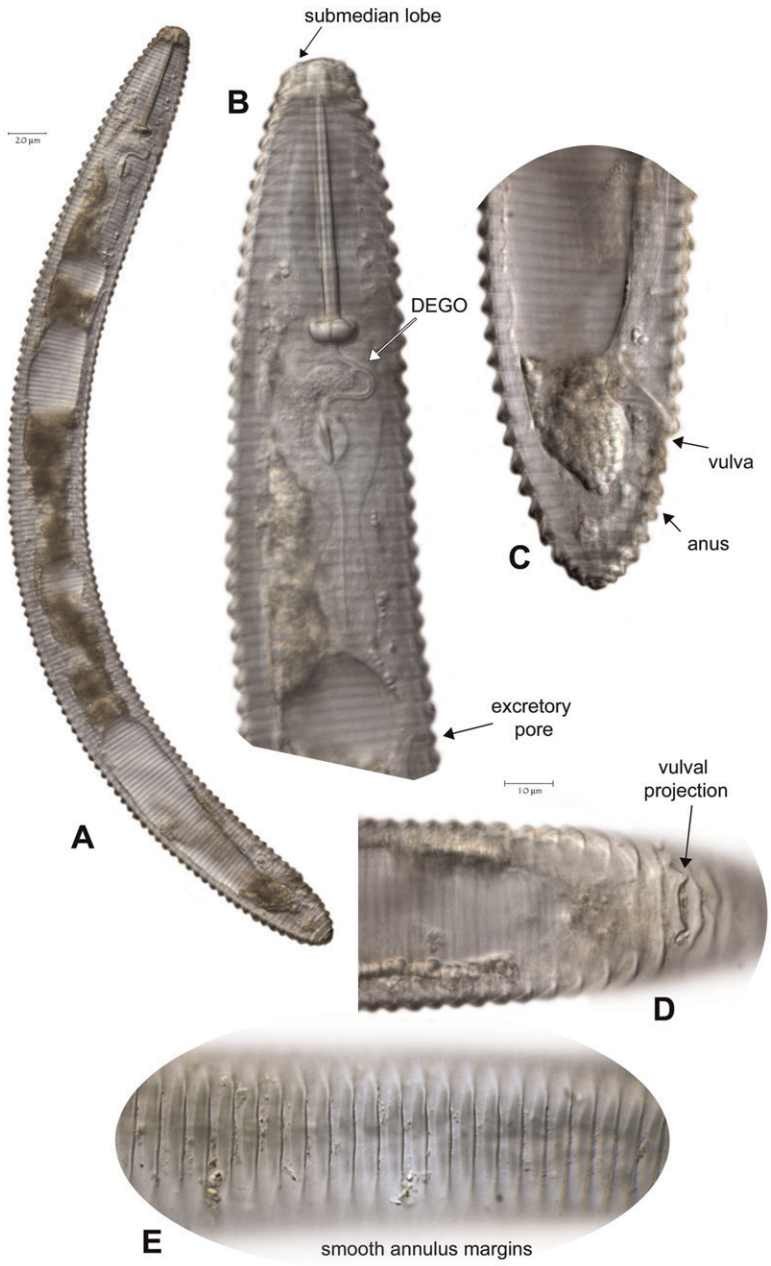

FIG. 8. Holotype specimen of *Mesocriconema nebraskense* n. sp., female, NID 7028 from Spring Creek Prairie, NE. A. Entire specimen. B. Anterior body with dorsal esophageal gland orifice and excretory pore labeled. C. Posterior region. D. Vulva with projections on anterior annulus. E. Midbody annuli with smooth margins.

located in the Central Tall Grasslands ecoregion of North America.

Type material deposition: Holotype tissue from NID 7028 has been deposited with collection number HWML 99848 in the Harold W. Manter Laboratory of Parasitology, W-529 Nebraska Hall, University of Nebraska State Museum curated by Dr. Scott Gardner. Four tubes of paratype tissue each containing residue of an adult female are also deposited in the University of Nebraska State Museum with collection numbers HWML 99845 (NID 1386), HWML 99846 (NID 3080), HWML 99847 (NID 3146), and HWML 99849 (NID 7037).

Ecology and distribution: Mesocriconema nebraskense n. sp. is known from the former native range of the tallgrass prairies of North America. The range extends eastward to a disjunct grassy site in the Great Smoky Mountains that may have been formed during a Holocene warming period when grasses migrated eastward as periods of drought grew longer and summer temperatures increased (Pielou, 1991). The species has not been found in the grassy balds of the Appalachian Mountains, the Black Earth Prairies of Georgia, coastal grasslands of Florida, or Longleaf Pine/wiregrass communities of the gulf coast region. Nor has it been recovered from mid and tallgrass prairies north of South Dakota. If this

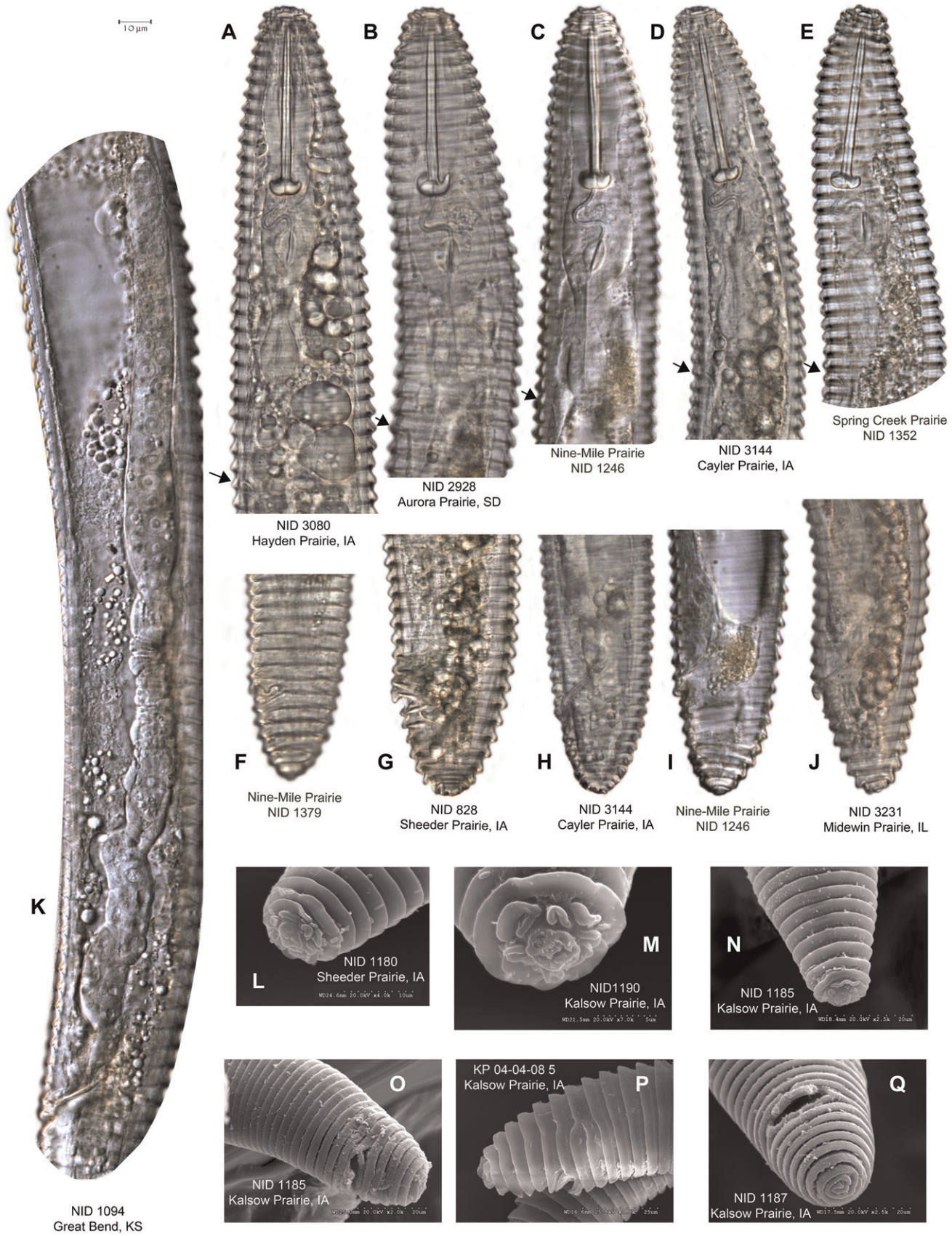

FIG. 9. Additional specimens of *Mesocriconema nebraskense* n. sp. identified by nematode identification number (NID) number and location. All specimens are females, A to K light micrographs, L to Q scanning electron micrographs, A to E anterior body region extending to excretory pore (arrow), F to J lateral view of posterior region, K reproductive tract, L to N face view with labial plates, submedian lobes, and oral disc, and O to Q posterior region with vulva and smooth annuli margins.

represents the northern border of *M. nebraskense* n. sp. distribution, it shows a lag time between parasite and host dispersal. Deeper nodes in the *Mesocriconema* phylogenetic tree indicate a possible southern U.S. origin of grassland nematode haplotypes (Noss, 2013). Grass hosts that are common to all collection sites and were frequently sampled include big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* (L.) Nash), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), and switchgrass (*Panicum virgatum* L.).

Discussion

There are an estimated 487 valid species in the family Criconematidae according to the latest review (Geraert, 2010). Assuming that morphological distinction was the criterion used to establish these species, the prospect of each morphospecies being composed of multiple cryptic species would suggest a dramatic increase in the number of species in a taxon already presumed to be "hyperdiverse" (Ehrlich and Wilson, 1991; Puillandre et al., 2012; Dey et al., 2013). The recognition of this added diversity has a direct bearing on estimates of global nematode biodiversity and concepts of nematode biogeography. Regional endemicity in plant-parasitic nematodes has seldom been recognized and cosmopolitan distributions in nematodes, like other microscopic organisms, are reportedly common (Finlay, 2002; Wouts, 2006). The large number of cosmopolitan taxa and the infrequent observation of endemicity may in fact be artifacts of coarse taxonomic resolution in species where delimitation has been exclusively based on morphological characters. Importantly, insufficient taxonomic resolution can also result from molecular analyses that rely on highly conserved genes for discrimination such as 18S small sub unit rDNA. An increasing number of reports note that closely related nematode species can have identical 18S nucleotide sequences (Tang et al., 2012; Armenteros et al., 2014).

This study of grassland *Mesocriconema* species illustrates the extensive diversity hidden within a widespread morphologically defined taxon. Our molecular approach detected significant differences in diversity and population structure between candidate species, suggestive of distinct evolutionary forces shaping these endemic lineages. The causes of these differences have yet to be determined. The patterns of diversity may be associated with subdivision and fragmentation of populations during episodic glacial periods within the 2 million years of the Pleistocene ice ages (Pielou, 1991; Knowles, 2000; Delcourt, 2007; Dejaco et al., 2016). The lack of sexual reproduction in these lineages may have accelerated the diversification among lineages (Fontaneto et al., 2009; Fonteneto and Barraclough, 2015). Specific host associations are known to occur in *Mesocriconema* (Powers et al., 2014), and changes in grassland plant species composition could dramatically and selectively alter the composition of plant-parasitic nematode communities. Recolonization of recently glaciated land by nematodes may have been influenced by animals. One theory has advanced the possibility that historical bison migrations could have dispersed soil-dwelling nematodes through the movement of mud adhering to fur acquired during behavioral wallowing (Thorne and Malek, 1968). An understanding of the appropriate taxonomic units for analysis is key in determining which factors are responsible for patterns of nematode distribution.

Mesocriconema nebraskense n. sp. described in this report is an asexual, cryptic species sympatrically distributed with its cryptic counterpart, hg 24. This poses several difficulties with conventional taxonomic protocol. First, it is not possible to unequivocally select a holotype specimen without conducting DNA analysis which will destroy the specimen. To ensure fidelity between the holotype specimen and the species it represents, the holotype of *M. nebraskense* n. sp. is represented by DNA extracted from a verified member of the haplotype group through DNA sequencing and accompanied by photographic images of that individual specimen. DNA types in lieu of an entire specimen are permitted by the International Code of Zoological Nomenclature, "the name-bearing type can be an animal, or any part of an animal" (72.5.1 ICZN 1999) and in the International Code of Botanic Nomenclature (Strand and Sundberg, 2011; Kadereit et al., 2012). Similar cases of cryptic, sympatric species exist as in the recent description of nine species of meiofaunal sea-slugs in the genus *Pontohedyle* (Jörger et al., 2012; Jörger and Schrödl, 2013). Given the current rates of biodiversity loss and land conversion, we may never know the extent of nematode diversity, but by providing a means of recognition and names for cryptic species, we could accelerate a fundamental step in the documentation of that diversity (Pante et al., 2015; Dejaco et al., 2016; Morard et al., 2016).

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