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COI haplotype groups in *Mesocriconema* (Nematoda: Criconematidae) and their morphospecies associations

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Abstract

Without applying an *a priori* bias for species boundaries, specimen identities in the plant-parasitic nematode genus *Mesocriconema* were evaluated by examining mitochondrial COI nucleotide sequences, morphology, and biogeography. A total of 242 specimens that morphologically conformed to the genus were individually photographed, measured, and amplified by a PCR primer set to preserve the linkage between specimen morphology and a specific DNA barcode sequence. Specimens were extracted from soil samples representing 45 locations across 23 ecoregions in North America. Dendrograms constructed by neighbor-joining, maximum likelihood, and Bayesian Inference using a 721-bp COI barcode were used to group COI haplotypes. Each tree-building approach resulted in 24 major haplotype groups within the dataset. The distinctiveness of these groups was evaluated by node support, genetic distance, absence of intermediates, and several measures of distinctiveness included in software used for the exploration of species boundaries. Five of the 24 COI haplotype groups corresponded to morphologically characterized, Linnaean species. Morphospecies conforming to *M. discus*, *Discocriconemella inarata*, *M. rusticum*, *M. onoense*, and *M. kirjanovae* were represented by groups composed of multiple closely related or identical COI haplotypes. In other cases, morphospecies names could be equally applied to multiple haplotype groups that were genetically distant from each other. Identification based on morphology alone resulted in *M. curvatum* and *M. ornatum* species designations applied to seven and three groups, respectively. Morphological characters typically used for species level identification were demonstrably variable within haplotype groups, suggesting caution in assigning species names based on published compendia that solely consider morphological characters. Morphospecies classified as *M. xenoplax* formed a monophyletic group composed of seven genetically distinct COI subgroups. The spe-

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cies *Discocriconemella inarata* is transferred to *Mesocriconema inaratum* based on its phylogenetic position on the COI tree as well as previous phylogenetic analyses using 18S, ITS1, and cytochrome b nucleotide sequences. This study indicates that some of the species considered cosmopolitan in their distribution are actually multispecies polyphyletic groupings and an accurate assessment of *Mesocriconema* species distributions will benefit from molecular determination of haplotype relationships. The groups revealed by COI analysis should provide a useful framework for the evaluation of additional *Mesocriconema* species and will improve the reliability of designating taxonomic units in studies of nematode biodiversity.

Key words: DNA barcode, identification, North America, plant-parasitic nematode, taxonomy

Introduction

Nematodes are frequently cited as a major source of undiscovered biodiversity on earth (Creer *et al*. 2010; Wilson 2007). The vivid imagery evoked by N.A. Cobb (1915) of a world covered by a film of nematodes has permeated popular science literature and reinforces the impression of remarkable nematode abundance and diversity (White 1998). Yet in spite of the popular perception, there are surprisingly few described species when compared to other groups of invertebrates (Bik *et al.* 2012). A recent accounting of described species in the phylum Nematoda at 24,783 is conservatively believed to represent 5–10% of extant species (Hodda 2011). One contributing factor to this relatively low number of described species may be the actual process of species delimitation typically used in the description of a new nematode species. A large percentage of nematode species have been characterized solely on the basis of a comparatively small set of morphological characters and measurements. Species delimited in this fashion have been called morphospecies. The delimitation process in nematology is seldom articulated, although it generally involves the search for discriminating morphological characters. Discriminating or diagnostic characters often are identified by the authors as those that exceed the known range of a morphometric variable, or combine measurements in a novel form to satisfy implicit criteria of diagnosability. If no morphologically diagnosable characters are identified, the species is assumed to be conspecific with an existing nominal species. Given the limited set of available morphological characters easily resolved by light microscopy, this approach has resulted in the recognition of a large number of cosmopolitan species (Artois *et al*. 2011).

The addition of DNA characters to the discovery process has revealed that many of these cosmopolitan species may actually consist of multiple genetically distinct subgroups (Kiontke *et al.* 2011; Nadler, S.A. & Pérez-Ponce De León, G. 2012). In this study we examined North American species in the plant-parasitic nematode genus *Mesocriconema* Andrássy, 1965. Worldwide this genus has 90 valid species (Geraert 2010). Approximately 24 species are reported from North America. Among the North American species are five morphospecies that could be considered worldwide in distribution. These species, *M. curvatum* (Raski, 1952) Loof & DeGrisse, 1989, *M. ornatum* (Raski, 1958) Loof & DeGrisse, 1989, *M. rusticum* (Micoletzky, 1915) Loof & DeGrisse, 1989, *M. sphaerocephalum* (Taylor, 1936) Loof & DeGrisse, 1989, and *M. xenoplax* (Raski, 1952) Loof & DeGrisse, 1989 are predominantly associated with agricultural plant hosts, and it is this association that is assumed responsible for their present-day cosmopolitan distribution (Wouts 2006). These species, however, are not confined to agricultural hosts, and surveys of native plant communities also record their presence. Another potential factor contributing to their perceived cosmopolitan distribution is the relatively early dates of their descriptions. All five species were described prior to 1952, a time when only 21 species were listed as belonging to the genus *Criconemoides* Taylor, 1936. Early records of occurrence leading to present-day perception of global distributions may actually reflect the limited options available in early keys, “fuzzy” species boundaries, or assumptions of identity biased by plant host association. For example, a criconematid species associated with peach in the southeastern U.S. might automatically be assumed to be *M. xenoplax*, based on that species’ role in the disease complex called peach tree short-life (Nyczepir *et al*. 1985).

Our study encompasses collections from within 23 of the 118 North American World Wildlife Federation (WWF) ecoregions (Olson *et al*. 2004) including both native plant communities and cultivated soils (Table 1). From these soils we have extracted and analyzed specimens morphologically conforming to the genus *Mesocriconema*. Each specimen is measured, photographed, and then processed for PCR by primers that permit nucleotide sequencing of a 721-bp portion of the mitochondrial COI gene. This approach preserves the link between specimen morphology and COI haplotype, and allows us to link morphologically distinct juvenile and adult stages. Males are rare in *Mesocriconema* and have not been included in the analysis. Of the 89 *Mesocriconema* species descriptions in Geraert (2010), males are unknown for 72. Criconematid males have a degenerate morphology and differ little among the various genera in this family. It is likely that *Mesocriconema* males play little or no role in reproduction and that mitochondrial and nuclear genomes are inherited clonally; parthenogenesis is assumed to be the primary form of reproduction in *Mesocriconema*. No *Mesocriconema* males were encountered in the collections examined in this study.

The goals of this study were to construct a gene tree from the 721-bp portion of COI, use it as a framework to evaluate nematode morphology, and assess congruence of morphospecies designations with COI-derived groups. This study is not a revision of *Mesocriconema* and is not intended to address issues of higher classification. Included among the morphospecies in the dataset are four of the five cosmopolitan species commonly observed in North America. Two species were sampled at their type localities (*Discocriconemella inarata* Hoffman, 1974 and *Mesocriconema discus* (Thorne & Malek, 1968) Loof & DeGrisse, 1989) and two other species were sampled from type hosts within the vicinity of a type locality (*M. ornatum* and *M. xenoplax*). Defining characteristics of the genus *Mesocriconema* are the presence of four submedian lobes surrounding the oral disc on the cephalic region and an open vulva, often preceded by cuticular modifications or projections of the annule anterior to the vulva. This modification is sometimes termed the anterior vulval lip or vulval flap. In general, *Mesocriconema* species, like all Criconematidae, are recognized by the distinct transverse annulations that give the nematode a segmented appearance and hence their common name of ring nematodes. Nomenclature of the genus has been considered unstable (Hunt 2008). Some nematode taxonomists use *Macroposthonia* de Man, 1880, in the place of *Mesocriconema* (Siddiqi 2000; Wouts 2006) and others recognize neither genus name, preferring to assign species to the genus *Criconemoides* Taylor, 1936 (Decraemer & Hunt 2006). In using *Mesocriconema*, we are following two recent comprehensive taxonomic treatments of terrestrial nematodes (Andrássy 2007; Geraert 2010).

Species descriptions of criconematid nematodes have historically used an implicit morphological species concept, although it has been noted that during the last 25 years of taxonomic research in nematology "nematode descriptions are typically devoid of reference to explicit species concepts or details and procedures of delimitation" (Nadler 2002). In this report we apply a lineage species concept as articulated by De Queiroz (2007) using an integrated taxonomic approach for species delimitation (De Salle *et al.* 2005; Gibbs 2009; Padial *et al.* 2010). Molecular criteria used to evaluate the distinctiveness of haplotype groups in this study included Rosenberg's measure of reciprocal monophyly (Rosenberg *et al.* 2007), absence of genetic intermediates, genetic distance, and the Rodrigo *et al.* (2008) measure of taxon distinctiveness. Most sampling localities were georeferenced and locality information appended to GenBank accession numbers. The morphology of individual specimens was reassessed and discussed in light of molecular-based haplotype groupings.

Material and methods

Collection information about each of the specimens examined in this study is given in Table 1. Each specimen is represented by a unique Nematode Identification Number (NID) and that number is affixed to the terminal tips on the phylogenetic trees and to the figures depicting nematode morphology. The figures and text in the results section were organized according to the haplotype groups generated by neighbor-joining analysis of the entire 247-specimen dataset using the COI gene region. Haplotype groups were sequentially numbered starting with the group arranged closest to the outgroup taxa on the radial version of the neighbor-joining tree (Fig. 1).

Nematode specimens were extracted from soil samples by a modified flotation-sieving and centrifugation method (Jenkins 1964). Morphological analysis was generally conducted on living specimens, or specimens heat-relaxed on glass microscope slides. Living specimens were more likely to provide views of ventral body parts and occasionally *en face* views useful for evaluating submedian lobes and labial plates. Each nematode was photographed with a Leica DC300 video camera and measured on a Leica DMLB light microscope with Differential Interference Contrast. A set of standard measurements were obtained from each nematode. Measurements for adults are illustrated on Figure 3 and include the following: L = body length, eso = length of esophagus (pharynx) from anterior end to junction with intestine, R = number of annules, Rex = number of annules from anterior end of body to excretory pore, Rv = number of annules from tail terminus to vulva, V = position of the vulva expressed as a percentage of total body length, number of anastomoses on the body cuticle, length of stylet, width of stylet knobs, width of annules at midbody, midbody width, vulval body width and width of first labial annule.

Following photo-documentation the nematode specimen was removed from the glass slide, placed on a coverslip in an 18 µl drop of sterile water, and ruptured with a transparent micropipette tip. The macerated nematode in water was transferred to a 0.25-ml PCR reaction tube and stored at -20ºC.

The COI primer sequences were COI-F5—5'-AATWTWGGTGTTGGAACTTCTTGAAC-3' and COI-R9—5'-CTTAAAACATAATGRAAATGWGCWACWACATAATAAGTATC-3' which in PCR reactions produced an approximately 790-bp amplification product, providing 721 bp of sequence for genetic analysis. The primers are located on the mitochondrial COI gene at positions 1822–2612 on the *Drosophila yakuba* reference sequence (GenBank Accession # X03240) (Clary & Wolstenholme 1985). PCR amplification reactions, conducted in a 30.0-µl total volume within 0.6-ml reaction tubes, consisted of 9.0 µl of template from the ruptured nematode specimen, 2.4 µl of each 20µM primer solution for a 1.6 µM final primer concentration, 1.2 µl ddH20, and 15 µl of 2x JumpStart REDTaq ReadyMix (Sigma-Aldrich) for a 0.03U/µl final enzyme concentration. PCR conditions included a hotstart and 5-minute treatment at 94ºC followed by 50 cycles of 30 seconds at 94ºC denaturation, 30 seconds at 48ºC annealing, and 1.5 minutes at 72ºC with a ramping rate of 0.5ºC/second for the elongation step. A final 5-minute extension at 72ºC completed the process. Following amplification, an initial check gel was run followed by cleaning of the PCR product by gel fragment extraction from a 0.7% agarose gel, using Gel/PCR DNA Fragment Extraction Kit (IBI Scientific). DNA templates were sequenced by either the sequencing center at the University of Arkansas for Medical Services or by Davis Sequencing Services. Nearly all amplification products were sequenced in both directions (three were sequenced in one direction only). CodonCode Aligner Version 4.2 (http://www.codoncode.com/) was used for sequence editing prior to alignment using Clustal W in MEGA version 5 (Tamura *et al.* 2011).

Neighbor-joining analysis was conducted in MEGA5 using p-distance values and 2,000 bootstrap replications. Maximum-likelihood analyses conducted in MEGA5 with selection of best substitutions model by the Akaike Information Criterion (Akaike 1974) resulted in the selection of the General Time Reversible Model with Gamma-distributed rates plus invariant sites (GTR+G+I). Bayesian analyses were conducted using the Mr. Bayes 3.2.1 Plugin through Geneious R7. The selected substitution model was GTR and posterior probability was used in assessment of clade credibility (Bayesian trees not presented).

Nucleotide and haplotype diversity, and other features of the haplotype groups were evaluated using DnaSP Version 5 (Librado & Rozas 2009). Mean pairwise intergroup p-distances were calculated using MEGA5. Measures of haplotype group distinctiveness were evaluated with the Species Delimitation plug-in to the Geneious software package (Masters *et al*. 2011). The plug-in options include assessments of reciprocal monophyly and Intra/Inter, the ratio of within-group genetic differentiation to the distance to the nearest neighbor. This ratio, together with the known number of taxa in the reference group, was used in determining the probability of correct identification under strict or relaxed cladistic criteria (P ID (Strict) or P ID (liberal)) (Ross *et al*. 2008). Under the liberal criteria, the unknown member of the group must fall within or be a sister to the group, and under the strict criteria the unknown member must fall within the group and not in the sister group. These probabilities are reported with 95% confidence intervals. Rosenberg's test (Rosenberg 2007) for reciprocal monophyly (P(AB)) and the statistic for calculating clade distinctiveness by Rodrigo *et al*. (2008) were also applied to the haplotype groups. The latter two measures assess the probability that the observed patterns were due to random coalescent processes.

DNA sequences were submitted to GenBank (GB Acc #s KJ787823–KJ788069) with associated GPS coordinates when available. Both females and juveniles were used in molecular analyses. Only adult females were used in the morphological analyses presented in Table 5.

Nematodes were prepared for scanning electron microscopy (SEM) by fixation in 4% formalin followed by dehydration in a graded series of alcohol to 100% ETOH, critical point drying, mounting on SEM specimen stubs, and coating with gold. Images were obtained on a Hitachi S-3000N scanning electron microscope. Nematodes prepared for SEM were selected from conspecific specimens from the same soil sample, which were measured and analyzed molecularly. Although care was taken in the microscopic selection of specimens for SEM analysis, the possibility exists that non-target specimens were unintentionally selected.

TABLE 1. Specimen origin, COI haplotype group, morphospecies designation and Nematode Identification Number (NID)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
1	500	*Mesocriconema*	*ornatum*	F	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787823
1	502	*Mesocriconema*	*ornatum*	F	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787824
1	505	*Mesocriconema*	*ornatum*	J	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787825
1	1275	*Mesocriconema*	*ornatum*	F	Chilton County, AL	Southeastern Mixed Forests	NA0413	Y	KJ787826
1	1281	*Mesocriconema*	*ornatum*	F	Chilton County, AL	Southeastern Mixed Forests	NA0413	Y	KJ787827
1	1283	*Mesocriconema*	*ornatum*	F	Chilton County, AL	Southeastern Mixed Forests	NA0413	Y	KJ787828
1	1284	*Mesocriconema*	*ornatum*	F	Chilton County, AL	Southeastern Mixed Forests	NA0413	Y	KJ787829
1	1285	*Mesocriconema*	*ornatum*	J	Chilton County, AL	Southeastern Mixed Forests	NA0413	Y	KJ787830
1	P183088	*Mesocriconema*	*ornatum*	J	Heredia Province, Costa Rica	Isthmian-Atlantic Moist Forests	NT0129	Y	KJ787831
1	P183090	*Mesocriconema*	*ornatum*	J	Heredia Province, Costa Rica	Isthmian-Atlantic Moist Forests	NT0129	Y	KJ787832
1	P190087	*Mesocriconema*	*ornatum*	J	Frio County, TX	Tamaulipan Mezquital	NA1312	Y	KJ787833
2	1242	*Mesocriconema*	*onoense*	F	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787834
2	1249	*Mesocriconema*	*onoense*	F	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787835
2	1250	*Mesocriconema*	*onoense*	F	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787836
2	1253	*Mesocriconema*	*onoense*	F	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787837
2	1254	*Mesocriconema*	*onoense*	J	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787838
3	1059	*Mesocriconema*	sp.	F	Gallatin National Forest, MT	South Central Rockies Forests	NA0528	N	KJ787839
3	1096	*Mesocriconema*	sp.	F	Gallatin National Forest, MT	South Central Rockies Forests	NA0528	N	KJ787840
3	1100	*Mesocriconema*	sp.	J	Gallatin National Forest, MT	South Central Rockies Forests	NA0528	N	KJ787841
3	1101	*Mesocriconema*	sp.	F	Gallatin National Forest, MT	South Central Rockies Forests	NA0528	N	KJ787842
4	122	*Mesocriconema*	*curvatum*	F	MT	Northern Tall Grasslands	NA0812	Y	KJ787843
4	123	*Mesocriconema*	*curvatum*	F	MT	Northern Tall Grasslands	NA0812	Y	KJ787844
4	124	*Mesocriconema*	*curvatum*	J	Boone County, MO	Central US Hardwood Forests	NA0404	Y	KJ787845
4	149	*Mesocriconema*	*curvatum*	F	Big Horn County, WY	Wyoming Basin Shrub Steppe	NA1313	Y	KJ787846
4	362	*Mesocriconema*	*curvatum*	F	Treasure County, MT	Northwestern Mixed Grasslands	NA0810	Y	KJ787847
4	1087	*Mesocriconema*	*curvatum*	F	Saunders County, NE	Central Tall Grasslands	NA0805	Y	KJ787848
4	1088	*Mesocriconema*	*curvatum*	F	Saunders County, NE	Central Tall Grasslands	NA0805	Y	KJ787849
4	1090	*Mesocriconema*	*curvatum*	J	Saunders County, NE	Central Tall Grasslands	NA0805	Y	KJ787850

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
4	1116	*Mesocriconema*	*curvatum*	J	Arena Pines and Sand Barrens State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ787851
5	99	*Mesocriconema*	*rusticum*	F	Summit County, UT	Wasatch and Uinta Montane Forests	NA0530	Y	KJ787852
5	447	*Mesocriconema*	*rusticum*	F	Douglas County, NE	Central Tall Grasslands	NA0805	Y	KJ787853
5	691	*Mesocriconema*	*rusticum*	F	UNL East Campus, NE	Central Tall Grasslands	NA0805	Y	KJ787854
5	742	*Mesocriconema*	*rusticum*	J	Shimek State Forest, IA	Central Tall Grasslands	NA0805	N	KJ787855
5	1140	*Mesocriconema*	*rusticum*	F	Avoca Prairie and Savanna State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ787856
5	1141	*Mesocriconema*	*rusticum*	J	Avoca Prairie and Savanna State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ787857
5	P155078	*Mesocriconema*	*rusticum*	F	Konza Prairie Biological Station , KS	Flint Hills Tall Grasslands	NA0807	N	KJ787858
5	P228021	*Mesocriconema*	*rusticum*	U	UNL East Campus, NE	Central Tall Grasslands	NA0805	Y	KJ787859
6	432	*Mesocriconema*	sp.	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787860
6	753	*Mesocriconema*	sp.	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787861
6	1257	*Mesocriconema*	sp.	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787862
6	1258	*Mesocriconema*	sp.	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787863
6	1259	*Mesocriconema*	sp.	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787864
6	1260	*Mesocriconema*	sp.	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787865
7	431	*Mesocriconema*	*discus*	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787866
7	433	*Mesocriconema*	*discus*	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787867
7	443	*Mesocriconema*	*discus*	J	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787868
7	444	*Mesocriconema*	*discus*	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787869
7	756	*Mesocriconema*	*discus*	F	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787870
7	757	*Mesocriconema*	*discus*	J	Brookings County, SD	Central Tall Grasslands	NA0805	N	KJ787871
7	844	*Mesocriconema*	*discus*	J	Doolittle Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787872
8	728	*Mesocriconema*	*xenoplax*	F	Pickens County, SC	Southeastern Mixed Forests	NA0413	Y	KJ787873
8	729	*Mesocriconema*	*xenoplax*	J	Pickens County, SC	Southeastern Mixed Forests	NA0413	Y	KJ787874
8	730	*Mesocriconema*	*xenoplax*	J	Pickens County, SC	Southeastern Mixed Forests	NA0413	Y	KJ787875
8	1294	*Mesocriconema*	*xenoplax*	F	Clemson University, Musser Fruit Research Farm, SC	Southeastern Mixed Forests	NA0413	Y	KJ787876
8	1297	*Mesocriconema*	*xenoplax*	J	Clemson University, Musser Fruit Research Farm, SC	Southeastern Mixed Forests	NA0413	Y	KJ787877

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
8	1298	*Mesocriconema*	*xenoplax*	F	Clemson University, Musser Fruit Research Farm, SC	Southeastern Mixed Forests	NA0413	Y	KJ787878
8	P231030	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787879
9	583	*Mesocriconema*	*xenoplax*	F	Wakulla County, FL	Southeastern Conifer Forests	NA0529	N	KJ787880
9	607	*Mesocriconema*	*xenoplax*	F	Schluckebier Prairie State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ787881
9	733	*Mesocriconema*	*xenoplax*	J	Washington County, AR	Ozark Mountain Forests	NA0412	N	KJ787882
9	735	*Mesocriconema*	*xenoplax*	J	Washington County, AR	Ozark Mountain Forests	NA0412	N	KJ787883
9	1217	*Mesocriconema*	*xenoplax*	F	Sauk County, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ787884
10	584	*Mesocriconema*	*xenoplax*	F	Columbia County, FL	Southeastern Conifer Forests	NA0529	N	KJ787885
10	588	*Mesocriconema*	*xenoplax*	F	Columbia County, FL	Southeastern Conifer Forests	NA0529	N	KJ787886
10	736	*Mesocriconema*	*xenoplax*	F	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787887
10	P231026	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787888
10	P231028	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787889
10	P231031	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787890
10	P231032	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787891
10	P231034	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787892
10	P231035	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787893
10	P231036	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787894
10	P231037	*Mesocriconema*	*xenoplax*	U	USDA Southeastern Fruit and Nut Research Laboratory, GA	Southeastern Mixed Forests	NA0413	Y	KJ787895
11	999	*Mesocriconema*	*xenoplax*	F	GRSM, TN	Appalachian Blue Ridge Forests	NA0403	N	KJ787896
11	1024	*Mesocriconema*	*xenoplax*	F	GRSM, TN	Appalachian Blue Ridge Forests	NA0403	N	KJ787897
11	1025	*Mesocriconema*	*xenoplax*	F	GRSM, TN	Appalachian Blue Ridge Forests	NA0403	N	KJ787898
11	1028	*Mesocriconema*	*xenoplax*	J	GRSM, TN	Appalachian Blue Ridge Forests	NA0403	N	KJ787899
11	1215	*Mesocriconema*	*xenoplax*	F	Accotink Creek Watershed, Fairfax County, VA	Southeastern Mixed Forests	NA0413	N	KJ787900

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
12	3	*Mesocriconema*	*xenoplax*	F	Cass County, NE	Central Tall Grasslands	NA0805	N	KJ787901
12	173	*Mesocriconema*	*xenoplax*	F	Hooker County, NE	Nebraska Sand Hills Mixed Grasslands	NA0809	N	KJ787902
12	944	*Mesocriconema*	*xenoplax*	J	Cass County, NE	Central Tall Grasslands	NA0805	N	KJ787903
12	945	*Mesocriconema*	*xenoplax*	J	Cass County, NE	Central Tall Grasslands	NA0805	N	KJ787904
12	947	*Mesocriconema*	*xenoplax*	J	Cass County, NE	Central Tall Grasslands	NA0805	N	KJ787905
13	724	*Mesocriconema*	*xenoplax*	F	GRSM, TN	Appalachian Blue Ridge Forests	NA0403	N	KJ787906
13	746	*Mesocriconema*	*xenoplax*	J	British Columbia, CAN	Cascade Mountains Leeward Forests	NA0507	N	KJ787907
13	747	*Mesocriconema*	*xenoplax*	F	British Columbia, CAN	Cascade Mountains Leeward Forests	NA0507	N	KJ787908
13	1267	*Mesocriconema*	*xenoplax*	J	GWMP, VA	Southeastern Mixed Forests	NA0413	N	KJ787909
13	1276	*Mesocriconema*	*xenoplax*	F	GWMP, VA	Southeastern Mixed Forests	NA0413	N	KJ787910
13	P74053	*Mesocriconema*	*xenoplax*	J	Fresno County, CA	California Central Valley Grasslands	NA0801	Y	KJ787911
13	P194033	*Mesocriconema*	*xenoplax*	F	Konza Prairie Biological Station, KS	Flint Hills Tall Grasslands	NA0807	N	KJ787912
14	1072	*Mesocriconema*	*xenoplax*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787913
14	1073	*Mesocriconema*	*xenoplax*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787914
14	1368	*Mesocriconema*	*xenoplax*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787915
14	1375	*Mesocriconema*	*xenoplax*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787916
15	1067	*Mesocriconema*	*ornatum*	F	Beaufort County, SC	Middle Atlantic Coastal Forests	NA0517	Y	KJ787917
15	1104	*Mesocriconema*	*ornatum*	F	Beaufort County, SC	Middle Atlantic Coastal Forests	NA0517	Y	KJ787918
15	1105	*Mesocriconema*	*ornatum*	J	Beaufort County, SC	Middle Atlantic Coastal Forests	NA0517	Y	KJ787919
15	1106	*Mesocriconema*	*ornatum*	F	Beaufort County, SC	Middle Atlantic Coastal Forests	NA0517	Y	KJ787920
15	1107	*Mesocriconema*	*ornatum*	F	Beaufort County, SC	Middle Atlantic Coastal Forests	NA0517	Y	KJ787921
15	1248	*Mesocriconema*	*ornatum*	F	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787922
15	1251	*Mesocriconema*	*ornatum*	J	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787923
15	1252	*Mesocriconema*	*ornatum*	J	Auburn University Turf Unit, AL	Southeastern Mixed Forests	NA0413	Y	KJ787924
15	1299	*Mesocriconema*	*ornatum*	F	Lowndes County, MS	Southeastern Mixed Forests	NA0413	Y	KJ787925
15	1300	*Mesocriconema*	*ornatum*	F	Lowndes County, MS	Southeastern Mixed Forests	NA0413	Y	KJ787926
15	1301	*Mesocriconema*	*ornatum*	J	Lowndes County, MS	Southeastern Mixed Forests	NA0413	Y	KJ787927
16	244	*Mesocriconema*	sp.	J	NMSU, Leyendecker Plant Science Research Center, NM	Chihuahuan Desert	NA1302	Y	KJ787928
16	245	*Mesocriconema*	sp.	F	NMSU, Leyendecker Plant Science Research Center, NM	Chihuahuan Desert	NA1302	Y	KJ787929

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
17	9	*Mesocriconema*	*inaratum*	F	Kalsow Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787930
17	12	*Mesocriconema*	*inaratum*	F	Kalsow Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787931
17	759	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787932
17	760	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787933
17	1134	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787934
17	1135	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787935
17	1138	*Mesocriconema*	*inaratum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787936
17	1153	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787937
17	1154	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787938
17	1172	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787939
17	1244	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787940
17	1266	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787941
17	1312	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787942
17	1319	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787943
17	1320	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787944
17	1321	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787945
17	1322	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787946
17	1323	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787947
17	1363	*Mesocriconema*	*inaratum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787948
17	1364	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787949
17	1366	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787950
17	1367	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787951
17	1369	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787952
17	1373	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787953
17	1402	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787954
17	1403	*Mesocriconema*	*inaratum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787955
17	1410	*Mesocriconema*	*inaratum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787956
17	1422	*Mesocriconema*	*inaratum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787957
17	1424	*Mesocriconema*	*inaratum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787958

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
18	600	*Mesocriconema*	*curvatum*	F	Aurora Prairie, SD	Central Tall Grasslands	NA0805	N	KJ787959
18	825	*Mesocriconema*	*curvatum*	F	Sheeder Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787960
18	827	*Mesocriconema*	*curvatum*	J	Sheeder Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787961
18	828	*Mesocriconema*	*curvatum*	F	Sheeder Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787962
18	829	*Mesocriconema*	*curvatum*	F	Sheeder Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787963
18	832	*Mesocriconema*	*curvatum*	F	Kalsow Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787964
18	833	*Mesocriconema*	*curvatum*	F	Kalsow Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787965
18	835	*Mesocriconema*	*curvatum*	J	Kalsow Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787966
18	836	*Mesocriconema*	*curvatum*	F	Kalsow Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787967
18	842	*Mesocriconema*	*curvatum*	F	Doolittle Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787968
18	843	*Mesocriconema*	*curvatum*	J	Doolittle Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787969
18	845	*Mesocriconema*	*curvatum*	F	Doolittle Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787970
18	884	*Mesocriconema*	*curvatum*	F	Doolittle Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ787971
18	888	*Mesocriconema*	*curvatum*	F	Reichelt Unit, Rock Creek State Park, IA	Central Tall Grasslands	NA0805	N	KJ787972
18	1050	*Mesocriconema*	*curvatum*	J	Stafford County, KS	Central and Southern Mixed Grasslands	NA0803	N	KJ787973
18	1074	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787974
18	1091	*Mesocriconema*	*curvatum*	J	Stafford County, KS	Central and Southern Mixed Grasslands	NA0803	N	KJ787975
18	1092	*Mesocriconema*	*curvatum*	F	Stafford County, KS	Central and Southern Mixed Grasslands	NA0803	N	KJ787976
18	1093	*Mesocriconema*	*curvatum*	J	Stafford County, KS	Central and Southern Mixed Grasslands	NA0803	N	KJ787977
18	1094	*Mesocriconema*	*curvatum*	F	Stafford County, KS	Central and Southern Mixed Grasslands	NA0803	N	KJ787978
18	1112	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787979
18	1246	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787980
18	1265	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787981
18	1313	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787982
18	1315	*Mesocriconema*	*curvatum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787983
18	1318	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787984
18	1343	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787985
18	1352	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787986
18	1374	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787987

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
18	1378	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787988
18	1379	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787989
18	1380	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787990
18	1386	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787991
18	1413	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787992
18	1415	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787993
18	1429	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787994
18	1430	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787995
18	1433	*Mesocriconema*	*curvatum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787996
18	1434	*Mesocriconema*	*curvatum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ787997
18	P77025	*Mesocriconema*	*curvatum*	F	Konza Prairie Biological Station, KS	Flint Hills Tall Grasslands	NA0807	N	KJ787998
19	919	*Mesocriconema*	*kirjanovae*	J	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ787999
19	920	*Mesocriconema*	*kirjanovae*	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788000
19	943	*Mesocriconema*	*kirjanovae*	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788001
19	1168	*Mesocriconema*	*kirjanovae*	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788002
19	1169	*Mesocriconema*	*kirjanovae*	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788003
19	1170	*Mesocriconema*	*kirjanovae*	J	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788004
20	1270	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788005
20	1278	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788006
20	1303	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788007
20	1351	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788008
21	918	*Mesocriconema*	*curvatum*	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788009
21	942	*Mesocriconema*	*curvatum*	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788010
22	321	*Mesocriconema*	*curvatum*	F	Baltimore County, MD	Southeastern Mixed Forests	NA0413	N	KJ788011
22	363	*Mesocriconema*	*curvatum*	F	Baltimore County, MD	Southeastern Mixed Forests	NA0413	N	KJ788012
22	1167	*Mesocriconema*	*curvatum*	J	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788013
23	609	*Mesocriconema*	*curvatum*	F	Avoca Prairie and Savanna State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ788014
23	956	*Mesocriconema*	*curvatum*	F	Schluckebier Prairie State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ788015
24	608	*Mesocriconema*	*curvatum*	F	Walworth County, SD	Northern Short Grasslands	NA0811	Y	KJ788016

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
24	741	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788017
24	855	*Mesocriconema*	*curvatum*	J	Doolittle Prairie State Preserve, IA	Central Tall Grasslands	NA0805	N	KJ788018
24	1051	*Mesocriconema*	*curvatum*	F	Stafford County, KS	Central and Southern Mixed Grasslands	NA0803	N	KJ788019
24	1053	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788020
24	1054	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788021
24	1055	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788022
24	1056	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788023
24	1057	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788024
24	1058	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788025
24	1064	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788026
24	1066	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788027
24	1075	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788028
24	1109	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788029
24	1127	*Mesocriconema*	*curvatum*	F	Avoca Prairie and Savanna State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ788030
24	1129	*Mesocriconema*	*curvatum*	F	Avoca Prairie and Savanna State Natural Area, Iowa County, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ788031
24	1142	*Mesocriconema*	*curvatum*	F	Avoca Prairie and Savanna State Natural Area, WI	Upper Midwest Forest-Savanna Transition Zone	NA0415	N	KJ788032
24	1166	*Mesocriconema*	*curvatum*	F	Roth Prairie Natural Area, AR	Mississippi Lowland Forests	NA0409	N	KJ788033
24	1238	*Mesocriconema*	*curvatum*	F	Hitchcock County, NE	Central and Southern Mixed Grasslands	NA0803	N	KJ788034
24	1239	*Mesocriconema*	*curvatum*	F	Hitchcock County, NE	Central and Southern Mixed Grasslands	NA0803	N	KJ788035
24	1240	*Mesocriconema*	*curvatum*	J	Hitchcock County, NE	Central and Southern Mixed Grasslands	NA0803	N	KJ788036
24	1245	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788037
24	1264	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788038
24	1271	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788039
24	1277	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788040
24	1286	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788041
24	1287	*Mesocriconema*	*curvatum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788042
24	1289	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788043
24	1314	*Mesocriconema*	*curvatum*	J	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788044

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TABLE 1. (Continued)

Haplotype Group*	NID	Genus	Species	Stage**	Locality Name	Ecoregion Name (WWF)	Eco Code	Site Agri-cultural	Accession Number
24	1317	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788045
24	1342	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788046
24	1359	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788047
24	1360	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788048
24	1370	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788049
24	1371	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788050
24	1383	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788051
24	1384	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788052
24	1388	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788053
24	1389	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788054
24	1390	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788055
24	1391	*Mesocriconema*	*curvatum*	J	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788056
24	1393	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788057
24	1394	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788058
24	1427	*Mesocriconema*	*curvatum*	F	Spring Creek Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788059
24	1428	*Mesocriconema*	*curvatum*	F	Nine-Mile Prairie, NE	Central Tall Grasslands	NA0805	N	KJ788060
O	570	*Mesocriconema*	*sp.*	J	Jonathan Dickinson State Park, FL	Florida Sand Pine Scrub	NA0513	N	KJ788061
O	1068	*Mesocriconema*	*ornatum*	J	Beaufort County, SC	Middle Atlantic Coastal Forests	NA0517	Y	KJ788062
O	1451	*Mesocriconema*	*xenoplax*	F	GWMP, VA	Southeastern Mixed Forests	NA0413	N	KJ788063
O	P151049	*Nothocriconemoides*	sp.	F	La Selva Biological Station, Costa Rica	Isthmian-Atlantic Moist Forests	NT0129	N	KJ788064
OG	449	*Hemicycliophora*	*typica*	F	Greece	Aegean and Western Turkey Sclerophyllous and Mixed Forests	PA1201	Y	KJ788065
OG	1261	*Hemicycliophora*	*macristhmus*	F	GWMP, VA	Southeastern Mixed Forests	NA0413	N	KJ788066
OG	1292	*Hemicycliophora*	*thornei*	F	GWMP, VA	Southeastern Mixed Forests	NA0413	N	KJ788067
OG	1305	*Hemicycliophora*	sp.	F	GWMP, VA	Southeastern Mixed Forests	NA0413	N	KJ788068
OG	P184030	*Discocriconemella*	*limitanea*	F	Las Cruces Biological Station, Costa Rica	Isthmian-Pacific Moist Forests	NT0130	N	KJ788069

*O—Orphan (single individual belonging to no haplotype group); OG—Outgroup

**U— Undetermined

Results

COI haplotype groups in *Mesocriconema* dataset. A neighbor-joining analysis of all 242 *Mesocriconema* specimens in the dataset and 5 outgroup specimens was prepared as a radial tree (Fig. 1). The bars outside the terminal tips in Figure 1 identify 24 well-supported groups based on calculated genetic distance (mean intergroup p-distance 12.4%). A matrix of pairwise genetic p-distance values with the closest values for each pairing highlighted is presented in Table 3. Pairwise p-distance values of the haplotype groups range from a low of 0.027 (2.7% for groups 12x13) to 0.227 (22.7% for groups 17x1). Haplotype diversity and nucleotide diversity for the 24 groups are presented in Table 2. Four singletons present in the dataset (N1068, P151049, N570, N1451) were not readily accommodated by any group and were excluded from these analyses.

A maximum-likelihood tree from the *Mesocriconema* dataset was generated after redundant sequences were removed (Fig. 2). Eight of the groups in Figure 1 were collapsed to a single sequence due to the lack of haplotype diversity within those groups, reducing the dataset to 82 sequences, excluding outgroup taxa. These eight haplotypes were still considered as haplotype "groups" for morphological analyses due to the existence of multiple specimens included within the groups and the large genetic distance between groups. In the case of Group 1, a single COI haplotype was collected from five locations ranging geographically from Georgia, Alabama, and Texas in the United States, to Costa Rica in Central America. Six of the other haplotype groups lacking diversity were collected from single locations indicating the need for additional sampling.

TABLE 2. COI haplotype, nucleotide diversity, and mean intragroup distance.

Haplotype Group	n	Number of Mutations	Number of Haplotypes	Haplotype Diversity	Nucleotide Diversity	P-Distance	
						Range	Mean
1	11	0	1	0	0	0	0
2	5	0	1	0	0	0	0
3	4	0	1	0	0	0	0
4	9	1	2	0.556	0.00077	0–0.001	0.0008
5	8	11	5	0.857	0.00446	0–0.012	0.0045
6	6	0	1	0	0	0	0
7	7	1	2	0.286	0.0004	0–0.001	0.0004
8	7	1	2	0.286	0.0004	0–0.001	0.0004
9	5	37	4	0.9	0.02469	0–0.043	0.0247
10	11	0	1	0	0	0	0
11	5	2	2	0.4	0.00111	0–0.003	0.0011
12	5	8	4	0.9	0.0043	0–0.008	0.0043
13	7	3	3	0.524	0.00119	0–0.004	0.0012
14	4	0	1	0	0	0	0
15	11	2	3	0.636	0.00101	0–0.001	0.001
16	2	0	1	0	0	0	0
17	29	8	6	0.719	0.00292	0–0.01	0.0029
18	40	31	9	0.706	0.00539	0–0.024	0.0054
19	6	33	3	0.6	0.02413	0–0.046	0.0234
20	4	2	3	0.833	0.00139	0–0.003	0.0014
21	2	0	1	0	0	0	0
22	3	24	3	1	0.02219	0.004–0.033	0.0222
23	2	15	2	1	0.0208	0.0208	0.0208
24	45	53	15	0.788	0.01293	0–0.029	0.0129

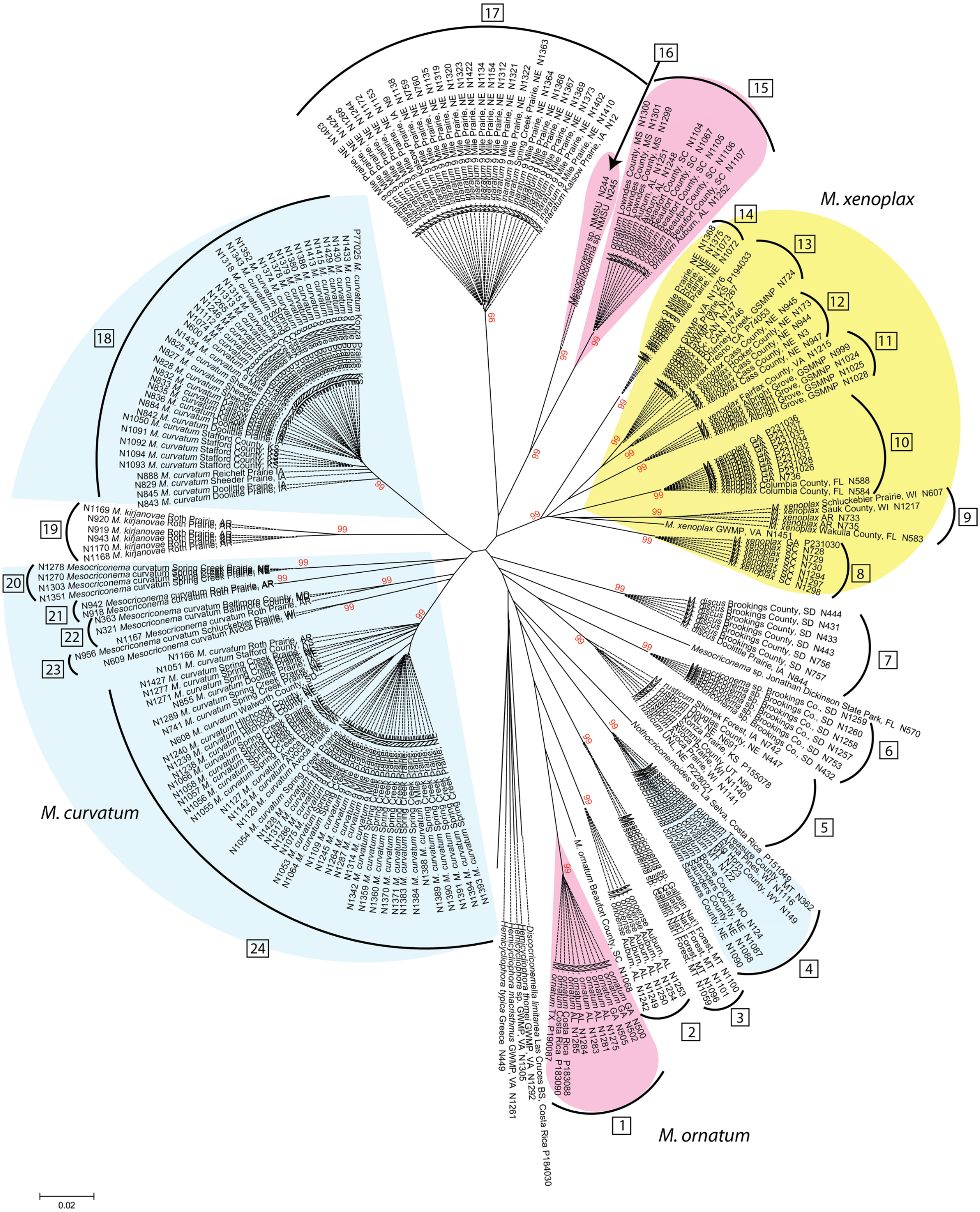

FIGURE 1. Neighbor-joining tree of COI nucleotide sequence from 242 *Mesocriconema* specimens. Each terminal node includes a Nematode Identification Number (NID), taxon name, and location information. Haplotype groups have been bracketed and given a group number. Groups shaded by color indicate haplotype groups and their potential morphospecies identification (blue=*M. curvatum*, pink=*M. ornatum*, yellow=*M. xenoplax*). Bootstrap support values are provided for each haplotype group.

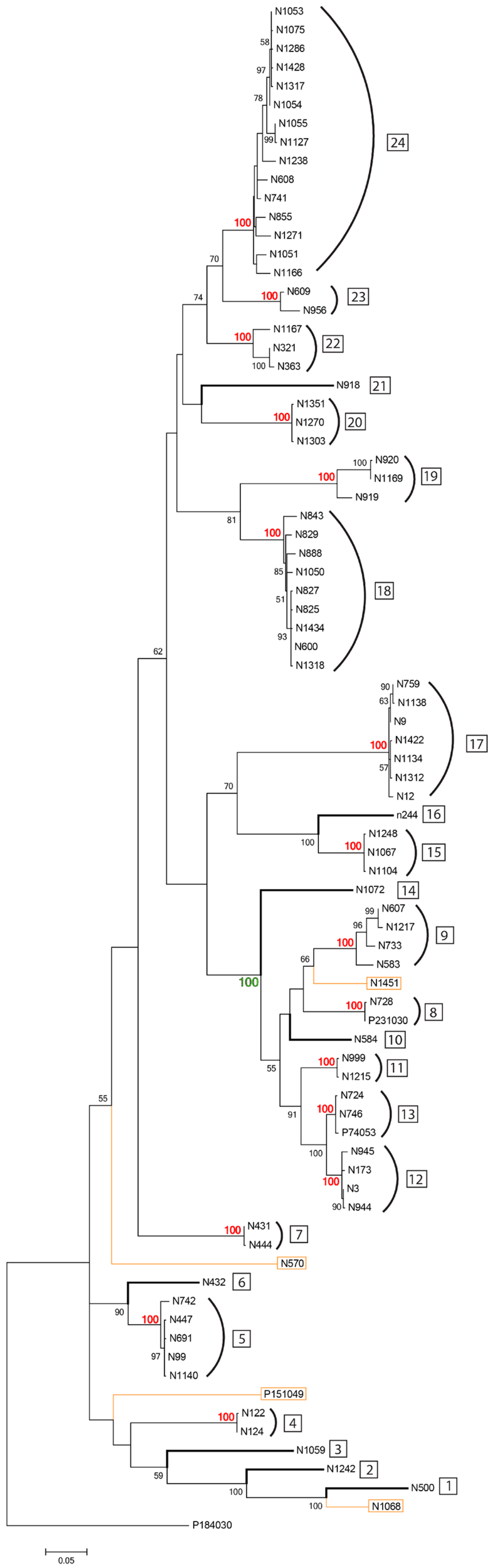

FIGURE 2. Maximum-likelihood tree of dataset reduced to 81 unique Mesocriconema haplotypes and including 1 outgroup sequence. Haplotype groups are indicated by the boxed numbers by the terminal branch tips. Eight haplotype groups without haplotype diversity are indicated by bolded branches. Four singleton specimens of uncertain taxonomic status are indicated by tan branches and boxed NID numbers. Bootstrap support values represent 100 replications. The tree was rooted with *Discocriconemella limitanea*.

TABLE 3. Interspecific mean pairwise distance (P-distance). Lowest distance-values bolded.

Group #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1		**0.138**	0.174	0.165	0.167	0.179	0.196	0.204	0.219	0.213	0.196	0.201	0.199	0.226	0.216	0.204	0.227	0.198	0.189	0.207	0.225	0.199	0.203	0.21
2			**0.136**	0.143	0.144	0.15	0.163	0.193	0.202	0.188	0.195	0.191	0.192	0.207	0.219	0.19	0.204	0.174	0.173	0.179	0.19	0.173	0.184	0.175
3				0.139	**0.125**	0.143	0.142	0.164	0.179	0.172	0.18	0.179	0.172	0.185	0.213	0.193	0.189	0.16	0.174	0.159	0.181	0.153	0.169	0.144
4					**0.114**	0.121	0.147	0.173	0.182	0.187	0.182	0.169	0.174	0.18	0.195	0.174	0.179	0.158	0.17	0.166	0.179	0.163	0.17	0.148
5						**0.078**	**0.11**	0.143	0.163	0.154	0.153	0.143	0.137	0.153	0.18	0.171	0.169	0.131	0.149	0.144	0.154	0.117	0.126	0.109
6							0.128	0.165	0.173	0.161	0.152	0.156	0.153	0.157	0.194	0.179	0.178	0.152	0.163	0.169	0.171	0.138	0.158	0.131
7								0.114	0.151	0.134	0.133	0.121	0.121	0.146	0.17	0.165	0.159	0.13	0.144	0.144	0.145	0.118	0.129	0.112
8									0.097	0.089	0.097	0.081	**0.079**	0.11	0.158	0.17	0.157	0.148	0.149	0.159	0.178	0.131	0.143	0.122
9										0.097	0.097	0.094	**0.091**	0.128	0.184	0.188	0.174	0.166	0.156	0.166	0.184	0.148	0.16	0.146
10											0.096	0.091	**0.087**	0.103	0.165	0.164	0.173	0.147	0.147	0.152	0.161	0.128	0.147	0.134
11												0.069	**0.06**	0.113	0.16	0.168	0.172	0.143	0.122	0.158	0.17	0.128	0.144	0.127
12													**0.027**	0.1	0.149	0.165	0.167	0.149	0.133	0.153	0.171	0.12	0.143	0.127
13														**0.1**	0.151	0.161	0.17	0.146	0.131	0.151	0.164	0.116	0.142	0.126
14															0.156	0.157	0.17	0.156	0.151	0.157	0.176	0.144	0.149	0.141
15																**0.098**	0.158	0.149	0.161	0.16	0.169	0.147	0.163	0.158
16																	0.163	0.135	0.148	0.138	0.172	0.144	0.16	0.15
17																		0.158	0.181	0.175	0.178	0.157	0.167	**0.155**
18																			**0.108**	0.121	0.147	**0.115**	0.123	**0.107**
19																				0.148	0.16	0.124	0.136	0.125
20																					0.14	**0.109**	0.131	0.114
21																						0.136	0.134	**0.124**
22																							0.093	**0.079**
23																								**0.085**
24																								

TABLE 4. Species delimitation analyses by Geneious plug-in software (Masters *et al.* 2011). Calculation based on Tamura-Nei distances.

Haplotype Group	n	Closest Group	Monophyletic	Intraspecific Distance	Interspecific Distance to Closest Group	Intra/ Inter	P ID (Strict)	P ID (Liberal)	Av (MRCA-tips)	P (Randomly Distinct)	Group Support	Rosenberg's P
1	11	2	yes	-	0.155	-	0.99 (0.90,1.0)	1.00 (0.96,1.0)	-	-	100%	0.02
2	5	1	yes	-	0.155	-	0.93 (0.81,1.0)	0.98 (0.88,1.0)	-	-	100%	2.00E-05
3	4	5	yes	-	0.148	-	0.87 (0.73,1.0)	0.98 (0.87,1.0)	-	-	100%	NAN
4	9	5	yes	0.001	0.141	0.01	0.98 (0.90,1.0)	1.00 (0.95,1.0)	0.0015	0.05	100%	0.02
5	8	6	yes	0.006	0.089	0.06	0.92 (0.81,1.0)	0.98 (0.92,1.0)	0.0069	0.05	100%	5.10E-05
6	6	5	yes	-	0.089	-	0.93 (0.81,1.0)	0.98 (0.88,1.0)	-	-	100%	5.10E-05
7	7	5	yes	6.11E-04	0.125	4.91E-03	0.94 (0.84,1.0)	1.00 (0.94,1.0)	3.43E-04	0.05	100%	NAN
8	7	10	yes	4.17E-04	0.096	4.36E-03	0.94 (0.84,1.0)	1.00 (0.95,1.0)	2.34E-04	0.05	100%	9.70E-05
9	5	9	yes	0.026	0.11	0.24	0.77 (0.65,0.90)	0.95 (0.84,1.0)	0.0208	0.05	100%	0.07
10	11	9	yes	-	0.096	-	0.99 (0.90,1.0)	1.00 (0.96,1.0)	-	-	100%	3.40E-08
11	5	13	yes	0.001	0.071	0.02	0.92 (0.80,1.0)	0.98 (0.88,1.0)	0.0024	0.05	100%	2.00E-05
12	5	13	yes	0.005	0.031	0.16	0.83 (0.70,0.96)	0.96 (0.86,1.0)	0.0025	0.05	100%	2.20E-04
13	7	12	yes	0.001	0.031	0.05	0.92 (0.82,1.0)	0.99 (0.93,1.0)	0.0027	0.05	99.30%	2.20E-04
14	4	13	yes	-	0.119	-	0.87 (0.73,1.0)	0.98 (0.87,1.0)	-	-	100%	3.00E-07
15	11	16	yes	0.002	0.114	0.02	0.98 (0.89,1.0)	1.00 (0.95,1.0)	0.0018	0.05	100%	2.14E-03
16	2	15	yes	-	0.114	-	0.59 (0.44,0.74)	0.98 (0.83,1.0)	-	-	100%	2.14E-03
17	29	16	yes	0.004	0.186	0.02	0.99 (0.94,1.0)	1.00 (0.97,1.0)	0.0034	0.05	100%	1.90E-12
18	40	19	yes	0.006	0.125	0.05	0.98 (0.92,1.0)	1.00 (0.97,1.0)	0.0129	0.05	100%	4.70E-09
19	6	18	yes	0.026	0.125	0.21	0.80 (0.67,0.92)	0.95 (0.85,1.0)	0.0223	0.05	100%	4.70E-09
20	4	22	yes	0.001	0.134	0.01	0.86 (0.72,1.0)	0.98 (0.87,1.0)	0.0012	0.05	100%	0.03
21	2	22	yes	-	0.152	-	0.59 (0.44,0.74)	0.98 (0.83,1.0)	-	-	100%	0.03
22	3	24	yes	0.023	0.092	0.25	0.62 (0.44,0.80)	0.86 (0.72,1.0)	0.0163	0.05	100%	2.00E-06
23	2	24	yes	0.021	0.094	0.23	0.47 (0.32,0.63)	0.84 (0.69,0.99)	0.0107	0.05	100%	4.00E-05
24	45	22	yes	0.014	0.092	0.15	0.94 (0.89,0.99)	0.98 (0.95,1.0)	0.0197	0.05	99.90%	4.00E-05

TABLE 5. Comparison of haplotype groups for standard morphological characters.

Haplotype Group		Body annules (R)					Annules from vulva to tail tip (Rv)					Annules from anterior end to excretory pore (Rex)					Body length (L) (µm)					Stylet length (S) (µm)					Anterior end to vulva as percentage of body length (V)				
#	N	N	Mean	Std Dev	Min	Max	N	Mean	Std Dev	Min	Max	N	Mean	Std Dev	Min	Max	N	Mean	Std Dev	Min	Max	N	Mean	Std Dev	Min	Max	N	Mean	Std Dev	Min	Max
1	6	6	90	3	86	94	6	7	1	6	8	6	25	1	24	27	6	536	52	450	593	6	56	2	53	59	6	93	1	92	94
2	4	4	140	4	137	146	4	9	1	8	9	4	38	1	37	40	4	616	53	550	663	4	60	2	59	62	4	93	1	92	94
3	3	3	74	5	69	78	3	6	1	6	7	3	22	2	20	23	3	544	56	480	585	3	72	6	65	76	3	93	1	93	94
4	6	6	79	4	76	87	6	5	1	5	6	5	22	1	21	23	5	396	14	378	413	5	55	2	53	59	5	94	1	93	95
5	4	4	98	6	89	104	4	7	2	5	10	4	28	2	26	30	4	487	40	450	535	4	54	1	53	55	3	93	3	90	95
6	6	6	97	4	92	103	6	7	1	7	8	6	29	1	28	30	6	477	44	410	520	6	54	3	48	57	6	94	3	92	99
7	4	4	98	3	96	102	4	7	1	6	8	4	26	2	23	27	4	556	84	480	650	4	73	4	67	76	3	95	1	94	96
8	3	3	99	3	97	102	3	7	1	7	8	3	29	1	28	30	3	598	43	552	638	3	84	2	82	86	3	93	1	92	93
9	3	3	93	7	87	100	3	7	0	7	7	3	26	2	24	27	3	641	105	563	760	3	84	10	77	95	3	93	1	92	94
10	3	3	104	4	100	108	3	7	1	6	8	3	30	3	28	33	3	489	154	365	662	3	79	10	72	91	3	94	0	94	94
11	4	4	100	4	96	105	4	8	1	7	9	3	27	2	25	29	4	528	26	505	565	4	71	2	70	73	4	94	1	93	95
12	2	2	90	0	90	90	2	7	0	7	7	1	23		23	23	2	551	18	538	563	2	77	2	75	78	2	93	0	93	93
13	3	3	100	6	93	105	3	7	2	5	8	3	27	2	25	28	3	606	16	593	624	3	77	3	75	80	3	93	1	92	94
14	3	3	102	5	98	107	3	8	1	7	8	3	28	2	26	30	3	653	38	615	690	3	72	1	71	72	3	92	2	90	93
15	7	7	93	3	88	97	7	7	1	6	8	4	26	2	24	27	7	558	23	528	605	7	53	3	51	59	7	92	1	91	93
16	1	1	105		105	105	1	7		7	7	1	30		30	30	1	553		553	553	1	55		55	55	0				
17	25	25	95	5	88	106	25	7	1	6	8	24	25	2	23	29	25	496	66	363	613	25	60	3	55	65	25	93	2	88	97
18	29	29	103	8	87	118	29	8	1	6	10	26	27	2	23	31	29	509	59	393	670	29	53	3	45	60	28	93	1	91	98
19	4	4	107	6	101	113	4	10	1	9	11	4	27	1	26	28	4	495	25	470	530	4	56	3	54	60	4	91	1	90	92
20	3	3	97	5	93	103	3	6	1	5	7	3	24	3	21	27	3	529	35	500	568	3	55	1	54	56	3	94	1	94	95
21	2	2	93	0	93	93	2	8	1	7	8	2	24	1	23	24	2	590	21	575	605	2	62	1	61	62	2	93	0	93	93
22	2	2	87	2	85	88	2	8	1	7	8	2	22	1	21	23	1	550		550	550	1	62		62	62	1	94		94	94
23	2	2	87	3	85	89	2	7	1	6	7	2	24	0	24	24	2	563	53	525	600	2	62	3	60	64	2	94	0	94	94
24	29	29	91	7	80	104	29	7	1	6	10	25	24	2	20	32	29	537	65	405	655	29	57	4	48	66	27	93	1	91	95

Different tree building methods on the reduced 82-taxa dataset produced strong node support values for haplotype groups. In fifteen of the sixteen haplotypes groups with haplotype diversity, maximum likelihood and neighbor-joining trees had bootstrap values of 100. Group 13 had a bootstrap value of 99 in both analyses. Bayesian inference produced trees with posterior probabilities of 1.0 for 14 groups, with groups 24 and 13 expressing values of 0.99.

Additional measures of group distinctiveness (Table 4) were tested on the full dataset with the species delimitation plug-in from Geneious (Masters *et al.* 2011). Rosenberg's test (Rosenberg 2007) for reciprocal monophyly (P(AB)) and the statistic for calculating clade distinctiveness by Rodrigo *et al.* (2008) both assess the probability that the observed patterns were due to random coalescent processes. Both statistics strongly supported the distinctiveness of the haplotype groups, rejecting as unlikely distinctiveness and monophyly arising as a result of chance. Similarly, the probability of correctly identifying unknown members of the haplotype groups as a function of the ratio of intraspecific differentiation to the distance to the nearest haplotype group was calculated under strict and liberal criteria (P ID (Strict) and P ID (Liberal)) in Table 4. Under the liberal criteria where the unknown member of the group must fall within or sister to the group, all probabilities were 95% or higher except for groups 22 and 23. Under the strict criteria where the unknown member must fall within the group and not in the sister group, 19 of the groups had mean probabilities of 80% or better of being correctly identified (Table 4).

TABLE 6. Diagnostic characteristics of seven species of Criconematidae from Brzeski *et al.* 2002a, 2002b.

Species	Stylet length	R	Rex	RV	RVan	Ran	V	VL/VB	Anast-omoses
Mesocriconema curvatum	47–68	74–106	20–29	5–10	0–3	2–6	92–96	0.7–1.2	0–few
M. discus	65–72	94–106	(29)	7	1	5	94–95	(1.1)	few
M. kirjanovae	49–74	71–105	20–27	7–14	0–5	4–10	85–93	1.0–2.0	few
M. onoense	40–63	111–138	27–36	8–14	0–5	4–10	89–94	0.9–1.4	0–few
M. ornatum	44–56	78–94	25–27	7–9	0–2	5–8	92–96	0.7–1.2	0–few
M. rusticum	50–60	81–107	24–32	7–10	0–2	4–9	92–95	0.7–1.2	0–few
M. xenoplax	54–87	77–114	26–30	6–11	0–4	4–7	92–96	0.7–1.3	0
Criconemoides informis	57–83	48–77	15–24	6–9	1–3	3–6	86–94	1.0–1.6	0–few

TABLE 6. (Continued)

Species	Annule margin	Vagina	Anterior vulval lip	Tail shape	L	Submedian lobes
Mesocriconema curvatum	smooth	straight	lobes	rounded	0.29–0.56	+
M. discus		sigmoid		conical-rounded	0.45–0.65	+
M. kirjanovae	smooth to crenate	straight	lobes	conical-acute	0.35–0.79	+
M. onoense	smooth	straight	flap-lobes	rounded	0.37–0.67	+
M. ornatum	smooth	straight	sharp-lobes	conical-rounded	0.33–0.52	+
M. rusticum	smooth	straight	simple	rounded	0.34–0.52	flattened
M. xenoplax	smooth to irregular	sigmoid	lobes	conical-rounded	0.40–0.75	+
Criconemoides informis	irregular to smooth			conical	0.32–0.62	

Evidence from morphology, ecology, and geography. Key morphological characteristics of the 24 haplotype groups in the dataset are summarized and illustrated in Figure 3. Scanning electron microscopy is often used as a tool to resolve character states that are difficult to interpret under light microscopy, but since SEM preparation is a destructive process, it is not possible to obtain DNA from the identical specimens used in SEM. Emphasis in this study is placed on those diagnostic characters that are likely to be used in routine species identification. Based on specific diagnostic traits or measurements, five of the haplotype groups generated by COI analysis each appear to be associated with a discrete morphospecies:

Haplotype group 2— *Mesocriconema onoense* (Luc, 1959) Loof & De Grisse, 1989
Haplotype group 5—*M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989
Haplotype group 7—*M. discus* (Thorne & Malek, 1968) Loof & De Grisse, 1989
Haplotype group 17—*M. inaratum* (Hoffmann, 1974) **n.comb.**
Haplotype group 19—*M. kirjanovae* (Andrássy, 1962) Loof & De Grisse, 1989

Haplotypes groups 8–14 generally conform morphologically to *Mesocriconema xenoplax* (Raski, 1952) Loof & De Grisse, 1989 and collectively belong to a monophyletic group (Figs. 1 & 2). The remaining haplotype groups (1, 3, 4, 6, 15, 16, 18, 20–24) did not associate with any single morphospecies description. For example, specimens in haplotype groups 1, 15, and 16 conform in part to *M. ornatum* (Raski, 1958) Loof & De Grisse, 1989. Similarly, groups 4, 18, and 20–24 could be placed in either morphospecies *M. curvatum* (Raski, 1952) Loof & De Grisse, 1989 or *M. ornatum* depending on the degree of annule crenation on juvenile cuticles and the complexity of the labial plates. A ninth morphospecies, *M. sphaerocephalum* did not group with other members of the in-group *Mesocriconema* and is the subject of a separate study (T. Powers, in prep). Table 6 presents morphological measurements from the compendia of Brzeski *et al*. 2002a and Brzeski *et al*. 2002b in which were compiled some of the published species characteristics discussed in the section below.

Haplotype groups

Groups 1, 15 and 16

Morphological evidence. These three haplotype groups conform, in part, to the morphospecies *Mesocriconema ornatum* (Raski, 1958) Loof & De Grisse, 1989. In the original description of *M. ornatum*, described by Raski (1952) under the name *Criconemoides cylindricum*, the species was diagnosed by the absence of labial plates, possession of small submedian lobes, and the outline of the anterior vulval flap, with two distinct "points". Markings on the annule margins of juveniles were also identified as a differentiating character when compared to *M. curvatum* and *M. rusticum*, which possess smooth annule margins in the juvenile stages. Subsequent investigators have differed in the interpretation of these characters. Loof & De Grisse, (1973, Fig. 2) presented line-drawings of face views with submedian lobes arranged from the smallest (*M. ornatum*) to the largest (*M. surinamensis*). Later the same authors show *M. ornatum* with small labial plates and four relatively large submedian lobes approximately the same size as *M. xenoplax* (Loof & DeGrisse, 1989, Fig. 2b). In an examination of African *M. ornatum* specimens from four populations, Luc (1970) also observed large submedian lobes with considerable variation in the size of the labial plates, and illustrated a range of vulval projections from undulating to sharply pointed. SEM face views of *M. ornatum* in Mulawarman & Geraert (1997) show large submedian lobes with a longitudinal groove as frequently observed in *M. curvatum*, labial plates flanking the labial disc, and the anterior annule of the vulva with minute points. The key to *Mesocriconema* spp. in Geraert (2010) requires decisions about the size of submedian lobes and the relative development of the labial plates, both problematic decisions for this seemingly polymorphic species.

In this study isolates from four southeastern US states, Texas, New Mexico and Costa Rica, contained adult specimens with measurements that conformed to *M. ornatum* and had associated juveniles in the population with crenate margins on the annules (Figs. 4F, G, I, J, 17D). Analysis of COI placed these specimens in three separate and distinct haplotype groups. Haplotype group 1 (Figs. 4G–M) included specimens from Byron, Georgia, a locality approximately 100 miles (160 km) north of the type locality in Albany, Georgia. Adult females in group 1 have moderately sized submedian lobes and lateral labial plates (Figs. 4I, K, L), and virtually no projections on the annule forming the anterior lip of the vulva (Fig. 4H). Haplotype group 15, which also conformed to *M. ornatum*, was nearly identical morphologically to haplotype group 1, the only exception being the presence of moderately pointed projections on the anterior vulval annule (Fig. 4D). Both haplotype groups include specimens with stylet and body lengths that slightly exceeded the upper range of stylet length (56 µm) and body length (520 µm) reported in the compendium of Brzeski *et al*. (2002b) (Table 6). Lateral labial plates could be observed in both haplotype groups by SEM and light microscopy, and were present in adults and juveniles. The "bluntly rounded almost truncate tail" as described and illustrated by Raski (1952) is similar in form to many of the *Mesocriconema* specimens collected in this study. We suspect that nematodes identified as "short-stylet" forms of *M. xenoplax* from bentgrass in South and North Carolina reported by Zeng *et al*. (2012) and those associated with Bermuda grass and river cane in Arkansas (Cordero *et al*. 2012) may be members of either haplotype group 1 or 15. Only two specimens, a juvenile and adult female from New Mexico, were included in Group 16. Crenate annule margins on the juvenile (Fig. 17D), female morphometrics, and a shared ancestry with Group 15 support its identity as *M. ornatum.*

Ecological and geographic evidence. All members of groups 1, 15, and 16 were obtained from agricultural sites. The majority of the *M. ornatum* specimens were collected from managed turf sites or turf associated with the understory of orchards. The lack of haplotype diversity in group 1 associated with a relatively broad geographic range suggests that the haplotype was spread recently, possibly through the movement of turf or other agricultural commodities.

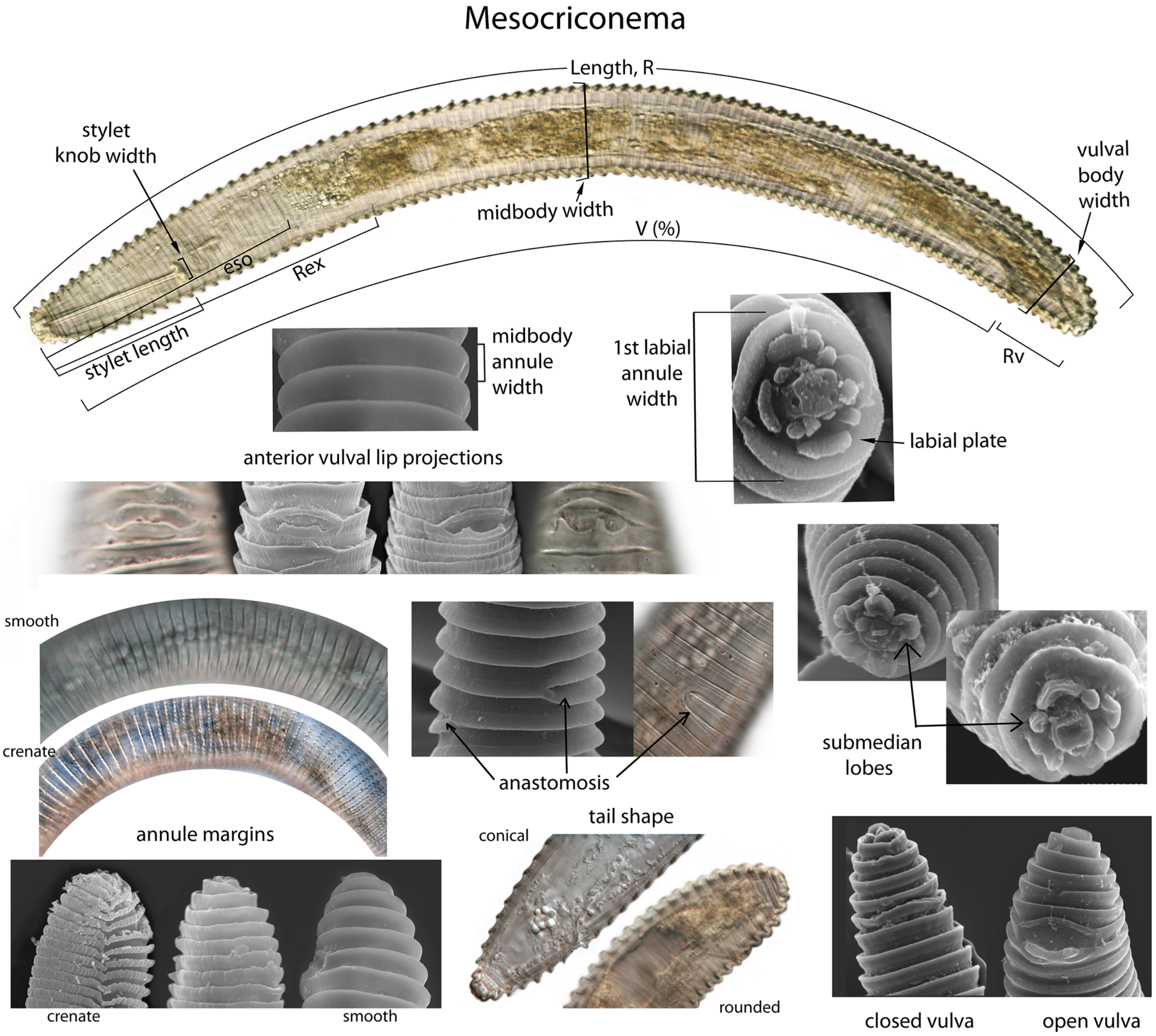

FIGURE 3. Key morphological characteristics of *Mesocriconema*. Images from scanning electron microscopy and light microscopy are combined to enhance understanding of the morphological characters. Measurements of specimens of Criconematidae are generally expressed in micrometers and in terms of the number of annules (R) between two morphological landmarks. R=total number of annules from anterior to posterior end or the body. Rex=number of annules from anterior end to the excretory pore. Rv=number of annules from the vulva opening to the posterior end of the body. V=position of the vulva expressed as a percentage of total body length, and eso=length of the esophagus (pharynx) from anterior end to the esophageal/ intestinal junction.

Fig. 4

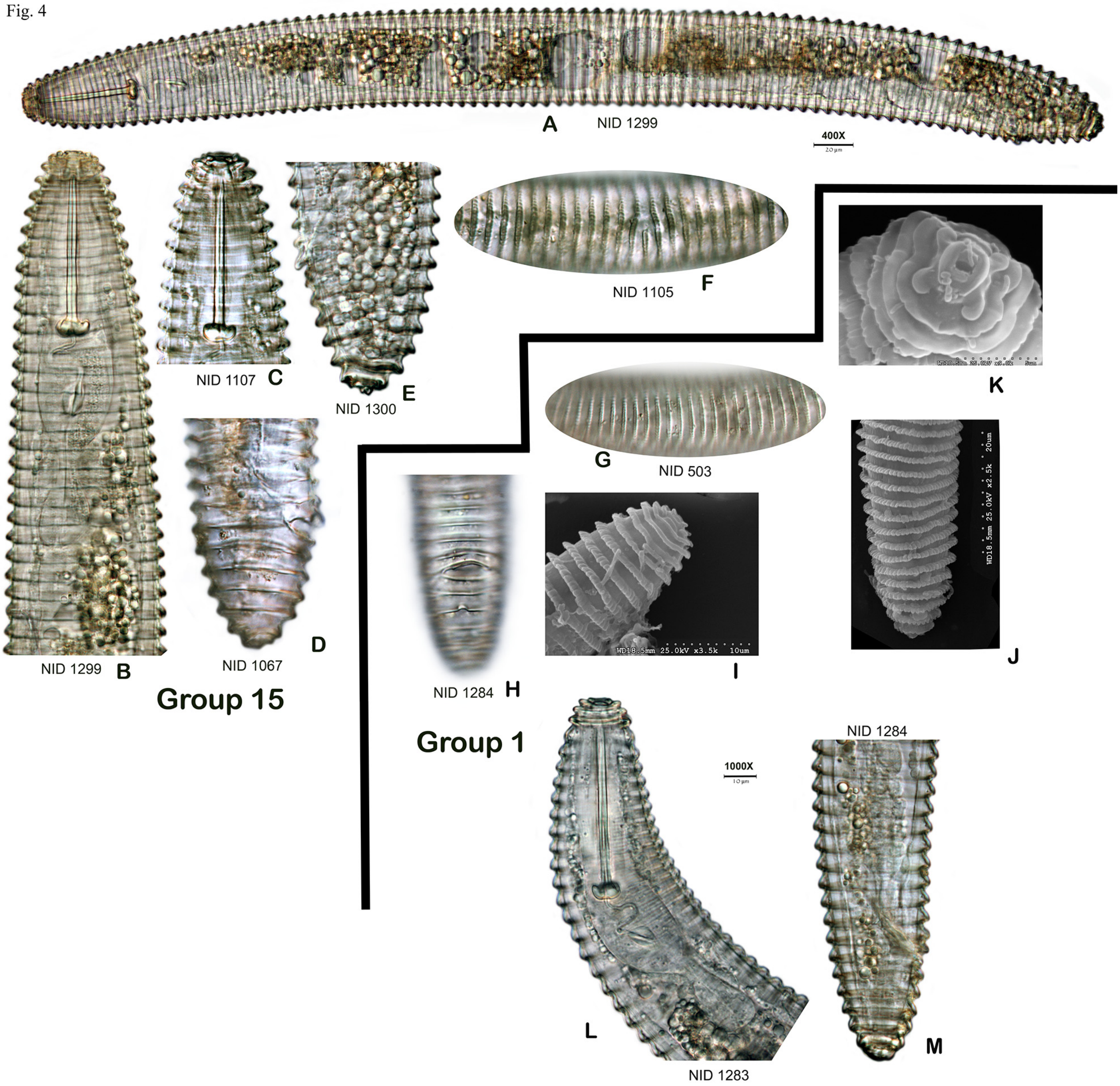

FIGURE 4. Haplotype Groups 1 (G, H, I, J, K, L, M) and 15 (A, B, C, D, E, F). Specimens conforming to morphospecies *Mesocriconema ornatum*. Location data for NID numbers are in Table 1. Adult females=A, B, C, D, E, H, L, M. Juveniles=F, G, I, J, K. Specimens for SEM images I, J, K were from a peach orchard in Alabama.

Group 2: *M. onoense* (Luc, 1959) Loof & De Grisse, 1989

Morphological evidence. A single isolate collected from a turf research facility in Alabama contained specimens notable by the large number of annules on the body, with all specimens having more than 137 annules (Fig. 5A; Table 5). Other discriminating features included the large number of annules anterior to the excretory pore (Rex) (Figs. 5C, D) and the number of annules between the vulva and tail tip (Rv) (Fig. 5B). Only two *Mesocriconema* spp. reported from North America possess similar numbers of annules: *M. onoense* (Luc, 1959) Loof & De Grisse, 1989 and *M. canadense* (Ebsary, 1981) Loof & De Grisse, 1989. *M. canadense* differs from haplotype 2 and *M. onoense* in its lack of submedian lobes, crenate female annuli, and short stylet. *Mesocriconema onoense* has been reported from rice in Louisiana (Hollis 1977) and recently from grass and sugar maple (*Acer saccharum*) in Arkansas (Cordero *et al*. 2012). The original description published in 1959 was based on six specimens recovered from the roots of sugar cane (*Saccharum* spp.) in Venezuela. In the original description the anterior lip of the vulva is described as rounded with a central bulge. NID 1250 (Fig. 5F) has small, pointed projections, whereas NID 1249 and 1253 (Figs. 5E, G) have a smooth anterior lip with no projections. Adult and juvenile annule margins are smooth (Figs. 5H, I).

Ecological and geographic evidence. The reported distribution of *M. onoense* in North America is limited to the south-central states of Alabama, Louisiana, and Arkansas. We also have recorded the presence of the morphospecies in Bermuda grass turf plots in Alabama and from Big Thicket National Preserve in Texas.

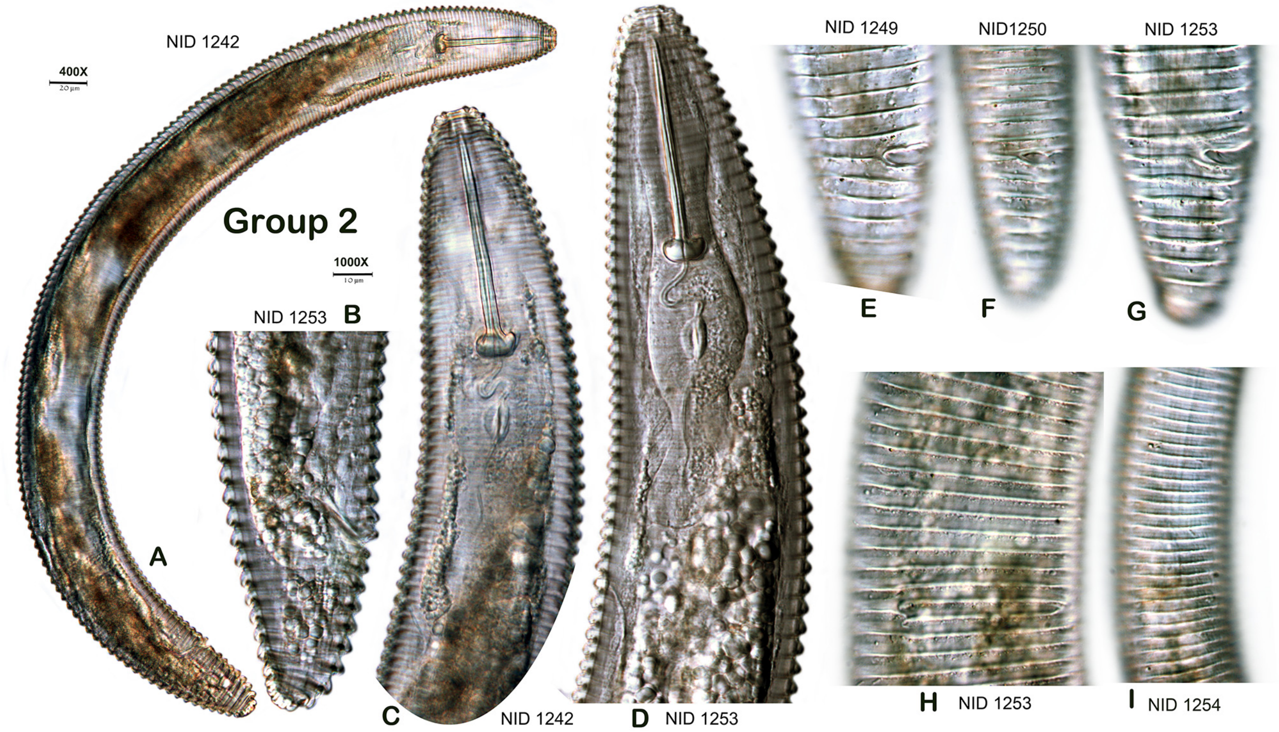

FIGURE 5. Haplotype Group 2 (A, B, C, D, E, F, G, H, I). Specimens conforming to morphospecies *Mesocriconema onoense*. Adult females=A–H. Juvenile=I.

Group 3: *Mesocriconema* sp.

Morphological evidence. Uncertainty exists regarding the placement of this haplotype group within *Mesocriconema*. From a morphological perspective, rounded submedian lobes appear in lateral view of NID 1101 (Fig. 6B). Submedian lobes were not clearly evident in the other adult specimens (Figs. 6C, D). The vulva was similarly ambiguous since specimens were only observed in the lateral view and the annule immediately anterior to the vulva was not markedly different from other body annules (Figs. 6A, E). The body annules were not strongly retrorse and the single juvenile observed exhibited faintly crenate margins (Fig. 6G). The specimens conformed in morphometrics, with the exception of a larger body length, to *M. maritimum* (De Grisse, 1964) Loof & De Grisse, 1989, a species originally described from Belgium. Line drawings in Geraert (2010, Fig. 31), particularly of the variation in the labial region, bear a striking resemblance to the Montana specimens examined in this study. If upon further investigation the vulva is shown to be closed, the specimens could be considered *Criconemoides informis* (Micoletzky, 1922) Taylor, 1936, a presumed cosmopolitan species (Geraert 2010). Support values strongly supported group 3 as a distinct lineage, but support at the higher nodes linking this haplotype group with others in the *Mesocriconema* data set was lacking in all three tree building methods.

Ecological and geographic evidence. Specimens belonging to this group were only collected from an alpine meadow in Gallatin County, Montana.

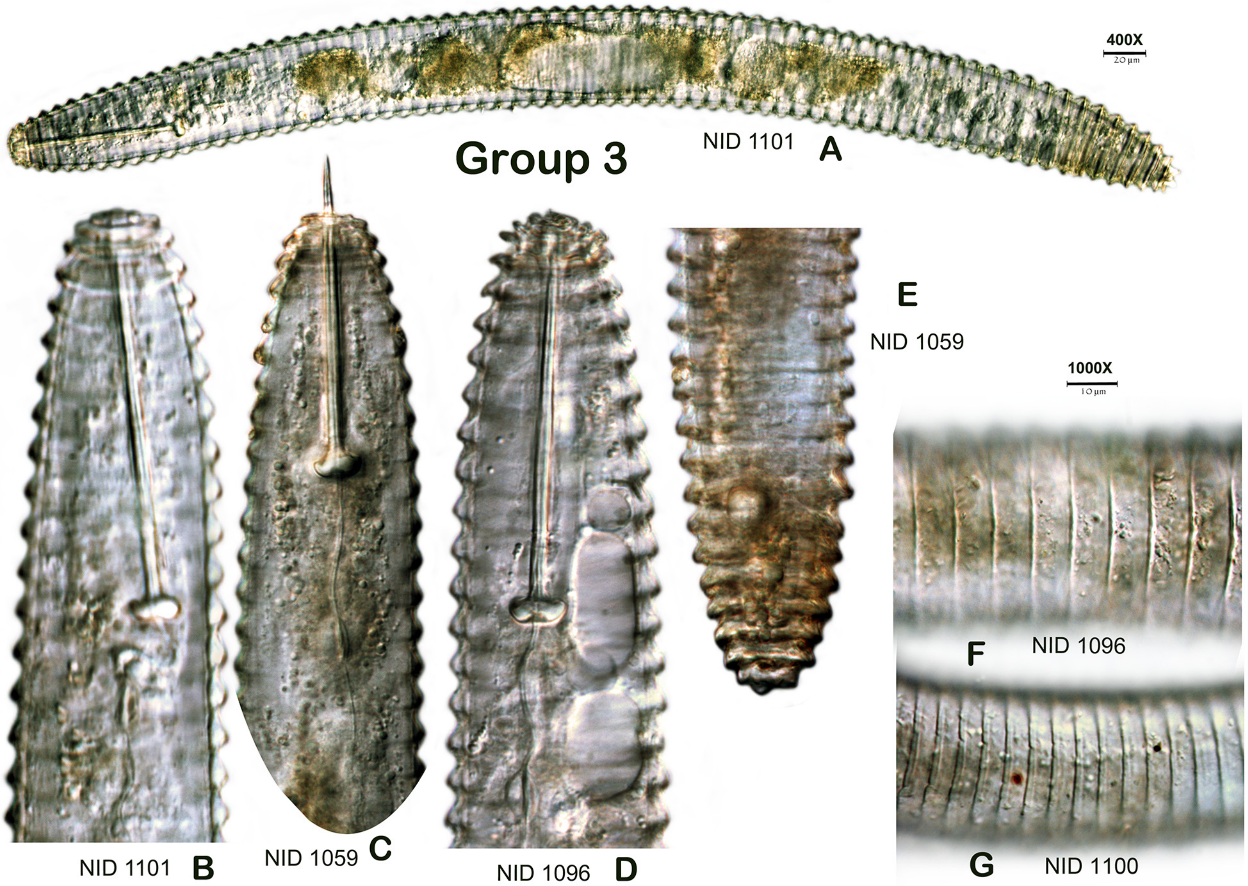

FIGURE 6. Haplotype Group 3 (A, B, C, D, E, F, G). Specimens of uncertain morphospecies designation. Adult females= A–F. Juvenile=G.

Groups 4, 18, 20–24: *M. curvatum* morphotypes

Morphological evidence. *Mesocriconema* specimens that conform, in part, to the morphological description of *M. curvatum* are widespread in North American grasslands. Fifteen grasslands from 10 states sampled in this study contained specimens exhibiting the morphological features of the species. However, it is not always clear from the taxonomic literature what diagnostic features delimit *M. curvatum*. The Commonwealth Institute of Helminthology (C.I.H.) description emphasizes "four well-developed, separate submedian lobes with rounded anterior margins" with the "first annule broken up into labial plates, often irregularly" (Loof 1974). Separation from *M. xenoplax* is based on the shorter stylet of *M. curvatum* and the straight vagina of *M. curvatum* versus the sigmoid vagina shape associated with *M. xenoplax* (although see below for additional commentary on this character). In Geraert (2010) the submedian lobes are described as "conical in shape, lateral plates present but not very pronounced" and the "anterior vulva lip variable usually with two rounded lobes". The tail is described as variable, more or less rounded with the tip single or sometimes multi-lobed. Geraert (2010) also reported that young juveniles have irregular annuli margins, but the larger juveniles have smooth margins. Recent published assessments of *M. curvatum* diagnostic characters have emphasized that *M. curvatum* lacks an elevated labial disc, and the labial plates are described as "minute or obvious" (Cordero *et al*. 2012) or irregular (Zeng *et al*. 2012). The full range of morphological variability is evident within the COI haplotype groups that conform and generally key to *M. curvatum* in Geraert (2010) or Brzeski *et al*. (2002b).

Group 4 stands out among the seven *Mesocriconema curvatum*-like haplotype groups by being the only group solely represented by specimens collected from agricultural samples. This may be a significant clue to its identity and origin since the type host of *M. curvatum* from Colma, California, was snapdragon (*Antirrhinum* sp.), an old world plant species. Other hosts mentioned in the original description included lupine (*Lupinus* sp.), apple (*Malus* *sylvestris*), an unidentified plant species associated with a home garden, grassy field, vegetable garden, and a potato field (Raski 1952). Group 4 has the shortest mean body length (396.2 µm), smallest Rv value (5.3), and the second smallest R value (79.3) among all 24 haplotype groups. One of the three juveniles included in group 4 had crenate annules (Fig. 7G), whereas the others appeared smooth.

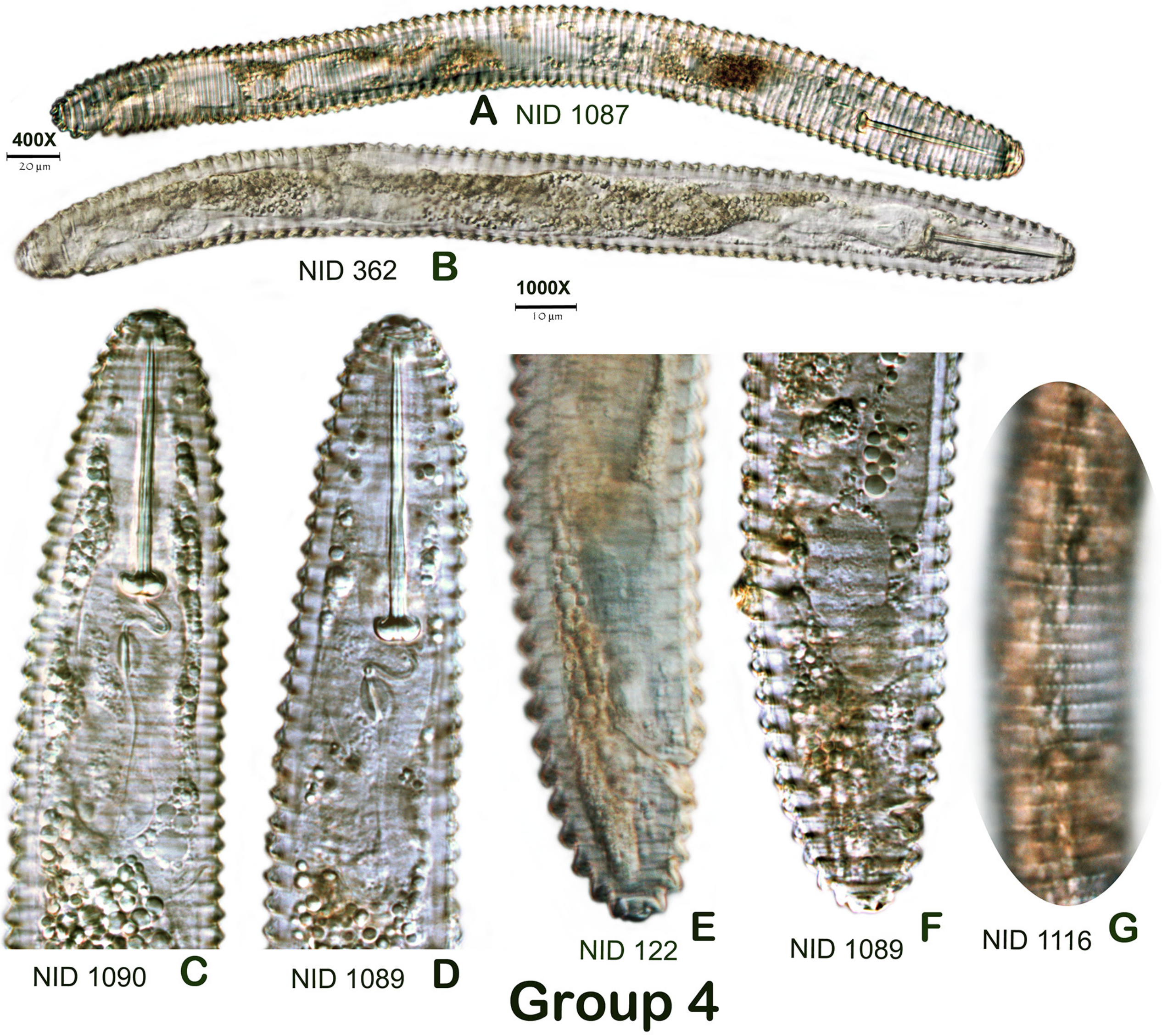

FIGURE 7. Haplotype Group 4 (A, B, C, D, E, F, G). Specimens conforming to morphospecies *Mesocriconema curvatum*. Adult females=A, B, D–F. Juveniles=C, G.

The two largest groups in the dataset, Group 18 and 24 represented by 40 and 45 specimens, respectively, were collected only from central U.S. grasslands and savannas and never from agricultural soils (Table 1). Mean morphometric values of body length and annule number fell within reported ranges of previous studies as compiled in the compendium of Brzeski *et al*. (2002b), however, select individual specimens in both haplotype groups exceeded previously reported upper limits for both characters (Table 5). The anterior regions of the two groups are compared in Figs. 19A–D and 26A–F. Both groups possess a relatively robust stylet with strong stylet knobs. The labial region in group 18 appears to have more prominent submedian lobes than group 24. SEM images (Figs. 19E–G) derived from individuals selected from Kalsow Prairie in Iowa, where only haplotype 18 has been observed, display rounded submedian lobes with a central indentation, and labial plates with varying degrees of fragmentation. In several cases the lateral plates fuse to form half a labial annule, which complicates interpretations of lateral views when using light microscopy. Lateral and ventral views of the tail region of both groups can be compared in Figures 20 and 27. One obvious feature is that the vagina in both groups can appear either straight, as a diagonal line from midbody/uterus to the vulva, or it can assume a sigmoid shape, with the appearance of a bend in the vagina either at the point it connects to the uterus or as it nears the vulva. Interpretation of this taxonomic character may have led to the recognition of short-stylet forms of *M. xenoplax* as discussed above (Hoffman 1974). A second taxonomic character within both haplotypes is the size and shape of the projections extending from the anterior vulval annule. The projections range from low and blunt (Figs. 20G, H; Figs. 27G, I, K) to high and pointed (Figs. 20 L, 27J). All specimens of *M. curvatum* collected from Kalsow Prairie belong to haplotype group 18. SEM views of pointed vulval projections from the Kalsow Prairie population are seen in Figs. 21 A–D. Another taxonomic feature sometimes used to discriminate *M. curvatum* from *M. ornatum* is the presence or absence of crenation on the annule margins of the juveniles. Crenate juvenile cuticle was observed in both groups, which tended to be more prominent on the posterior portion of the body, although the degree of crenation ranged from faint to strong (Figs. 21E–J; Figs. 28A–C). Adults in groups 4, 18 and 24 all possessed smooth annule margins. Haplotype groups 20–23 (Figs. 23–25) each included 2–4 specimens. The specimens in these groups usually keyed to *M. curvatum* in Geraert (2010) but too few individuals were available to assess morphological variation.

Ecological and geographic evidence. Colma, CA, just south of San Francisco, is the type locality for *Mesocriconema curvatum*. The original description mentioned other locations for the species in California, North Carolina, New York, Vermont and Nevada, generally associated with agricultural hosts. Haplotype groups 18 and 24 have never been recovered from agricultural hosts nor have they been recorded outside of native grasslands in the central U.S. Therefore, in spite of their morphological similarity it is unlikely that they belong to *M. curvatum*. Previous records of *M. curvatum* in native central U.S. grasslands probably refer to these haplotype groups (Schmitt & Norton 1972; Norton & Ponchillia 1968; Powers *et al*. 2010). Group 4 specimens were recovered from managed agricultural ecosystems in Missouri, Montana, Nebraska, Wisconsin, and Wyoming. Additional studies are necessary to determine if this haplotype group is actually *M. curvatum*.

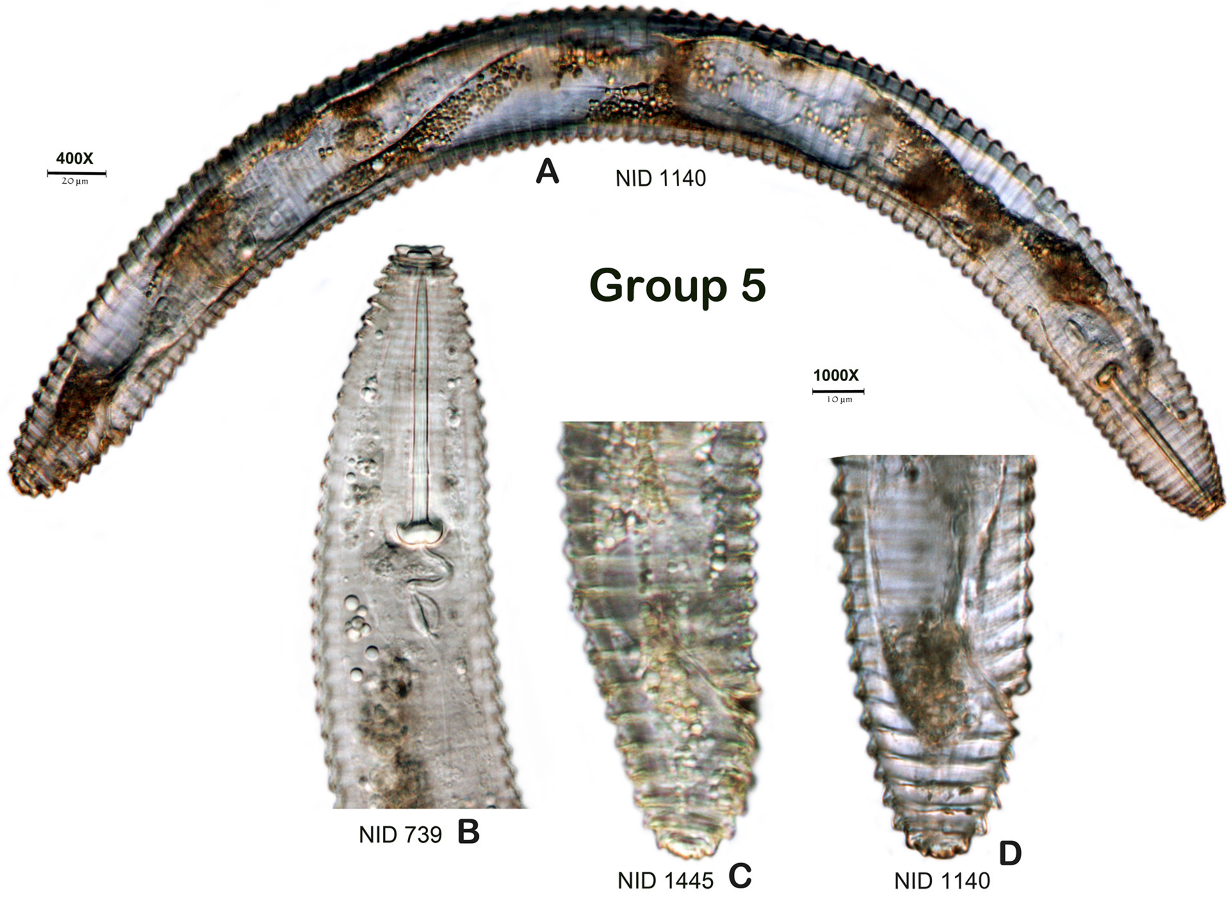

FIGURE 8. Haplotype Group 5 (A, B, C, D). Specimens conforming to morphospecies *Mesocriconema rusticum*. Adult females=A–D.

Group 5: *M. rusticum* (Micoletzky, 1915) Loof & De Grisse, 1989

Morphological evidence. Eight specimens in the dataset formed group 5, which morphologically conformed to *Mesocriconema rusticum*. Half of the specimens were collected from agricultural ecosystems and were isolated from soils in five states (Table 1). *M. rusticum* is a cosmopolitan species that is commonly found in turf and vegetable production (Wouts 2006). Like *M. discus* it is a species morphologically identifiable by its large submedian lobes, which when viewed in lateral profile with light microscopy present a truncated appearance to the anterior end (Figs. 8A, B). Several authors also have considered the blunt or multi-lobed, truncated tail terminus with a slight dorsal bend (Fig. 8C) as a diagnostic character (Raski 1952; Wouts 2006; Geraert 2010). In describing *Criconemoides lobatum*, later synonymized with *M. rusticum* by Loof (1965), Raski (1952) illustrated the vulva without projections on the anterior lip. No vulva projections were observed in the females in group 5. SEM face views of *M. rusticum* from Vermont were provided in Powers *et al.* (2010).

Ecological and geographic evidence. Wouts (2006) noted that *Mesocriconema rusticum* has a wide distribution in New Zealand, was not very common, and was mainly associated with cultivated plants. The specimens in the current study were also widespread, but included a mixture of cultivated plants and native grasslands. Despite its wide geographic range in North America, relatively little within-group COI variation was detected for this lineage (Table 4).

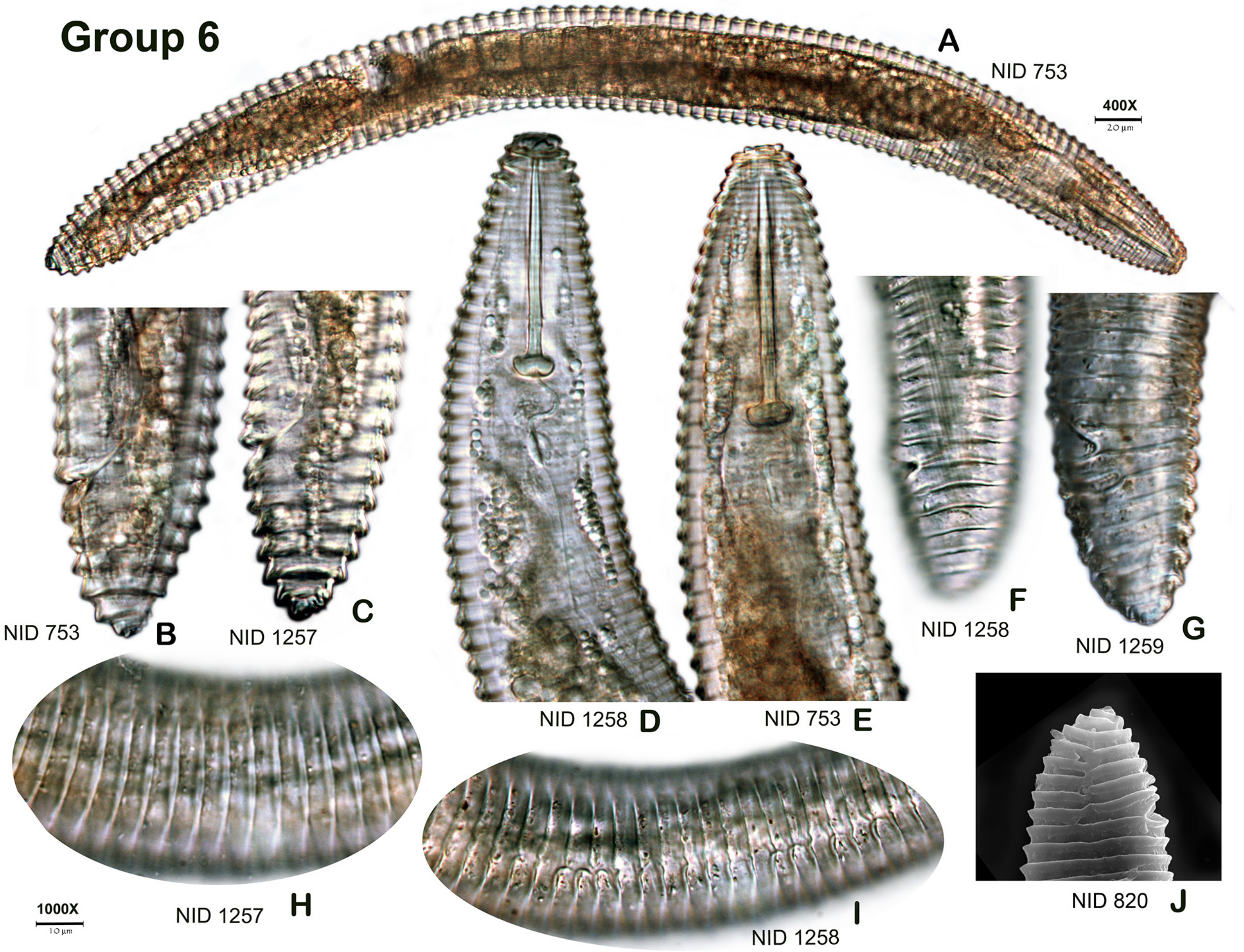

FIGURE 9. Haplotype Group 6 (A, B, C, D, E, F, G, H, I, J). Specimens of uncertain morphospecies designation. Adult females=A–J. Specimen J is from Brookings, South Dakota.

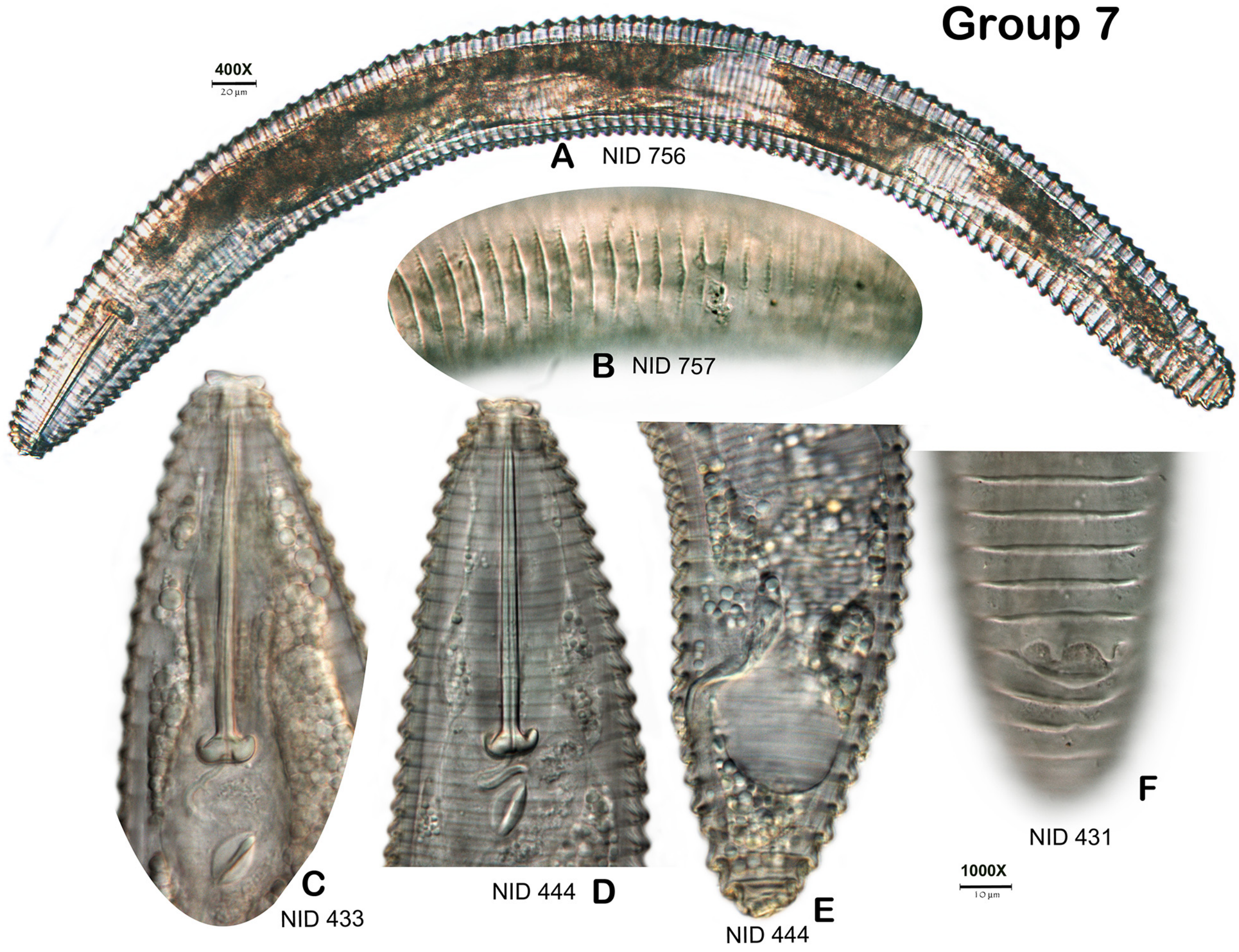

FIGURE 10. Haplotype Group 7 (A, B, C, D, E, F). Specimens conforming to morphospecies *Mesocriconema discus.* All specimens from type locality in Brookings, South Dakota. Adult females=A, C–F. Juvenile=B.

Group 6: *Mesocriconema* sp.

Morphological evidence. Specimens included in haplotype group 6 were recovered only from a single location in South Dakota. The specimens key to *Mesocriconema denoudeni* (De Grisse, 1967) Loof & De Grisse, 1989 in Geraert (2010). The first couplet of that key requires decisions about the number of anastomoses in the lateral field, which varied from zero to more than ten in the midbody of adult females in this collection (Figs. 9H, I). The labial region possessed moderately developed submedian lobes (Figs. 9D, E) and the anterior annule of the vulva lacked projections (Figs. 9B, C, F, G, J). Mean values of R and Rex for specimens in group 6 do not fit within the range of *M. denoudeni* in the Brzeski *et al.* (2002b) compendium.

Ecological and geographic evidence. Group 6 specimens were obtained around the roots of thistle (*Cirsium* sp.). The collection site was also the type locality of *M. discus*. No COI haplotype diversity was observed among the six specimens analyzed.

Group 7: *M. discus* (Thorne & Malek, 1968) Loof & De Grisse, 1989

Morphological evidence. *Mesocriconema discus* (Thorne & Malek, 1968) Loof & De Grisse, 1989 was recovered from its type locality "2 miles west of Brookings, S. Dak." in a lowland, wet slough. A second discovery of *M. discus* occurred in Doolittle Prairie, a wet, pothole prairie in central Iowa. The species also was reported previously from Kalsow Prairie, a native tallgrass prairie in northeast Iowa (Hoffmann 1974). In the original description and in the present study, no specific plant host was identified.

Taxonomically *M. discus* has been variously interpreted, in part because of the ambiguous original description of the labial region which mentioned “four broad flat lobes” but illustrated the labial region as a single continuous disc (Ebsary 1982; Brzeski 2002b; Powers *et al*. 2010). An earlier 18S barcode analysis grouped *M. discus* together with several GenBank accessions of *M. xenoplax* collected from Europe, but existing outside of a clade of *M. xenoplax* specimens from North America which included specimens from the type locality of *M. xenoplax* (Powers *et al*. 2011). COI analysis strongly supports *M. discus* as a distinct group (Table 2). Only two haplotypes separated by a single nucleotide substitution were observed among the seven COI group 7 sequences, whereas the smallest mean genetic distance (P-value) to another *Mesocriconema* sequence was over 11% (Table 3).

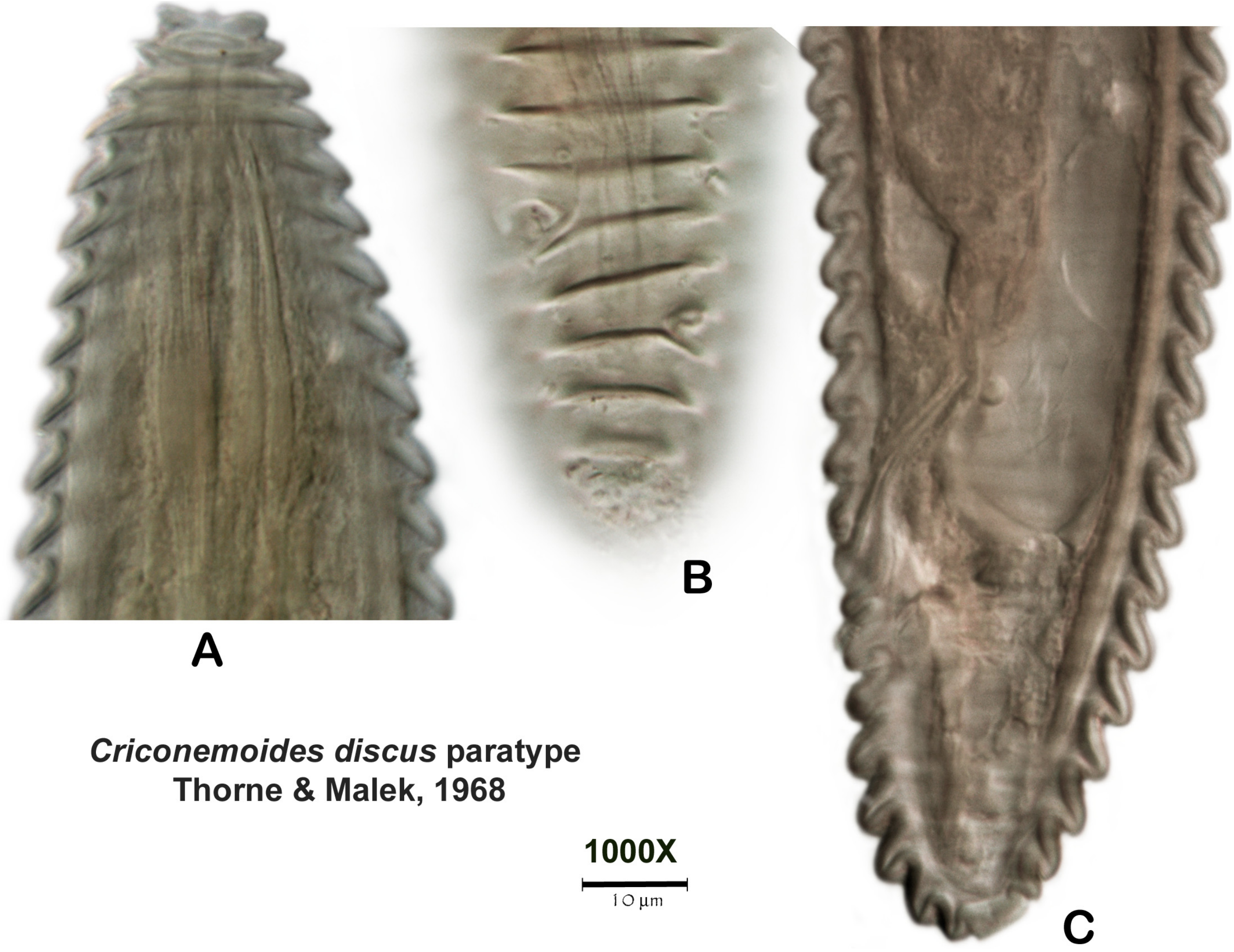

FIGURE 11. Haplotype Group 7. Adult female paratype specimens of *Mesocriconema discus* (A, B, C).

Topotype specimens are similar to a paratype specimen (Figs. 10–12). Seen in light microscopy, the adult female cephalic profile exhibits large flattened lobes, often projecting above the central labial disc (Figs. 10C, D; Fig. 11A). SEM face views of topotype specimens show four greatly enlarged submedian lobes surrounding a round labial disc (Figs. 12A–E). The submedian lobes may obscure the first labial annule, which in some specimens is reduced to two lateral labial plates, and in others there exists a connection between the lateral plates and a portion of the first annule. The next annule is usually entire, but irregular in shape. Not mentioned in the original description but present on all topotypes are two large pointed projections located on the anterior vulval annule (Fig. 10F; Figs. 12F–H). One projection can be seen clearly on the paratype specimen (Fig. 11B). A sigmoid vagina was illustrated in the original description. Paratype (Fig. 11C) and topotype (Fig. 10E) specimens display varying degrees of this character. The annule margins of the adult female cuticle are smooth. Juveniles have crenate margins over the entire body; these crenations are most strongly expressed in the posterior two-thirds of the body (Fig. 10B).

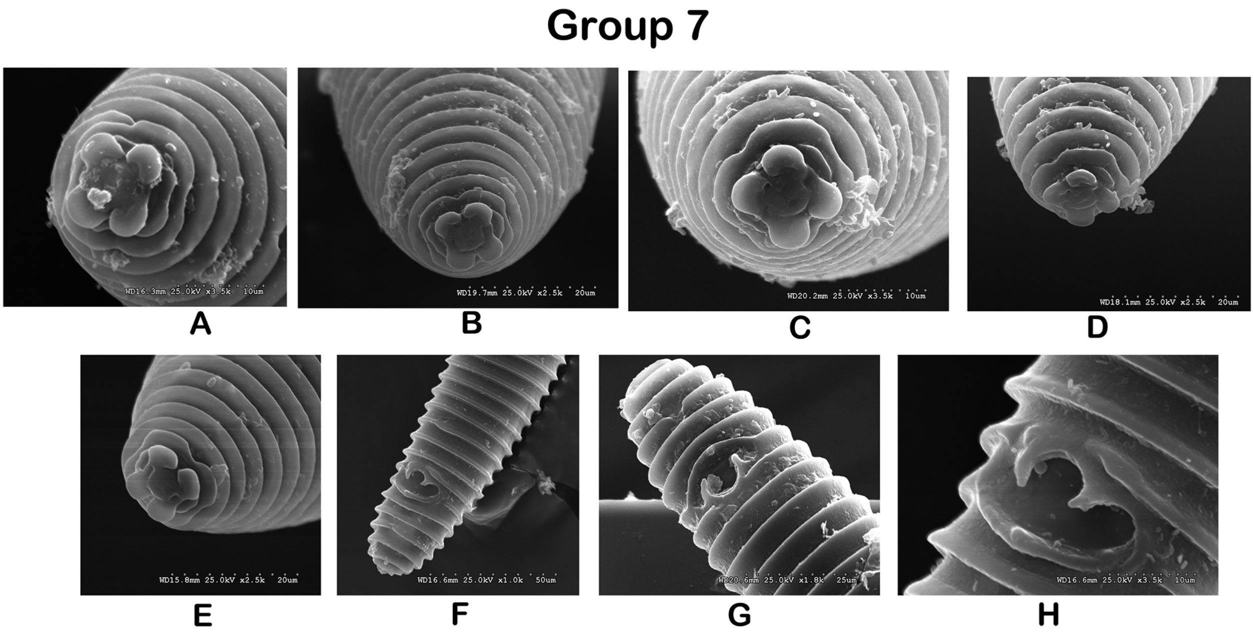

FIGURE 12. Haplotype Group 7. SEM images of topotype adult female specimens of *Mesocriconema discus*. Labial region with large submedian lobes (A, B, C, D, E), vulva with anterior annule projections (F–H).

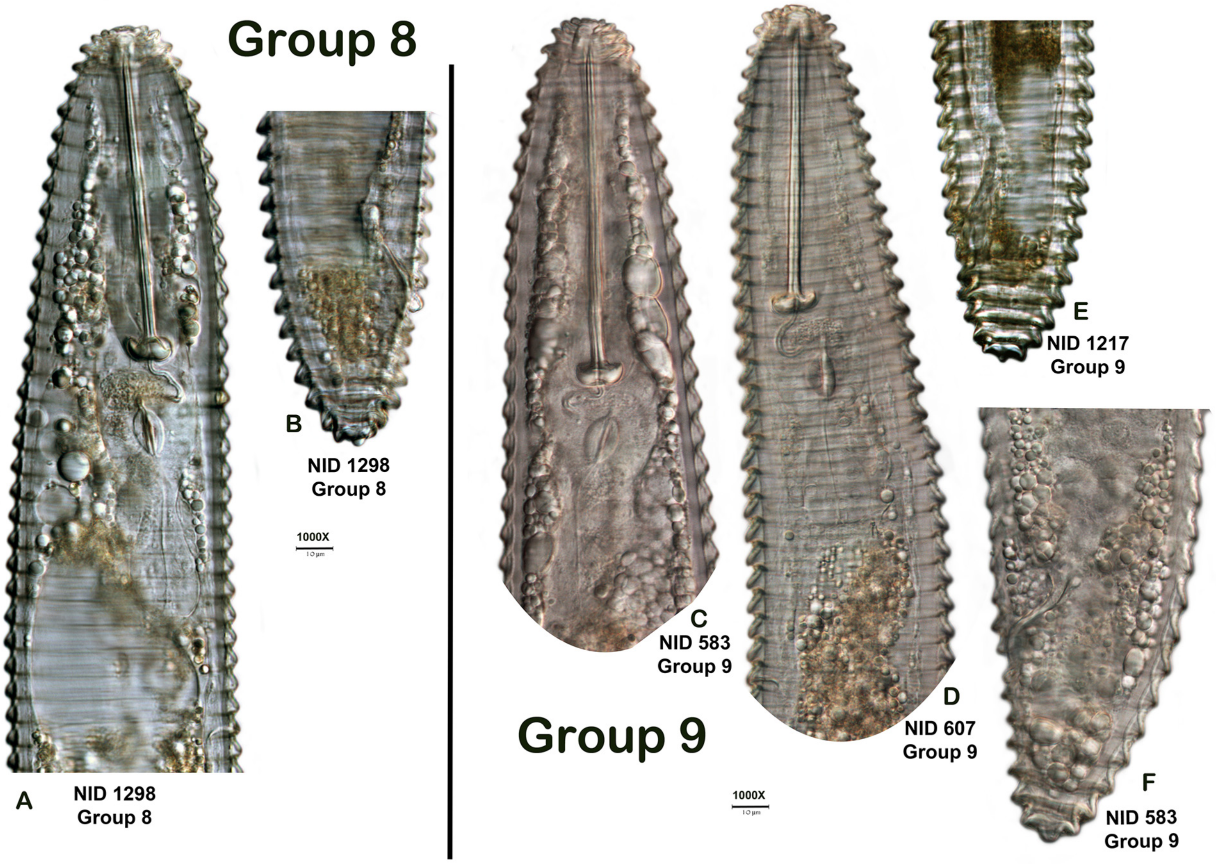

FIGURE 13. Haplotype Groups 8 (A, B) and 9 (C, D, E, F). Specimens conforming to morphospecies *Mesocriconema xenoplax*. Adult females=A–F.

Other species with large submedian lobes are *M. juliae* Crozzoli & Lamberti, 2001, *M. napoense* (Talavera & Hunt, 1997) Luc & Baujard, 1998, *M. planilobatum* (Talavera & Hunt, 1997) Luc & Baujard, 1998, *M. rusticum*, *M. sphaerocephaloides* (De Grisse, 1967) Loof & De Grisse, 1989, *M. surinamense* (De Grisse & Maas, 1970) Loof & De Grisse, 1989, *M. talensis* (Chaves, 1984) Loof & De Grisse, 1989, and *M. yossifovichi* (Krnjaic,1968) Luc & Raski, 1981. None of these species appear to possess large vulva projections. Cordero *et al.* (2012) described a collection of *M. surinamense* with small vulva projections from grass and maple in the Ozark National Forest and Savoy, Arkansas. Otherwise, morphological features of the Arkansas specimens closely match those of *M. discus*.

Ecological and geographic evidence. Based on collections in this study, *Mesocriconema discus* appears to be a species endemic to central North American grasslands.

Groups 8–14: *M. xenoplax* morphotypes

Morphological evidence. Groups 8–14 constitute seven lineages that are collectively supported as a monophyletic group by COI. They are also united by mean stylet lengths that exceed 70 µm. As a group they tend to be among the longest nematodes in the dataset, with some adult females exceeding 700 µm in body length (Table 5). The labial region in lateral view often gives the impression of a complex pattern of labial plates and divided annules. SEM supports this interpretation (Fig. 14C, Fig. 15F), but some individuals display a simple pattern of four rounded submedian lobes surrounding a rectangular oral disk, without any labial plates (Figs. 15G, H). Variability can also be observed in the shape of the vagina, which ranges from distinctly sigmoidal (Figs. 13F, 14H) to relatively straight (Figs. 13B, 15B, D). With allowances for variability in the shape of the vagina, the specimens in groups 8–14 key to *M. xenoplax* in Geraert (2010).

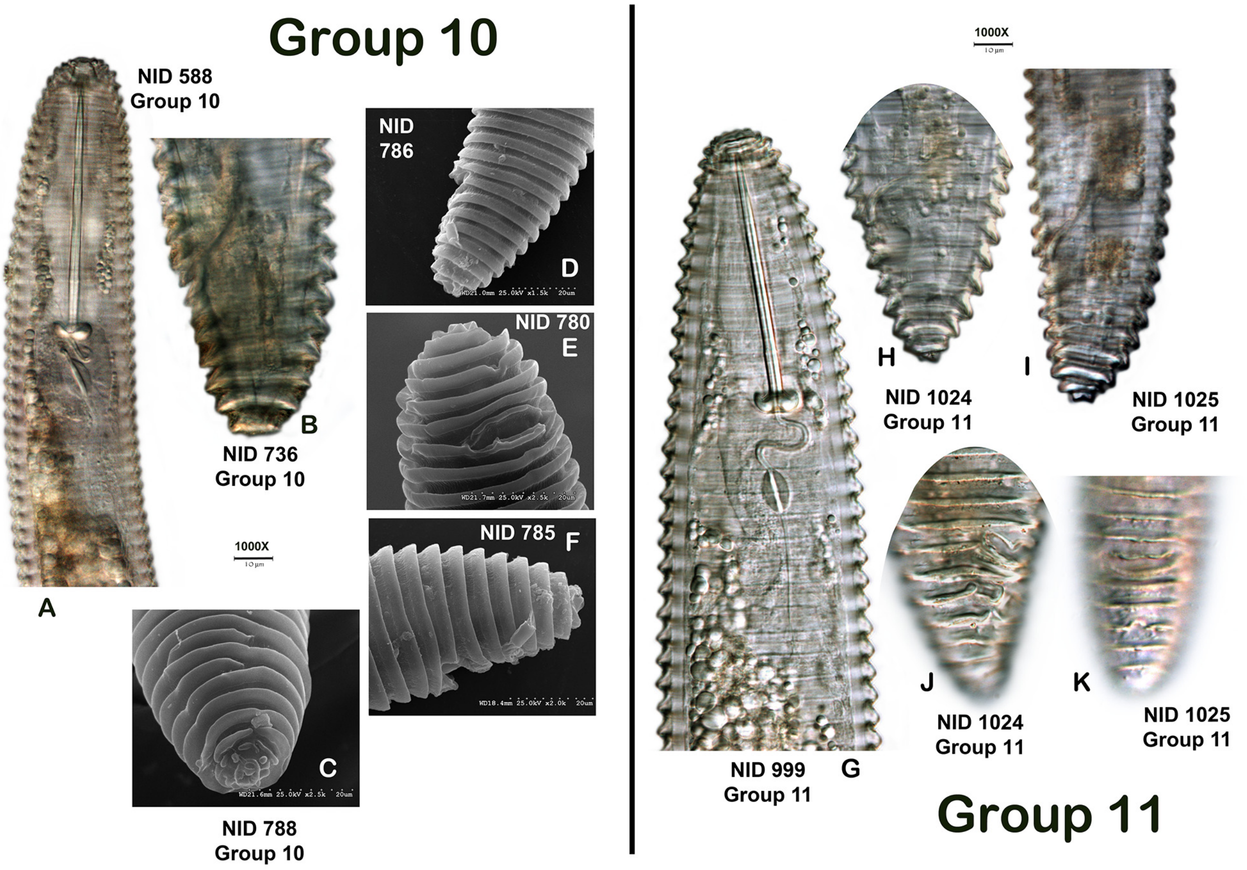

FIGURE 14. Haplotype Groups 10 (A, B, C, D, E, F) and 11 (G, H, I, J, K). Specimens conforming to morphospecies *Mesocriconema xenoplax*. Juvenile=A, Adult females=B–K. SEM images of specimens from a peach orchard in Georgia.

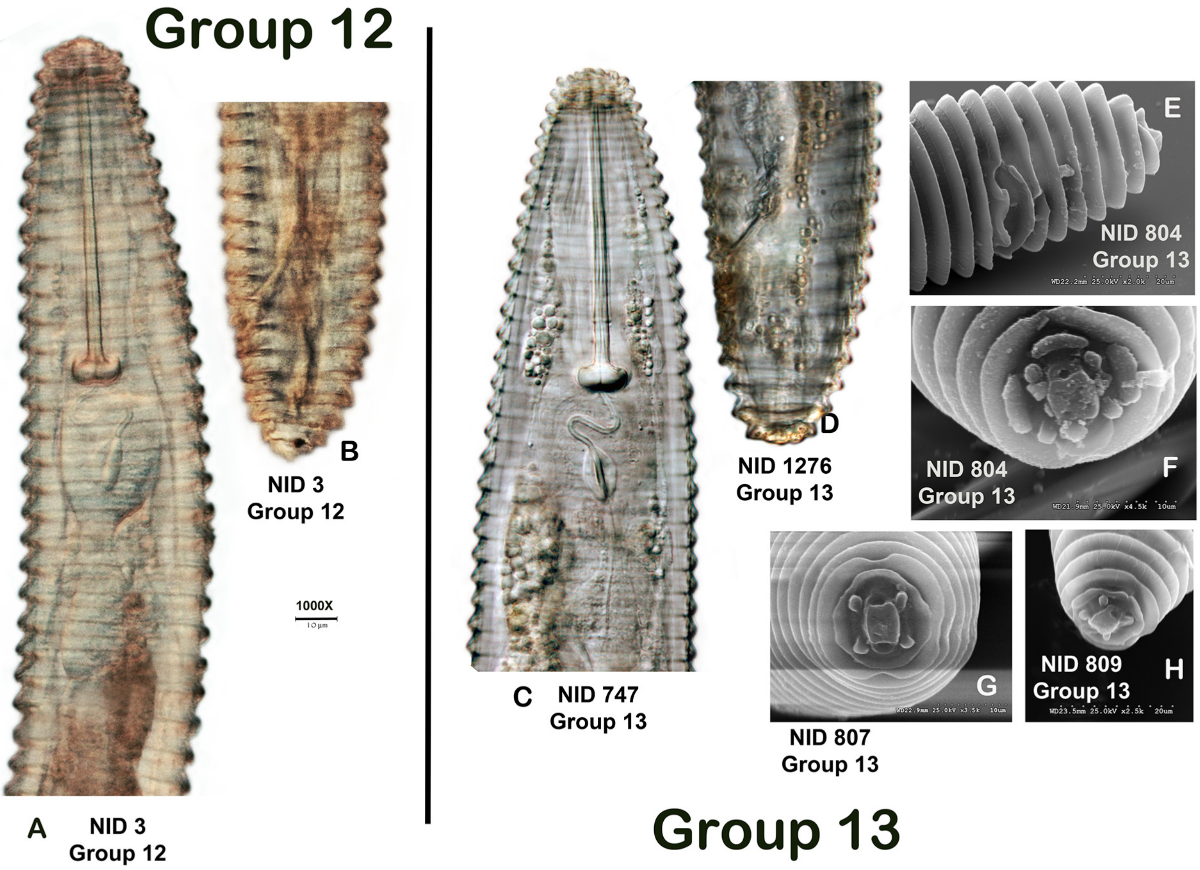

FIGURE 15. Haplotype Groups 12 (A, B) and 13 (C, D, E, F, G, H). Specimens conforming to morphospecies *Mesocriconema xenoplax*. Adult females=A–H. SEM images of specimens from British Columbia.

The seven well-supported subgroups within the morphospecies exhibit mean genetic distances of 2.7–12.8%. Interestingly, two isolates from commercial peach production, groups 8 and 10 from South Carolina and Georgia, respectively, fall into separate subgroups with a pairwise mean genetic distance of 8.9%. The peach isolate COI sequences from Byron, Georgia (Group 10) identically match specimens from native hardwood forest in Northern Florida. Some regional geographic patterns may exist among *M. xenoplax* COI subgroups.

Ecological and geographic evidence. *Mesocriconema xenoplax* is widely acknowledged as a cosmopolitan species (Andrássy 2007; Peneva *et al*. 2000; Geraert 2010; Wouts 2006). In North America *M. xenoplax* is the most frequently reported species in its genus (Norton *et al*. 1984). Reported host associations of *M. xenoplax* range from grasses to hardwood trees in both agricultural and native habitats. As mentioned above, the recognition of "short-stylet" forms of *M. xenoplax* from monocot hosts should be confirmed by haplotype analysis (also see Thorne 1961 regarding recognition of these variant specimens). All of the specimens in haplotype groups 8–14 were associated with woody vegetation. Group 14 specimens collected from Nine-Mile Prairie in Nebraska were associated with smooth sumac (*Rhus glabra)* encroaching into the grassland from bordering riparian vegetation. The type host for *M. xenoplax* is cultivated grape *Vitis vinifera* var. *sulanina* (*Vitis longii* rootstock) near Fresno, California. It is possible that *M. xenoplax* was transported to Europe on native North American *Vitis* rootstock used for grafting resistance to Phylloxera. Conversely, *M. xenoplax* could have accompanied the initial introduction of European *Vitis vinifera* into North America. Undoubtedly, global viticulture provided an opportunity for spread of the species. Another possibility is that host associations of an *M. xenoplax* ancestor extended across plant species of the late Cretaceous (70–65 mya) when North American and Eurasian flora communities exhibited a high degree of similarity (Graham 1999). Testing these biogeographic hypotheses will require additional sampling.

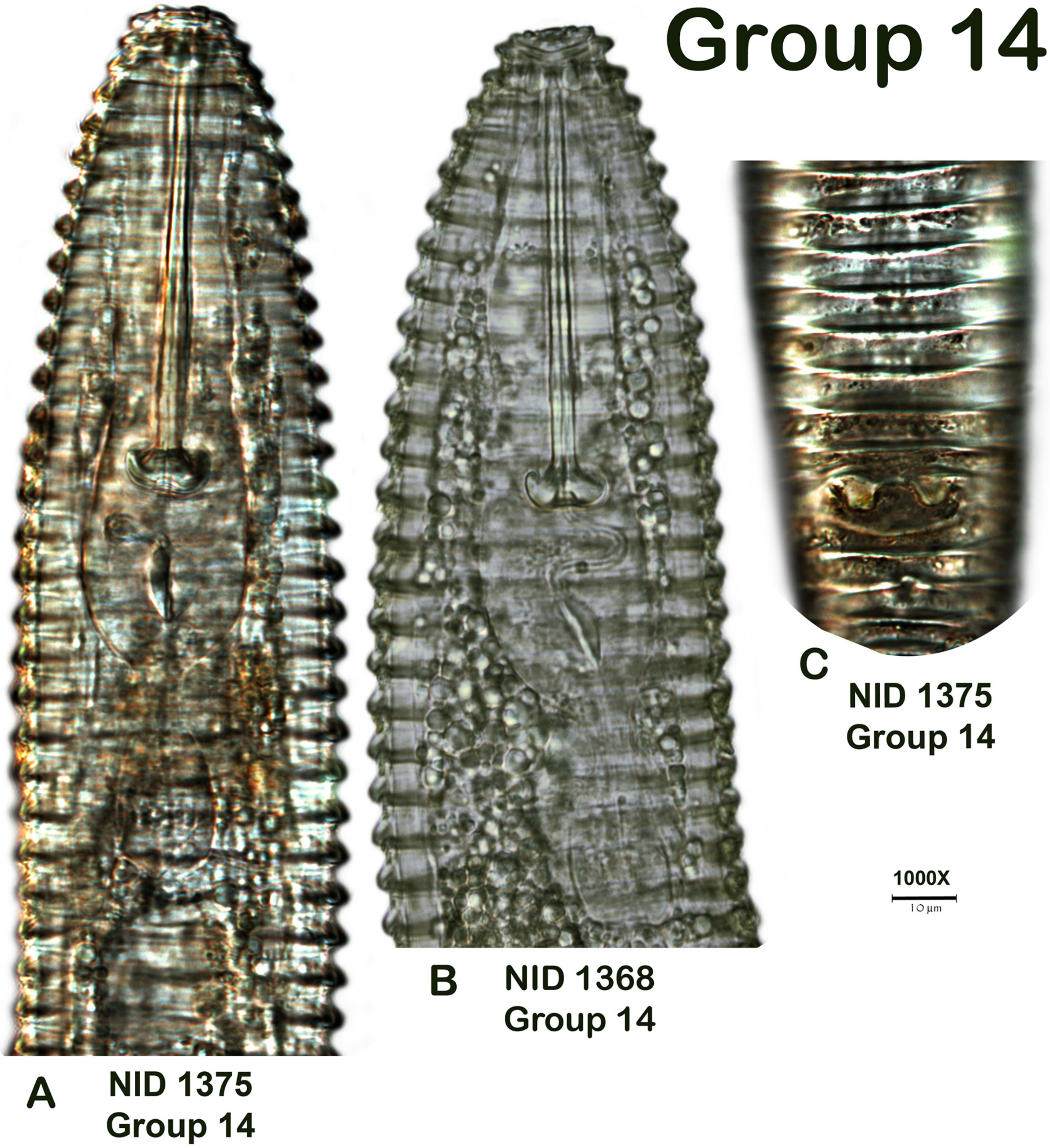

FIGURE 16. Haplotype Group 14 (A, B, C). Specimens conforming to morphospecies *Mesocriconema xenoplax*. Adult females=A–C.

Group 17: *Mesocriconema inaratum* new combination

= *Discocriconemella inaratus* Hoffman, 1974: 212; Powers *et al.*, 2010: 35 (as *D. inarata).*

Morphological evidence. This species stands out among the *Meoscriconema* species examined in this study based on its large, expanded cephalic annule and lack of submedian lobes as seen in SEM and light micrographs of specimens from Nebraska prairies (Figs. 18I–M). In lateral view at X1000 magnification (Figs. 18N, O), the broad, continuous first labial annule helps differentiate this species from other *Mesocriconema* groups that are typically sympatric in North American tallgrass prairies. The open vulva generally possesses two moderately-sized projections on the anterior annule (Figs. 18A–E). The systematics of this species, including comparative morphology and a phylogenetic analysis with 18SrDNA, ITS1, and cytochrome b nucleotide sequences, was addressed by Powers *et al*. (2010). The addition of COI data in the current study strengthens the previous contention that *M. inaratum* does not belong in the genus *Discocriconemella*. Additional evidence of its position within *Mesocriconema* can be seen on the COI amino acid tree (http://nematode.unl.edu/COI-ProteinTree_4-25-14.html). Therefore, we transfer *D. inarata* to *Mesocriconema* as *M. inaratum*.

Ecological and geographic evidence. In our collections this species is commonly associated with prairie and rough dropseed, *Sporobolus heterolepsis* and *S. asper,* respectively. *Mesocriconema inaratum* was found in Nine-Mile and Spring Creek Prairies, two remnant tallgrass prairies in eastern Nebraska, and Kalsow Prairie, the type locality in northwestern Iowa.

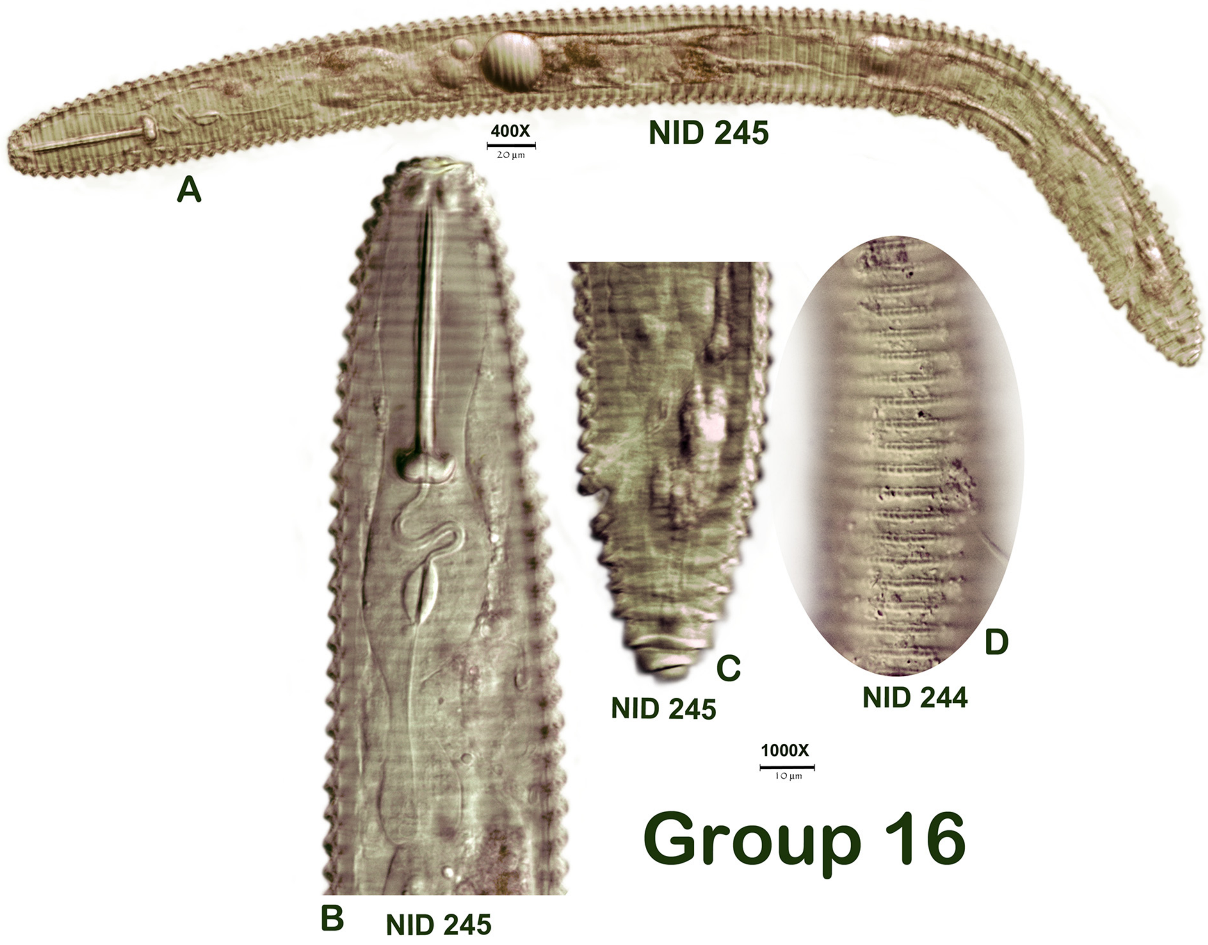

FIGURE 17. Haplotype Group 16 (A, B, C, D). Specimens conforming to morphospecies *Mesocriconema ornatum*. Adult female=A–C. Juvenile=D.

Group 19: *M. kirjanovae* (Andrássy, 1962) Loof & De Grisse, 1989

Morphological evidence. Specimens from Roth Prairie, Arkansas, stood out among other *Mesocriconema* in the dataset by their relatively acute, pointed tail (Figs. 22A, C, D). Additionally, large mean Rv values and other morphological characters (Table 5), as well as a recent report of *M. kirjanovae* from Arkansas (Cordero *et al.* 2012), lead to a tentative identification of these specimens as *M. kirjanovae*. Geraert (2010) reported that the annule projections anterior to the vulva were not large, but the projections observed in group 19 could be considered large (Fig. 22B).

Ecological and geographic evidence. *Mesocriconema kirjanovae* was recently reported for the first time from North America (Cordero *et al.* 2012), at Pine Tree, Arkansas, approximately 108 km from Roth Prairie, where the *M. kirjanovae* specimens in this dataset were obtained. Host information from Cordero *et al*. (2012) included an unidentified grass, young pines, and hickory (*Carya* sp.). The Roth Prairie sample was obtained from soil near unidentified prairie grasses. The type locality of *M. kirjanovae* is listed as soil from marshy meadow grass near Budapest, Hungary (Loof & De Grisse, 1989). Additional specimens collected, but not used in COI analyses, were found at Big Thicket National Preserve in Texas.

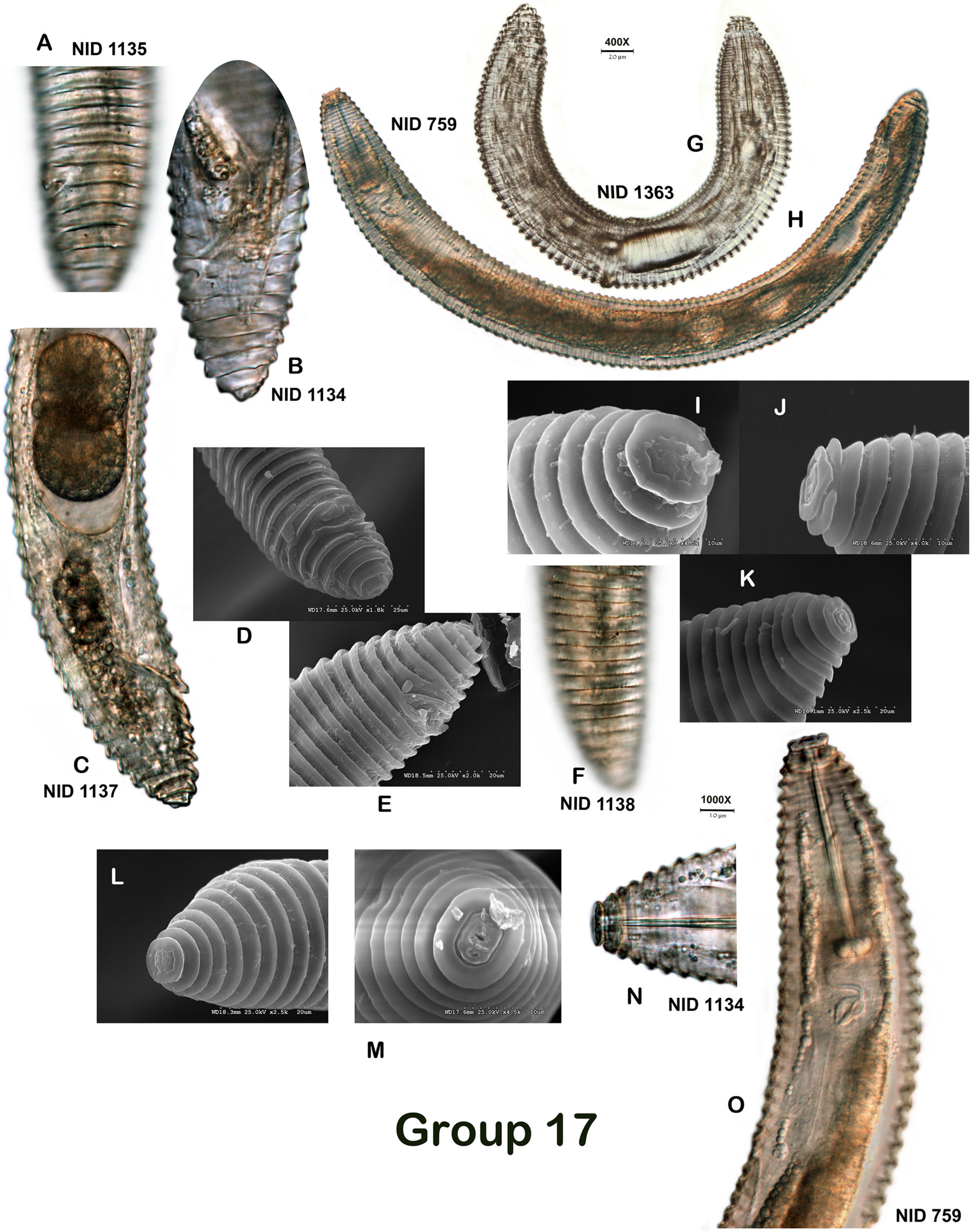

FIGURE 18. Haplotype Group 17 (A, B, C, D, E, F, G, H, I, J, K, L, M, N, O). Specimens conforming to morphospecies *Mesocriconema inaratum*. Adult females=A–E, G–O. Juvenile=F. Specimens G,O=from type locality. SEM images of specimens from Nine-mile Prairie, NE.

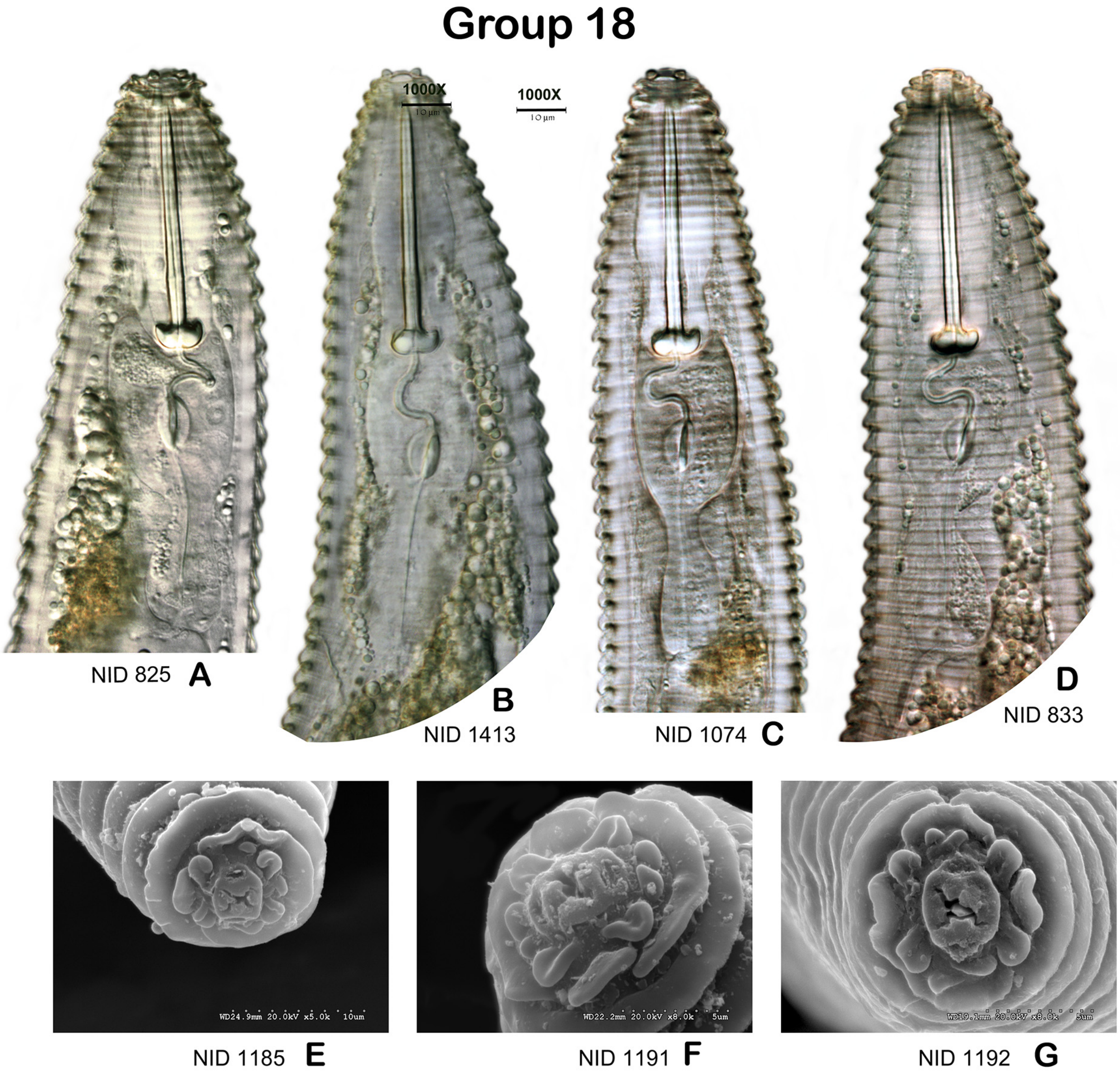

FIGURE 19. Haplotype Group 18 (A, B, C, D, E, F, G). Specimens conforming to morphospecies *Mesocriconema curvatum*. Adult females=A–G. SEM specimens from Kalsow Prairie, IA.

Discussion

The observations made in this study have bearing on two frequently mentioned features of nematodes, their potentially large contribution to overall animal biodiversity on earth and the cosmopolitan distribution ascribed to many species. The analysis of COI for the *Mesocriconema* dataset illustrates that the determination and recognition of taxonomic units could strongly influence interpretations of nematode biodiversity and biogeography. Specimens contributing to seven of the COI groups recognized in this dataset could be morphologically identified as *Mesocriconema curvatum*. That broad recognition, however, obscures differences in genetics, distribution, and host associations. The 40 specimens of group 18, which included 9 COI haplotypes, all came from central U.S. tallgrass prairies with the exception of a single haplotype collected from the mixed grassland region of central Kansas. Similarly the 45 specimens and 15 haplotypes of group 24 were distributed across a wider range of native grasslands from southern Wisconsin to Roth Prairie, a rare tallgrass remnant of the former Grand Prairie in eastern Arkansas. In contrast, group 4, also potentially recognized as a member of morphospecies *M. curvatum*, was collected from Montana to Missouri but only from agricultural soils. These biogeographic and host associations would be obscured under a broad morphological definition of *M. curvatum* as suggested in the compendium of Brzeski (2002b). It is clear that while a morphospecies recognized as *M. curvatum* could be considered widespread in North America, the COI lineages display a more nuanced view of the morphospecies distribution.

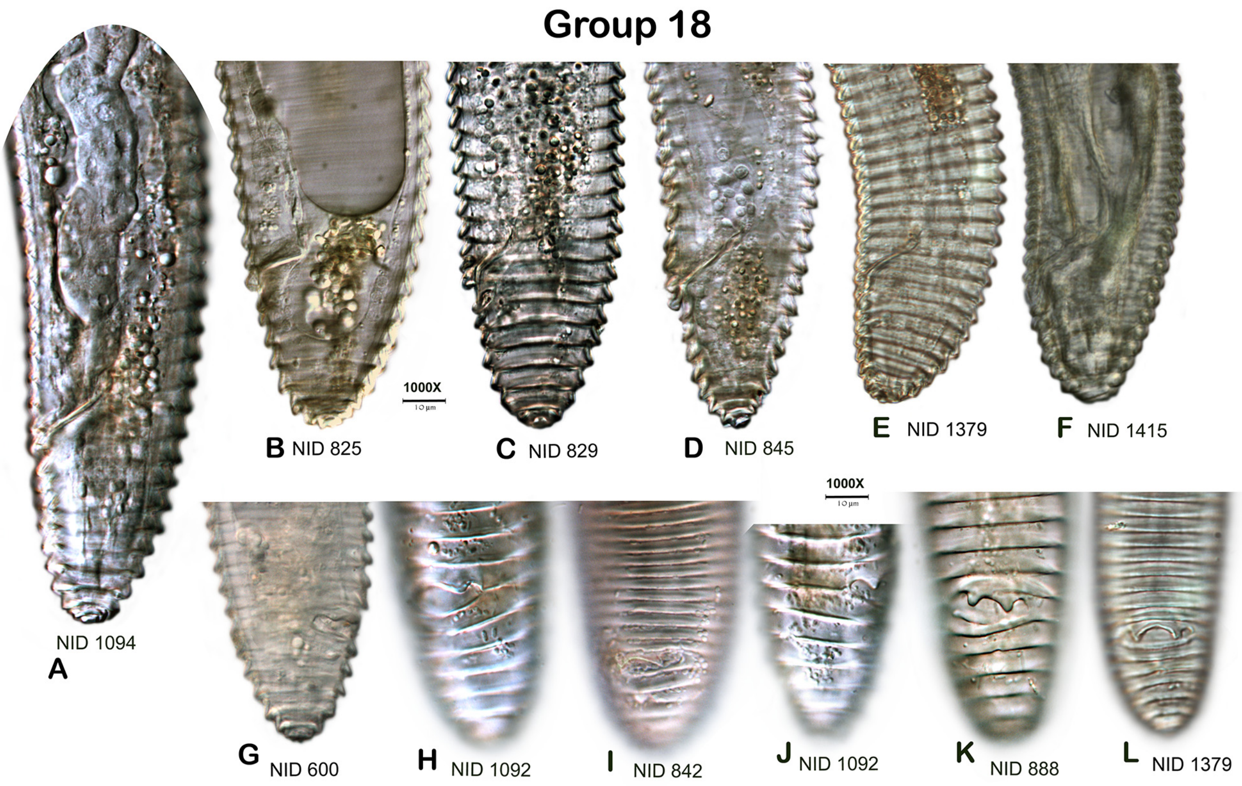

FIGURE 20. Haplotype Group 18 (A, B, C, D, E, F, G, H, I, J, K, L). Specimens conforming to morphospecies *Mesocriconema curvatum*. Adult females=A–L.

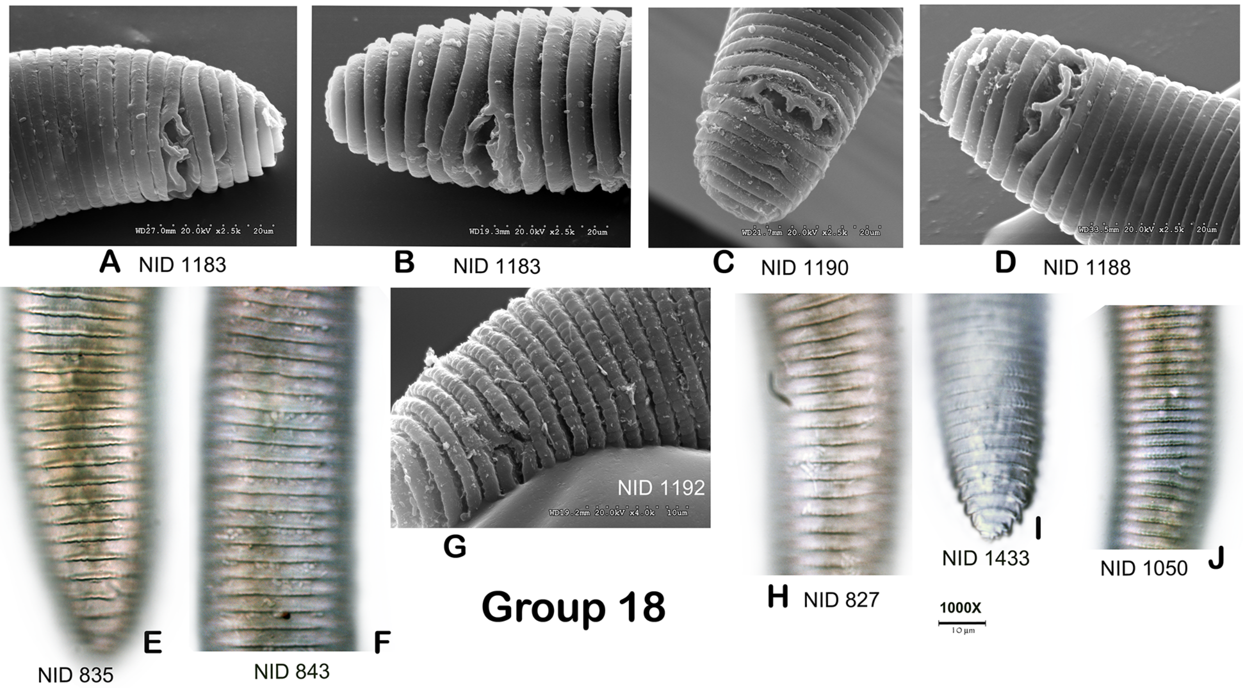

FIGURE 21. Haplotype Group 18 (A, B, C, D, E, F, G). Specimens conforming to morphospecies *Mesocriconema curvatum*. Adult females=A–D. Juveniles=E–J. SEM specimens A, B from Sheeder Prairie, IA, C, D, G from Kalsow Prairie, IA.

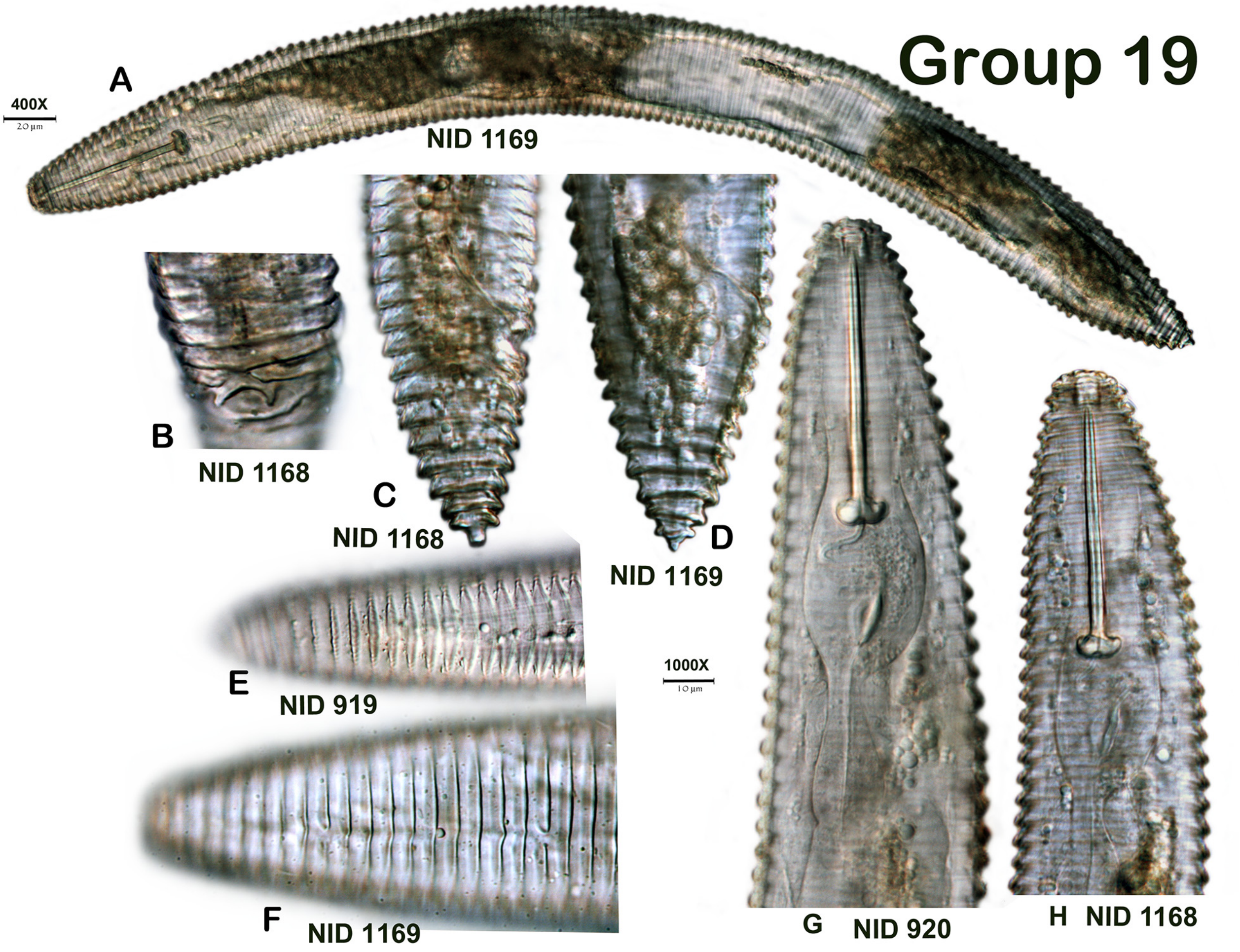

FIGURE 22. Haplotype Group 19 (A, B, C, D, E, F, G, H). Specimens conforming to morphospecies *Mesocriconema kirjanovae*. Adult females=A–D, F–H. Juvenile=E.

Unrecognized variation in *Mesocriconema ornatum* may have implications for management of pest species. Groups 1 and 15 both conformed to the morphospecies description and were only isolated from agricultural soils, yet the mean pairwise p-distance of the COI haplotypes of the two groups was 21.6%. This genetic distance, which is almost certainly an underestimate of the actual genetic distance (Srivathsan & Meier 2012), signals a possible difference in nematode physiology that could compromise management approaches such as biological control or resistant varieties which are based on highly specific host-parasite interactions. Furthermore, the low nucleotide diversity of both groups and their absence from native plant communities suggest an exotic origin of these two haplotype groups. *M. xenoplax*, best known as a cosmopolitan pest species of vineyards and orchards, might also be expected to have haplotype distributions heavily influenced by agricultural commerce (Wouts 2006). Haplotype groups 8–14 formed a monophyletic group and conformed to the morphospecies definition of *M. xenoplax*, but deep divisions in the clade present a complex pattern of both geographic localization and possible long distance dispersal associated with agriculture. It is possible that these specimens exhibit genetic breaks consistent with a phylogeographic structure reflecting ancient host associations. Overlying this ancient geographic structure may be a subset of haplotypes associated with agricultural commodities and recent anthropogenic dispersal. More intensive sampling of this group will be necessary for an understanding of its phylogenetic and biogeographic patterns.

It is quite likely that additional sampling of *Mesocriconema* will continue to reveal what appear to be cryptic species within Linnaean morphospecies. This is not unexpected. Molecular analyses in numerous well-studied nematode taxa such as *Caenorhabditis* (Kiontke *et al*. 2011), *Trichuris* (Callejón *et al*. 2013), *Pristionchus* (Kanzaki *et al*. 2012), and *Globodera* (Handoo *et al*. 2012) have discovered genetic variation that has forced reconsideration of diagnostic characters. In the case of *Mesocriconema,* morphological variation in key diagnostic characters within a haplotype group and overlap in morphological characters between groups creates major

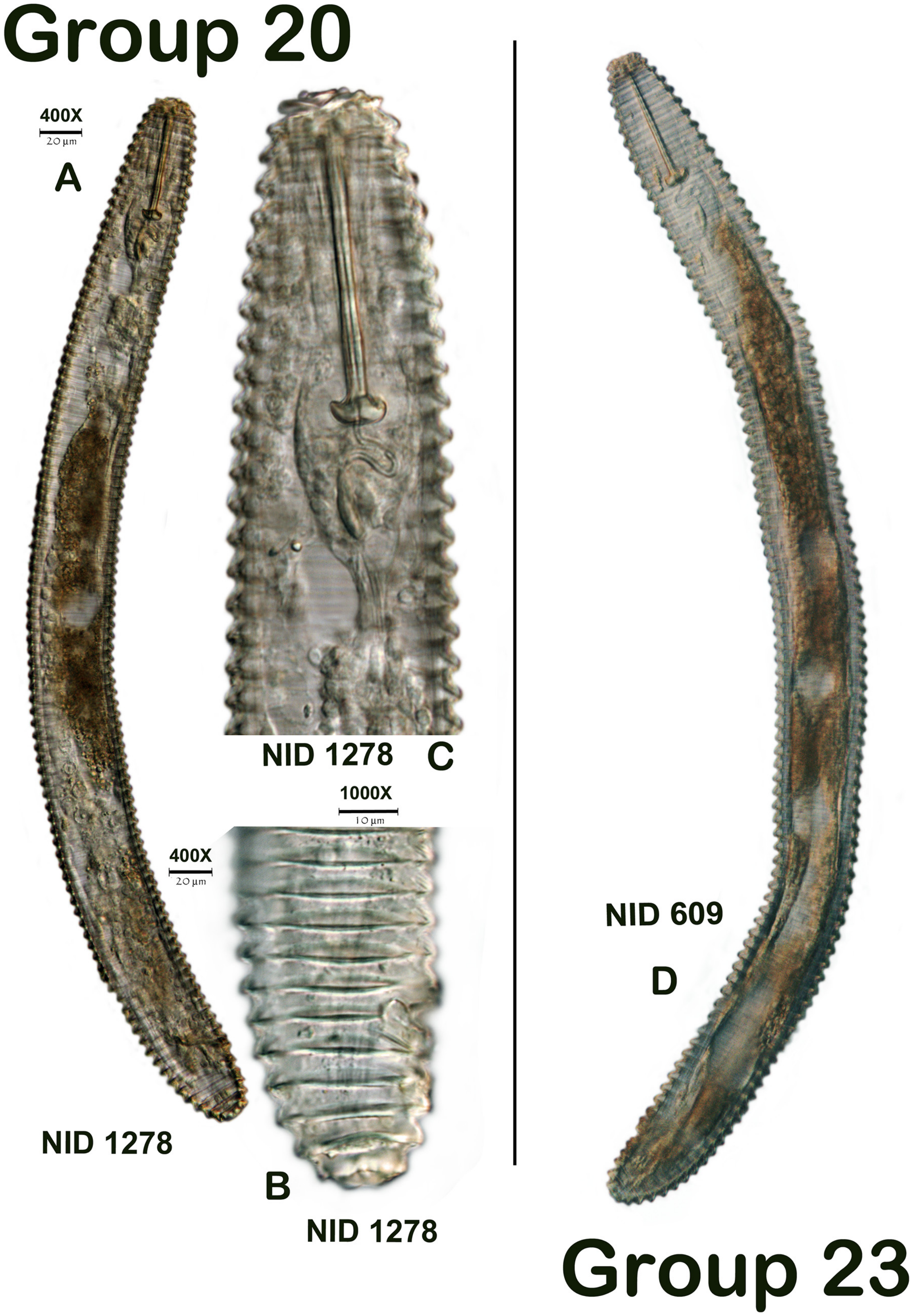

FIGURE 23. Haplotype Groups 20 (A, B, C) and 23 (D). Specimens conforming to morphospecies *Mesocriconema curvatum*. Adult females=A–D.

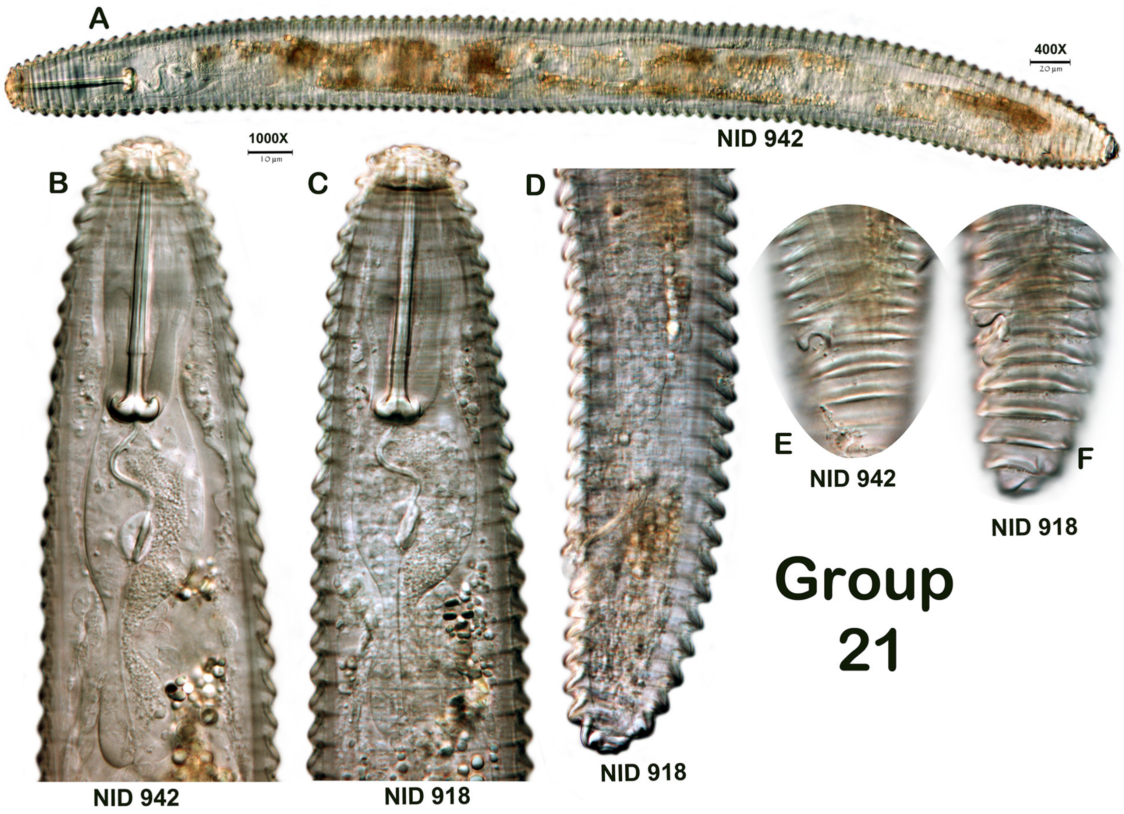

FIGURE 24. Haplotype Group 21 (A, B, C, D, E, F). Specimens conforming to morphospecies *Mesocriconema curvatum.* Adult female=A–F.

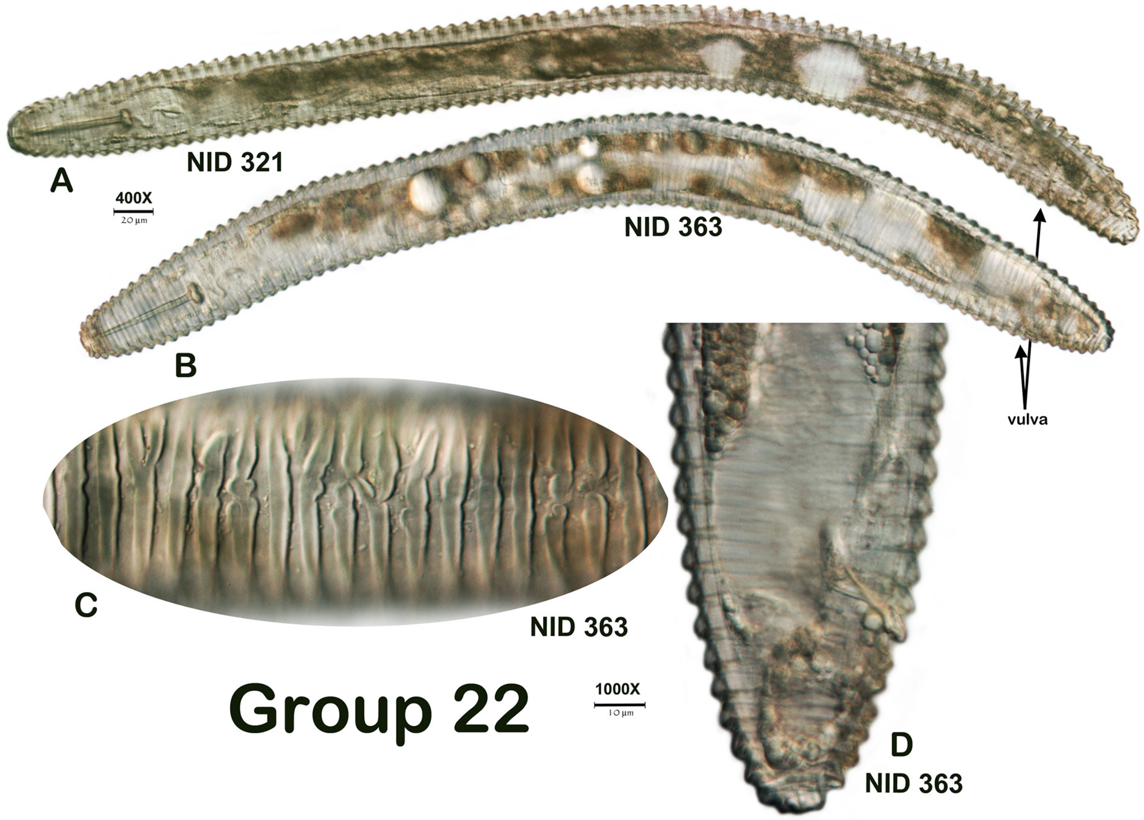

FIGURE 25. Haplotype Group 22 (A, B, C, D). Specimens conforming to morphospecies *Mesocriconema curvatum*. Adult females=A–D.

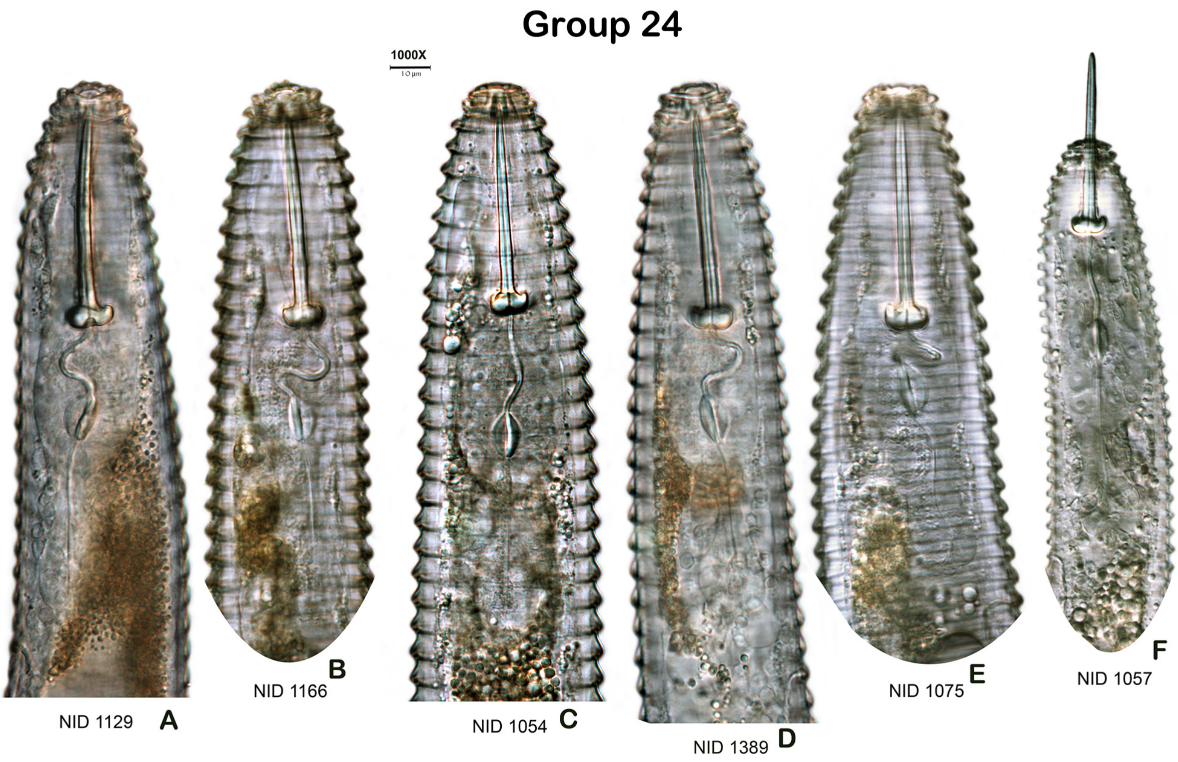

FIGURE 26. Haplotype Group 24 (A, B, C, D, E, F). Specimens conforming to morphospecies *Mesocriconema curvatum.* Adult females=A–E. Juvenile=F.

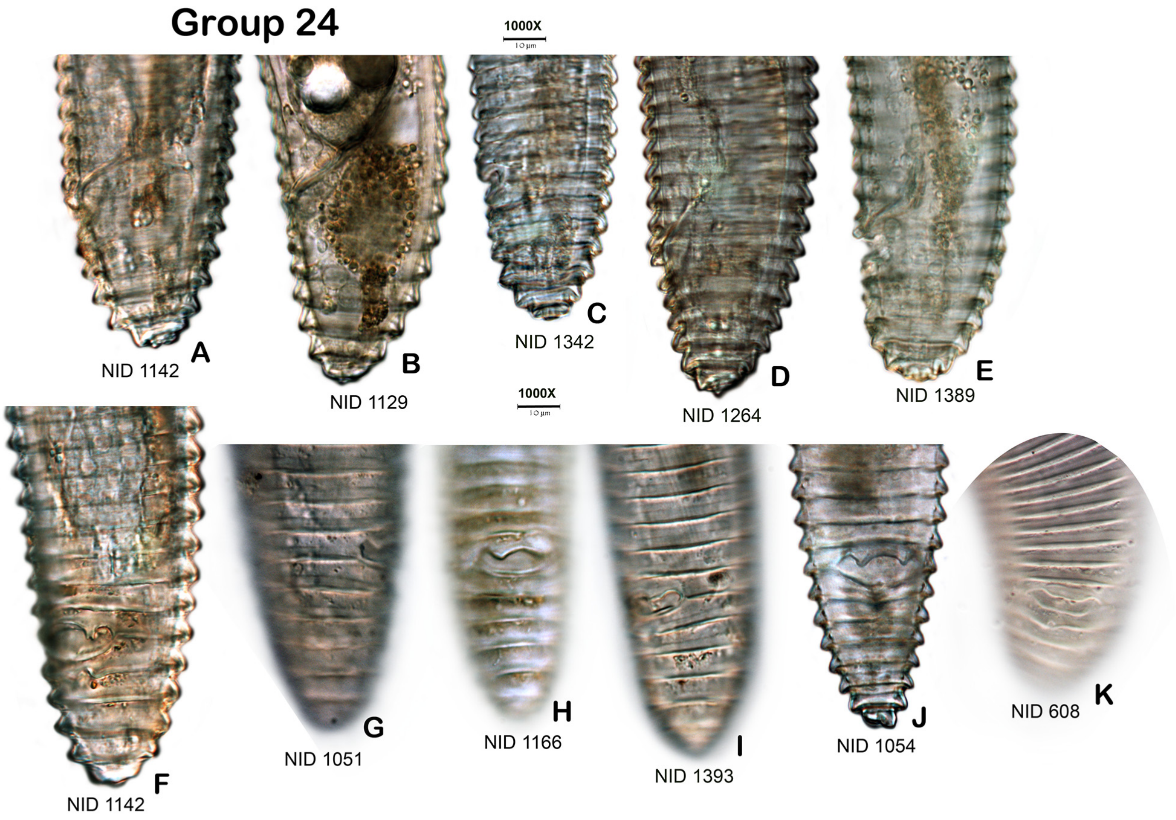

FIGURE 27. Haplotype Group 24 (A, B, C, D, E, F, G, H, I, J, K). Specimens conforming to morphospecies *Mesocriconema curvatum.* Adult females=A–K.

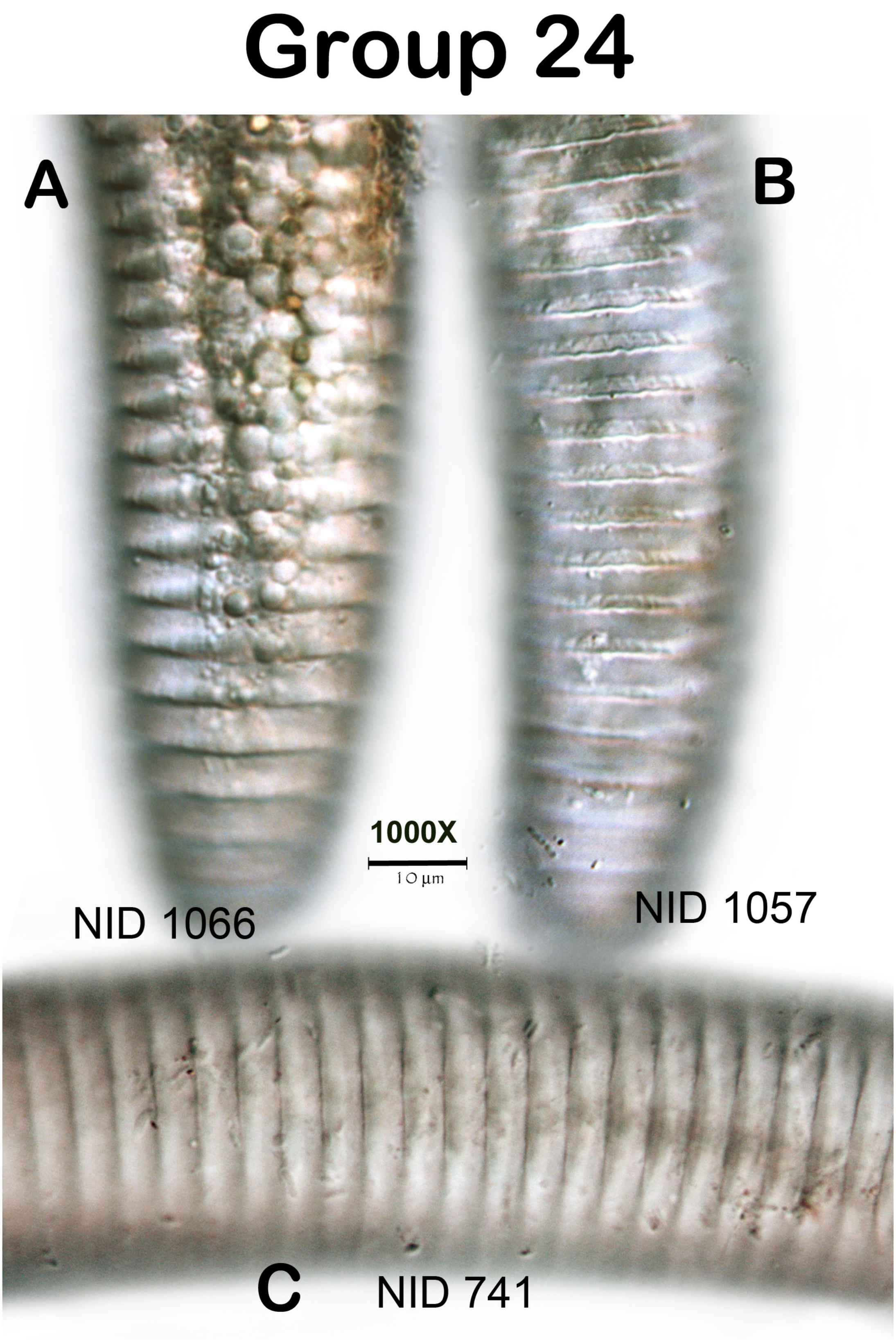

FIGURE 28. Haplotype Group 24 (A, B, C). Specimens conforming to morphospecies *Mesocriconema curvatum*. Juvenile=A–C.

difficulties in identification and the recognition of species boundaries. Obvious examples include the variation in labial plates in SEM face views, the degree of crenation on annule margins of juveniles, the size of the projections in the anterior lip of the vulva, and the interpretation of the shape of the vagina. These diagnostic morphological characters need to be systematically re-evaluated within the context of molecularly derived groupings in order to fully realize the information content of the taxonomic units. That realization will, in turn, allow nematode taxonomy to better integrate with and contribute to larger questions of global biodiversity.

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