



Species discovery and diversity in *Lobocriconema* (Criconeematidae: Nematoda) and related plant-parasitic nematodes from North American ecoregions

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Abstract

There are many nematode species that, following formal description, are seldom mentioned again in the scientific literature. *Lobocriconema thornei* and *L. incrassatum* are two such species, described from North American forests, respectively 37 and 49 years ago. In the course of a 3-year nematode biodiversity survey of North American ecoregions, specimens resembling *Lobocriconema* species appeared in soil samples from both grassland and forested sites. Using a combination of molecular and morphological analyses, together with a set of species delimitation approaches, we have expanded the known range of these species, added to the species descriptions, and discovered a related group of species that form a monophyletic group with the two described species. In this study, 148 specimens potentially belonging to the genus *Lobocriconema* were isolated from soil, individually measured, digitally imaged, and DNA barcoded using a 721 bp region of cytochrome oxidase subunit 1 (COI). One-third of the specimens were also analyzed using amplified DNA from the 3' region of the small subunit ribosomal RNA gene (18SrDNA) and the adjacent first internal transcribed spacer (ITS1). Eighteen mitochondrial haplotype groups, falling into four major clades, were identified by well-supported nodes in Bayesian and maximum likelihood trees and recognized as distinct lineages by species delimitation metrics. Discriminant function analysis of a set of morphological characters indicated that the major clades in the dataset possessed a strong morphological signal that decreased in comparisons of haplotype groups within clades. Evidence of biogeographic and phylogeographic patterns was apparent in the dataset. COI haplotype diversity was high in the southern Appalachian Mountains and Gulf Coast states and lessened in northern temperate forests. *Lobocriconema* distribution suggests the existence of phylogeographic patterns associated with recolonization of formerly glaciated regions by eastern deciduous forest, but definitive glacial refugia for this group of plant parasitic nematodes have yet to be identified. Unlike agricultural pest species of plant-parasitic nematodes, there is little evidence of long-distance dispersal in *Lobocriconema* as revealed by haplotype distribution. Most haplotype groups were characterized by low levels of intragroup genetic variation and large genetic distances between haplotype groups. The localization of nematode haplotypes together with their characteristic plant communities could provide insight into the historical formation of these belowground biotic communities.

Key words: Plant-parasitic nematodes, biogeography, glacial refugia, phylogeography, distribution, COI haplotypes, DNA barcoding, species delimitation

Introduction

Nematodes in the suborder Criconeematina Siddiqi, 1980 could provide a rich source of information about processes responsible for nematode biodiversity and distribution. The geographic patterns of contemporary species may reflect ancient processes as determined through a historical biogeographic perspective (Wiens 2012) or reveal the impact of more recent environmental and ecological events through an intraspecific, phylogeographic assessment (Avice 2000; Provan & Bennet 2008). Criconeematid nematodes are especially amenable for these analyses because of their global distribution, presence in a wide range of terrestrial habitats, their lack of

specialized dispersal stages, and their associations with numerous plant hosts that may have ancient, coevolutionary roots. They have a characteristic body form that is readily recognizable and easy to manipulate once isolated from the soil. There is, however, one feature that prevents them from becoming a model system for nematode biogeography: species boundaries and taxonomic groupings are obscure in spite of more than a century of taxonomic descriptions and classifications. The present study is a continuation of systematic investigations designed to construct a framework that integrates morphological and molecular characters in the suborder Criconematina with a goal of understanding nematode biodiversity and facilitating the study of terrestrial nematode biogeography (Powers *et al.* 2010; 2011; 2014).

Lobocriconema De Grisse & Loof, 1965 is a genus of plant-parasitic nematodes often found in eastern hardwood forests of North America. In some locations it may be a dominant member of the terrestrial plant parasitic nematode community (Knobloch & Bird 1978). More often in North American soils, it is recovered in relatively low numbers, generally in the presence of other members of Criconematidae Taylor, 1936 such as *Ogma* Southern, 1914, *Criconema* Hofmänner & Menzel, 1914, *Xenocriconemella* De Grisse & Loof, 1965, and *Mesocriconema* Andrassy, 1965 (Hoffmann 1974). Records of associated plant hosts include prominent members of the eastern deciduous forests such as black maple (*Acer nigrum* Michx. f.), sugar maple (*Acer saccharum* Marsh), box elder (*Acer negundo* L.), basswood (*Tilia americana* L.), smooth sumac (*Rhus glabra* L), eastern white pine (*Pinus strobus* L.), and oak (*Quercus* spp.) (Hoffmann 1974; Knobloch & Bird 1978). The genus has also been associated with false Virginia creeper, *Parthenocissus vitacea* (Knerr) Hitchc., a native North American woody vine commonly found in ravines and open woodlands (Hoffmann 1974). The genus has not, to the best of our knowledge, been associated with soils involved in intensive agricultural production.

The genus *Lobocriconema* was created by De Grisse & Loof (1965) in an effort to reduce the number of species in *Criconemoides* Taylor, 1936, which at that time numbered approximately 80. Five described species were transferred from *Criconema* and *Criconemoides* to the new genus *Lobocriconema* with *Lobocriconema crassiannulatum* (De Guiran, 1963) De Grisse & Loof, 1965 selected to serve as the type species of the genus. Key characters of the females used by De Grisse & Loof (1965) to define the genus were: “Lip region in females offset more or less distinctly, sometimes collar-like. Submedian lobes present. Number of body annules less than 50; they are retrorse in outline, the posterior edges are smooth or ornamented. Tail short with obscure anus. Vulva open. Juveniles with longitudinal rows of scales on posterior edges of annules. Males with bursa and four lateral lines.” Ironically, while the generic name refers to the presence of cephalic submedian lobes, the type species *L. crassiannulatum* was originally described as possessing flattened, indistinct lobes. Over time, species without submedian lobes or possessing a face pattern featuring six “pseudolips” have been accommodated in the genus (Geraert 2010).

In the recent taxonomic summary of Criconematidae by Geraert (2010), the genus is diagnosed as having very coarse annuli, 8–18 μm thick, that may be smooth, fringed, finely crenate, or with longitudinal scratches to regularly lobed. Differentiated cephalic annuli number 1 to 3. Submedian lobes are present but not distinct in some species. The stylet is stout, tail conoid- rounded, and the vulva either open or closed, sometimes with an overhanging anterior lip. Juveniles may have 8–24 rows of scales of different shapes, or in some cases no rows, or no scales as in *L. pilosum* (Van den Berg, 1984) Loof & De Grisse, 1989. There are 19 species listed in Geraert (2010). Since a male stage has been recorded for only three of the species, presumably most species in the genus reproduce by parthenogenesis, transmitting genetic material to offspring as an intact unit unaffected by outcrossing and recombination (Avisé 2000; Birky 2009). Biogeographically, ten of the species were originally described from Africa, four from India, three from Japan, and two from North America.

In our survey of criconematid nematodes from distinct ecoregions of North America initiated in 2012, nematodes were recovered that conformed to the descriptions of *Lobocriconema*, but did not possess distinct submedian lobes. Two species of *Lobocriconema* have been described from North America. *Lobocriconema thornei* Knobloch & Bird, 1978 was originally collected from a northern hardwood forest in East Lansing, Michigan (Knobloch & Bird 1978). This species has prominent submedian lobes, which were readily recognized in this study from type locality specimens as well as in soil samples from forests in Indiana, Ohio, and Virginia. The second species described from North America is *L. incrassatum* (Raski & Golden, 1966) Siddiqi, 1986. This species was originally characterized by strongly developed submedian lobes surrounded by a thin, narrow first labial annule and followed by a second labial annule that was thicker and wider. The type locality of this species was recorded as “Emigration Canyon, about 20 miles east of Salt Lake City, Utah, elevation between 7,000–8,000

ft.” The type host was maple, no species listed. This species was successfully recovered by the authors from the type locality from soil underneath mixed stands of canyon maple, *Acer grandidentatum* Nutt, and Gambel oak, *Quercus gambelii* Nutt at 1,890 m (6,200 ft) elevation. Specimens conforming, in part, to the morphometrics of *L. incrassatum* and *L. thornei* were also discovered in a nematode survey of Great Smoky Mountains National Park (GRSM), but features of the labial region left uncertainty about the species identification. Beyond morphology, other questions about generic membership of *Lobocriconema*-like specimens arose during the ecoregion survey with regard to plant community associations. For example, members of the genus are most often associated with tree hosts, yet *Lobocriconema*-like specimens were collected from central tall grasslands and savannas in the north-central plains states. Based on microscopic examination of key morphological features, these specimens appear to have a taxonomic affinity to members of the genus with indistinct submedian lobes. In order to resolve questions of taxonomic units in our ecoregion survey of North America, we have conducted an integrated taxonomic analysis of all the specimens collected in the survey that resemble *Lobocriconema*. This working definition generally encompassed nematodes with robust stylets and stylet knobs, 60 or fewer body annules which are wider than 8 µm, a vulva situated far posterior on the body, usually within 5 annules of the tail terminus, and juveniles with longitudinal scales that are lost in the adult molt.

A total of 148 *Lobocriconema*-like specimens and 10 related outgroup species constituted the criconematid dataset evaluated in this study. Our primary goal was to determine the taxonomic status of those specimens. When possible, at least five individual specimens of a morphospecies from each sampling site were measured, photographed, and processed for DNA sequencing. Previous studies of criconematid nematodes have demonstrated that mixtures of congeneric species or haplotype lineages are commonly found within a single soil sample emphasizing the necessity of conducting molecular analyses on individual specimens rather than pooled specimens (Powers *et al.* 2014). A second objective of the survey, to investigate factors responsible for present-day distribution, is partially addressed in this study.

Globally, *Lobocriconema* species appear to have geographically-restricted distributions with several disjunct regions of diversity. An examination of evolutionary lineages within *Lobocriconema* should provide insight into historical and ecological biogeographic factors responsible for present day diversity and distribution in North America. Resolving the taxonomic units within the genus is a necessary first step toward understanding those factors.

Materials and methods

Nematode collection. Collection sites of the 158 specimens in the *Lobocriconema* dataset are presented in Table 1. Geographic coordinates are associated with GenBank accession numbers (KU236486–KU236690) (Table 2). *Lobocriconema* specimens were obtained from 13 of 50 sampled North American terrestrial ecoregions as designated by the World Wildlife Fund (Olson *et al.* 2004). Sampling was relatively intense within five locations: Great Smoky Mountains National Park, TN and NC (54 soil samples), George Washington Memorial Parkway, VA (53 samples), Nine-Mile and Spring Creek Prairies, NE (47 samples), Big Thicket National Preserve, TX (28 samples), and Santa Cruz Island Reserve, CA (20 samples). Soil samples were collected using a standardized procedure to enhance recovery of nematode biodiversity (Neher *et al.* 1995). Typically a 40 x 40m grid was created recording GPS coordinates from each corner, and systematically sampled while walking a transect across the grid extracting a 20–30 cm-deep soil core every 6 meters using an Oakfield Tube with a 2 cm diameter. In some cases focal soil cores were taken from around a single plant species to better assess host-plant associations. Nematodes were extracted from soil by a modified flotation-sieving and centrifugation method (Jenkins 1964). The nematode extraction method is critical in the processing of criconematid nematodes, since methods that rely on active movement of the nematode such as Baermann trays or funnels may significantly underrepresent Criconematina (Viglierchio & Schmitt 1983).

Nematode DNA barcoding. Extracted nematodes were individually mounted on slides, measured, photographed, and prepared for PCR amplification and sequencing as described in Powers *et al.* (2014). Other than nematode rupture with a micropipette tip, there was no separate DNA extraction step. The COI primer sequences were COI-F5—5'-AATWTWGGTGTGGAAGTTCTTGAAC-3' and COI-R9—5'-CTTAAACATAATGRAAATGWGCWACWACATAATAAGTATC-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

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

COI Haplotype*	COI Clade	NID	Species	Stage	Locality	Ecoregion name	Plant community / host
1	A	288	<i>Lobocriconema</i> sp.	F	Sauk County, WI	Upper midwest forest-savanna transition zone	Bur oak- focal
1	A	1130	<i>Lobocriconema</i> sp.	F	Avoca Prairie and Savanna, WI	Upper midwest forest-savanna transition zone	Oak savannah
1	A	1149	<i>Lobocriconema</i> sp.	F	Avoca Prairie and Savanna, WI	Upper midwest forest-savanna transition zone	Oak savanna
1	A	1152	<i>Lobocriconema</i> sp.	F	Avoca Prairie and Savanna, WI	Upper midwest forest-savanna transition zone	Oak savanna
1	A	1205	<i>Lobocriconema</i> sp.	F	Governor Dodge State Park, WI	Upper midwest forest-savanna transition zone	Oak savanna
1	A	1206	<i>Lobocriconema</i> sp.	F	Governor Dodge State Park, WI	Upper midwest forest-savanna transition zone	Oak savanna
1	A	1214	<i>Lobocriconema</i> sp.	F	Accotink Creek, VA	Southeastern mixed forests	White oak, Yellow poplar, Virginia creeper
1	A	1462	<i>Lobocriconema</i> sp.	F	Roth Prairie, AR	Mississippi lowland forests	Mississippi alluvial plain
1	A	3265	<i>Lobocriconema</i> sp.	F	Ozark National Forest, AR	Central US hardwood forests	Wild cherry
1	A	3267	<i>Lobocriconema</i> sp.	F	Ozark National Forest, AR	Central US hardwood forests	Wild cherry
2	B	894	<i>Lobocriconema</i> sp.	F	Chimney Creek, GRSM, TN	Appalachian-Blue Ridge forest	Cove hardwood forest
2	B	899	<i>Lobocriconema</i> sp.	F	Chimney Creek, GRSM, TN	Appalachian-Blue Ridge forest	Cove hardwood forest
2	B	1465	<i>Lobocriconema</i> sp.	F	Purchase Knob, GRSM, NC	Appalachian-Blue Ridge forest	Cove hardwood forest
2	B	2582	<i>Lobocriconema</i> sp.	F	Purchase Knob, GRSM, NC	Appalachian-Blue Ridge forests	Cove hardwood forest
2	B	2739	<i>Lobocriconema</i> sp.	F	Purchase Knob, GRSM, NC	Appalachian-Blue Ridge forests	Cove hardwood forest
2	B	2791	<i>Lobocriconema</i> sp.	F	Goshen Prong, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
2	B	3001	<i>Lobocriconema</i> sp.	F	Goshen Prong, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
2	B	3003	<i>Lobocriconema</i> sp.	F	Goshen Prong, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
2	B	3420	<i>Lobocriconema</i> sp.	F	Snake Den, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
2	B	3421	<i>Lobocriconema</i> sp.	J	Snake Den, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
3	C	5560	<i>Lobocriconema incrassatum</i>	F	Emigration Canyon, UT	Wasatch and Uinta Montane forests	Oak, maple scrub
3	C	5562	<i>Lobocriconema incrassatum</i>	F	Emigration Canyon, UT	Wasatch and Uinta Montane forests	Oak, maple scrub
3	C	5563	<i>Lobocriconema incrassatum</i>	F	Emigration Canyon, UT	Wasatch and Uinta Montane forests	Oak, maple scrub
4	C	2805	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3243	<i>Lobocriconema</i> sp.	F	Oconaluftee, GRSM, NC	Appalachian-Blue Ridge forests	Montane alluvial forest
4	C	3248	<i>Lobocriconema</i> sp.	F	Oconaluftee, GRSM, NC	Appalachian-Blue Ridge forests	Montane alluvial forest
4	C	3282	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest

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TABLE 1. (Continued)

COI Haplotype*	COI Clade	NID	Species	Stage	Locality	Ecoregion name	Plant community / host
4	C	3283	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3284	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3288	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3290	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3304	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3306	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3315	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
4	C	3317	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian-Blue Ridge forests	Cove hardwood forest
5	C	1157	<i>Lobocriconema thornei</i>	F	Tippecanoe County, IN	Central forest-grassland transition zone	Red oak, Beech, Yellow poplar
5	C	1158	<i>Lobocriconema thornei</i>	F	Tippecanoe County, IN	Central forest-grassland transition zone	Red oak, Beech, Yellow poplar
5	C	1159	<i>Lobocriconema thornei</i>	F	Tippecanoe County, IN	Central forest-grassland transition zone	Red oak, Beech, Yellow poplar
5	C	1160	<i>Lobocriconema thornei</i>	F	Tippecanoe County, IN	Central forest-grassland transition zone	Red oak, Beech, Yellow poplar
5	C	1339	<i>Lobocriconema thornei</i>	F	Great Falls Park, GWMP, VA	Southeastern mixed forests	White oak forests (230 years old)
5	C	1340	<i>Lobocriconema thornei</i>	F	Great Falls Park, GWMP, VA	Southeastern mixed forests	White oak forests (230 years old)
5	C	1341	<i>Lobocriconema thornei</i>	F	Great Falls Park, GWMP, VA	Southeastern mixed forests	White oak forests (230 years old)
5	C	1344	<i>Lobocriconema thornei</i>	J	Great Falls Park, GWMP, VA	Southeastern mixed forests	White oak forests (230 years old)
5	C	1345	<i>Lobocriconema thornei</i>	F	Great Falls Park, GWMP, VA	Southeastern mixed forests	White oak forests (230 years old)
5	C	1381	<i>Lobocriconema thornei</i>	F	Turkey Run Trail, GWMP, VA	Southeastern mixed forests	Pawpaws, no groundcover
5	C	1414	<i>Lobocriconema thornei</i>	F	Turkey Run Trail, GWMP, VA	Southeastern mixed forests	Pawpaws, Tilia, Maple, various forbs
5	C	2524	<i>Lobocriconema thornei</i>	F	MSU, Ingham County, MI	Southern Great Lakes forests	Deciduous hardwood forest
5	C	2525	<i>Lobocriconema thornei</i>	F	MSU, Ingham County, MI	Southern Great Lakes forests	Deciduous hardwood forest
5	C	2526	<i>Lobocriconema thornei</i>	F	MSU, Ingham County, MI	Southern Great Lakes forests	Deciduous hardwood forest
5	C	2527	<i>Lobocriconema thornei</i>	J	MSU, Ingham County, MI	Southern Great Lakes forests	Deciduous hardwood forest
5	C	3366	<i>Lobocriconema thornei</i>	F	Crane Hollow Preserve, OH	Appalachian mixed mesophytic forest	Hemlock, Beech, Chestnut, Oak
5	C	3367	<i>Lobocriconema thornei</i>	F	Crane Hollow Preserve, OH	Appalachian mixed mesophytic forest	Hemlock, Beech, Chestnut, Oak

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TABLE 1. (Continued)

COI Haplotype*	COI Clade	NID	Species	Stage	Locality	Ecogeographic name	Plant community / host
5	C	3368	<i>Lobocriconema thornei</i>	F	Crane Hollow Preserve, OH	Appalachian mixed mesophytic forest	Hemlock, Beech, Chestnut, Oak
5	C	3369	<i>Lobocriconema thornei</i>	F	Crane Hollow Preserve, OH	Appalachian mixed mesophytic forest	Hemlock, Beech, Chestnut, Oak
6	D	1481	<i>Lobocriconema</i> sp.	F	Neches Bottom and Jack Gore Baygall Unit, BITH, TX	Piney woods forests	Cypress swamp
6	D	1491	<i>Lobocriconema</i> sp.	F	Neches Bottom and Jack Gore Baygall Unit, BITH, TX	Piney woods forests	Cypress swamp
6	D	2074	<i>Lobocriconema</i> sp.	F	Roy E. Larsen Sandyland Sanctuary, TX	Piney woods forests	Sandhills
6	D	3214	<i>Lobocriconema</i> sp.	F	Big Sandy Creek Unit, BITH, TX	Piney woods forests	Wetland pine savannah-
6	D	3215	<i>Lobocriconema</i> sp.	F	Big Sandy Creek Unit, BITH, TX	Piney woods forests	Wetland pine savannah-
6	D	3645	<i>Lobocriconema</i> sp.	F	Neches Bottom and Jack Gore Baygall Unit, BITH, TX	Piney woods forests	Cypress Swamp
6	D	3668	<i>Lobocriconema</i> sp.	F	Canyonlands Unit, BITH, TX	Piney woods forests	Swampy lowlands with hardwood
7	D	5583	<i>Criconema warrenense</i>	F	St. Francis National Forest, AR	Mississippi lowland forests	Oak rhizosphere
7	D	5584	<i>Criconema warrenense</i>	F	St. Francis National Forest, AR	Mississippi lowland forests	Oak rhizosphere
7	D	5585	<i>Criconema warrenense</i>	F	St. Francis National Forest, AR	Mississippi lowland forests	Oak rhizosphere
7	D	577	<i>Lobocriconema</i> sp.	F	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Floodplain marsh.
7	D	587	<i>Lobocriconema</i> sp.	F	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Floodplain marsh
7	D	589	<i>Lobocriconema</i> sp.	M	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
7	D	3194	<i>Lobocriconema</i> sp.	F	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
7	D	3195	<i>Lobocriconema</i> sp.	F	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
7	D	3197	<i>Lobocriconema</i> sp.	F	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
7	D	3203	<i>Lobocriconema</i> sp.	F	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
8	D	2273	<i>Lobocriconema</i> sp.	F	Roy E. Larsen Sandyland Sanctuary, TX	Piney woods forests	Relic Longleaf pines
8	D	2293	<i>Lobocriconema</i> sp.	M	Roy E. Larsen Sandyland Sanctuary, TX	Piney woods forests	Relic Longleaf pines
8	D	2862	<i>Lobocriconema</i> sp.	F	Konza Prairie, KS	Flint Hills tall grasslands	Tallgrass prairie
9	D	938	<i>Lobocriconema</i> sp.	J	Spring Creek Prairie, NE	Central tall grasslands	Tallgrass prairie
9	D	1108	<i>Lobocriconema</i> sp.	F	Nine-Mile Prairie, NE	Central tall grasslands	Tallgrass prairie
9	D	1124	<i>Lobocriconema</i> sp.	F	Nine-Mile Prairie, NE	Central tall grasslands	Switchgrass- focal
9	D	1155	<i>Lobocriconema</i> sp.	F	Nine-Mile Prairie, NE	Central tall grasslands	Leadplant- focal
9	D	1156	<i>Lobocriconema</i> sp.	F	Nine-Mile Prairie, NE	Central tall grasslands	Leadplant- focal
9	D	1243	<i>Lobocriconema</i> sp.	M	Nine-Mile Prairie, NE	Central tall grasslands	Leadplant- focal
9	D	1291	<i>Lobocriconema</i> sp.	F	Spring Creek Prairie, NE	Central tall grasslands	Rose- focal
9	D	1310	<i>Lobocriconema</i> sp.	F	Nine-Mile Prairie, NE	Central tall grasslands	Leadplant- focal

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TABLE 1. (Continued)

COI Haplotype*	COI Clade	NID	Species	Stage	Locality	Ecoregion name	Plant community / host
9	D	1362	<i>Lobocriconema</i> sp.	J	Spring Creek Prairie, NE	Central tall grasslands	Tallgrass prairie
9	D	1372	<i>Lobocriconema</i> sp.	F	Spring Creek Prairie, NE	Central tall grasslands	Tallgrass prairie
9	D	1382	<i>Lobocriconema</i> sp.	J	Spring Creek Prairie, NE	Central tall grasslands	Tallgrass prairie
9	D	2861	<i>Lobocriconema</i> sp.	F	Konza Prairie, KS	Flint Hills tall grasslands	Tallgrass prairie
9	D	2914	<i>Lobocriconema</i> sp.	F	Aurora Prairie, SD	Central tall grasslands	Tallgrass prairie
10	D	3249	<i>Lobocriconema</i> sp.	F	Oconaluftee, GRSM, NC	Appalachian-Blue Ridge forests	Montane alluvial forest
10	D	3257	<i>Lobocriconema</i> sp.	F	Ozark National Forest, AR	Central US hardwood forests	Hackberry- focal
10	D	3258	<i>Lobocriconema</i> sp.	F	Ozark National Forest, AR	Central US hardwood forests	Hackberry- focal
10	D	3259	<i>Lobocriconema</i> sp.	F	Ozark National Forest, AR	Central US hardwood forests	Hackberry- focal
11	D	3213	<i>Lobocriconema</i> sp.	F	Big Sandy Creek Unit, BITH, TX	Piney woods forests	Wetland pine savannah
11	D	3247	<i>Lobocriconema</i> sp.	M	Oconaluftee, GRSM, NC	Appalachian-Blue Ridge forests	Montane alluvial forest
11	D	3250	<i>Lobocriconema</i> sp.	F	Oconaluftee, GRSM, NC	Appalachian-Blue Ridge forests	Montane alluvial forest
11	D	3256	<i>Lobocriconema</i> sp.	F	Ozark National Forest, AR	Central US hardwood forests	Hackberry- focal
11	D	3293	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Upland hardwood forest
11	D	3295	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Upland hardwood forest
11	D	3296	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Upland hardwood forest
11	D	3301	<i>Lobocriconema</i> sp.	J	Torrey State Park, FL	Southeastern conifer forests	Upland hardwood forest
11	D	3310	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
11	D	3311	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
11	D	3312	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Upland mixed forest
11	D	3334	<i>Lobocriconema</i> sp.	F	Sapelo Island Coastal LTER, GA	Middle Atlantic coastal forest	Upland maritime forest
11	D	3341	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Alluvial forest (cypress)
11	D	3615	<i>Lobocriconema</i> sp.	F	Canyonlands Unit, BITH, TX	Piney woods forests	Upland and stream
11	D	3658	<i>Lobocriconema</i> sp.	F	Lance Rosier Unit, BITH, TX	Piney woods forests	Oak- focal
11	D	3663	<i>Lobocriconema</i> sp.	F	Lance Rosier Unit, BITH, TX	Piney woods forests	Oak- focal
11	D	5586	<i>Lobocriconema</i> sp.	F	Tuskegee National Forest, AL	Southeastern mixed forests	Pine, beech, oak, wild grape, sweetgum, greenbriar
11	D	5631	<i>Lobocriconema</i> sp.	F	Tuskegee National Forest, AL	Southeastern mixed forests	Pine, beech, oak, wild grape, sweetgum, greenbriar
11	D	5632	<i>Lobocriconema</i> sp.	F	Tuskegee National Forest, AL	Southeastern mixed forests	Pine, beech, oak, wild grape, sweetgum, greenbriar
11	D	5633	<i>Lobocriconema</i> sp.	F	Tuskegee National Forest, AL	Southeastern mixed forests	Pine, beech, oak, wild grape, sweetgum, greenbriar
11	D	5647	<i>Lobocriconema</i> sp.	F	Beech Creek Unit, BITH, TX	Piney woods forests	Magnolia, hardwood
11	D	5653	<i>Lobocriconema</i> sp.	F	Big Sandy Creek Unit, BITH, TX	Piney woods forests	Pine savannah
12	D	3057	<i>Lobocriconema</i> sp.	F	Tunica Hills, LA	Mississippi lowland forests	Marshland
12	D	3090	<i>Lobocriconema</i> sp.	F	Tunica Hills, LA	Mississippi lowland forests	Marshland
12	D	3091	<i>Lobocriconema</i> sp.	F	Tunica Hills, LA	Mississippi lowland forests	Marshland

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TABLE 1. (Continued)

COI Haplotype*	COI Clade	NID	Species	Stage	Locality	Ecoregion name	Plant community / host
12	D	3121	<i>Lobocriconema</i> sp.	J	Tunica Hills, LA	Mississippi lowland forests	Marshland
13	D	3068	<i>Lobocriconema</i> sp.	F	Cataloochee, GRSM, NC	Appalachian-Blue Ridge forests	Montane Oak-Hickory
13	D	3129	<i>Lobocriconema</i> sp.	F	Cataloochee, GRSM, NC	Appalachian-Blue Ridge forests	Montane Oak-Hickory
13	D	3176	<i>Lobocriconema</i> sp.	F	Gregory Bald, GRSM, NC	Appalachian-Blue Ridge forests	Grassy bald
13	D	3400	<i>Lobocriconema</i> sp.	F	Gregory Bald, GRSM, NC	Appalachian-Blue Ridge forests	Grassy bald
13	D	3401	<i>Lobocriconema</i> sp.	F	Gregory Bald, GRSM, NC	Appalachian-Blue Ridge forests	Grassy bald
13	D	3402	<i>Lobocriconema</i> sp.	F	Gregory Bald, GRSM, NC	Appalachian-Blue Ridge forests	Grassy bald
13	D	3403	<i>Lobocriconema</i> sp.	F	Gregory Bald, GRSM, NC	Appalachian-Blue Ridge forests	Grassy bald
14	D	3297	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Upland hardwood forest
14	D	3328	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Alluvial forest (cypress)
14	D	3329	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Alluvial forest (cypress)
14	D	3343	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Alluvial forest (cypress)
14	D	3347	<i>Lobocriconema</i> sp.	F	Torrey State Park, FL	Southeastern conifer forests	Alluvial forest (cypress)
15	D	3196	<i>Lobocriconema</i> sp.	F	Ichetuknee Springs State Park, FL	Southeastern conifer forests	Suwannee Riverbank
15	D	3321	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Floodplain Cypress swamp
15	D	3322	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Floodplain Cypress swamp
15	D	3323	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Floodplain Cypress swamp
15	D	3324	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Floodplain Cypress swamp
15	D	3325	<i>Lobocriconema</i> sp.	F	Wakulla Springs State Park, FL	Southeastern conifer forests	Floodplain Cypress swamp
16	C	5575	<i>Lobocriconema incrassatum</i>	F	Providence Canyon, UT	Wasatch and Uinta Montane forests	Bigtooth maple and evergreen shrub
16	C	5576	<i>Lobocriconema incrassatum</i>	J	Providence Canyon, UT	Wasatch and Uinta Montane forests	Bigtooth maple and evergreen shrub
16	C	5577	<i>Lobocriconema incrassatum</i>	F	Providence Canyon, UT	Wasatch and Uinta Montane forests	Bigtooth maple and evergreen shrub
17	C	3667	<i>Lobocriconema</i> sp.	F	Canyonlands Unit, BITH, TX	Piney woods forests	Swampy lowlands with hardwood
17	C	3675	<i>Lobocriconema</i> sp.	F	Canyonlands Unit, BITH, TX	Piney woods forests	Swampy lowlands with hardwood
17	C	3677	<i>Lobocriconema</i> sp.	F	Canyonlands Unit, BITH, TX	Piney woods forests	Swampy lowlands with hardwood
18	C	3381	<i>Lobocriconema thornei</i>	F	Porcupine Mountains State Park, MI	Western Great Lakes forest	Hemlock forest
18	C	3382	<i>Lobocriconema thornei</i>	F	Porcupine Mountains State Park, MI	Western Great Lakes forest	Hemlock forest
18	C	3414	<i>Lobocriconema thornei</i>	F	Parfrey's Glen, WI	Upper midwest forest/savanna transition zone	Mixed hardwood forest floodplain
18	C	3415	<i>Lobocriconema thornei</i>	F	Parfrey's Glen, WI	Upper midwest forest/savanna transition zone	Mixed hardwood forest floodplain
S	D	1	<i>Lobocriconema</i> sp.	F	Cass County, NE	Central tall grasslands	Red oak- focal

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TABLE 1. (Continued)

COI Haplotype*	COI Clade	NID	Species	Stage	Locality	Ecoregion name	Plant community / host
S	D	1395	<i>Lobocriconema</i> sp.	F	Turkey Run Trail, GWMP, VA	Southeastern mixed forests	Tilia, Maple, Pawpaw
S	C	3229	<i>Lobocriconema</i> sp.	F	Ozark National Forest, AR	Central US hardwood forests	Maple- focal
S	C	3260	<i>Lobocriconema</i> sp.	J	Ozark National Forest, AR	Central US hardwood forests	Hackberry- focal
S	D	3291	<i>Lobocriconema</i> sp.	F	Twin Creeks, GRSM, TN	Appalachian/Blue Ridge forests	Cove hardwood forest
O		1327	<i>Mesocriconema xenoplax</i>		Central Valley, CA	California central valley grasslands	
O		278	<i>Bakernema inaequale</i>		Grundy State Forest, TN	Appalachian mixed mesophytic forests	Oak and magnolia
O		3268	<i>Criconema</i> sp.	F	No Boh forest, Thailand	Kayah-Karen Montane rain forests	Montane rain forest, overgrazed
O		238	<i>Neolobocriconema serratum</i>	F	Douglas County, NE	Central tall grasslands	Commercial nursery
O		1454	<i>Mesocriconema sphaerocephalum</i>		Puerto Rico	Puerto Rican dry forests	Corn
O		1328	<i>Criconema mutabile</i>		Central Valley, CA	California central valley grasslands	
O		3096	<i>Criconema permistum</i>		Tunica Hills, LA	Mississippi lowland forests	Marshland
O		2966	<i>Criconema sphagni</i>		West Point, GRSM, TN	Appalachian-Blue Ridge forests	
O		2993	<i>Criconema petasum</i>		Gifford Woods State Park, VT	New England Acadian forests	Oldgrowth forest
O		1490	<i>Criconema loofi</i>		Neches Bottom and Jack Gore Baygall Unit, BITH, TX	Piney woods forests	Cypress swamp

*S- Singleton, O- Outgroup

TABLE 2. GenBank accession numbers for study specimens. 158 specimens are represented by COI sequence, 44 specimens are represented by both COI and ITS1 sequences and 3 specimens are represented only by ITS1 sequence.

COI Haplotype	COI Clade	NID	Species	Stage	Primer	GenBank accession #
1	A	288	<i>Lobocriconema</i> sp.	F	COI	KU236486
1	A	1130	<i>Lobocriconema</i> sp.	F	COI	KU236487
1	A	1149	<i>Lobocriconema</i> sp.	F	COI	KU236488
1	A	1152	<i>Lobocriconema</i> sp.	F	COI	KU236489
1	A	1205	<i>Lobocriconema</i> sp.	F	COI	KU236490
1	A	1206	<i>Lobocriconema</i> sp.	F	COI	KU236491
1	A	1214	<i>Lobocriconema</i> sp.	F	COI	KU236492
1	A	1462	<i>Lobocriconema</i> sp.	F	COI	KU236493
1	A	3265	<i>Lobocriconema</i> sp.	F	COI	KU236494
1	A	3265	<i>Lobocriconema</i> sp.	F	ITS1	KU236644
1	A	3267	<i>Lobocriconema</i> sp.	F	COI	KU236495
1	A	3267	<i>Lobocriconema</i> sp.	F	ITS1	KU236645
2	B	894	<i>Lobocriconema</i> sp.	F	COI	KU236496
2	B	899	<i>Lobocriconema</i> sp.	F	COI	KU236497
2	B	1465	<i>Lobocriconema</i> sp.	F	COI	KU236498
2	B	2582	<i>Lobocriconema</i> sp.	F	COI	KU236499
2	B	2739	<i>Lobocriconema</i> sp.	F	COI	KU236500
2	B	2791	<i>Lobocriconema</i> sp.	F	COI	KU236501
2	B	3001	<i>Lobocriconema</i> sp.	F	COI	KU236502
2	B	3001	<i>Lobocriconema</i> sp.	F	ITS1	KU236646
2	B	3003	<i>Lobocriconema</i> sp.	F	COI	KU236503
2	B	3003	<i>Lobocriconema</i> sp.	F	ITS1	KU236647
2	B	3420	<i>Lobocriconema</i> sp.	F	COI	KU236504
2	B	3421	<i>Lobocriconema</i> sp.	J	COI	KU236505
3	C	5560	<i>Lobocriconema incrassatum</i>	F	COI	KU236506
3	C	5562	<i>Lobocriconema incrassatum</i>	F	COI	KU236507
3	C	5563	<i>Lobocriconema incrassatum</i>	F	COI	KU236508
3	C	5563	<i>Lobocriconema incrassatum</i>	F	ITS1	KU236654
4	C	2805	<i>Lobocriconema</i> sp.	F	COI	KU236509
4	C	3243	<i>Lobocriconema</i> sp.	F	COI	KU236510
4	C	3248	<i>Lobocriconema</i> sp.	F	COI	KU236511
4	C	3282	<i>Lobocriconema</i> sp.	F	COI	KU236512
4	C	3283	<i>Lobocriconema</i> sp.	F	COI	KU236513
4	C	3284	<i>Lobocriconema</i> sp.	F	COI	KU236514
4	C	3288	<i>Lobocriconema</i> sp.	F	COI	KU236515
4	C	3290	<i>Lobocriconema</i> sp.	F	COI	KU236516
4	C	3290	<i>Lobocriconema</i> sp.	F	ITS1	KU236649
4	C	3304	<i>Lobocriconema</i> sp.	F	COI	KU236517
4	C	3305	<i>Lobocriconema</i> sp.	F	COI	KU236518
4	C	3306	<i>Lobocriconema</i> sp.	F	COI	KU236519

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TABLE 2. (Continued)

COI Haplotype	COI Clade	NID	Species	Stage	Primer	GenBank accession #
4	C	3315	<i>Lobocriconema</i> sp.	F	COI	KU236520
4	C	3317	<i>Lobocriconema</i> sp.	F	COI	KU236521
4	C	3317	<i>Lobocriconema</i> sp.	F	ITS1	KU236650
5	C	1157	<i>Lobocriconema thornei</i>	F	COI	KU236522
5	C	1158	<i>Lobocriconema thornei</i>	F	COI	KU236523
5	C	1159	<i>Lobocriconema thornei</i>	F	COI	KU236524
5	C	1160	<i>Lobocriconema thornei</i>	F	COI	KU236525
5	C	1339	<i>Lobocriconema thornei</i>	F	COI	KU236526
5	C	1340	<i>Lobocriconema thornei</i>	F	COI	KU236527
5	C	1341	<i>Lobocriconema thornei</i>	F	COI	KU236528
5	C	1344	<i>Lobocriconema thornei</i>	J	COI	KU236529
5	C	1345	<i>Lobocriconema thornei</i>	F	COI	KU236530
5	C	1381	<i>Lobocriconema thornei</i>	F	COI	KU236531
5	C	1414	<i>Lobocriconema thornei</i>	F	COI	KU236532
5	C	2524	<i>Lobocriconema thornei</i>	F	COI	KU236533
5	C	2525	<i>Lobocriconema thornei</i>	F	COI	KU236534
5	C	2526	<i>Lobocriconema thornei</i>	F	COI	KU236535
5	C	2527	<i>Lobocriconema thornei</i>	J	COI	KU236536
5	C	3366	<i>Lobocriconema thornei</i>	F	COI	KU236537
5	C	3367	<i>Lobocriconema thornei</i>	F	COI	KU236538
5	C	3367	<i>Lobocriconema thornei</i>	F	ITS1	KU236651
5	C	3368	<i>Lobocriconema thornei</i>	F	COI	KU236539
5	C	3368	<i>Lobocriconema thornei</i>	F	ITS1	KU236652
5	C	3369	<i>Lobocriconema thornei</i>	F	COI	KU236540
6	D	1481	<i>Lobocriconema</i> sp.	F	COI	KU236541
6	D	1491	<i>Lobocriconema</i> sp.	F	COI	KU236542
6	D	2074	<i>Lobocriconema</i> sp.	F	COI	KU236543
6	D	3214	<i>Lobocriconema</i> sp.	F	COI	KU236544
6	D	3214	<i>Lobocriconema</i> sp.	F	ITS1	KU236667
6	D	3215	<i>Lobocriconema</i> sp.	F	COI	KU236545
6	D	3215	<i>Lobocriconema</i> sp.	F	ITS1	KU236668
6	D	3645	<i>Lobocriconema</i> sp.	F	COI	KU236546
6	D	3668	<i>Lobocriconema</i> sp.	F	COI	KU236547
		5583	<i>Criconema warrenense</i>	F	ITS1	KU236684
		5584	<i>Criconema warrenense</i>	F	ITS1	KU236685
		5585	<i>Criconema warrenense</i>	F	ITS1	KU236686
7	D	577	<i>Lobocriconema</i> sp.	F	COI	KU236548
7	D	587	<i>Lobocriconema</i> sp.	F	COI	KU236549
7	D	589	<i>Lobocriconema</i> sp.	M	COI	KU236550
7	D	3194	<i>Lobocriconema</i> sp.	F	COI	KU236551

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TABLE 2. (Continued)

COI Haplotype	COI Clade	NID	Species	Stage	Primer	GenBank accession #
7	D	3195	<i>Lobocriconema</i> sp.	F	COI	KU236552
7	D	3195	<i>Lobocriconema</i> sp.	F	ITS1	KU236664
7	D	3197	<i>Lobocriconema</i> sp.	F	COI	KU236553
7	D	3197	<i>Lobocriconema</i> sp.	F	ITS1	KU236665
7	D	3203	<i>Lobocriconema</i> sp.	F	COI	KU236554
7	D	3203	<i>Lobocriconema</i> sp.	F	ITS1	KU236666
8	D	2273	<i>Lobocriconema</i> sp.	F	COI	KU236555
8	D	2273	<i>Lobocriconema</i> sp.	F	ITS1	KU236658
8	D	2293	<i>Lobocriconema</i> sp.	M	COI	KU236556
8	D	2293	<i>Lobocriconema</i> sp.	M	ITS1	KU236659
8	D	2862	<i>Lobocriconema</i> sp.	F	COI	KU236557
9	D	938	<i>Lobocriconema</i> sp.	J	COI	KU236558
9	D	1108	<i>Lobocriconema</i> sp.	F	COI	KU236559
9	D	1124	<i>Lobocriconema</i> sp.	F	COI	KU236560
9	D	1155	<i>Lobocriconema</i> sp.	F	COI	KU236561
9	D	1156	<i>Lobocriconema</i> sp.	F	COI	KU236562
9	D	1243	<i>Lobocriconema</i> sp.	M	COI	KU236563
9	D	1291	<i>Lobocriconema</i> sp.	F	COI	KU236564
9	D	1310	<i>Lobocriconema</i> sp.	F	COI	KU236565
9	D	1362	<i>Lobocriconema</i> sp.	J	COI	KU236566
9	D	1372	<i>Lobocriconema</i> sp.	F	COI	KU236567
9	D	1382	<i>Lobocriconema</i> sp.	J	COI	KU236568
9	D	2861	<i>Lobocriconema</i> sp.	F	COI	KU236569
9	D	2861	<i>Lobocriconema</i> sp.	F	ITS1	KU236660
9	D	2914	<i>Lobocriconema</i> sp.	F	COI	KU236570
9	D	2914	<i>Lobocriconema</i> sp.	F	ITS1	KU236661
10	D	3249	<i>Lobocriconema</i> sp.	F	COI	KU236571
10	D	3257	<i>Lobocriconema</i> sp.	F	COI	KU236572
10	D	3257	<i>Lobocriconema</i> sp.	F	ITS1	KU236672
10	D	3258	<i>Lobocriconema</i> sp.	F	COI	KU236573
10	D	3259	<i>Lobocriconema</i> sp.	F	COI	KU236574
10	D	3259	<i>Lobocriconema</i> sp.	F	ITS1	KU236673
11	D	3213	<i>Lobocriconema</i> sp.	F	COI	KU236575
11	D	3247	<i>Lobocriconema</i> sp.	M	COI	KU236576
11	D	3247	<i>Lobocriconema</i> sp.	M	ITS1	KU236669
11	D	3250	<i>Lobocriconema</i> sp.	F	COI	KU236577
11	D	3250	<i>Lobocriconema</i> sp.	F	ITS1	KU236670
11	D	3256	<i>Lobocriconema</i> sp.	F	COI	KU236578
11	D	3256	<i>Lobocriconema</i> sp.	F	ITS1	KU236671
11	D	3293	<i>Lobocriconema</i> sp.	F	COI	KU236579

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TABLE 2. (Continued)

COI Haplotype	COI Clade	NID	Species	Stage	Primer	GenBank accession #
11	D	3295	<i>Lobocriconema</i> sp.	F	COI	KU236580
11	D	3296	<i>Lobocriconema</i> sp.	F	COI	KU236581
11	D	3296	<i>Lobocriconema</i> sp.	F	ITS1	KU236674
11	D	3301	<i>Lobocriconema</i> sp.	J	COI	KU236582
11	D	3310	<i>Lobocriconema</i> sp.	F	COI	KU236583
11	D	3310	<i>Lobocriconema</i> sp.	F	ITS1	KU236675
11	D	3311	<i>Lobocriconema</i> sp.	F	COI	KU236584
11	D	3312	<i>Lobocriconema</i> sp.	F	COI	KU236585
11	D	3334	<i>Lobocriconema</i> sp.	F	COI	KU236586
11	D	3334	<i>Lobocriconema</i> sp.	F	ITS1	KU236678
11	D	3341	<i>Lobocriconema</i> sp.	F	COI	KU236587
11	D	3341	<i>Lobocriconema</i> sp.	F	ITS1	KU236679
11	D	3615	<i>Lobocriconema</i> sp.	F	COI	KU236588
11	D	3658	<i>Lobocriconema</i> sp.	F	COI	KU236589
11	D	3658	<i>Lobocriconema</i> sp.	F	ITS1	KU236682
11	D	3663	<i>Lobocriconema</i> sp.	F	COI	KU236590
11	D	3663	<i>Lobocriconema</i> sp.	F	ITS1	KU236683
11	D	5586	<i>Lobocriconema</i> sp.	F	COI	KU236591
11	D	5586	<i>Lobocriconema</i> sp.	F	ITS1	KU236687
11	D	5631	<i>Lobocriconema</i> sp.	F	COI	KU236592
11	D	5631	<i>Lobocriconema</i> sp.	F	ITS1	KU236688
11	D	5632	<i>Lobocriconema</i> sp.	F	COI	KU236593
11	D	5632	<i>Lobocriconema</i> sp.	F	ITS1	KU236689
11	D	5633	<i>Lobocriconema</i> sp.	F	COI	KU236594
11	D	5633	<i>Lobocriconema</i> sp.	F	ITS1	KU236690
11	D	5647	<i>Lobocriconema</i> sp.	F	COI	KU236595
11	D	5653	<i>Lobocriconema</i> sp.	F	COI	KU236596
12	D	3057	<i>Lobocriconema</i> sp.	F	COI	KU236597
12	D	3090	<i>Lobocriconema</i> sp.	F	COI	KU236598
12	D	3091	<i>Lobocriconema</i> sp.	F	COI	KU236599
12	D	3091	<i>Lobocriconema</i> sp.	F	ITS1	KU236662
12	D	3121	<i>Lobocriconema</i> sp.	J	COI	KU236600
12	D	3121	<i>Lobocriconema</i> sp.	J	ITS1	KU236663
13	D	3068	<i>Lobocriconema</i> sp.	F	COI	KU236601
13	D	3129	<i>Lobocriconema</i> sp.	F	COI	KU236602
13	D	3176	<i>Lobocriconema</i> sp.	F	COI	KU236603
13	D	3400	<i>Lobocriconema</i> sp.	F	COI	KU236604
13	D	3400	<i>Lobocriconema</i> sp.	F	ITS1	KU236680
13	D	3401	<i>Lobocriconema</i> sp.	F	COI	KU236605
13	D	3402	<i>Lobocriconema</i> sp.	F	COI	KU236606

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TABLE 2. (Continued)

COI Haplotype	COI Clade	NID	Species	Stage	Primer	GenBank accession #
13	D	3402	<i>Lobocriconema</i> sp.	F	ITS1	KU236681
13	D	3403	<i>Lobocriconema</i> sp.	F	COI	KU236607
14	D	3297	<i>Lobocriconema</i> sp.	F	COI	KU236608
14	D	3328	<i>Lobocriconema</i> sp.	F	COI	KU236609
14	D	3329	<i>Lobocriconema</i> sp.	F	COI	KU236610
14	D	3329	<i>Lobocriconema</i> sp.	F	ITS1	KU236677
14	D	3343	<i>Lobocriconema</i> sp.	F	COI	KU236611
14	D	3347	<i>Lobocriconema</i> sp.	F	COI	KU236612
15	D	3196	<i>Lobocriconema</i> sp.	F	COI	KU236613
15	D	3321	<i>Lobocriconema</i> sp.	F	COI	KU236614
15	D	3321	<i>Lobocriconema</i> sp.	F	ITS1	KU236676
15	D	3322	<i>Lobocriconema</i> sp.	F	COI	KU236615
15	D	3323	<i>Lobocriconema</i> sp.	F	COI	KU236616
15	D	3324	<i>Lobocriconema</i> sp.	F	COI	KU236617
15	D	3325	<i>Lobocriconema</i> sp.	F	COI	KU236618
16	C	5575	<i>Lobocriconema incrassatum</i>	F	COI	KU236619
16	C	5575	<i>Lobocriconema incrassatum</i>	F	ITS1	KU236655
16	C	5576	<i>Lobocriconema incrassatum</i>	J	COI	KU236620
16	C	5576	<i>Lobocriconema incrassatum</i>	J	ITS1	KU236656
16	C	5577	<i>Lobocriconema incrassatum</i>	F	COI	KU236621
16	C	5577	<i>Lobocriconema incrassatum</i>	F	ITS1	KU236657
17	C	3667	<i>Lobocriconema</i> sp.	F	COI	KU236622
17	C	3675	<i>Lobocriconema</i> sp.	F	COI	KU236623
17	C	3677	<i>Lobocriconema</i> sp.	F	COI	KU236624
18	C	3381	<i>Lobocriconema thornei</i>	F	COI	KU236625
18	C	3382	<i>Lobocriconema thornei</i>	F	COI	KU236626
18	C	3382	<i>Lobocriconema thornei</i>	F	ITS1	KU236653
18	C	3414	<i>Lobocriconema thornei</i>	F	COI	KU236627
18	C	3415	<i>Lobocriconema thornei</i>	F	COI	KU236628
S	D	1	<i>Lobocriconema</i> sp.	F	COI	KU236629
S	D	1395	<i>Lobocriconema</i> sp.	F	COI	KU236630
S	C	3229	<i>Lobocriconema</i> sp.	F	COI	KU236631
S	C	3229	<i>Lobocriconema</i> sp.	F	ITS1	KU236648
S	C	3260	<i>Lobocriconema</i> sp.	J	COI	KU236632
S	D	3291	<i>Lobocriconema</i> sp.	F	COI	KU236633
O		1327	<i>Mesocriconema xenoplax</i>	F	COI	KU236636
O		278	<i>Bakernema inaequale</i>	F	COI	KU236635
O		3268	<i>Criconema</i> sp.	F	COI	KU236643
O		238	<i>Neolobocriconema serratum</i>	F	COI	KU236634
O		1454	<i>Mesocriconema sphaerocephalum</i>	U	COI	KU236638

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TABLE 2. (Continued)

COI Haplotype	COI Clade	NID	Species	Stage	Primer	GenBank accession #
O		1328	<i>Criconema mutabile</i>	F	COI	KU236637
O		3096	<i>Criconema permistum</i>	F	COI	KU236642
O		2966	<i>Criconema sphagni</i>	F	COI	KU236640
O		2993	<i>Criconema petasum</i>	F	COI	KU236641
O		1490	<i>Criconema loofi</i>	F	COI	KU236639

*S—Singleton, O—Outgroup

at positions 1822–2612 on the *Drosophila yakuba* Burla, 1954 reference sequence (GenBank Accession #X03240) amplifying the center one-half of the gene (Clary & Wolstenholme 1985). PCR amplification reactions, conducted in a 30.0 µl total volume within 0.6 ml reaction tubes and on a Techne Prime thermocycler, consisted of 9.0 µl of template from the ruptured nematode specimen, 2.4 µl of each 20 µM primer solution for a 1.6 µM final primer concentration, 1.2 µl ddH₂O, and 15 µl of 2x JumpStart REDTaq ReadyMix (Sigma-Aldrich) for a 0.05 U/µl final enzyme concentration. PCR conditions included a modified hotstart and 5 minute treatment at 94°C followed by 45 cycles of 30 seconds at 94°C denaturation, 30 seconds at 48°C annealing, and 1.5 minutes at 72°C with a ramping rate of 0.5°C/second for the elongation step. A final 5-minute extension at 72°C completed the process. Following amplification, an initial check gel was run followed by cleaning of the PCR product by gel fragment excision from a 0.7% agarose TAE gel, using the Gel/PCR DNA Fragment Extraction Kit (IBI Scientific). Amplification products were sequenced in both directions by the sequencing center at the University of Arkansas for Medical Services, Davis Sequencing Services, or by UCD DNASEq Facility. ITS1 primer sequences were rDNA2—5'-TTGATTACGTCCCTGCCCTTT-3' and rDNA1.58Sa—5'-ACGAGCCGAGTGATC CACC-3' (Cherry *et al.* 1997). Amplification conditions were similar with the following modifications: 1.8 µl of 20 µM working stock of each primer (for a 1.2 µM final concentration) per reaction, 5.0 µl of template per reaction, 6.4 µl ddH₂O, denaturation at 94°C for 15 seconds, annealing at 55°C for 15 seconds and elongation at 72°C for 1 minute.

Morphological characters - morphometrics. Living nematodes were measured when possible. Images were taken with a Leica DC300 video camera mounted on a Leica DMLB light microscope with Differential Interference Contrast optics. Each nematode in the analyses received a unique Nematode IDentification number (NID) which is linked to voucher images. Nematodes viewed by Scanning Electron Microscopy (SEM) also received a NID number. These SEM specimens were not analyzed by nucleotide sequencing and could potentially include morphologically similar, but genetically distinct species in mixed species populations. SEM images were obtained on a Hitachi S-3000N microscope using preparation methods described in Powers *et al.* (2014).

Females, males, and juveniles were used in molecular analyses. Only adult females were used in the morphological analyses. Morphometric analysis included some characters that are specifically applied to the suborder: the number of annules on the body (R), the distance from the anterior end to the excretory pore expressed in number of annules (Rex), and the number of annules from the vulva to tail terminus (RV) are illustrated in Powers *et al.* (2014, Fig. 3) together with other key morphological features.

Morphological data—multivariate analyses. We recorded 22 morphometric variables for 148 *Lobocriconema* specimens. Measurements were those (Geraert 2010) typically taken when observing Criconematina; R, RV, Rex, Ran, Rvan, Body Annule Width (BAW), Tail Length, MV, Length (L), Shaft, Stylet (STY), Stylet Knob Width (SKW), DEGO, Vulva position (V), MidBody Width (MBW), Vulval Body Width (VBW), Anal Body Width (ABW), and Pharyngeal (Esophagus) Length (ESO) and ratios PV (length of the post-vulval portion of the body)/VBW, V% (L/V), *a* (L/MBW), and *b* (L/ESO).

For the morphometric analyses, we included only the 134 females and used a simplified dataset of 12 measurements: R, RV, Rex, BAW, L, STY, SKW, V, MBW, VBW, ESO and V%. Missing data were replaced with mean values from their respective molecular groupings. Prior to analysis, the data were screened for outliers. Univariate analysis of variance (ANOVA) was performed to test whether samples showed significant differences in specimen size using L as an indicator across the geographic range. Post-hoc least significant differences (LSD)

tests were used to identify which group means differed significantly. We screened for highly correlated variables and then selected only the morphometric variables with correlation coefficients under 0.8. This selection identified R, RV, Rex, BAW, L, STY and MBW as variables suitable for the determination of linkages between molecular groupings and potential diagnostic morphological characters.

Discriminant function analysis was used to evaluate the morphological characters within the *a priori* genetic groupings based on COI nucleotide sequences, and to assess morphological discrimination between groups with a minimum misclassification risk. The genetic groupings were either well-supported, distinct haplotypes groups based on phylogenetic analyses and species delimitation assessments, or deeper nodes in the phylogenetic tree representing clades that include multiple haplotype groups in the 148-*Lobocriconema* dataset. In some cases low, unequal group counts and a large number of groups created statistical hurdles that required special tools to obtain reliable parameter estimates. Regularized discriminant analysis (RDA) was employed to classify observations into their genetic groups while estimating regularization parameters (Friedman 1989). Linear Discriminant Analysis (LDA) was also employed to assess the most accurate model. Model assessment also included evaluation of equal and proportional prior probabilities. Before running the discriminant models, stepwise classification variable selection provided the most parsimonious combination of covariates that adequately account for the differences between the genetic groups. An improvement of less than 5% separation ability between steps justified the cutoff. Parameter estimates were obtained using 117-fold cross-validation in the klaR (Weihs *et al.* 2005) and MASS (Venables & Ripley 2002) R (R Core Team 2015) packages for RDA and LDA, respectively.

Linear discriminant analysis (LDA) with proportional priors was used for the major groups of clades A–D and LDA with equal priors was used for the 18-group Bayesian dataset. Proportional prior probabilities equal to the observed specimen occurrences in each molecular grouping were used according to their frequency in the data. For the regularized discriminant analysis, cross-validation estimates of the misclassification rates were minimized with $\lambda=1$ and $\gamma=0.1$ after searching a grid for $0<\lambda<1$ and $0<\gamma<1$.

Species delimitation and gene trees. Unique COI sequences (haplotypes) were arranged into haplotype groups based on assessments of monophyly, node support, and genetic distance in an evaluation of gene trees. Maximum likelihood (PHYML [Guindon & Gascuel 2003]), neighbor-joining, and Bayesian trees (MrBayes plugin [Huelsenbeck & Ronquist 2001]) were constructed using the Geneious 8.0.5 program and MEGA 6 (Tamura *et al.* 2013). DNA editing was done with CodonCode Aligner (CodonCode Corp, Dedham, Massachusetts). DNA alignments were constructed using Muscle (Edgar 2004). J-Model Test (Posada 2008) selected GTR+I+G as the appropriate substitution model for maximum likelihood and Bayesian analyses. Bootstrap support for maximum likelihood and neighbor-joining trees was estimated for 100 and 5,000 replicates respectively. Bayesian analyses were based on 4 heated chains running for 1,100,000 generations with a sampling frequency of 1,000 and a burn-in length of 500,000.

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 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 criterion 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 of 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.

Networks and AGBD. TCS network analyses (Clement *et al.* 2000) were used to assess haplotype relationships associated with population level divergences that allow for nonbifurcating genealogical information. The statistical parsimony analysis by TCS used an absolute distance matrix from pairwise comparisons of haplotypes to calculate the probability of parsimony at a 95% connection level. The Automatic Barcode Gap Discovery (ABGD) method is an automated procedure that groups genetic sequences into candidate species without *a priori* species hypotheses. This method operates under the assumption that a "gap" exists between intra- and interspecific diversity in the distribution of pairwise differences for any set of genetic sequences. Unlike other species delimitation methods (i.e., statistical parsimony networks) that conform to a predetermined, fixed threshold, ABGD determines an optimal threshold based on the given data set. Comprised of two steps,

ABGD first divides sequences into candidate species based on a statistically inferred barcode gap, and then conducts a second recursive partition on the initial partitions. The data are partitioned so that the distance between any two sequences derived from unique groups will always be greater than the determined barcode gap. The analysis was conducted on the ABGD web-server (<http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html>). Default values of $P_{min} = 0.001$ and $P_{max} = 0.1$ for prior maximum divergence of intraspecific diversity (i.e., species divergence) were used. The full dataset of 148 specimens was analyzed using the Kimura (K80) distance model.

Results

COI haplotype groups and major clades A–D. Specimens conforming to the *Lobocriconema* phenotype were extracted from soil collected at 47 separate localities representing 15 North American ecoregions and 17 U.S. states (Table 1). The geographic range of the *Lobocriconema* dataset extended from northern Florida to the Porcupine Mountains on the northwestern edge of Michigan's Upper Peninsula. Isolates from George Washington Memorial Parkway in Virginia represented the eastern-most border and Emigration Canyon, east of Salt Lake City, Utah established the western-most border. Emigration Canyon, Utah, the type locality of *L. incrassatum*, and Providence Canyon 90 km north were the only sites west of the Great Plains region that yielded *Lobocriconema* specimens.

The dataset, exclusive of outgroups, consisted of 148 specimens represented by 134 females, 10 juveniles and 4 males. Maximum likelihood, Bayesian analysis and neighbor-joining analyses each identified four major COI clades labeled A–D in the Bayesian majority rule consensus tree (Fig. 1). Clades C and D, which included most of the *Lobocriconema* specimens, were characterized by strongly-supported monophyletic haplotype subgroups labeled 3–18 in Fig. 1. The estimated mean genetic distances (P-values) between the haplotype subgroups within clades C and D, were 9.8% and 10.1% respectively. Mean pairwise distances for all haplotype groups are presented in Table 3. Overall mean pairwise distance (P-values) for the 148 *Lobocriconema* specimens was 12.0% (SE 0.006). Within the 18 designated haplotype groups, intragroup pairwise distances ranged from 0.0–6.6%, with only three groups exceeding 1.3% (Table 4). Haplotype group 6 (4.6%), group 8 (6.6%), and group 11 (4.2%) had the highest levels of intragroup variability.

Bootstrap, posterior probability values, and genetic distance strongly supported the genetic distinctiveness of the haplotype groups. One exception is group 8 in which the low number of specimens ($n=3$) and high intragroup distances challenged the recognition of the group as a discrete taxonomic unit. The metrics in the species delimitation table derived from the 18-group maximum likelihood tree provide additional evidence of haplotype group distinctiveness (Table 5). All groups are recognized as monophyletic. Four of the groups (3, 4, 16, and 18) exhibit no haplotype COI diversity. Two of the groups characterized by a single haplotype, groups 4 and 18, included specimens that were found at more than a single geographic location.

Both of the described *Lobocriconema* species from North America, *L. thornei* and *L. incrassatum* were located in clade C (Fig. 2). Haplotype group 5 included topotype specimens of *L. thornei* from East Lansing, Michigan (Fig. 2C, E, F, I) and closely related haplotypes from Indiana and Ohio. Group 18 shared the closest neighbor distance with group 5 (2.5%), the smallest neighbor distance in the dataset, and the two groups displayed no measurable morphological difference, although a slight crenation of the female body annule margins was noted in group 18 specimens and not in members of group 5. We have considered both haplotype groups as belonging to *L. thornei*. Group 4 from GRSM, however, has a closest neighbor distance to group 18 of 3.8% and had mean stylet lengths that moderately differed (96.5 μm in group 4 vs 90.5 μm in group 5) (Table 3). On the combined basis of that morphological character, reciprocal monophyly and genetic distance, group 4 was tentatively not considered taxonomically a member of *L. thornei*, although the close relationship is notable.

Specimens of *L. incrassatum* collected from the type locality at Emigration Canyon in Utah formed group 3. The morphological distinctiveness of the topotype *L. incrassatum* specimens left little doubt of species identity. Species diagnosis is primarily based on the relatively large body and stylet size of the species (Fig. 2A, D, G) and the series of labial plates surrounding the labial disk and submedian lobes as seen in SEM (Fig. 3C). This latter characteristic was interpreted by the original authors as a narrow, thin first annule "closely surrounding sublateral lobes and labial disc" (Raski & Golden 1966). A second population of *Lobocriconema* from Utah, group 16, collected from the same plant community and located in Providence Canyon approximately 90 kilometers north of

Emigration Canyon, was morphologically nearly identical to *L. incrassatum* (Fig. 2B, E, H). However, the pairwise COI haplotype distance between the two Utah populations was 8.9%. This relatively large genetic distance forced the reconsideration of species assignment for this population. Images of other specimens in clade C, representing haplotype groups 4, 17 and 18 are presented in Fig. 4.

TABLE 3. Mean interspecific distance (P-value) of 18 *Lobocriconema* COI haplotype groups.

	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9
Gp 1									
Gp 2	0.133								
Gp 3	0.135	0.130							
Gp 4	0.125	0.114	0.079						
Gp 5	0.123	0.107	0.083	0.044					
Gp 6	0.152	0.144	0.161	0.139	0.143				
Gp 7	0.158	0.150	0.164	0.133	0.145	0.092			
Gp 8	0.160	0.160	0.171	0.157	0.159	0.106	0.117		
Gp 9	0.138	0.139	0.158	0.135	0.138	0.086	0.093	0.087	
Gp 10	0.154	0.140	0.149	0.133	0.138	0.093	0.094	0.113	0.092
Gp 11	0.158	0.160	0.173	0.146	0.152	0.097	0.106	0.119	0.093
Gp 12	0.181	0.159	0.162	0.151	0.158	0.110	0.117	0.122	0.111
Gp 13	0.156	0.155	0.162	0.135	0.144	0.108	0.110	0.128	0.108
Gp 14	0.156	0.150	0.166	0.140	0.142	0.104	0.096	0.118	0.090
Gp 15	0.160	0.149	0.165	0.144	0.151	0.101	0.108	0.121	0.087
Gp 16	0.124	0.127	0.089	0.079	0.087	0.156	0.162	0.163	0.149
Gp 17	0.188	0.177	0.169	0.149	0.159	0.193	0.200	0.213	0.184
Gp 18	0.115	0.105	0.075	0.038	0.025	0.135	0.137	0.154	0.130

continued.

	Gp 10	Gp 11	Gp 12	Gp 13	Gp 14	Gp 15	Gp 16	Gp 17	Gp 18
Gp 1									
Gp 2									
Gp 3									
Gp 4									
Gp 5									
Gp 6									
Gp 7									
Gp 8									
Gp 9									
Gp 10									
Gp 11	0.090								
Gp 12	0.089	0.102							
Gp 13	0.092	0.103	0.098						
Gp 14	0.090	0.094	0.090	0.097					
Gp 15	0.095	0.098	0.100	0.100	0.089				
Gp 16	0.156	0.170	0.172	0.160	0.170	0.164			
Gp 17	0.198	0.205	0.213	0.206	0.208	0.205	0.156		
Gp 18	0.131	0.150	0.149	0.135	0.137	0.145	0.082	0.149	

TABLE 4. Intraspecific distance (P-value) for 18 *Lobocriconema* COI haplotype groups.

	Distance	Standard Error
Gp 1	0.015	0.003
Gp 7	0.001	0.001
Gp 2	0.002	0.001
Gp 9	0.012	0.002
Gp 5	0.009	0.002
Gp 6	0.046	0.005
Gp 8	0.066	0.008
Gp 4	0.000	0.000
Gp 12	0.001	0.001
Gp 13	0.004	0.002
Gp 15	0.013	0.002
Gp 11	0.042	0.004
Gp 10	0.006	0.002
Gp 14	0.001	0.001
Gp 18	0.000	0.000
Gp 3	0.000	0.000
Gp 16	0.000	0.000
Gp 17	0.002	0.001

Clade B specimens, all members of haplotype group 2, were localized to four collection sites within Great Smoky Mountains National Park. The specimens morphologically conform to *Criconema lamellatum* (Raski & Golden, 1966) Raski & Luc, 1985. In addition to morphological similarity, the presumed species identity of group 2 is supported by geographic proximity to the topotype locality, and host association. Fig. 5 displays images of the single female of *C. lamellatum* on paratype slide T-384p from the USDA Nematology Lab collection, collected on October 17, 1957 in Florence, South Carolina. The specimen is similar in general body form, head, and tail shape to the specimens collected from the locations within Great Smoky Mountains National Park (Fig. 6A–L). The original description stated that the head had “one labial annule about 18 μ m wide, rounded not retrorse, well set off and distinct from succeeding annules...lips form a simple, rounded, concave outline anteriorly...sublateral lobes absent”. The SEM images of labial features of a female group 2 specimen agree with the original description (Fig. 7I). The tail was considered to be bluntly rounded by Raski & Golden (1966) and this feature can be observed in specimens from Great Smoky Mountains National Park (Fig. 6). The geographic distance from the type locality in Florence, South Carolina, to the Purchase Knob GRSM sampling site is approximately 340 kilometers. *Quercus* sp. was mentioned as a host in one of the two collections associated with the original description, and oaks were prominent in all four of the GRSM collection sites. DNA barcoding of topotype material would help confirm this identification; unfortunately, urban development has greatly altered the original collection site. Group 2 formed a sister group to all other *Lobocriconema* groups in maximum likelihood and Bayesian trees (Fig. 1).

Clade A, like clade B, contains a single COI haplotype group and was designated group 1. Unlike clade B, clade A specimens were distributed across a wide geographic range (Table 1). The specimens in group 1 are morphologically distinct in the *Lobocriconema* dataset and most closely conform to *L. crassiannulatum* (de Guiran, 1963) De Grisse & Loof, 1965 (Fig. 8). *Lobocriconema crassiannulatum* was originally described from Ivory Coast associated with *Imperata cylindrica* (L.) P. Beauv., commonly known as cogon grass. Cogon grass is native to Southeast Asia, but in North America it is a widespread invasive species particularly in the southeastern U.S. It was introduced to North America on several occasions, including once from Japan to Alabama in 1912 for forage and erosion control (<http://www.fs.fed.us/database/feis/plants/>). A single collection record of *L. crassiannulatum* from North America exists in the USDA Nematology collection (slide G-9141, dated 5-14-84). These specimens are labeled as coming from an “apple orchard with grass” in Arentsville, Pennsylvania, and morphologically conform to members of group 1, including the distinctively tapered tail and body measurements (Table 6). Group 1 consists of specimens from Virginia, Arkansas, and Wisconsin.

TABLE 5. Delimitation of ML tree for 18 *Lobocriconema* groups. Analyses by Geneious plug-in software (Masters *et al.* 2011), calculation based on Tamura-Nei distances.

Species (Group)	Closest Species	Monophyletic?	Intraspecific Distance	Interspecific Distance to Closest Group	Intra/Inter	P ID (Strict)	P ID (Liberal)	Av (MRCAtips)	P (Randomly Distinct)	Clade Support	Rosenberg's P(AB)
1	18	yes	0.019	0.144	0.13	0.91 (0.82, 0.99)	0.97 (0.92, 1.0)	0.0204	1	100.00%	1.30E-17
2	18	yes	0.003	0.114	0.03	0.97 (0.88, 1.0)	1.00 (0.95, 1.0)	0.003	0.05	100.00%	8.20E-13
3	18	yes	0.00E+00	0.105	0.00E+00	0.79 (0.62, 0.97)	1.00 (0.86, 1.0)	0.00E+00	NA	100.00%	2.98E-03
4	18	yes	0.00E+00	0.038	0.00E+00	0.99 (0.92, 1.0)	1.00 (0.97, 1.0)	0.00E+00	NA	100.00%	2.40E-11
5	18	yes	0.011	0.025	0.45	0.86 (0.81, 0.91)	0.96 (0.93, 0.99)	0.0093	0.72	91.35%	1.00E-05
6	7	yes	0.049	0.098	0.5	0.69 (0.58, 0.79)	0.89 (0.83, 0.96)	0.0341	0.05	98.60%	4.40E-05
7	6	yes	0.002	0.098	0.02	0.94 (0.83, 1.0)	1.00 (0.94, 1.0)	0.0024	0.05	100.00%	4.40E-05
8	9	no	0.09	0.097	0.93	0.17 (0.00E+00, 0.35)	0.48 (0.33, 0.63)	0.0452	NA	NA	NA
9	8	yes	0.012	0.097	0.12	0.94 (0.87, 1.0)	0.98 (0.94, 1.0)	0.014	0.05	99.70%	0.01
10	14	yes	0.006	0.079	0.07	0.82 (0.68, 0.96)	0.97 (0.86, 1.0)	0.0042	0.05	100.00%	1.50E-04
11	10	yes	0.046	0.097	0.48	0.85 (0.80, 0.90)	0.96 (0.93, 0.99)	0.0327	0.05	99.75%	1.50E-11
12	10	yes	6.95E-04	0.093	0.01	0.86 (0.72, 1.0)	0.98 (0.87, 1.0)	3.81E-04	0.05	100.00%	1.06E-03
13	9	yes	0.004	0.115	0.03	0.93 (0.83, 1.0)	1.00 (0.93, 1.0)	0.0034	0.05	100.00%	7.10E-12
14	10	yes	0.004	0.079	0.05	0.90 (0.78, 1.0)	0.97 (0.87, 1.0)	0.002	0.05	100.00%	0.07
15	9	yes	0.014	0.116	0.12	0.85 (0.73, 0.98)	0.97 (0.86, 1.0)	0.0191	0.05	100.00%	0.05
16	18	yes	0.00E+00	0.1	0.00E+00	0.79 (0.62, 0.97)	1.00 (0.86, 1.0)	0.00E+00	NA	100.00%	0.02
17	18	yes	0.003	0.172	0.02	0.78 (0.61, 0.96)	1.00 (0.85, 1.0)	0.0017	0.05	100.00%	0.02
18	5	yes	1.89E-05	0.025	7.40E-04	0.87 (0.73, 1.0)	0.98 (0.87, 1.0)	9.50E-06	0.05	99.95%	1.00E-05

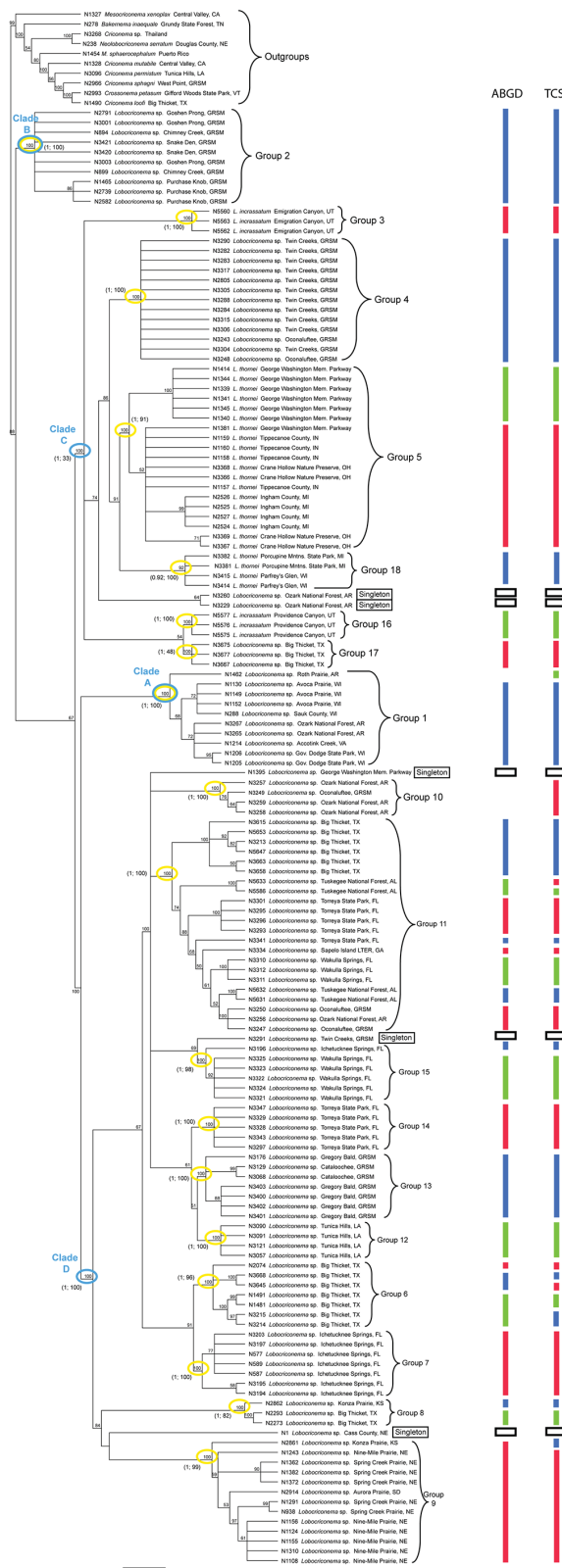


FIGURE 1. A 50% majority rule Bayesian Consensus tree constructed using 148 COI nucleotide sequences of *Lobocriconema* and outgroups. Eighteen groups are identified by brackets and group numbers. Five singletons, specimens not belonging to delineated groups, are indicated. Node support values in parentheses are posterior probability values for Bayesian analysis and bootstrap values for maximum likelihood, respectively. The letters A, B, C and D designate major clades. Each terminal branch includes a Nematode Identification number (NID) number, taxon and collection site. Colored bars indicate groupings according to species delimitation programs ABGD (Automatic Barcode Gap Discovery) and the Statistical Parsimony Program, TCS. TCS groupings were formed at the 95% similarity cutoff value.



FIGURE 2. Light microscope images of female *L. incrassatum* and *L. thornei* specimens from clade C (groups 3, 5, and 16). A, D, G) NID 5563 (group 3), *Lobocriconema incrassatum*, Emigration Canyon, Utah, A) entire, 400X, D) head, 1000X, G) tail, 1000X. B, E, H) NID 5575 (group 16), *Lobocriconema incrassatum*, Providence Canyon, Utah, B) entire, 400X, E) head, 1000X, H) tail, 1000X. C, F, I) NID 2525 (group 5), *Lobocriconema thornei*, Ingham County, Michigan, C) entire, 400X, F) head, 1000X, I) tail, 1000X.

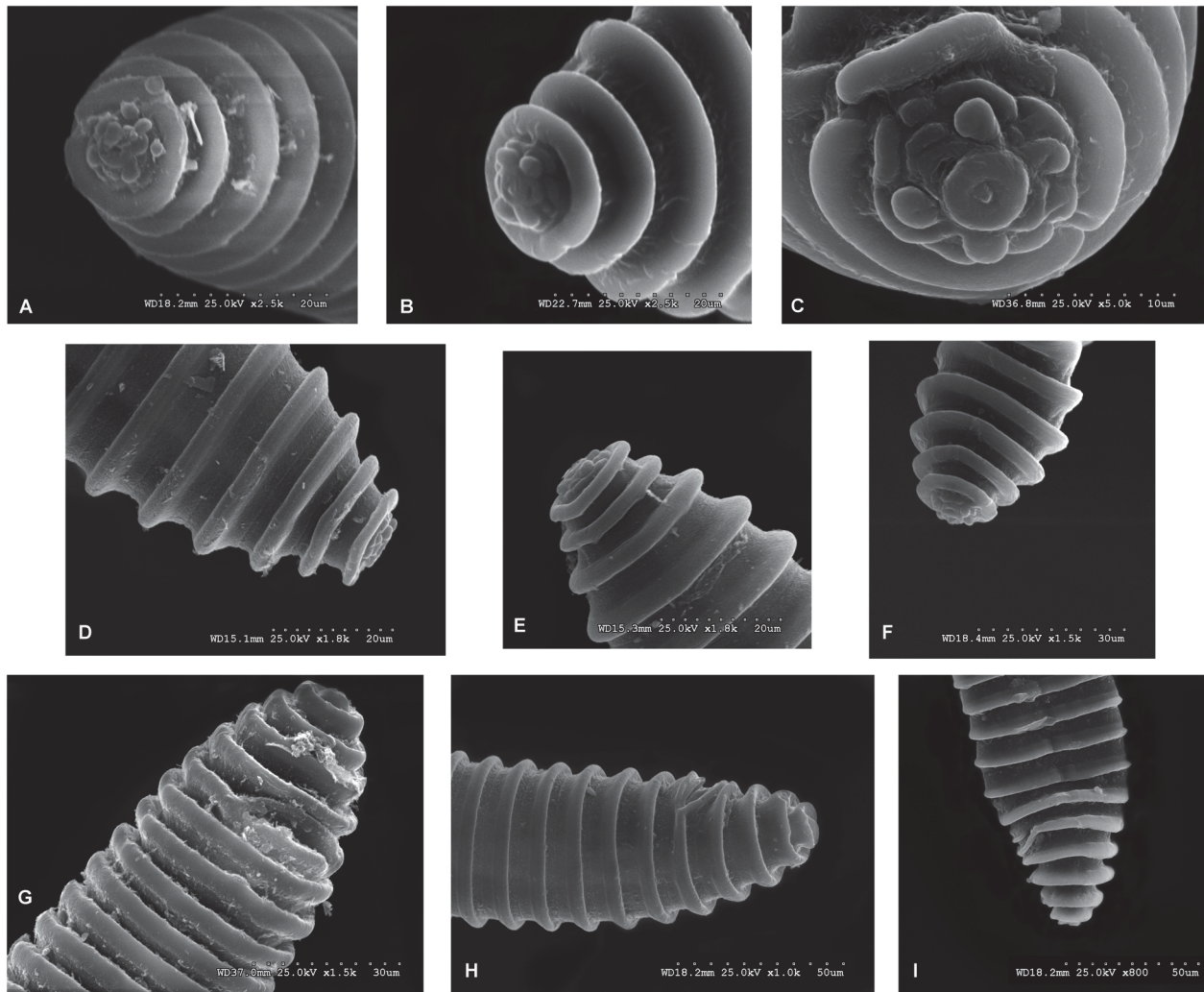


FIGURE 3. SEM images of specimens representing clade C. NID numbers are associated with unique specimens; all are females.

- A) *Lobocriconema thornei*, face view with oral disc and submedian lobes, George Washington Memorial Parkway, Virginia, NID 4517.
- B) *Lobocriconema thornei*, face view with submedian lobes surrounding oral disc and open amphid aperture, George Washington Memorial Parkway, Virginia, NID 4511.
- C) *Lobocriconema incrassatum*, face view with four prominent submedian lobes, discontinuous labial plates and notched first annule, Emigration Canyon, Utah (type locality), NID 4600.
- D) *Lobocriconema thornei*, head profile, George Washington Memorial Parkway, Virginia, NID 4520.
- E) *Lobocriconema thornei*, head profile with conspicuous submedian lobes, George Washington Memorial Parkway, Virginia, NID 4519.
- F) *Lobocriconema thornei*, head profile with submedian lobes, George Washington Memorial Parkway, Virginia, NID 4512.
- G) *Lobocriconema incrassatum*, tail with ventral view of vulva, Emigration Canyon, Utah (type locality), NID 4599.
- H) *Lobocriconema thornei*, lateral view of vulva, George Washington Memorial Parkway, Virginia, NID 4519.
- I) *Lobocriconema thornei*, lateral profile of tail with posteriorly directed post-vulval annule, George Washington Memorial Parkway, Virginia, NID 4511.

Potentially, a fifth described species was obtained from samples at several localities in the Arkansas National Forest that were associated with the original description of *Criconema arkaense* Cordero, Robbins & Szalanski, 2012 (Cordero *et al.* 2012). Eight specimens from *C. arkaense* collection sites were included in the *Lobocriconema* dataset. However, when we analyzed their COI sequences, the different specimens produced a mixture of haplotypes from the toptype localities making it difficult to determine which of the specimens actually represented *C. arkaense* (Fig. 9). Focal samples were taken under maple, wild cherry (*Prunus* spp.), and hackberry (*Celtis occidentalis* L.) by one of the original authors (RTR) in Cordero *et al.* (2012). Specimens from these sites

were placed into three different COI haplotype groups with an additional pair of singletons in clade C that were not accommodated by any of the designated haplotype groups. In the soil underneath the hackberry tree, there were four specimens from clade D (NIDs 3256–3259) and the singleton NID 3260 located in clade C. Underneath wild cherry were two specimens belonging to clade A and underneath the maple was singleton NID 3229 which grouped in clade C. Whereas the morphological measurements of the topotype specimens within clade D fit the original description of *C. arkaense*, the GenBank archived ITS1 sequences of that species were problematic (Fig. 10). All four of the ITS1 sequences were placed into clade D in neighbor-joining trees, but variability among those ITS1 sequences confounded the determination of which COI haplotype group best corresponds to *C. arkaense*. Mean ITS1 p-distance among the four *C. arkaense* GenBank sequences was 6.6% compared to 3.0% for 47 other *Lobocriconema* in the dataset representative of clades A–D. Nucleotide sequence alignment of the four GenBank sequences required the insertion of approximately nine times as many gaps as the alignment of the 47 other ITS1 sequences, suggesting potential complications in the editing of the original GenBank sequences. Unfortunately, the original ITS1 sequence traces from Cordero *et al.* (2012) were not archived. A similar situation exists with *Criconema warrenense*, also described in Cordero *et al.* (2012). Initial analyses comparing ITS1 sequence of topotype material of putative *C. warrenense* exhibit an identical match with NID 3214 and 3215, both members of *Lobocriconema* haplotype group 6 (Fig. 10). Future analyses with additional genetic markers will address the issue of their conspecificity.

Common to all trees is a deeper node that unites haplotype groups 6–15 with bootstrap and Bayesian analysis posterior probability values of 100 and 1.0 (Fig. 1). This clade was designated clade D and did not include any specimens with conspicuous submedian lobes (Fig. 11). The haplotype groups in clade D were largely geographically confined to the southern Gulf Coast states, with some distributional overlap with groups 1–5 in the lower Appalachian and Ozark Mountains (Fig. 12). Collections of *Lobocriconema* from the Gulf Coast states came from ecoregions characterized by broadleaf and coniferous evergreen forests, and were more common in wet, lowland forests. Two of the 10 haplotype groups in clade D are populated by specimens found outside the Gulf Coast states. Haplotype groups 8 and 9 have a distinctly different geographic distribution within the clade, with specimens recovered from a north–south tier of central states: South Dakota, Nebraska, Kansas, and Texas (Fig. 12).

Morphological characters. Figs 2–9 and 11 illustrate the range in body size and general morphological features of the female specimens in the *Lobocriconema* dataset. Most notable in images of the overall body is the coarse annulation and the relatively low number of body annules (R value in Table 6). The mean annule width ranges from 9.4 μm in group 1 to 12.1 μm in group 5. Group 1, the most morphologically distinct group, also has the lowest mean R value in the dataset (R=44.7) compared to the largest (R=61.7) found in group 3. Group 3 (*L. incrassatum*) has the largest mean values of body length (658.3 μm), stylet length (99.0 μm), and Rex (19.7). Also notable in the dataset is the range of tail shapes. Specimens in clade B have near hemispherical tails (Fig. 6) whereas clade A specimens (Fig. 8) have conical tails that are the most pointed and tapered in the dataset.

A second aspect to *Lobocriconema* tails in the dataset is the location and structure of the vulva. All specimens possess what would be considered a “closed” vulva without a well-developed anterior vulval flap. Generally the vulva appears as a separation of the cuticle along the annule margin (Figs 3A, 7G). Interpreting the position of the opening can sometimes be difficult in light microscopy due to the posteriorly directed vulva margins. In lateral view these can create the impression of a vulva opening anterior to the actual opening and may account for some historical observation of *Lobocriconema* with open vulvas. Similarly in ventral view, focusing through the cuticle can give the impression of an oval, open aperture when in fact the vulva is inclined posteriorly. No clear instances of a well-developed vulva “flap” as seen in many *Criconema* species have been observed in the *Lobocriconema* dataset.

At the anterior end of the body, the most important feature for determining membership within *Lobocriconema* has been the presence of four rounded submedian lobes that bracket the oral disc. Specimens in clade C (Figs 2–4) have relatively large submedian lobes, which are clearly seen in SEM images of the labial region (Fig. 3A–F). When viewed with light microscopy, a lateral cephalic profile is sufficient to determine lobe presence (Figs 2D–F, 4D–F). However, specimens in clades A, B and D have either reduced submedian lobes or lack lobes entirely. Adult specimens from haplotype group 9 (Fig. 7A, D, E) have reduced lobes, while juveniles of the group appear to possess distinct lobes (Fig. 7C), suggesting that lobe size may be reduced in the final molt. SEM and DIC light microscopy show that many face views in the dataset show no evidence of submedian lobes.

TABLE 6. Morphometric data of 18 *Lobocriconema* COI haplotype groups and singletons.

COI Haplotype Groups and Singletons (S)	Length (L)					# of body annules (R)					# of annules from vulva to tail terminus (Rv)					# of annules anterior to excretory pore (Rex)				
	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	10	389.3	47.7	320	445	10	44.7	1.4	42	46	10	5.6	0.5	5	6	10	12.8	0.6	12	14
2	9	598.0	55.5	530	704	9	51.2	3.0	48	57	9	3.7	0.5	3	4	9	17.0	1.7	14	19
3	3	658.7	44.7	610	698	3	61.7	1.2	61	63	3	5.0	0.0	5	5	3	19.7	1.2	19	21
4	13	538.2	47.1	455	600	13	47.2	2.0	44	51	13	3.8	0.4	3	4	13	15.3	0.6	14	16
5	17	559.6	47.5	475	620	17	49.2	1.8	46	53	17	4.8	0.4	4	5	17	15.7	1.0	13	17
6	7	531.6	38.0	503	612	7	51.9	2.9	49	57	7	4.7	0.5	4	5	7	16.0	1.2	15	18
7	6	484.0	59.8	413	555	6	45.7	0.8	45	47	6	3.8	0.4	3	4	6	14.7	0.8	14	16
8	2	562.5	17.7	550	575	2	46.0	4.2	43	49	2	5.0	0.0	5	5	2	15.0	0.0	15	15
9	9	520.2	46.8	463	585	9	47.9	3.3	42	52	9	4.3	0.9	3	6	9	16.1	1.2	15	19
10	4	536.3	38.8	482	568	4	52.3	3.6	47	55	4	4.3	0.5	4	5	4	19.0	0.0	19	19
11	20	492.0	54.6	385	563	20	47.3	2.6	44	54	20	4.4	0.5	4	5	20	16.1	1.0	14	18
12	3	565.3	52.5	513	618	3	52.3	0.6	52	53	3	4.3	1.2	3	5	3	17.3	0.6	17	18
13	7	456.6	57.0	385	555	7	45.4	2.1	43	49	7	4.0	0.6	3	5	7	15.7	0.5	15	16
14	5	508.6	40.6	475	577	5	53.4	4.0	50	60	5	4.8	0.4	4	5	5	18.0	0.7	17	19
15	6	539.3	41.4	483	600	6	51.2	1.2	50	53	6	4.7	0.5	4	5	6	16.2	0.8	15	17
16	2	628.0	91.9	563	693	2	63.5	4.9	60	67	2	5.0	0.0	5	5	2	23.5	0.7	23	24
17	3	464.7	33.1	439	502	3	72.3	5.7	66	77	3	6.3	0.6	6	7	3	21.7	1.5	20	23
18	4	488.3	40.3	430	523	4	49.8	1.3	48	51	4	4.8	0.5	4	5	4	16.3	0.5	16	17
S1	1	473.0	.	473	473	1	55.0	.	55	55	1	4.0	.	4	4	1	18.0	.	18	18
S2	1	558.0	.	558	558	1	58.0	.	58	58	1	3.0	.	3	3	1	17.0	.	17	17
S3	1	643.0	.	643	643	1	56.0	.	56	56	1	6.0	.	6	6	1	18.0	.	18	18
S4	1	490.0	.	490	490	1	56.0	.	56	56	1	4.0	.	4	4	0

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TABLE 6. (Continued)

COI Haplotype Groups and Singletons (S)	ESO										Stylet knob width										MBW									
	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									
1	10	10	98.4	7.8	90	113	10	57.8	2.1	55	60	10	8.0	0.0	8	8	10	44.0	4.8	37	54									
2	9	9	153.0	21.6	120	182	9	96.8	6.6	90	108	9	15.7	1.4	13	18	9	57.7	10.5	45	77									
3	3	3	157.7	7.5	150	165	3	99.0	5.3	93	103	3	14.0	1.0	13	15	3	57.7	3.2	54	60									
4	13	13	148.0	8.9	133	160	13	96.5	4.0	88	103	13	13.7	0.9	12	15	13	46.7	5.1	35	54									
5	17	17	144.6	7.6	125	155	17	90.8	2.7	87	97	17	14.4	0.8	13	16	17	57.2	4.5	46	64									
6	7	7	133.0	6.6	123	145	7	79.3	4.6	73	86	7	12.1	1.2	10	13	7	50.0	2.0	48	54									
7	6	6	117.2	11.9	95	128	6	72.0	3.6	68	77	6	11.2	0.8	10	12	6	43.2	6.0	36	53									
8	2	2	137.0	12.7	128	146	2	77.0	4.2	74	80	2	11.5	0.7	11	12	2	55.0	0.0	55	55									
9	9	9	123.8	14.6	105	155	9	72.8	5.2	67	83	9	11.8	0.8	11	13	9	55.8	5.3	48	64									
10	4	4	124.0	13.8	105	138	4	80.0	1.8	78	82	4	11.8	1.3	10	13	4	44.3	6.9	37	52									
11	20	20	123.9	8.0	105	142	20	79.2	4.5	68	89	20	11.8	1.0	10	14	20	46.2	4.8	35	55									
12	3	3	138.7	1.2	138	140	3	86.3	2.3	85	89	3	13.0	1.0	12	14	3	54.0	3.6	50	57									
13	7	7	116.7	7.9	100	125	7	73.4	3.3	70	77	7	11.0	0.6	10	12	7	36.6	7.5	26	49									
14	5	5	127.2	7.0	121	138	5	85.2	3.3	81	90	5	13.2	0.4	13	14	5	40.4	5.1	35	46									
15	6	6	124.3	5.2	117	130	6	79.8	3.9	73	84	6	12.2	1.0	11	14	6	46.2	2.6	44	50									
16	2	2	166.5	16.3	155	178	2	95.0	7.1	90	100	2	13.5	0.7	13	14	2	55.0	2.8	53	57									
17	3	3	139.0	9.2	131	149	3	70.3	3.2	68	74	3	12.3	0.6	12	13	3	47.7	3.1	45	51									
18	4	4	138.5	6.1	130	143	4	89.3	2.5	86	92	4	14.5	0.6	14	15	4	52.8	1.9	50	54									
S1	1	1	103.0	.	103	103	1	81.0	.	81	81	0	1	50.0	.	50	50									
S2	1	1	137.0	.	137	137	1	82.0	.	82	82	1	11.0	.	11	11	1	47.0	.	47	47									
S3	1	1	128.0	.	128	128	1	92.0	.	92	92	1	17.0	.	17	17	1	57.0	.	57	57									
S4	1	0	.	.	.	0	1	78.0	.	78	78	1	11.0	.	11	11	1	36.0	.	36	36									

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TABLE 6. (Continued)

COL Haplotype Groups and Singletons (S)	VUL					V					VBW					Body Annule Width						
	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	
1	10	10	346.0	44.3	288	405	10	89.0	2.6	83	92	10	31.2	3.1	27	36	10	9.5	1.0	8	11	
2	9	9	556.6	49.4	500	660	9	93.0	2.4	87	95	9	44.3	9.2	30	59	9	12.1	1.3	10	14	
3	3	3	610.3	42.4	563	645	3	92.3	0.6	92	93	3	41.0	6.2	34	46	3	11.7	1.5	10	13	
4	13	13	496.6	45.4	427	561	13	92.3	1.3	90	94	13	33.9	4.9	25	43	13	11.6	1.2	10	13	
5	17	17	512.9	44.9	445	565	17	91.6	1.6	89	94	17	44.6	3.4	36	50	17	11.9	1.2	10	14	
6	7	7	492.7	37.6	463	573	7	92.7	1.1	91	94	7	38.6	1.3	37	40	7	11.6	1.1	10	13	
7	6	6	451.2	56.7	383	518	6	92.7	1.4	90	94	6	33.5	3.3	30	38	6	10.8	1.2	9	12	
8	2	2	522.0	25.5	504	540	2	93.0	1.4	92	94	2	42.0	4.2	39	45	2	11.5	2.1	10	13	
9	9	9	479.0	40.7	423	535	9	91.9	1.8	90	95	9	41.7	5.1	35	52	9	11.4	1.1	10	13	
10	4	4	497.8	35.3	452	528	4	92.8	1.3	91	94	4	37.0	6.7	27	41	4	11.3	1.0	10	12	
11	20	20	455.2	52.6	355	520	20	92.7	1.2	91	95	20	35.2	4.2	24	42	20	11.4	1.2	9	13	
12	3	3	522.0	41.5	480	563	3	92.3	1.5	91	94	3	42.7	2.5	40	45	3	12.0	0.0	12	12	
13	7	7	425.3	55.7	354	520	7	93.1	1.3	91	95	7	29.0	6.1	18	39	7	10.7	1.3	9	13	
14	5	5	473.4	42.5	430	539	5	93.0	1.9	91	96	5	30.6	4.4	25	35	5	10.6	1.1	9	12	
15	6	6	500.7	38.6	443	550	6	93.0	0.9	92	94	6	35.8	2.1	34	40	6	11.3	0.8	10	12	
16	2	2	589.0	93.3	523	655	2	94.0	1.4	93	95	2	43.5	2.1	42	45	2	10.5	2.1	9.0	12	
17	3	3	444.0	41.9	408	490	3	93.0	0.0	93	93	3	38.7	3.5	35	42	3	7.7	1.2	7.0	9	
18	4	4	447.8	37.1	393	475	4	91.8	1.0	91	93	4	43.0	3.2	39	46	4	11.5	1.0	11	13	
S1	1	0	0	0	0
S2	1	1	523.0	.	523	523	1	94.0	.	94	94	1	38.0	.	38	38	1	9.0	.	9	9	
S3	1	1	590.0	.	590	590	1	92.0	.	92	92	1	45.0	.	45	45	1	12.0	.	12	12	
S4	1	1	464.0	.	464	464	1	95.0	.	95	95	1	27.0	.	27	27	1	10.0	.	10	10	

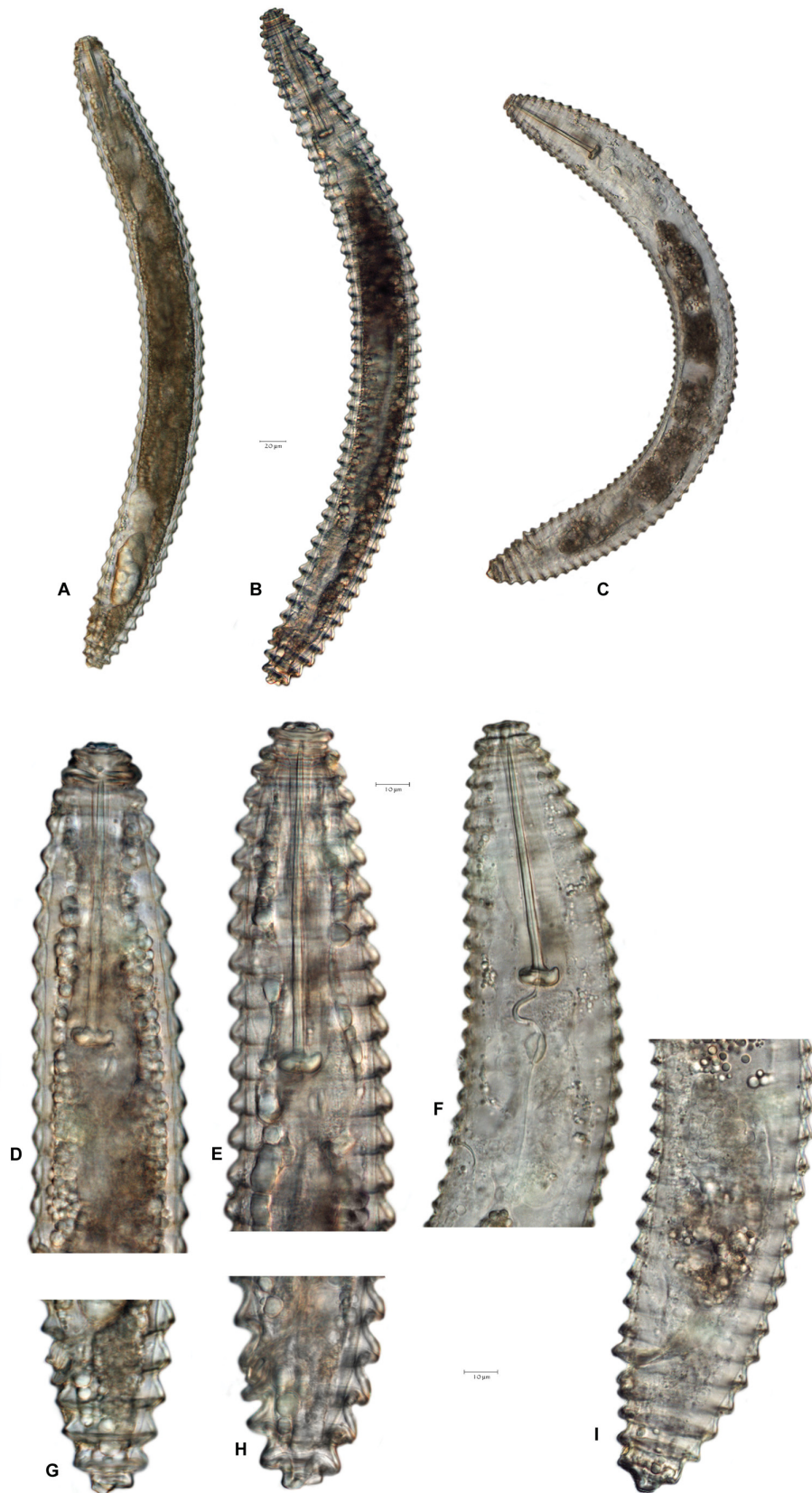


FIGURE 4. Light microscope images of female *Lobocriconema* sp. specimens from clade C. A, D, G) NID 3414 (group 18), Parfrey's Glen, Wisconsin, A) entire, 400X, D) head, 1000X, G) tail, 1000X. B, E, H) NID 3243 (group 4), Oconaluftee, Great Smoky Mountains National Park, North Carolina, B) entire, 400X, E) head, 1000X, H) tail, 1000X. C, F, I) NID 3675 (group 17), Big Thicket National Preserve, Texas, C) entire, 400X, F) head, 1000X, I) tail, 1000X.

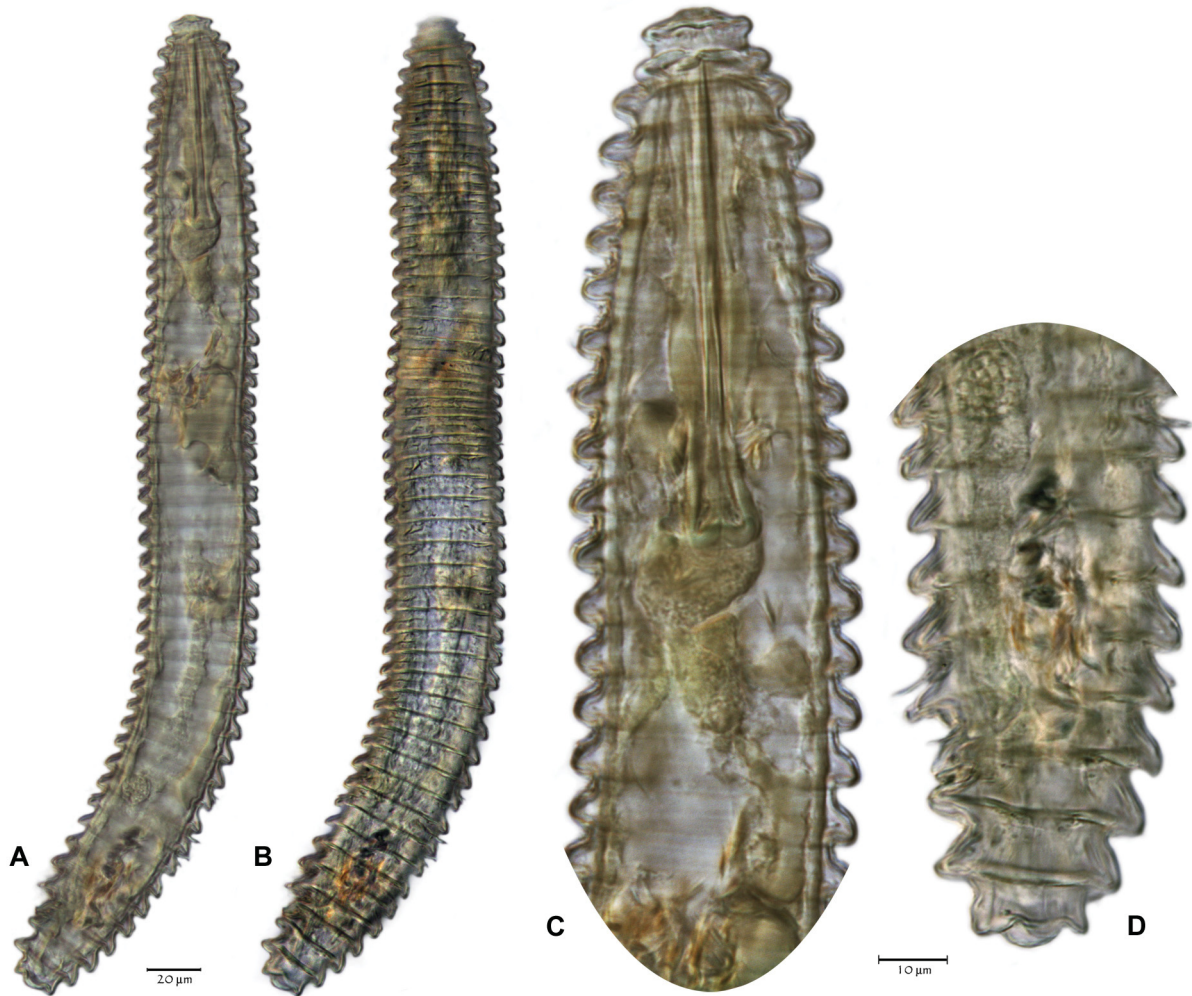


FIGURE 5. Light microscope images of *Criconema lamellatum* paratype, a single female collected from Florence, South Carolina in October 17, 1957. Hosts were recorded as fern, grass and trees. A, B) entire 400X, C) head 1000X, D) tail 1000X.

When viewing living nematodes the flexible nature of the first labial annule is clearly apparent, particularly when drawn back from the oral disc. Often the underlying cephalic structure is revealed as a series of ridges radiating from the oral disc. SEM confirms that some haplotype groups do not possess submedian lobes (Fig. 7B, I). One other notable characteristic observed in haplotype group 3 and some specimens in haplotype group 5 is the discontinuous structure of the lip annule (Figs 2E, 3C). In the original description of *L. incrassatum*, the authors referred to this discontinuity as a “notch” and interpreted the irregular labial plates circumscribed by that annule as a thin first annule. In some specimens the notched annule is considerably thickened and can resemble an upturned collar surrounding and sometimes obscuring the submedian lobes and labial plates.

Stylets in the dataset are generally robust and long. Stylet knobs are drawn out laterally with anterior projections that range from slight in clade A (Fig. 8E–H) to moderately prominent in clades B, C, and D. Stylet length falls into three general classes. In group 1 (clade A) mean stylet length is 57.8 µm, the shortest of all haplotype groups (Table 6). In groups 2–5, 16 and 18 mean stylet lengths range between 89.3–99.0 µm. In groups 6–15 (clade D) there are intermediate mean stylet lengths of 72–86.3 µm. Group 17, comprised of three specimens in clade C collected from Big Thicket National Preserve, was an anomaly within the clade with respect to generalizations about morphological traits. It was also anomalous geographically as it was the only haplotype group in clade C collected south of 36°30' latitude.

Few juveniles or males were collected. Only four males from groups 7, 8, 9, and 11, all in clade D, were included in the dataset. The male in Fig. 13E–H conforms to the general morphological description of males in *Lobocriconema* with an undifferentiated labial region, the absence of a stylet, a degenerate pharyngeal region, a

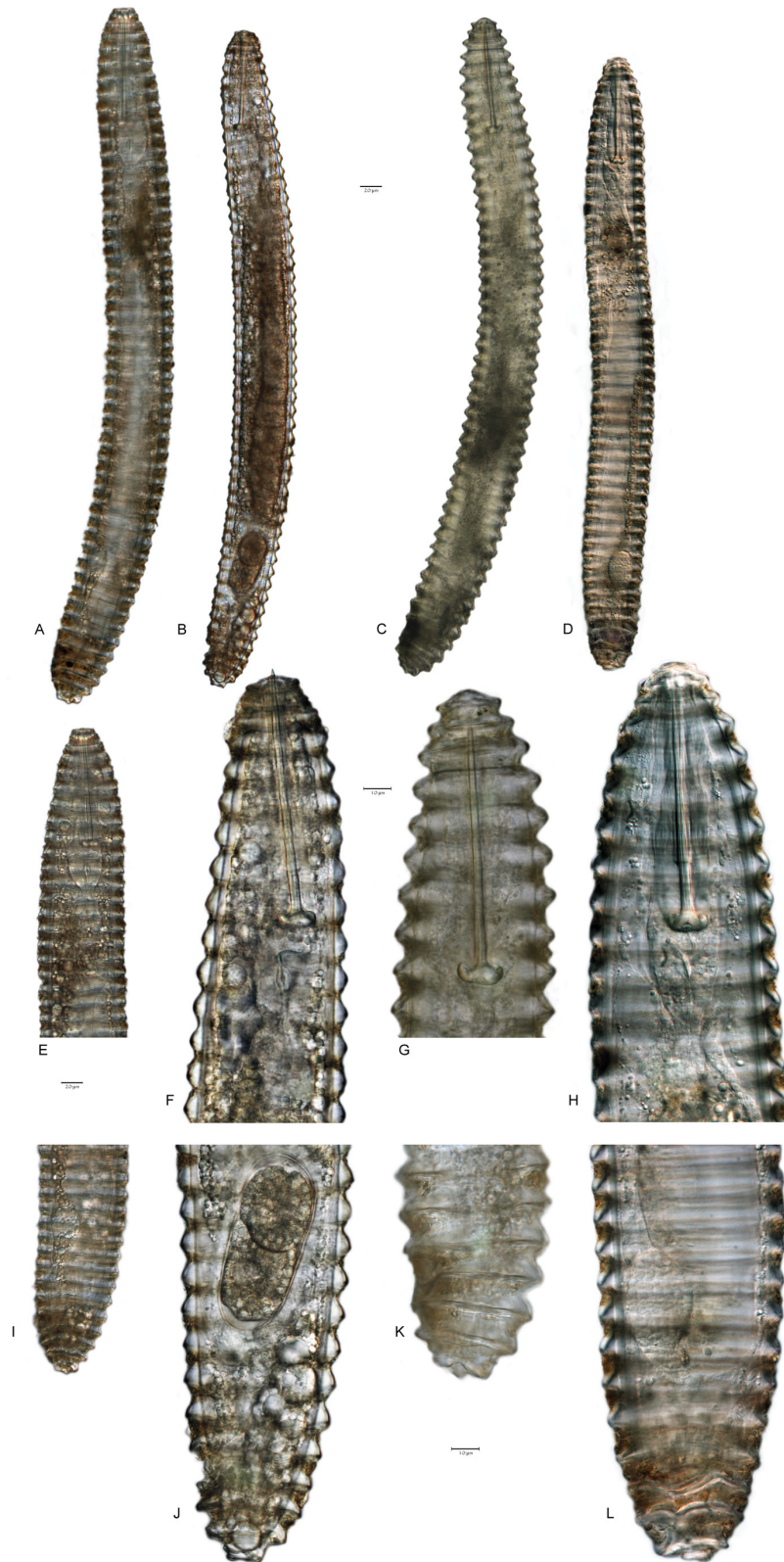


FIGURE 6. Light microscope images of female specimens in group 2, provisionally identified as *Criconema lamellatum*. A, E, I) NID 1465, *Lobocriconema* sp., Purchase Knob, GSMNP, A) entire, 400X, E) head, 400X, I) tail, 400X. B, F, J) NID 2582, *Lobocriconema* sp., Purchase Knob, GRSM, B) entire, 400X, F) head, 1000X, J) tail, 1000X. C, G, K) NID 3001, *Lobocriconema* sp., Goshen Prong, GRSM. C) entire, 400X, G) head, 1000X, K) tail, 1000X. D, H, L) NID 899, *Lobocriconema* sp., Chimney Creek, GRSM D) entire, 400X, H) head, 1000X, L) tail, 1000X.

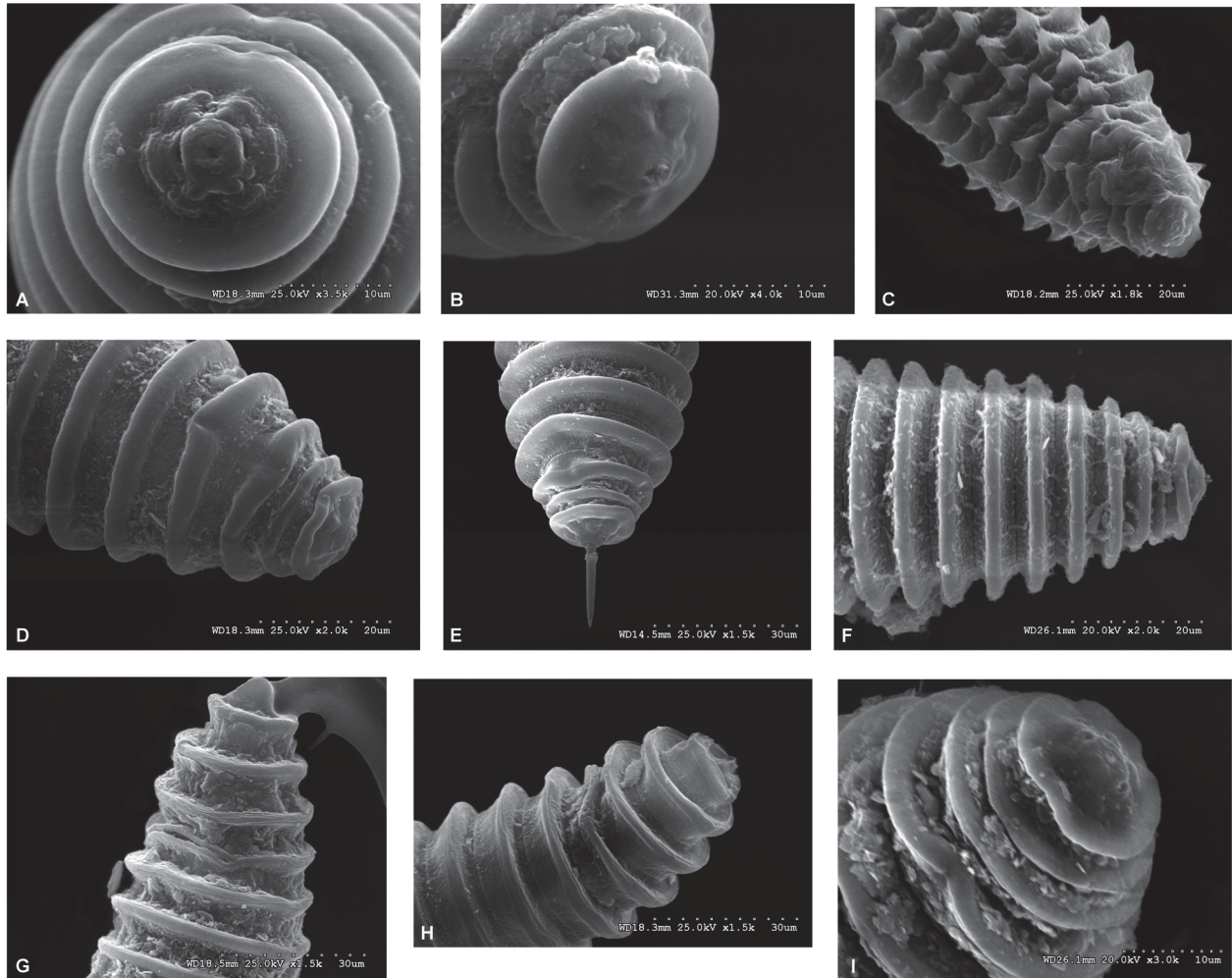


FIGURE 7. SEM images of specimens representing clades D (A–H) and B (I). NID numbers are associated with unique specimens, all are females except image C.

- A) *Lobocriconema* sp., face view with conspicuous labial disc surrounded by irregular labial structure, Nine-Mile Prairie, Nebraska, NID 4533.
 B) *Lobocriconema* sp., face view lacking submedian lobes and displaying subcuticular labial structure, Big Thicket National Preserve, Texas, NID 4560.
 C) *Lobocriconema* sp., juvenile, head with visible submedian lobes, body scales with fine terminal projections, Spring Creek Prairie, Nebraska, NID 4514.
 D) *Lobocriconema* sp., face view lacking submedian lobes and displaying subcuticular labial structure, Nine-Mile Prairie, Nebraska, NID 4527
 E) *Lobocriconema* sp., cephalic profile with protruding stylet, Nine-Mile Prairie, Nebraska, NID 4529.
 F) *Lobocriconema* sp., head profile lacking submedian lobes, Tunica Hills, Louisiana, NID 4574.
 G) *Lobocriconema* sp., tail with closed vulva, Nine-Mile Prairie, Nebraska, NID 4533.
 H) *Lobocriconema* sp., tail with closed vulva, Nine-Mile Prairie, Nebraska, NID 4526.
 I) *Lobocriconema* sp., face view lacking submedian lobes, Great Smoky Mountains National Park, Purchase Knob, NID 4570.

reduced bursa and four faint lateral lines (Geraert 2010). The males typically have twice the number of body annules (R) as the corresponding females. Juveniles are characterized by simple scales arranged longitudinally on the body, with a maximum of 8–12 rows at midbody (Fig. 13A–D). In some specimens fine projections were observed lining the margin of the scales (Fig. 13D).

Discriminant Function Analysis of morphological traits. DFA was conducted on the four major clades first, followed by an analysis of the 18 haplotype groups in the COI dataset. For clades A–D stylet length and stylet knob width accounted for approximately 80% of the discriminatory ability as determined through a stepwise classification variable selection (Fig. 14A). Clade A specimens were classified with 100% accuracy, whereas

clades D and C were correctly classified 90% and 88% of the time. Only two-thirds of the time were members of clade B correctly classified by the discriminant model, with misclassification within clade C occurring one-third of the time. A mapping of classifications into discriminant space is presented in Fig. 14B. The cross-validation accuracies of the models are in Table 7.

TABLE 7. Summaries of the Stepwise-selected Discriminant Models—Clades

##	APER	crossval	gamma	lambda
## rda.eq.mcr	0.1020672	0.11285714	0.02366162	1
## rda.prop.mcr	0.1044776	0.09628465	0.01401976	1
## lda.eq.mcr	0.1343284	0.13432836	0.00000000	1
## lda.prop.mcr	0.1119403	0.11194030	0.00000000	1

Less accuracy in classification was observed in the analysis of the 18 haplotype groups. The best model, a linear discriminant analysis with equal priors had a 45% misclassification rate. The characters stylet length, number of body annules (R), mid-body width and location of the vulva expressed in number of annules from the tail terminus (Rv) provided the bulk of the morphological signal (Fig. 15A). Only two haplotype groups were classified with 100% accuracy, groups 1 and 17. Four other groups 3, 4, 12, and 13 were classified correctly at least two-thirds of the time, with a majority of the groups with 50% or less correct classification. The mapping of the classification is presented in Fig. 15B, with the cross-validation accuracies of the models in Table 8.

TABLE 8. Summaries of the Stepwise-selected Discriminant Models—18 groups

##	APER	crossval	gamma	lambda
## rda.eq.mcr	0.3547899	0.7605263	0.53942456	1
## rda.prop.mcr	0.3731343	0.6893362	0.02263992	1
## lda.eq.mcr	0.3507463	0.4477612	0.00000000	1
## lda.prop.mcr	0.3731343	0.4552239	0.00000000	1

Distribution and Phylogeography. The geographic distribution of haplotype diversity appeared to be strongly influenced by latitude (Fig. 12). The 49 northern specimens collected between 38.96° and 46.81° latitude populated 6 different haplotype groups and included 21 unique haplotypes. Eighty-five southern specimens collected between 29.95° and 36.13° latitude represented 16 haplotype groups and 43 unique haplotypes. Using a statistical parsimony approach and a 95% connectivity criterion, only groups 1 and 5 displayed haplotype connectivity across broad geographic regions (Fig. 12). Group 5 connected haplotypes from Michigan, Ohio, and Indiana with 1–3 mutational steps. Loosening the connectivity restrictions to 90% and 16 mutational steps added specimens from the Upper Peninsula of Michigan, central Wisconsin (group 18) and George Washington Memorial Parkway in northern Virginia. It is notable that the haplotypes in group 18 from the Upper Peninsula of Michigan and central Wisconsin were identical genetically, yet were both separated by 10–12 mutational steps from haplotypes east and south of Lake Michigan located in Indiana, Ohio, and the lower peninsula of Michigan.

Haplotype distribution in the Gulf Coast states presented a complex matrix of patterns. In an east–west transect from southern Georgia, across the Florida peninsula to Tunica Hills, Louisiana, there was a mix of shared and unique haplotype groups (Fig. 12). In Torreya State Park, Florida, there were two distinct haplotype groups, 11 and 14. In mean group distance (p-values) they are 9.4% genetically distant from each other. Approximately 73 kilometers to the southeast in Wakulla State Park, Florida, haplotype group 11 exists together with members of haplotype group 15, with a genetic distance of 9.8% separating the two. Additionally, at Ichetucknee Springs State Park, Florida, 150 km to the east of Wakulla SP, a variant haplotype of group 15 was found with two haplotypes comprising the geographically localized group 7. Haplotype groups 7 and 15 are 10.8% distant. However, haplotypes from each site share a more closely related haplotype with a geographically adjacent site. Specimens of haplotype group 11 at Torreya have an average 0.8% genetic distance from haplotype group 11 at Wakulla. Similarly, specimens of haplotype group 15 at Wakulla are 0.7% distant from the variant haplotype group 15 at

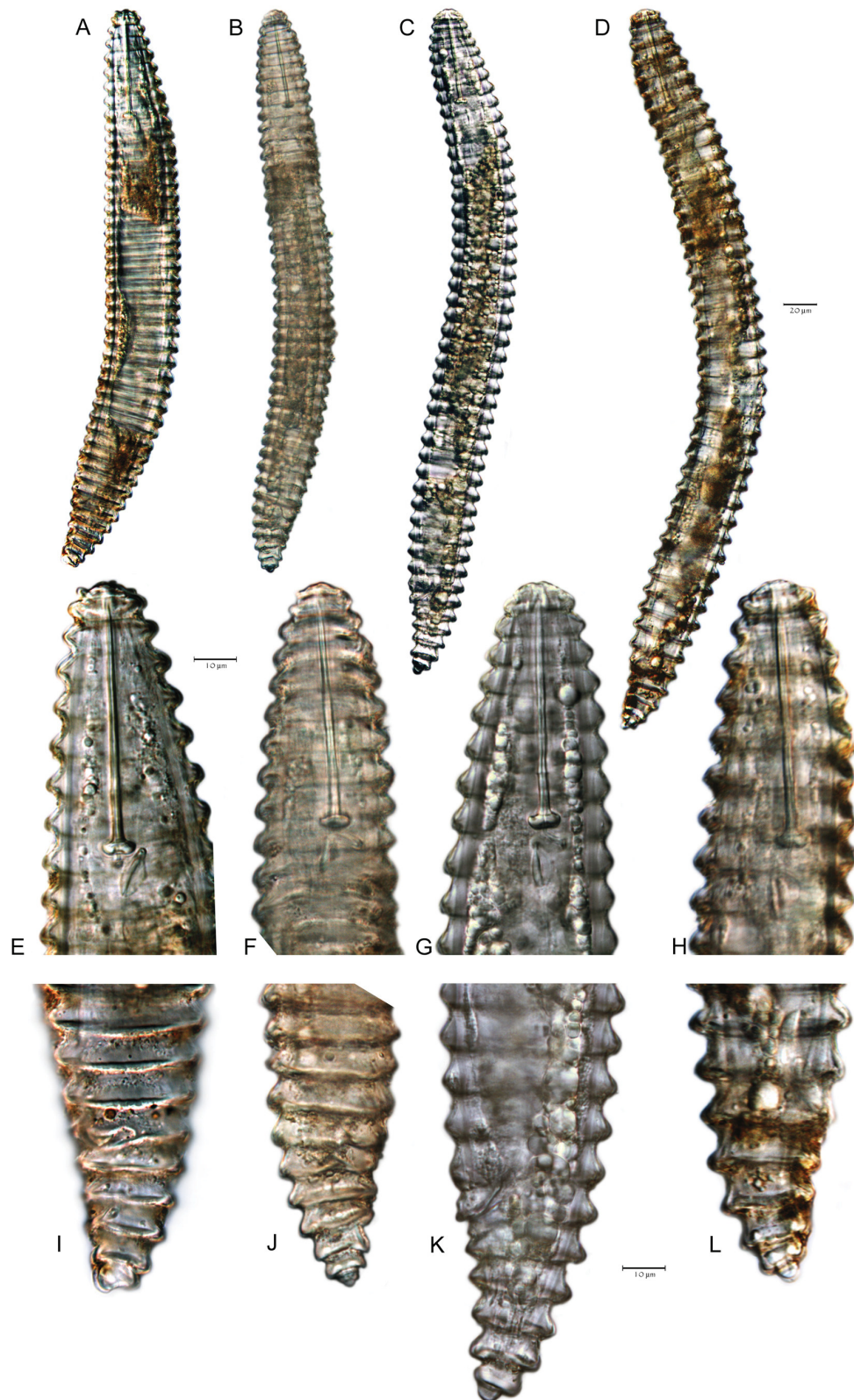


FIGURE 8. Light microscope images of female specimens in clade A (group 1) provisionally identified as *Lobocriconema crassiannulatum*. A, E, I) NID 1149, *Lobocriconema* sp., Avoca Prairie, Wisconsin, A) entire, 400X, E) head, 1000X, I) tail, 1000X. B, F, J) NID 3267, *Lobocriconema* sp., Ozark National Forest, Arkansas. B) entire, 400X, F) head, 1000X, J) tail, 1000X. C, G, K) NID 1462, *Lobocriconema* sp., Roth Prairie, Arkansas. C) entire, 400X, G) head, 1000X, K) tail, 1000X. D, H, L) NID 1214, *Lobocriconema* sp., Fairfax County, Virginia. D) entire, 400X, H) head, 1000X, L) tail, 1000X.

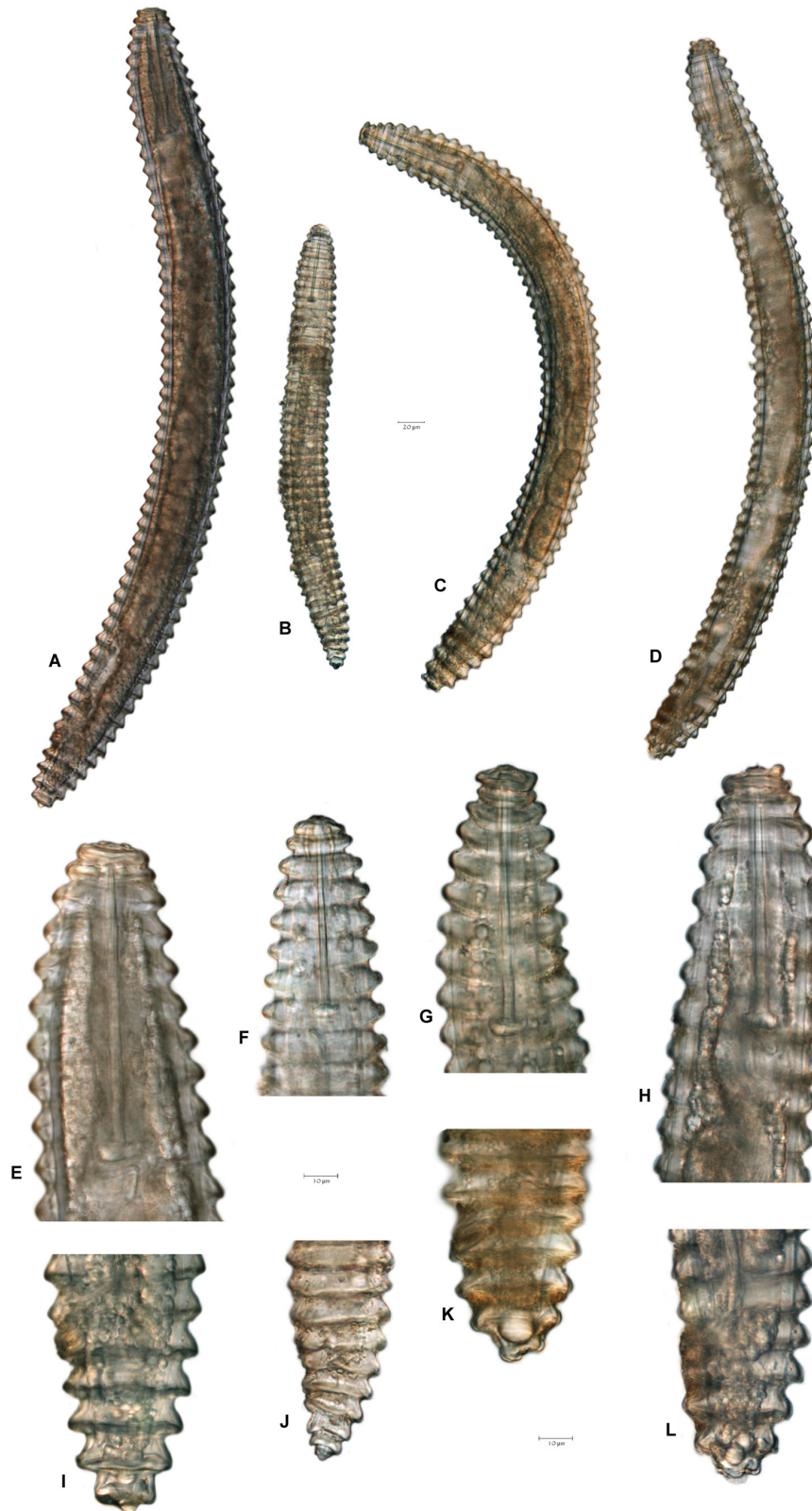


FIGURE 9. Light microscope images of female specimens from *Criconema arkaense* topotype and paratype localities. A, E, I) NID 3229 (clade C, singleton), host maple, Ozark National Forest, Arkansas, A) entire, 400X, E) head, 1000X, I) tail, 1000X. B, F, J) (clade A, group 1) host wild cherry, Ozark National Forest, Arkansas, B) NID 3267, entire, 400X, F) NID 3265, head, 1000X, J) NID 3267, tail, 1000X. C, G, K) (clade D, group 10), host hackberry, Ozark National Forest, Arkansas, C) NID 3259, entire, 400X, G) NID 3259, head, 1000X, K) NID 3257, tail, 1000X. D, H, L) NID 3256 (clade D, group 11), host hackberry, Ozark National Forest, Arkansas D) entire, 400X, H) head, 1000X, L) tail, 1000X.

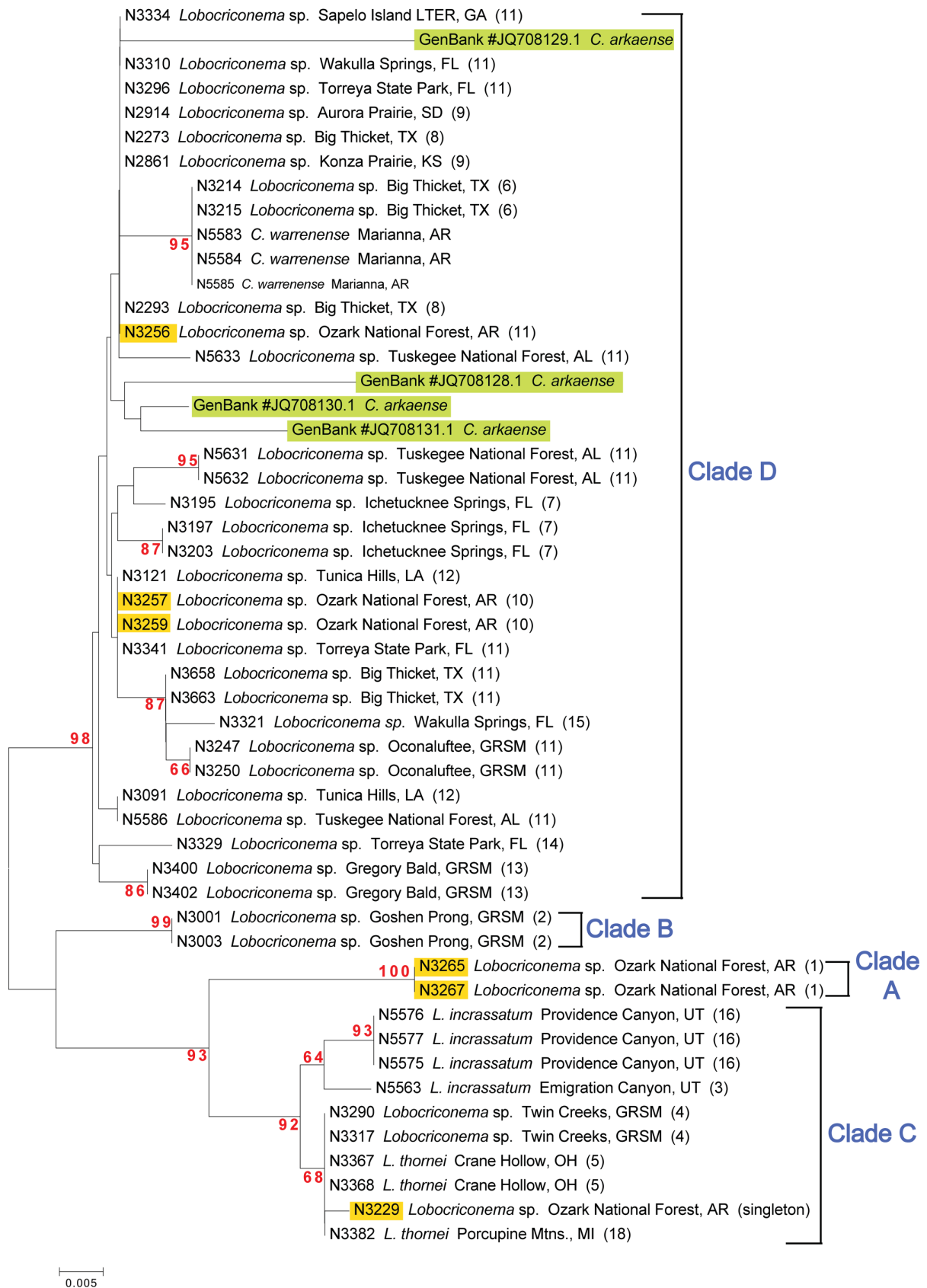


FIGURE 10. Neighbor-joining ITS1 tree (Internal Transcribed Spacer 1). Terminal branches identified by NID numbers, taxon, location information and COI group. GenBank Accession sequences are highlighted in green, NID numbers from *Criconema arkaense* collection sites in Cordero *et al.* (2012) are highlighted in orange. Clade designation follows the COI tree structure. Red bootstrap values of 5,000 replications.

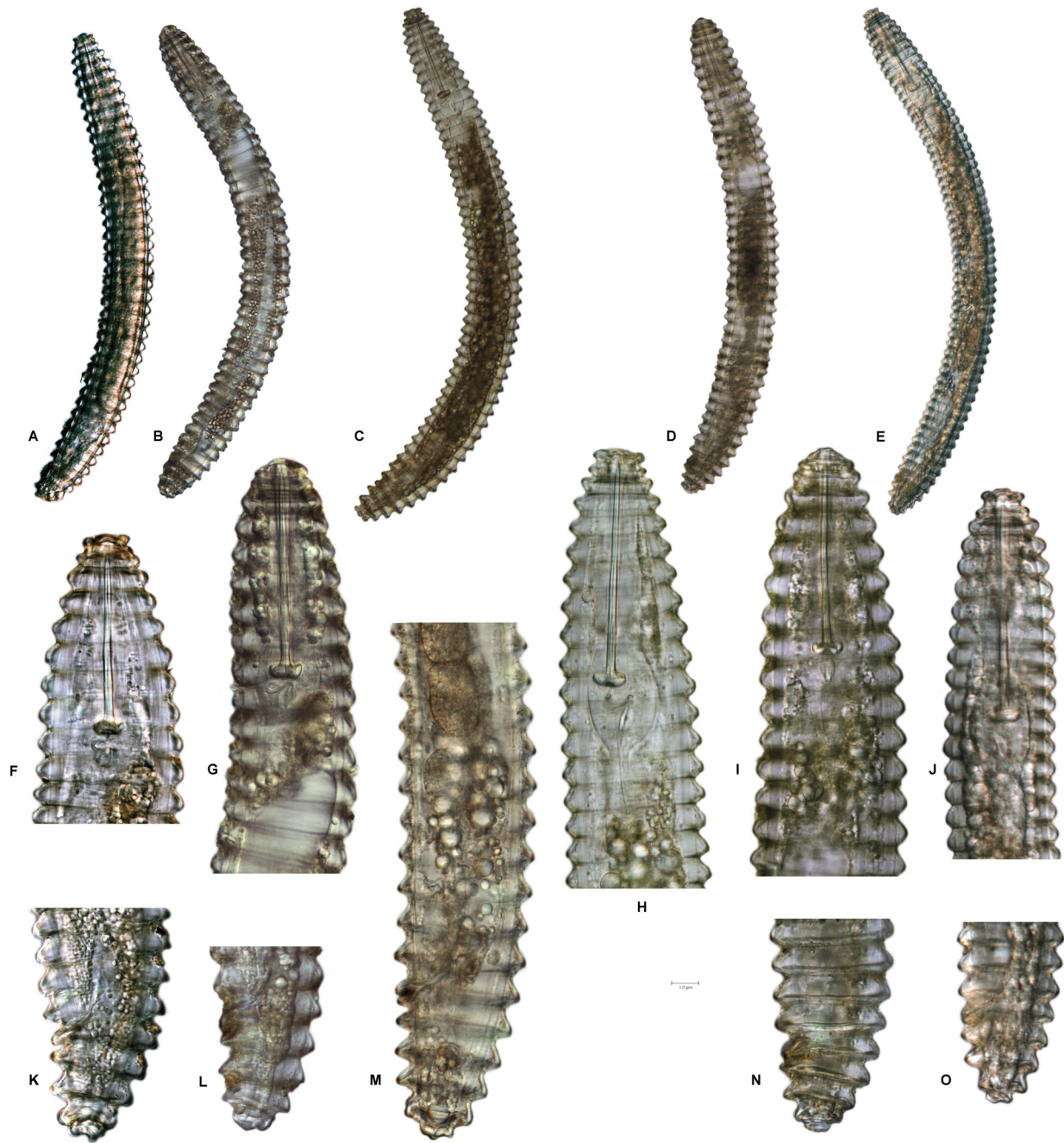


FIGURE 11. Light microscope images of female *Lobocriconema* sp. specimens from clade D. A, F, K (group 9), Nine-Mile Prairie, Nebraska, A) NID 1155, entire, 400X, F) NID 1156, head, 1000X, K) NID 1156, tail, 1000X. B, G, L (group 11), Big Thicket National Preserve, Texas, B) NID 5647, entire, 400X, G) NID 5647, head, 1000X, L) NID 5653, tail, 1000X. C, H, M (group 12), Tunica Hills, Louisiana, C) NID 3057, entire, 400X, H) NID 3057, head, 1000X, M) NID 3090, tail, 1000X. D, I, N) NID 3403 (group 13), Gregory Bald, Great Smoky Mountains National Park, North Carolina, D) entire, 400X, I) head, 1000X, N) tail, 1000X. E, J, O) NID 3297 (group 14), Torreya State Park, Florida, E) entire, 400X, J) head, 1000X, O) tail, 1000X.

Ichetucknee. These sampling sites are separated from each other by rivers that have been hypothesized as Pleistocene barriers to gene flow for various organisms (Soltis *et al.* 2006), but the patterns among these morphologically conservative haplotype groups also appear to involve divergences that occurred at much earlier time periods.

In Great Smoky Mountains National Park, three haplotype groups, (2, 4, and 13) could be considered geographically restricted endemics based on existing genetic data. Two other GRSM haplotype groups, 10 and 11,

share members with specimens from the Ozark Mountains. Interestingly, none of the haplotype groups span the geographic distance from the Gulf Coast to northern temperate forests of New England or the Great Lakes region. This restricted distribution is surprising given that associated host species of *Lobocriconema* such as maples and oaks do span the latitudinal gradient of collection sites.

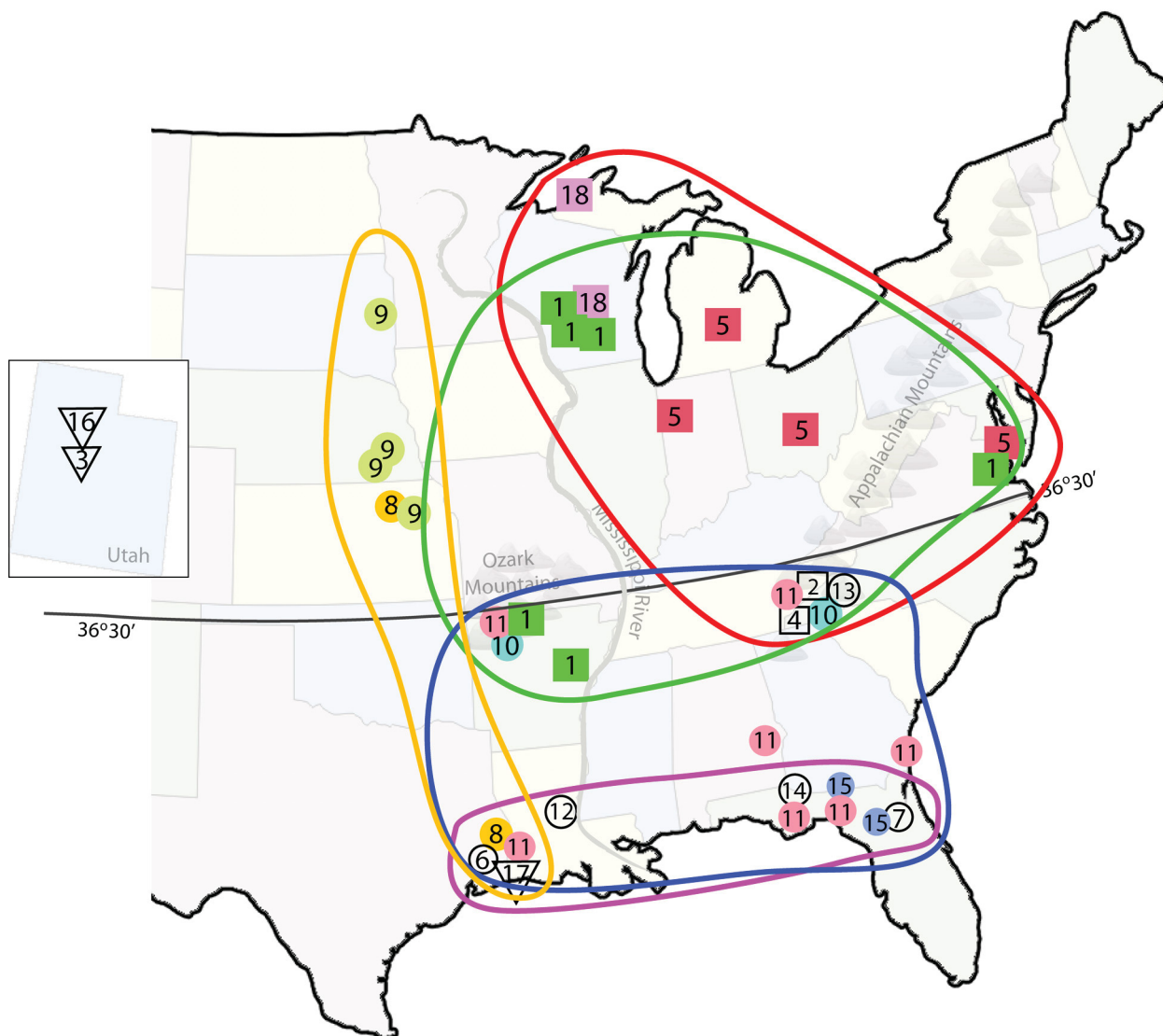


FIGURE 12. Distribution of 18 *Lobocriconema* haplotype groups. Major clades A, B and C are enclosed by boxes, members of clade D are encircled. Triangles identify geographic outliers in the dataset. Boxes and circles without color represent putative endemics, recovered only from that specific location. Colors identify shared group membership. Green outline indicates the geographic range of clade A. Red outline indicates clade C minus the Utah outliers, (groups 3 and 16), group 17, and the endemic clade B (group 2). Blue, magenta, and orange outlines indicate distributions within clade D, Gulf Coast and southern Ozark and Appalachian, Gulf Coast, and north–south corridor respectively.

Discussion

Taxonomic records presently list two *Lobocriconema* species on the North American continent. The molecular evidence presented in this study indicates that there are many more, especially when specimens lacking submedian lobes are incorporated into the group. This result raises the taxonomic question: can a species be a member of *Lobocriconema* without lobes? Both COI and ITS1 sequences support the existence of a monophyletic group

within Criconematidae that can be subdivided into four major clades. Clade C, with groups 3–5 and 16–18, includes the two described species in North America, *L. incrassatum* and *L. thornei*, both of which possess prominent submedian lobes. The second major clade (D) contains haplotype groups 6–15. This clade lacks specimens with prominent lobes; includes some specimens with indistinct or reduced lobes, but the majority of specimens lack lobes entirely. Groups 1 and 2 constitute separate clades with deep nodes that connect them to the other *Lobocriconema* clades. They appear to lack lobes, are morphologically recognized by discriminant function analysis, and their genetic relationships within the *Lobocriconema* dataset are not clearly resolved. Yet many other morphological characters that help define *Lobocriconema* are shared between the four clades. These characters include a low number of total body annules (generally less than 60), annules that are usually 10 μm or more in width, and vulva placement within 3–6 annules from the tail terminus. The first labial annule is usually smaller or the same size as the subsequent annule. The vulva is closed and does not possess a well-developed flap as is seen in many *Criconema* species. The tails may be conoid-rounded or tapered to more of a point, but they are never long, drawn-out, pointed tails as seen in many species of *Criconema* and *Ogma*. These characters by themselves may not always be sufficient to accurately classify specimens, but combined with COI analysis, they provide an example of a morphologically conservative assemblage of nematodes with distinct haplotype groups that are non-randomly distributed and largely geographically restricted to the forests of eastern North America. The formal recognition of these clades defined by deeper nodes on the tree is a taxonomic issue raised in this study that will benefit from an examination of specimens outside North America.

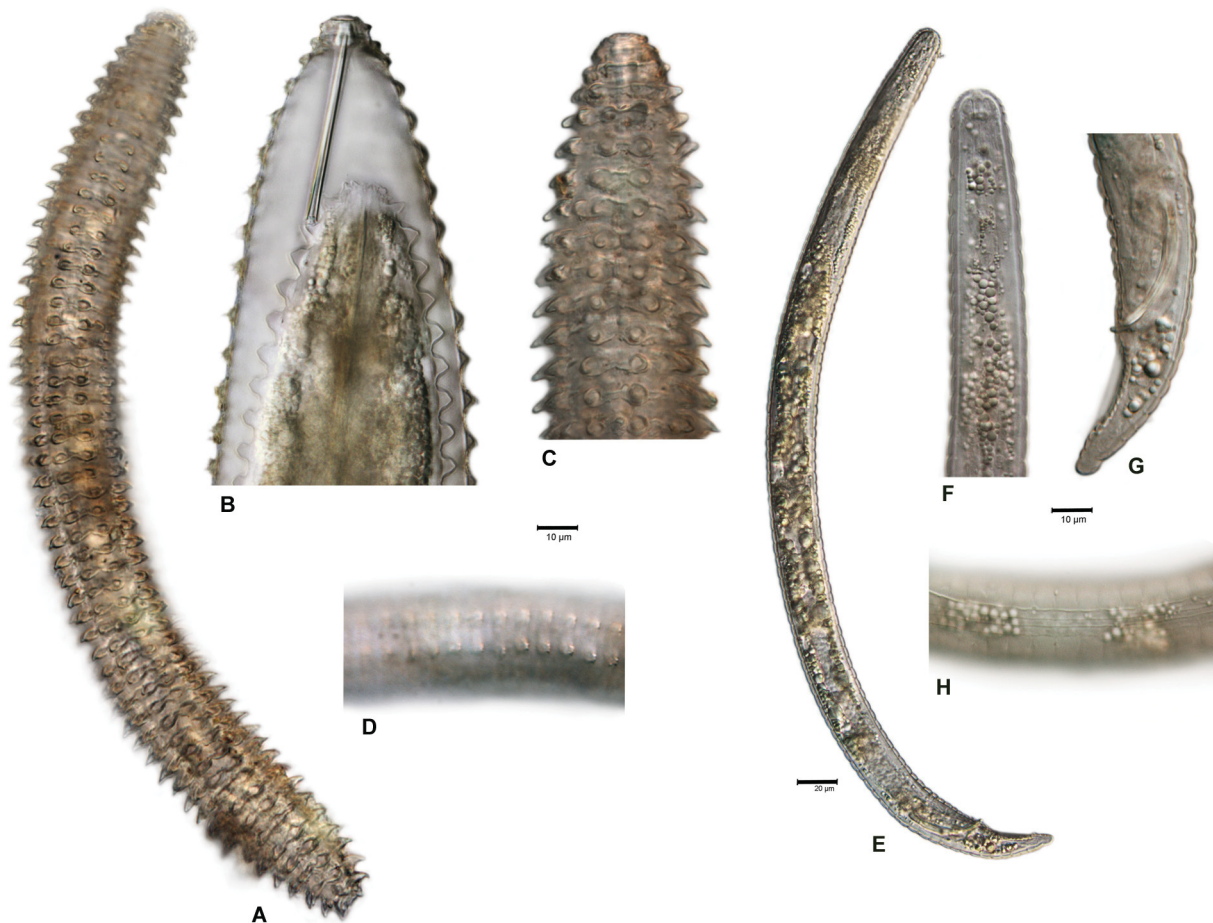
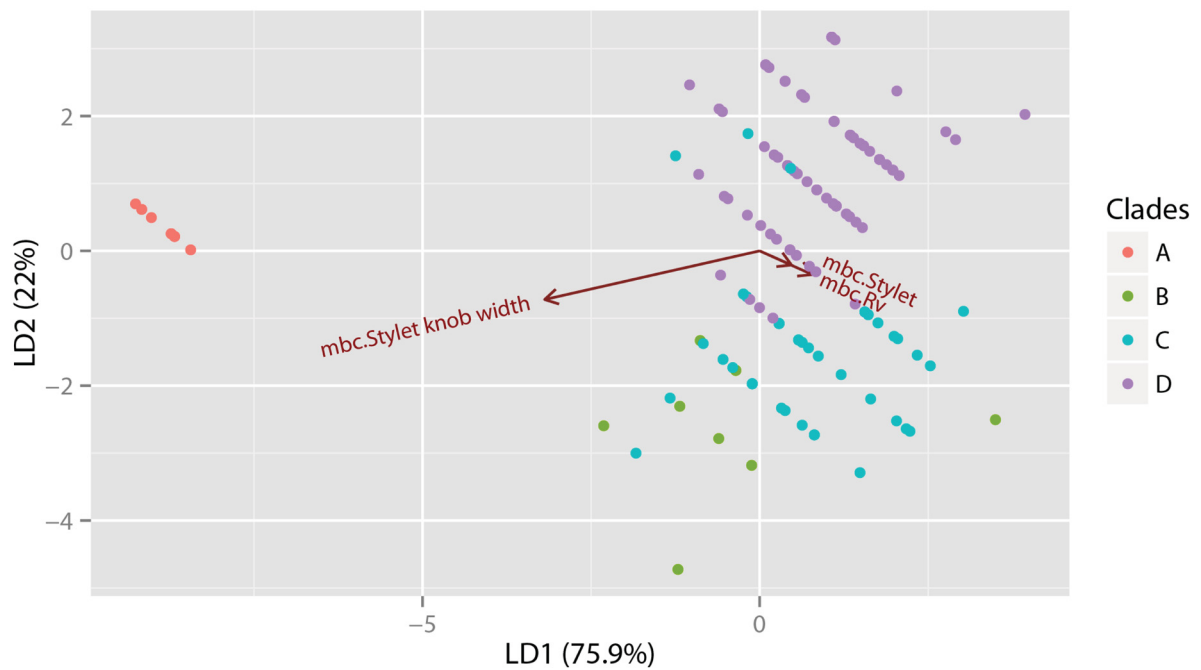
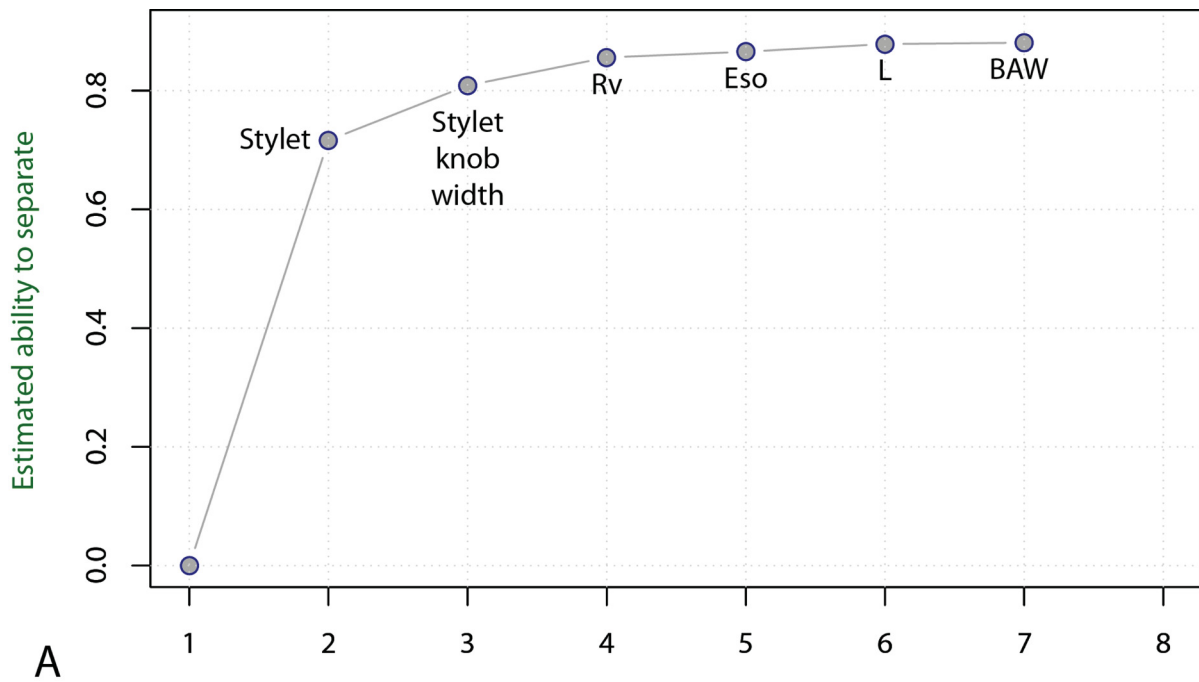
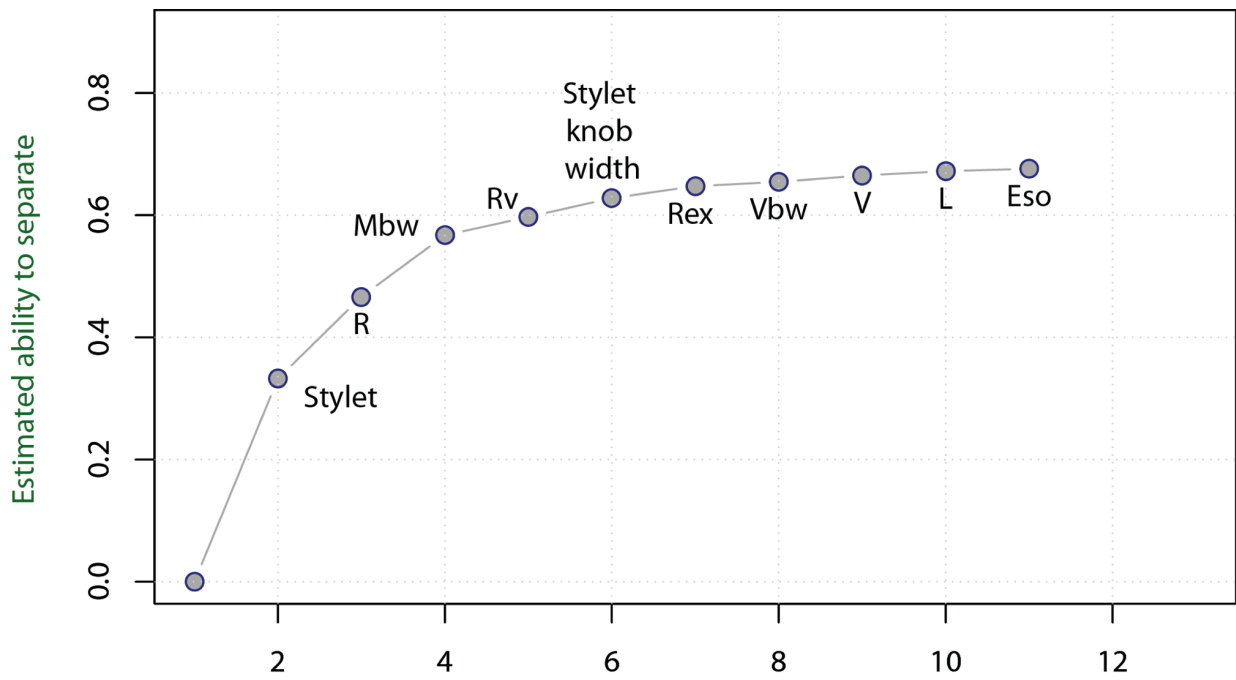


FIGURE 13. Images of juveniles and males. A) NID 3121 (group 12), *Lobocriconema* sp., juvenile, entire, 1000X, Tunica Hills, Louisiana. B) NID 2527 (group 5), *Lobocriconema thornei*, moulting juvenile, head, 1000X, Ingham County, Michigan - type locality. C) NID 3301 (group 11), *Lobocriconema* sp., juvenile, head, 1000X, Torreya State Park, Florida. D) NID 938 (group 9), *Lobocriconema* sp., juvenile, cuticle with scales and fine projections, 1000X, Spring Creek Prairie, Nebraska. E–H) NID 589 (group 7), male, Ichetucknee River, Florida, hardwood forest, E) entire, 400X, F) head, 1000X, G) tail region with spicule and subterminal bursa, 1000X, H) midbody cuticle with lateral lines, 1000X.

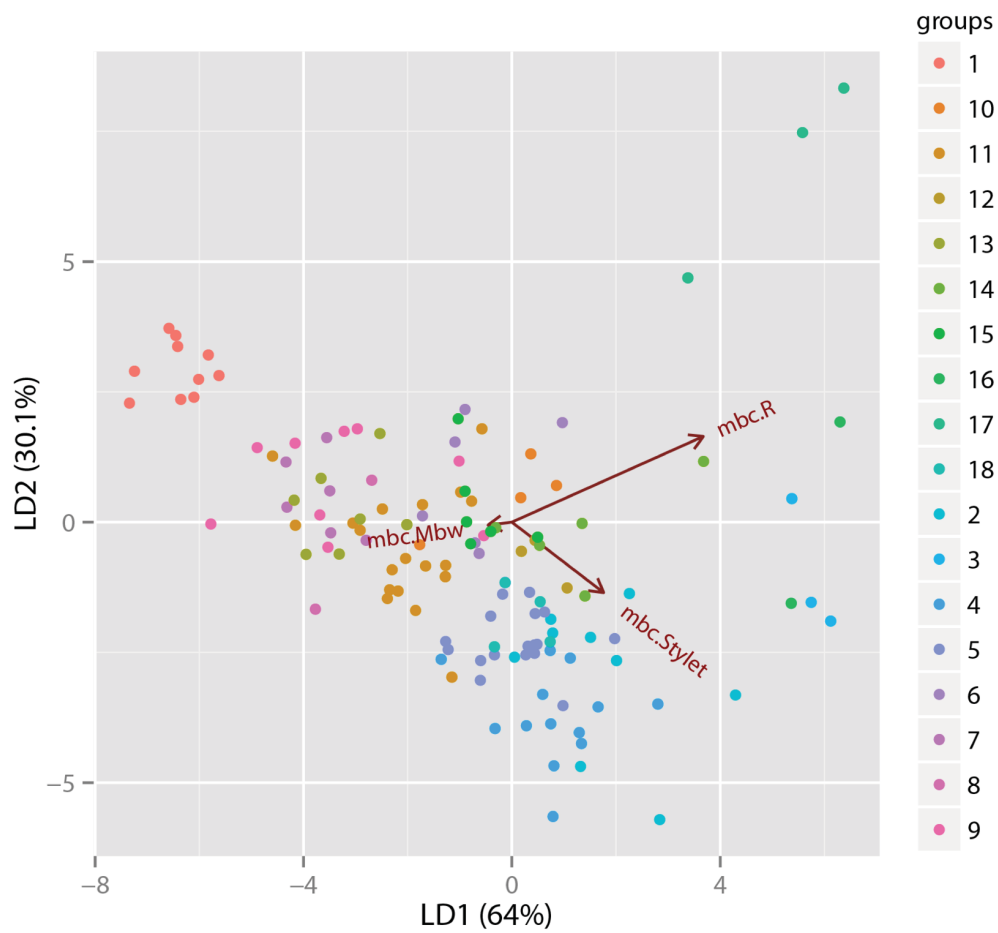


B

FIGURE 14. Discriminant function analysis of the 4 major clades. A) Stepwise selection of morphological variables and estimated ability to separate major clades. B) Mapping of classifications into discriminant space.



A



B

FIGURE 15. Discriminant function analysis of the 18 haplotype groups. A) Stepwise selection of morphological variables and estimated ability to separate haplotype groups. B) Mapping of classifications into discriminant space.

Haplotype distribution is another issue that will benefit from increased sampling density. Presently most haplotype groups identified in the dataset exist as genetically distinct isolated islands, suggesting populations which have been subjected to genetic bottlenecks and founder effects possibly associated with glacial oscillations during the Pleistocene (Hewitt 2000; Widmer & Lexer 2001). The presence of putative nematode endemics and greater haplotype diversity found in the southern Gulf Coast states and the Great Smoky Mountains is consistent with these regions serving as possible glacial refugia for plant hosts such as maples, oaks, and beeches (Saeki *et al.* 2011; Kitamura & Kawano 2001; Jackson *et al.* 2000). However, missing from this scenario for nematodes are haplotypes linking the putative southern refugia to northern, formerly glaciated regions that have been recolonized by plant hosts. Group 5 displays a population structure expected from such a scenario in eastern deciduous forests, but the haplotype linkages suggest an east-coast glacial refuge or possibly a northern glacial refuge in Wisconsin's Driftless Area (Hansen 1939; Clayton *et al.* 2001). The genetic distance between the putative GRSM endemic group 4 and group 5, an average mean distance of 4.4% (Table 3), indicates an older divergence possibly stemming from early Pleistocene glaciations.

Group 9 displays an unusual distribution, with haplotypes collected from tallgrass prairies in Kansas, Nebraska, and South Dakota. Subsequent focal sampling of those prairies has revealed a host association with native wild rose (*Rosa woodsii* Lindl.), smooth sumac (*Rhus aromatica* Aiton), and wild plum (*Prunus americana* Marsh.). Sumac and plum are both common native shrubs of riparian habitats in the central plains and could provide a woody host corridor for extending the range of *Lobocriconema* from forests into grasslands. Generally, host associations in this study were indirectly inferred due to a sampling design that emphasized comparative diversity by processing bulked soil samples from within a 40×40 m grid. Nonetheless, it is possible to speculate about host relationships that might play a significant role in the geographic structure of haplotype groups. The mixture of haplotypes in the southern Gulf Coast states is suggestive of host associations with plant species found in habitats characteristic of the Southeastern Conifer Forest Ecoregion (Olson *et al.* 2004). For example, at Torreya and Wakulla State Parks in northwest Florida, we sampled plots in both sites that were classified as upland forest and lowland/cypress bogs (Table 1). The “upland” forest at Wakulla is only 2.7 m in elevation higher and 570 m distant from the lowland site. At Torreya the elevation difference was 59 meters with a separation of 466 m between the two plant communities. The upland sites were characterized by a plant community that included Loblolly pine (*Pinus taeda* L.), magnolia (*Magnolia grandiflora* L.), beech (*Fagus grandifolia* Ehrh.), holly (*Ilex* spp.), and white oak (*Quercus alba* L.). The lowland communities were characterized by bald cypress (*Taxodium distichum* (L.) Rich.), sweet gum (*Liquidambar styraciflua* L.), saw-tooth palmetto (*Serenoa repens* (W. Bartram) Small), red buckeye (*Aesculus pavia* L.), and holly (*Ilex* spp.). Haplotype group 11 from Torreya and from Wakulla consists of *Lobocriconema* specimens collected from upland forest soils. Haplotype group 14 contains four *Lobocriconema* from the Torreya lowland site and one from the nearby upland site. Haplotype group 15 includes all five of the *Lobocriconema* collected from the Wakulla lowland site and a single specimen from a lowland site in Ichetucknee Springs, Florida. Detailed focal sampling should help resolve the extent to which host specificity influences nematode community composition.

There is little evidence in this dataset of long distance dispersal (LDD) processes. Only two haplotypes were shared across long distances. The haplotype of NID 1381 from Alexandria, Virginia was identical to haplotypes in West Lafayette, Indiana, a distance of 865 km, and NID 3256 from the Ozark National Forest west of Fayetteville, Arkansas displayed the same haplotype as specimens from Oconaluftee in Great Smoky Mountains National Park, a distance of 998 km. Numerous factors could explain the infrequency of LDD in *Lobocriconema* including the lack of dispersal stages (*e.g.*, cysts or dauer stages), unknown ecological requirements associated with colonization, or a limited geographic range of potential host species. Lack of evidence for widespread long-distance dispersal contrasts with explanations of distribution for nematodes associated with agricultural commodities. It is well-documented that agricultural commerce has spread species of plant-parasitic nematodes (O'Bannon 1977; Steiner *et al.* 1951; Van den Berg *et al.* 2014). This study of *Lobocriconema* species illustrates that plant-parasitic nematodes can have a complex phylogeographic structure similar to other plants and animals. Using the taxonomic tools and framework developed in this study, it should be possible to explore the various factors responsible for geographic patterns and diversity observed in *Lobocriconema*.

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