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MOTUs, Morphology, and Biodiversity Estimation: A Case Study Using Nematodes of the Suborder Criconematina and a Conserved 18S DNA Barcode

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Abstract: DNA barcodes are increasingly used to provide an estimate of biodiversity for small, cryptic organisms like nematodes. Nucleotide sequences generated by the barcoding process are often grouped, based on similarity, into molecular operational taxonomic units (MOTUs). In order to get a better understanding of the taxonomic resolution of a 3' 592-bp 18S rDNA barcode, we have analyzed 100 MOTUs generated from 214 specimens in the nematode suborder Criconematina. Previous research has demonstrated that the primer set for this barcode reliably amplifies all nematodes in the Phylum Nematoda. Included among the Criconematina specimens were 25 morphologically described species representing 12 genera. Using the most stringent definition of MOTU membership, where a single nucleotide difference is sufficient for the creation of a new MOTU, it was found that an MOTU can represent a subgroup of a species (e.g. *Discocriconemella limitanea*), a single species (*Bakernema inaequale)*, or a species complex (MOTU 76). A maximum likelihood phylogenetic analysis of the MOTU dataset generated four major clades that were further analyzed by character-based barcode analysis. Fourteen of the 25 morphologically identified species had at least one putative diagnostic nucleotide identified by this character-based approach. These diagnostic nucleotides could be useful in biodiversity assessments when ambiguous results are encountered in database searches that use a distance-based metric for nucleotide sequence comparisons. Information and images regarding specimens examined during this study are available online.

Key words: Criconematidae, DNA taxonomy, phylogeny, barcode analysis, plant parasitic nematodes, nematode diversity.

The estimation of nematode biodiversity exemplifies the challenges in exploring a taxon with a major percentage of its diversity undescribed. In the phylum Nematoda, it is probably an overestimate to suggest that the approximately 27,000 described species represent 5-10% of the existing nematode taxa on the planet (Hugot et al., 2001; Creer et al., 2010; Fonseca et al., 2010). This "well-acknowledged biodiversity identification gap", the ratio of known species (described) to unknown species (not yet described), has been attributed to the small size of nematodes, their simple morphology, intraspecific variation, and the lack of nematode taxonomists (Creer et al., 2010). One study of nematode diversity in a tropical forest in Cameroon estimated that 6,000 scientist-hours of labor were required to sort and catalogue 431 morphologically identified nematode species, for a survey in which over 90% of the specimens could not be assigned to known species (Bloemers et al., 1997). It is no wonder that molecular approaches that can possibly expedite the process of species discovery and description have been actively pursued (Blaxter, 2004; Markmann and Tautz, 2005; Bhadury et al., 2006; Donn et al., 2008; Porazinska et al., 2009, 2010a, 2010b; Powers et al., 2009; Da Silva et al., 2010; Abebe et al., 2011).

Ironically, this identification gap will likely widen as molecular approaches increase in their application. With the advent of high throughput, next generation sequencing, an entire community of nematodes can be rapidly reduced to a single set of sequences (Creer et al. 2010; Porazinska et al., 2010b). These sequences, if they are derived from a common gene following PCR of pooled DNA from the nematode community, may be considered as a set of MOTUs (molecular operational taxonomic units) (Floyd et al., 2002; Blaxter et al., 2005; Caron et al., 2009; Creer et al. 2010; Jones et al. 2011). An MOTU can be defined as a cluster of sequences that fall within a designated cutoff value of sequence identity, the cutoff value being established by the author (Caron et al., 2009). The cutoff value could require 100% sequence identity, in which case each unique sequence is considered a separate MOTU. The taxonomic significance of a given MOTU depends on a number of factors such as the genetic region under analysis, the rate of evolution of that region, experimental error, and the congruence of gene trees and species trees. Since these factors are seldom completely understood, it is not a trivial question to ask, "What does an MOTU represent?"

In this study we explore the performance of a 3' 592 bp 18S barcode as a tool to generate MOTUs and assess nematode diversity. The term barcode in this case refers to the specific region of the 18S gene amplified by the 18S1.2a/18Sr2b PCR primer set, and the nucleotide sequence between them, but not including the primer sequence itself. The barcode was selected due to its evolutionarily conserved nature, exhibiting a balance between phylogenetic breadth and taxonomic resolution. It has previously been used in a phylum-wide molecular survey of nematode communities within a lowland Costa Rican rain forest (Powers et al., 2009), a metagenetic analysis of artificially constructed nematode communities (Porazinska et al., 2009) and a multi-phyla metagenetic survey replicating the aforementioned Costa Rican rain forest study (Porazinska et al., 2010a). MOTUs derived from 18S have the advantage of comparison to the 18S-based Nematode Tree of Life which in its most current published form includes 1215 taxa (van Megen et al., 2009). We assume that MOTUs that

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link with morphologically and taxonomically characterized entities are a richer source of systematic information and maximize information content from studies employing MOTUs unlinked from taxonomically characterized entities. Therefore, a second purpose of this study is to enlarge the reference database in order to facilitate future systematic studies.

To address the question of MOTU representation, we apply the 3' 592 bp 18S barcode to an analysis of a single, globally distributed suborder of plant-parasitic nematodes. The suborder Criconematina Siddiqi, 1980 ranges from the humid tropics to arctic and alpine habitats. There are an estimated 750 described species in the suborder (Subbotin et al., 2005). They are found on a wide range of hosts feeding on plants as diverse as hardwoods, conifers, bromeliads, grasses and moss. They are believed to have a high level of endemicity and are some of the most abundant soil-dwelling plant parasites in tropical forests (Wouts, 2006). Their high endemicity, poor dispersal capabilities, and apparent lack of specialized survival stages make them a potential subject for biogeographic analysis (Bernard and Schmitt, 2005; Wouts, 2006). They are potential indicators for soil disturbance (Bernard, 1992). While a few species appear to be adapted to disturbances associated with agricultural production, the vast majority are confined to native habitats with a relatively stable soil structure (Hoffman and Norton, 1976; Bernard, 1982; Peneva et al., 2000) and tend to disappear when these habitats are disrupted. It is widely believed that Criconematina constitutes a monophyletic group, although the relationships and composition of sub-groups are generally considered to be "taxonomically opaque" and in a perpetual state of taxonomic turmoil (Siddiqi, 2000; Subbotin et al., 2005, 2006; Bert et al., 2008; Hunt, 2008). Monophyly of the suborder has been supported by both molecular and morphological analysis (Holterman et al., 2006; Subbotin et al., 2006; Bert et al., 2008; van Megen et al., 2009).

The nematodes in this study have been obtained through a series of collections spanning the years 1999 to 2010 (Table 1). Nematodes were individually isolated from soil samples, many digitally photographed (most often while alive), measured, processed for PCR, amplified and sequenced for the 18S barcode. Collection localities included non-cultivated as well as cultivated soils, with approximately one-third of the specimens recovered from Costa Rica and the remaining specimens from the United States, Mexico, and Europe. An additional 21 sequences from GenBank were added to the analysis.

The specific objective of this study is to apply a phylogenetic and a character-based barcode analysis to a 100-MOTU dataset of Criconematina specimens. This dataset includes 25 *a priori* identified species, recognized by traditional morphological analysis. The dataset also includes specimens that could not be identified *a priori* to species with confidence. The unknown specimens may represent new species or specimens that do not provide sufficient information for an accurate identification. We attempt to determine if there exists any nucleotide sequence support for the morphologically identified taxa. This analysis should provide insight into the taxonomic resolution of the 18S barcode, which in turn should enhance studies of nematode biodiversity.

Materials and Methods

Nematode collections: The earliest collected specimens in this study, those collected between 1999 and 2005, tend to have less associated morphological data, as methods were being developed to obtain both molecular and morphological information from an individual specimen. Two biodiversity surveys contributed a significant number of specimens to this study; a 1999 nematode survey of Konza Prairie, a designated Long Term Ecological Reserve, and a 2005 survey of La Selva Biological Research Station operated by the Organization of Tropical Studies (NSF DEB 0640807) (NSF DEB 9806439). The geographic coverage in this study includes specimens from Atlantic and Pacific coasts, and Central Valley of Costa Rica. North American specimens were collected from 21 U.S. states and a single state in Mexico. Twenty one GenBank accessions were added to the analysis, all of which represent European collections. Four sampling sites represent type localities from which the targeted species was obtained.

Nematode morphological identification: Nematodes were observed by differential interference microscopy on a Leica DMLB microscope, images recorded by a Leica DC300 video camera, and measurements obtained using an eyepiece micrometer at 1000x magnification. Observations were made on living nematodes whenever possible. In some cases such as *Bakernema* specimens, the elaborate cuticular ornamentation is more visible in living than dead or fixed specimens. After nematode measurement, the slide was carefully dismantled by removing the cover slip, the nematode recovered using a fine insect pin pick, added to an 18ul drop of sterile water, and then smashed on a cover slip with a clear, sterile micropipette tip. Nematode residue was stored in PCR reaction tubes in a -20°C freezer until PCR amplification.

DNA amplicon characteristics, terminology and assumptions: The 18S1.2a/18Sr2b primer set typically amplifies a 635-bp region of the 18S ribosomal gene, with the 3'-most primer located 180-bp from the first internal transcribed spacer (ITS1). The primer set, 18S1.2a: 5'-CGATCAGATACCGCCCTAG-3' (forward) and 18Sr2b: 5'-TACAAAGGGCAGGGACGTAAT-3' (reverse) will amplify nematodes throughout the phylum and will amplify some non-nematode taxa. The term barcode in this study applies to that specific region of the 18S gene bounded by those primers. This barcode is distinct and does not overlap with the 5'-18S barcode region analyzed

TABLE 1. MOTUs and individual specimens used in this study.

MOTU	NID No.	Species ID	Stage[a]	Locality	Accession No.
M1	277	*Bakernema inaequale*	J	Grundy State Forest, TN	HM116036
M1	278	*Bakernema inaequale*	F	Grundy State Forest, TN	HM116037
M1	285	*Bakernema inaequale*	F	Grundy State Forest, TN	HM116038
M1	307	*Bakernema inaequale*	F	Pachaug State Forest, CT	HM116040
M1	309	*Bakernema inaequale*	M	Pachaug State Forest, CT	HM116042
M1	310	*Bakernema inaequale*	J	Pachaug State Forest, CT	HM116043
M1	311	*Bakernema inaequale*	J	Pachaug State Forest, CT	HM116044
M2	119005	*Criconema permistum*	F	Sheeder Prairie State Preserve, IA	FJ489519
M3	119010	*Criconema* sp.	J	Jasper County, MO	FJ489523
M3	119011	*Criconema* sp.	J	Jasper County, MO	FJ489524
M4	183021	*Mesocriconema crenatum*	F	Heredia Province, Costa Rica	FJ489543
M5	183030	*Criconema* sp.	F	Heredia Province, Costa Rica	FJ489545
M6	183035	*Criconemoides* sp.	J	Heredia Province, Costa Rica	FJ489546
M7	184034	*Mesocriconema* sp.		Puntarenas Province, Costa Rica	FJ489573
M8	184035	*Mesocriconema* sp.		Puntarenas Province, Costa Rica	FJ489574
M9	199012	*Criconema* sp.		Braulio Carillo National Park, Costa Rica	FJ489575
M9	199014	*Criconema* sp.		Braulio Carillo National Park, Costa Rica	FJ489577
M9	199017	*Criconema* sp.		Braulio Carillo National Park, Costa Rica	FJ489579
M9	214072	*Criconema* sp.	F	Braulio Carillo National Park, Costa Rica	HM115998
M9	214073	*Criconema* sp.	F	Braulio Carillo National Park, Costa Rica	HM115999
M10	199013	*Criconema* sp.		Braulio Carillo National Park, Costa Rica	FJ489576
M10	199015	*Criconema* sp.		Braulio Carillo National Park, Costa Rica	FJ489578
M11	214061	*Criconema* sp.	J	Las Cruces Biological Station, Costa Rica	HM115994
M12	214062	*Criconema* sp.	F	Las Cruces Biological Station, Costa Rica	HM115995
M13	214071	*Criconema* sp.	F	Braulio Carillo National Park, Costa Rica	HM115997
M14		*Criconema* sp.		GenBank	AJ966480
M15	289	*Criconema sphagni*	F	Governor Dodge State Park, WI	JF972462
M15	290	*Criconema sphagni*	J	Governor Dodge State Park, WI	JF972463
M15	291	*Criconema sphagni*	F	Governor Dodge State Park, WI	JF972464
M16	599	*Criconemoides annulatus*	F	Archuleta County, CO	JF972465
M16	603	*Criconemoides annulatus*	F	Archuleta County, CO	JF972466
M17	124095	*Criconemoides informis*		Perkins County, NE	FJ489532
M17	124097	*Criconemoides informis*		Perkins County, NE	FJ489533
M18	119007	*Criconemoides inusitatus*	F	Pammel Woods, Story County, IA	FJ489521
M18	119009	*Criconemoides inusitatus*	F	Pammel Woods, Story County, IA	FJ489522
M18	119012	*Criconemoides inusitatus*	J	Kent County, DE	FJ489525
M19	30	*Criconemoides* sp.	F	Larimer County, CO	HM116030
M20	214076	*Criconemoides* sp.	F	Xalatlaco, Mexico	FJ489591
M20	214077	*Criconemoides* sp.	F	Xalatlaco, Mexico	FJ489592
M21	132010	*Discocriconemella limitanea*	J	La Selva Biological Station, Costa Rica	EU879991
M22	132011	*Discocriconemella limitanea*	J	La Selva Biological Station, Costa Rica	EU879992
M23	138012	*Discocriconemella limitanea*	J	La Selva Biological Station, Costa Rica	EU880007
M24	138013	*Discocriconemella limitanea*	J	La Selva Biological Station, Costa Rica	EU880008
M25	138030	*Discocriconemella limitanea*	F	La Selva Biological Station, Costa Rica	EU880121
M26	151041	*Discocriconemella limitanea*	M	La Selva Biological Station, Costa Rica	FJ489535
M27	151059	*Discocriconemella limitanea*		La Selva Biological Station, Costa Rica	FJ489539
M28	183097	*Discocriconemella limitanea*	F	La Selva Biological Station, Costa Rica	FJ489549
M29	184026	*Discocriconemella limitanea*	F	Las Cruces Biological Station, Costa Rica	FJ489552
M30	184027	*Discocriconemella limitanea*	F	Las Cruces Biological Station, Costa Rica	FJ489553
M30	184030	*Discocriconemella limitanea*	F	Las Cruces Biological Station, Costa Rica	FJ489554
M31	184031	*Discocriconemella limitanea*	F	Las Cruces Biological Station, Costa Rica	FJ489555
M32	154100	*Hemicaloosia* sp.	J	Curtis Prairie, WI	HM116020
M32	164097	*Hemicaloosia* sp.	J	Curtis Prairie, WI	HM116021
M33		*Hemicriconemoides pseudobrachyurus*		GenBank	AY284622
M34		*Hemicriconemoides pseudobrachyurus*		GenBank	AY284623
M35		*Hemicriconemoides pseudobrachyurus*		GenBank	AY284624
M36	266	*Hemicriconemoides wessoni*	J	Archbold Biological Station, FL	HM116034
M37	267	*Hemicriconemoides wessoni*	F	Archbold Biological Station, FL	HM116035
M38	585	*Hemicriconemoides wessoni*	F	Ichetucknee River, Columbia County, FL	JF972467
M38	586	*Hemicriconemoides wessoni*	J	Ichetucknee River, Columbia County, FL	JF972468
M39		*Hemicycliophora conida*		GenBank	AJ966471
M40		*Hemicycliophora conida*		GenBank	EU669914
M41	571	*Hemicycliophora* sp.	F	Grundy State Forest, TN	JF972469
M42	574	*Hemicycliophora* sp.	F	Jonathan Dickinson State Park, FL	JF972470
M43	575	*Hemicriconemoides* sp.	F	Jonathan Dickinson State Park, FL	JF972471

(*continued*)

Table 1. Continued.

MOTU	NID No.	Species ID	Stage[a]	Locality	Accession No.
M44	597	*Hemicycliophora* sp.	F	Archuleta County, CO	JF972472
M44	598	*Hemicycliophora* sp.	F	Archuleta County, CO	JF972473
M44	118058	*Hemicycliophora gracilis*	J	Barta Bros. Ranch, NE	HM115993
M45	5091	*Hemicycliophora* sp.	J	Niobrara River, Cherry County, NE	HM116018
M45	5092	*Hemicycliophora* sp.	J	Niobrara River, Cherry County, NE	HM116019
M46	135032	*Hemicycliophora* sp.	F	La Selva Biological Station, Costa Rica	EU880119
M46	183098	*Hemicycliophora* sp.	J	La Selva Biological Station, Costa Rica	FJ489550
M47	214069	*Hemicycliophora* sp.	F	Las Cruces Biological Station, Costa Rica	FJ489588
M47	214070	*Hemicycliophora* sp.	F	Las Cruces Biological Station, Costa Rica	FJ489589
M48		*Hemicycliophora thienemanni*		GenBank	EU306341
M49	449	*Hemicycliophora typica*	F	Greece	JF972474
M49	450	*Hemicycliophora typica*	F	Greece	JF972475
M50	577	*Lobocriconema* sp.	F	Ichetucknee River, Columbia County, FL	JF972476
M51	1	*Lobocriconema thornei*	F	Cass County, NE	FJ489593
M51	2	*Lobocriconema thornei*	J	Cass County, NE	FJ489594
M51	226063	*Lobocriconema thornei*	F	Homestead Natl. Mon., NE	AY911948
M51	226069	*Lobocriconema thornei*	F	Nine-Mile Prairie, NE	AY911950
M51	226070	*Lobocriconema thornei*	F	Homestead Natl. Mon., NE	AY911949
M52		*Loofia thienemanni*		GenBank	AY284629
M53		*Loofia thienemanni*		GenBank	AY284628
M54	5	*Mesocriconema curvatum*	F	Kalsow Prairie State Preserve, IA	FJ489595
M54	18	*Mesocriconema curvatum*	F	Chase County, NE	HM116006
M54	19	*Mesocriconema curvatum*	F	Williams Prairie State Preserve, IA	HM116007
M54	23	*Mesocriconema curvatum*	F	Nance County, NE	HM116023
M54	24	*Mesocriconema curvatum*	F	Nance County, NE	HM116024
M54	25	*Mesocriconema curvatum*	F	Brookings County, SD	HM116025
M54	26	*Mesocriconema curvatum*	F	Brookings County, SD	HM116026
M54	119006	*Mesocriconema curvatum*	J	Sheeder Prairie State Preserve, IA	FJ489520
M54	124088	*Mesocriconema curvatum*		Polk County, NE	FJ489526
M54	124089	*Mesocriconema curvatum*		Polk County, NE	FJ489527
M54	155077	*Mesocriconema curvatum*	F	Konza Prairie, KS	AY919186
M54	223086	*Mesocriconema curvatum*	F	Lancaster County, NE	AY919190
M54	223087	*Mesocriconema curvatum*	F	Nine-Mile Prairie, NE	AY919191
M55	223083	*Mesocriconema* sp.	J	Nine-Mile Prairie, NE	AY919187
M55	223084	*Mesocriconema* sp.	F	Nine-Mile Prairie, NE	FJ489517
M56	223088	*Mesocriconema curvatum*	J	Nine-Mile Prairie, NE	FJ489518
M57	431	*Mesocriconema discus*	F	Brookings County, SD	HM116047
M57	433	*Mesocriconema discus*	F	Brookings County, SD	HM116048
M57	443	*Mesocriconema discus*	J	Brookings County, SD	HM116049
M57	444	*Mesocriconema discus*	F	Brookings County, SD	HM116050
M58	502	*Mesocriconema ornatum*	F	USDA Fruit and Nut Research Station, GA	JF972477
M58	183090	*Mesocriconema* sp.	J	Heredia Province, Costa Rica	FJ489548
M59	124090	*Mesocriconema rusticum*	F	Waldo County, ME	FJ489528
M59	124091	*Mesocriconema rusticum*		Waldo County, ME	FJ489529
M59	199022	*Mesocriconema rusticum*	F	Lamoille County, VT	FJ489580
M59	199026	*Mesocriconema rusticum*	F	Rich County, UT	FJ489582
M59	223085	*Mesocriconema rusticum*	F	Lancaster County, NE	AY919188
M59	228021	*Mesocriconema rusticum*		UNL East Campus, Lancaster County, NE	JF972478
M60	155078	*Mesocriconema rusticum*	F	Konza Prairie, KS	AY919189
M61	135026	*Mesocriconema* sp.	J	La Selva Biological Station, Costa Rica	EU880076
M62	151051	*Mesocriconema* sp.	J	La Selva Biological Station, Costa Rica	FJ489537
M62	184010	*Mesocriconema* sp.	F	La Selva Biological Station, Costa Rica	FJ489566
M62	184016	*Mesocriconema* sp.	F	La Selva Biological Station, Costa Rica	FJ489568
M62	184020	*Mesocriconema* sp.	F	La Selva Biological Station, Costa Rica	FJ489569
M63	4	*Mesocriconema xenoplax*	F	Cass County, NE	HM116002
M63	6	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	FJ489596
M63	7	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	HM116003
M63	9	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	FJ489597
M63	11	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	HM116011
M63	125027	*Discocriconemella inarata*		Kalsow Prairie State Preserve, IA	FJ489558
M63	125028	*Discocriconemella inarata*		Kalsow Prairie State Preserve, IA	FJ489559
M63	125029	*Discocriconemella inarata*		Kalsow Prairie State Preserve, IA	FJ489560
M63	150022	*Discocriconemella inarata*	J	Kalsow Prairie State Preserve, IA	FJ489561
M63	150023	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	FJ489562
M63	150032	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	FJ489563

(*continued*)

TABLE 1. Continued.

MOTU	NID No.	Species ID	Stage[a]	Locality	Accession No.
M63	150033	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	FJ489564
M63	150034	*Discocriconemella inarata*	F	Kalsow Prairie State Preserve, IA	FJ489565
M63	199025	*Mesocriconema* sp.	F	Reichelt Prairie, IA	FJ489581
M63	223080	*Mesocriconema xenoplax*	F	Konza Prairie, KS	AY919194
M63	223081	*Mesocriconema xenoplax*	F	Konza Prairie, KS	AY919193
M63	223082	*Mesocriconema xenoplax*	F	Fresno County, Fresno, CA	AY146454
M63	223089	*Mesocriconema xenoplax*	F	UC-Davis collection	AY919192
M64	17	*Mesocriconema* sp.	F	Williams Prairie State Preserve, IA	HM116005
M65		*Mesocriconema xenoplax*		GenBank	AY284625
M66		*Mesocriconema xenoplax*		GenBank	AY284626
M67		*Mesocriconema xenoplax*		GenBank	AY284627
M68	238	*Neolobocriconema serratum*	F	Douglas County, NE	HM116031
M68	124093	*Neolobocriconema serratum*		Boone County, MO	FJ489530
M68	124094	*Neolobocriconema serratum*		Boone County, MO	FJ489531
M68	150018	*Neolobocriconema serratum*	F	Boone County, MO	FJ489534
M69	151049	*Nothocriconemoides* sp.	F	La Selva Biological Station, Costa Rica	FJ489536
M69	151052	*Nothocriconemoides* sp.	F	La Selva Biological Station, Costa Rica	FJ489538
M70	155085	*Ogma decalineatum*	F	Konza Prairie, KS	AY919222
M70	226065	*Ogma decalineatum*	F	Nine-Mile Prairie, NE	AY919221
M70	226068	*Ogma decalineatum*	F	Nine-Mile Prairie, NE	AY919220
M71	226064	*Ogma fimbriatum*	F	Niobrara River, Cherry County, NE	AY911952
M72	720	*Ogma menzeli*	F	Great Smoky Mntns. Natl. Park, TN	JF972479
M72	721	*Ogma menzeli*	F	Great Smoky Mntns. Natl. Park, TN	JF972480
M72	722	*Ogma menzeli*	J	Great Smoky Mntns. Natl. Park, TN	JF972481
M73		*Ogma menzeli*		GenBank	EU669919
M74	27	*Ogma octangulare*	F	Mt. Philo State Park, VT	HM116027
M74	28	*Ogma octangulare*	F	Mt. Philo State Park, VT	HM116028
M74	29	*Ogma octangulare*	J	Mt. Philo State Park, VT	HM116029
M75	308	*Ogma seymouri*	F	Pachaug State Forest, CT	HM116041
M76	20	*Ogma* sp.	J	Marion County, OR	HM116022
M76	254	*Ogma* sp.	J	Butts County, GA	HM116032
M76	287	*Ogma* sp.	F	Sauk County, WI	HM116039
M76	314	*Ogma fimbriatum*	F	Baltimore County, MD	HM116045
M76	347	*Ogma fimbriatum*	J	Baltimore County, MD	HM116046
M76	83051	*Ogma* sp.	J	Manitoba, Canada	HM116008
M76	83052	*Ogma* sp.	J	Manitoba, Canada	HM116009
M76	83064	*Ogma* sp.	J	Lava Mountain, ID	HM116010
M76	214066	*Ogma* sp.	F	Braulio Carillo National Park, Costa Rica	FJ489585
M76	214067	*Ogma* sp.	F	Braulio Carillo National Park, Costa Rica	FJ489586
M76	214068	*Ogma* sp.	F	Braulio Carillo National Park, Costa Rica	FJ489587
M76	214078	*Criconema* sp.	F	UW Arboretum, Seattle, WA	HM116001
M76		*Ogma cobbi*		GenBank	EU669918
M77	257	*Ogma* sp.	J	Jasper County, SC	HM116033
M78	135038	*Ogma* sp.	M	La Selva Biological Station, Costa Rica	EU880151
M78	184013	*Ogma* sp.	J	La Selva Biological Station, Costa Rica	FJ489567
M78	214064	*Ogma* sp.	F	La Selva Biological Station, Costa Rica	FJ489583
M78	214065	*Ogma* sp.	F	La Selva Biological Station, Costa Rica	FJ489584
M79	184021	*Ogma* sp.	F	Las Cruces Biological Station, Costa Rica	FJ489570
M79	184022	*Ogma* sp.	F	Las Cruces Biological Station, Costa Rica	FJ489571
M79	184023	*Ogma* sp.	F	Las Cruces Biological Station, Costa Rica	FJ489572
M79	214063	*Ogma* sp.	J	Las Cruces Biological Station, Costa Rica	HM115996
M80	214074	*Ogma* sp.	F	Las Cruces Biological Station, Costa Rica	FJ489590
M80	214075	*Ogma* sp.	J	Las Cruces Biological Station, Costa Rica	HM116000
M81		*Paratylenchus dianthus*		GenBank	AJ966496
M82	155069	*Paratylenchus latescens*	F	Konza Prairie, KS	AY912039
M83		*Paratylenchus microdorus*		GenBank	AY284632
M84		*Paratylenchus microdorus*		GenBank	AY284633
M85		*Paratylenchus* cf. *neoamblicephalus*		GenBank	AY284634
M86	141021	*Paratylenchus* sp.	J	La Selva Biological Station, Costa Rica	EU880081
M87		*Paratylenchus straeleni*		GenBank	AY284630
M88		*Paratylenchus straeleni*		GenBank	AY284631
M89	226067	*Paratylenchus variatus*	F	Konza Prairie, KS	AY919230
M90	151054	*Trophotylenchulus* sp.	F	La Selva Biological Station, Costa Rica	FJ489540
M90	184002	*Trophotylenchulus* sp.	J	La Selva Biological Station, Costa Rica	FJ489551
M91	183016	*Trophotylenchulus* sp.	J	La Selva Biological Station, Costa Rica	FJ489541

(*continued*)

Table 1. Continued.

MOTU	NID No.	Species ID	Stage[a]	Locality	Accession No.
M92	183051	*Trophotylenchulus* sp.	J	La Selva Biological Station, Costa Rica	FJ489547
M93	226066	*Trophotylenchulus* sp.	J	Konza Prairie, KS	DQ080539
M93	226071	*Trophotylenchulus* sp.	J	Konza Prairie, KS	AY146455
M94	140015	*Tylenchocriconema alleni*	J	La Selva Biological Station, Costa Rica	EU880060
M95	183017	*Tylenchocriconema alleni*	F	La Selva Biological Station, Costa Rica	FJ489542
M96	183026	*Tylenchocriconema alleni*	F	La Selva Biological Station, Costa Rica	FJ489544
M97		*Tylenchulus semipenetrans*		GenBank	AJ966511
M98	14	*Xenocriconemella macrodora*	F	Minneapolis, MN	FJ489598
M98	15	*Xenocriconemella macrodora*	F	Minneapolis, MN	FJ489599
M98	16	*Xenocriconemella macrodora*	F	Minneapolis, MN	HM116004
M98	107	*Xenocriconemella macrodora*	F	Butler County, NE	JF972482
M98	164093	*Xenocriconemella macrodora*	J	Montgomery County, MD	HM116012
M98	201090	*Xenocriconemella macrodora*	F	Blue Mounds State Park, WI	HM116016
M99	193072	*Xenocriconemella macrodora*	F	CERA Woods, Grinnell, IA	FJ489556
M99	193073	*Xenocriconemella macrodora*	F	CERA Woods, Grinnell, IA	FJ489557
M99	201087	*Xenocriconemella macrodora*	F	CERA Woods, Grinnell, IA	HM116013
M99	201088	*Xenocriconemella macrodora*	F	Blue Mounds State Park, WI	HM116014
M99	201089	*Xenocriconemella macrodora*	F	Blue Mounds State Park, WI	HM116015
M100	201091	*Xenocriconemella macrodora*	F	Blue Mounds State Park, WI	HM116017

by Floyd et al., (2002). In this study, a single nucleotide difference is sufficient to designate a new MOTU. Both strands of the amplified product were sequenced in these analyses by direct sequencing at the University of Arkansas Medical Center Sequencing Facility.

We assume that each individual specimen is represented by a single 3'-18S barcode sequence. We know this is not the case among all nematodes species, as in the polyploid species of *Meloidogyne* and other select species (Abad et al., 2008; Lunt, 2008). Several specimens in this study produced nucleotide sequences that indicated heterogeneity within the barcode of that individual. Those specimens are noted in Tables 2 and 3. All sequences used in this study have been added to GenBank (Table 1).

Each specimen is supplied with a voucher identification number or Nematode ID (NID) number. These numbers have been applied sequentially and chronologically. In some cases NID numbers were applied retroactively. When multiple amplifications are made from a single specimen, a unique amplification number is associated with the NID number. MOTU designations were applied following the pooling of redundant sequences by the Redundant Taxa tool in Maclade.

DNA preparation, sequence alignment, phylogenetic and character-based analysis: DNA was amplified and sequenced as previously described (Powers et al., 2010). 18S sequences were edited and assembled using CodonCode Aligner (CodonCode Corp, Dedham, Massachusetts), DNA aligned by MUSCLE 3.7 (Edgar, 2004) and maximum likelihood analysis generated by PHYML 3.0 using approximate likelihood-ratio tests for the estimation of branch support (Anisimova et al., 2006). The FASTA file for the MOTU dataset is available in Dryad (DOI-pending).

Character-based barcode analysis of nucleotide sequences is an alternative approach to species diagnosis using DNA barcodes (DeSalle et al., 2005; Sarkar et al., 2008). It differs from the more traditional method of barcode analysis in that it is not a distance-based approach, but rather treats the nucleotide sites in a DNA sequence as characters and the different character states, A,T,C,G, are referred to as character attributes (CA) (Sarkar et al., 2002) or nucleotide diagnostics (ND) (Wong et al., 2009). A nucleotide diagnostic can be designated simple and pure when a particular nucleotide is fixed for a particular species, and found in all members of that species and no others. Compound nucleotide diagnostics consist of several nucleotide sites where the combination of nucleotides at those sites is only found in one species. In this study only pure, simple nucleotide diagnostics are analyzed. In large datasets, the first step in character-based barcode analysis is the generation of a phylogenetically derived guide tree which is subsequently examined node by node for the presence of diagnostic nucleotides. The computer program CAOS (Characteristic Attributes Organization System) is an automated method for the discovery of nucleotide diagnostics (Sarkar et al., 2008). The 100-MOTU 3'-18S barcode dataset was simple enough to conduct a manual analysis of nucleotide diagnostics using the maximum likelihood tree and its major clades as a guide tree.

Online access: Images and measurements of terminal taxa from the barcode tree are available online (http://nematode.unl.edu/CriconematidProject_Trees.htm). Individual specimens are listed by their NID numbers in Table 1.

Results

Barcode characteristics: This dataset is comprised of 100 18S barcode MOTUs derived from 214 sequences from nematodes in the suborder Criconematina (Table 1). The ClustalW alignment is 602 nucleotides in length

Table 2. Clade A polymorphic and diagnostic nucleotide positions with diagnostic characters. Diagnostic nucleotides are those shared by all individuals of a species and not found in any other species. The diagnostic nucleotide characters are shaded with a bold outline. Synapomorphic characters are shaded without a bold outline. Numbers after taxon labels refer to the number of specimens examined. GB refers to sequences obtained from GenBank. Numbering of the nucleotide position starts with 1 which is the first nucleotide following primer 18S1.2a.

MOTU	Taxa	Position 15	38	42	43	46	47	48	67	187	252	328	329	333	340	341	349	350	351	352	359	361	362	363	364	365	381	386	391	392	400	401	472	476	489	513	515	519	523	564	568	572	593
M2	*Criconema permistum* (1)	G	C	T	T	C	T	T	C	A	T	A	C	C	G	G	G	T	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A	A	C	C	G	A	G	G	A	C	G
M3	*Criconema* sp. (2)	G	T	T	T	C	T	A	C	A	T	A	C	C	G	G	G	T	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M5	*Criconema* sp. (1)	G	T	T	T	T	T	A	C	A	T	A	C	C	G	G	G	T	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M9	*Criconema* sp. (5)	G	T	T	T	T	T	T	C	A	T	A	C	C	G	G	G	T	T	T	C	T	G	—	—	T	T	T	T	C	C	A	A	A	C	T	G	A	G	G	A	C	G
M10	*Criconema* sp. (2)	G	C	T	T	T	G	C	C	A	T	A	C	C	G	G	G	C	T	T	C	C	G	—	—	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M11	*Criconema* sp. (1)	G	C	T	T	C	T	C	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A/G	G	C	T	G	A	G	G	A	C	G
M12	*Criconema* sp. (1)	G	T	T	T	C	T	T	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A	A	C	T	G	A	A/G	G	A	C	G
M13	*Criconema* sp. (1)	G	T	T	T	T	T	T	C	A	T	A	T	C	G	G	G	C	T	T	C	T	G	—	—	T	T	T	T	C	C	A	A	A	C	T	A	A	G	G	A	C	G
M14	*Criconema* sp. GB (1)	G	C	T	T	C	T	C	C	A	T	A	C	C	G	G	G	C	C	T	C	C	G	—	—	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M15	*Criconema sphagni* (3)	G	C	T	T	C	T	T	C	A	T	A	C	C	G	G	G	C	C	T	T	T	G	—	—	T	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M33	*Hemicriconemoides pseudobrachyurus* GB (1)	G	C	T	C	T	A	C	C	A	T	A	C	C	G	G	G	C	T	T	C	T	G	—	—	T	T	T	T	C	T	A	G	G	C	T	G	A	G	G	A	C	G
M34	*Hemicriconemoides pseudobrachyurus* GB (1)	G	C	T	C	T	A	C	C	A	T	A	C	C	G	G	G	C	T	T	C	T	G	—	—	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M35	*Hemicriconemoides pseudobrachyurus* GB (1)	G	C	T	C	T	A	C	C	A	A	A	C	C	G	G	G	C	T	T	C	T	G	—	—	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	T	C	G
M36	*Hemicriconemoides wessoni* (1)	G	T	T	C	T	T	T	C	A	T	A	C	C	T	A	G	C	C	T	C	C	T	—	—	G	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M37	*Hemicriconemoides wessoni* (1)	G	T	T	C	T	T	T	C	A	T	A	C	C	T	G	G	C	C	T	C	C	T	—	—	G	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M38	*Hemicriconemoides wessoni* (2)	G	T	T	C	T	T	T	C	A	T	A	C	C	G	G	G	C	C	T	C	T	T	—	—	G	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M43	*Hemicriconemoides* sp. (1)	G	T	T	C	T	T	T	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M70	*Ogma decalineatum* (3)	G	C	T	T	C	T	C	T	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	C	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M71	*Ogma fimbriatum* (1)	G	C	T	T	C	T	C	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	T	T
M72	*Ogma menzeli* (3)	G	C	T	T	C	T	C	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	G	A	C	T	G	A	G	G	A	C	G
M73	*Ogma menzeli GB* (1)	G	C	T	T	C	T	C	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	G	G	G	C	T	G	A	G	G	A	C	G
M74	*Ogma octangulare* (3)	G	C	T	T	C	T	C	T	A	T	A	C	C	G	G	G	C	T	T	C	T	G	—	—	T	T	T	C	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M75	*Ogma seymouri* (1)	G	C	T	T	C	T	C	C	G	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	C	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M76	*Ogma* sp. (13)	G	C	T	T	C	T	C	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M77	*Ogma* sp. (1)	G	T	T	A	C	A	A	C	A	T	A	C	C	G	G	G	T	T	T	C	C	G	—	—	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M78	*Ogma* sp. (4)	G	T	T	T	C	T	C	C	A	T	A	C	A	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A	G	C	T	G	A	G	T	A	C	G
M79	*Ogma* sp. (4)	A	T	C	T	C	T	C	C	G	T	G	C	C	G	G	G	C	C	T	C	T	G	—	—	T	C	C	T	T	C	A	A	G	T	T	G	G	G	G	A	C	G
M80	*Ogma* sp. (2)	G	C	T	T	C	T	C	C	A	T	A	C	C	G	G	G	C	C	T	C	T	G	—	—	T	T	T	T	C	C	A	A	G	C	T	G	A	G	G	A	C	G
M98	*Xenocriconemella macrodora* (6)	G	C	T	C	T	A	A	C	G	T	G	C	C	G	G	A	C	C	G	C	C	G	T	C	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M99	*Xenocriconemella macrodora* (5)	G	T	T	C	T	A	A	C	G	T	G	C	C	G	G	A	C	C	G	C	C	G	T	C	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G
M100	*Xenocriconemella macrodora* (1)	G	C/T	T	C	T	A	A	C	G	T	G	C	C	G	G	A	C	C	G	C	C	G	T	C	T	T	T	T	C	C	A	G	G	C	T	G	A	G	G	A	C	G

TABLE 3. Clade B polymorphic and diagnostic nucleotide positions with diagnostic characters. Diagnostic nucleotides are those shared by all individuals of a species and not found in any other species. The diagnostic nucleotide characters are shaded with a bold outline. Numbers after taxon labels refer to the number of specimens examined. GB refers to sequences obtained from GenBank. Numbering of the nucleotide position starts with 1 which is the first nucleotide following primer 18S1.2a.

		Position																								
MOTU	Taxa	38	43	44	45	46	53	212	333	349	350	351	353	361	363	364	367	400	476	488	489	491	500	502	503	512
M4	*Mesocriconema crenatum* (1)	T	A	—	—	T	C	G	C	G	C	T	C	T	C	T	A	C	A	G	A	A	T	C	C	C
M21	*Discocriconemella limitanea* (1)	T	G	—	—	C	T	G	C	G	T	C	C	C	T	C	A	C	G	T	G	G	C	T	C	C
M22	*Discocriconemella limitanea* (1)	C	C	T	A	C	C	G	A	G	C	C	T	C	T	—	A	C	G	T	G	G	C	T	G	A
M23	*Discocriconemella limitanea* (1)	C	C	T	A	C	C	G	A	G	C	T	C	C	T	—	A	C	G	T	G	G	C	T	G	A
M24	*Discocriconemella limitanea* (1)	C	C	T	A	C	C	G	A	G	C	C	C	C	T	—	A/G	C	G	T	G	G	C	T	G	A
M25	*Discocriconemella limitanea* (1)	C	G	—	—	C	C	A	C	G	T	C	C	C	T	C	A	C	G	T	G	G	T	C	G	C
M26	*Discocriconemella limitanea* (1)	C	C	T	A	C	C	G	A	G	C	C	C/T	C	T	—	A	C	G	T	G	G	C	T	G	A
M27	*Discocriconemella limitanea* (1)	T	G	—	—	C	C	G	C	G	T	C	C/T	C	T	C	A	C	G	T	G	G	C	T	C/G	C
M28	*Discocriconemella limitanea* (1)	C	C	T	A	C	C	G	A	G	C	C	C	C	T	—	A	C	G	T	G	G	C	T	G	A
M29	*Discocriconemella limitanea* (1)	C	G	—	—	C	T	G	C	C	T	C	C	C	T	C	A	A	G	T	G	G	C	T	C	C
M30	*Discocriconemella limitanea* (2)	C	G	—	—	C	T	G	C	G	T	C	C	C	T	C	A	C	G	T	G	G	C	C	C	C
M31	*Discocriconemella limitanea* (1)	C	C	T	T	C	C	G	A	G	C	C	T	C	T	—	A	C	G	T	G	G	C	T	G	A

which includes 10 hypothesized sites of nucleotide insertion or deletion (indels). There are 470 (78%) invariant and 132 polymorphic nucleotide sites in the dataset. Among the polymorphic nucleotide sites, 56 (42%) are singletons, positions where a single MOTU has a nucleotide not shared by any others in the dataset.

Barcode species analysis: The dataset includes 25 nominal species identified by the authors through microscopic examinations of morphological characteristics. A maximum likelihood tree for the 100 MOTUs is presented in Figure 1. Four clusters with moderate support values (0.80-0.93) have been identified and were labeled A-D for character-based barcode analysis.

Within clade A, there are nine morphologically identified nominal species not considering GenBank entries (Fig. 1, Table 2). Included in this clade are species that morphologically fall within the genera *Ogma*, *Xenocriconemella*, *Criconema*, and *Hemicriconemoides*. Five of the *Ogma* species possessed morphological characters that permitted assignment to known species. However, neither phylogenetic analysis nor character-based barcode analysis recognized all *Ogma* MOTUs as collectively comprising a natural group exclusive of the other genera in the clade. *Ogma decalineatum* and *O. octangulare* shared a T at nucleotide 67 to the exclusion of all other MOTUs in clade A (Table 2). Another nucleotide character (C) at position 391 provides evidence for relatedness of these two species to *O. seymouri*. The *O. menzeli* MOTU from Tennessee (M72) differs by two nucleotides from the European *O. menzeli* in GenBank (M73). M76 is a broadly distributed MOTU, one of only two MOTUs found in both Costa Rica and the United States. Additionally, it shares 100% identity with GenBank accession EU669918, an *O. cobbi* reported from Europe. Morphologically, the adult females that represent M76 include a range of phenotypes, particularly in the arrangement of scales on the adult female cuticle.

Xenocriconemella macrodora is represented by 12 specimens and three MOTUs (M98, M99, M100) collected from five U.S. states. There are four diagnostic nucleotide sites, including two insertions, which are observed in every specimen of this species. These are found at nucleotide positions 349, 352, 363, and 364. *Hemicriconemoides wessoni* was collected at two sites in Florida, one site within 60 miles of the type locality. Three MOTUs were observed for this species, each diagnosable by nucleotides T and G at positions 362 and 365 respectively. *Criconema permistum* and *C. sphagni* were represented by one and three specimens respectively, each containing a single, unique fixed nucleotide. Other *Criconema* species in clade A are not united by shared derived characters, reflecting a lack of phylogenetic support for the genus.

Clade B, with the exception of a single MOTU (M4), is exclusively represented by *Discocriconemella limitanea* from Costa Rica (Table 3). The clade is well-supported phylogenetically. *D. limitanea* is represented by 12 specimens and 11 MOTUs which break into two discrete subgroups. There are six nucleotide sites that separate the two subgroups. Morphologically, however, there are no characters that appear to discriminate between the subgroups, and both subgroups are found in Las Cruces and La Selva Biological Research Stations, geographically distinct rainforest habitats of Costa Rica. MOTU M4 was recovered from cultivated passionfruit in Costa Rica and conforms morphologically to *Mesocriconema crenatum* (Loof, 1964) De Grisse & Loof, 1965.

Clade C includes six nominal species identified by morphology (Table 4). Both phylogenetic analysis and character-based barcode analysis support *Mesocriconema rusticum* and *M. curvatum* as diagnosable species within this clade. Nucleotide sites at 472 and 488 diagnose *M. rusticum*, and an additional two synapomorphic characters at sites 46 and 503 support a sister group relationship with *M. ornatum*. *Mesocriconema rusticum* was

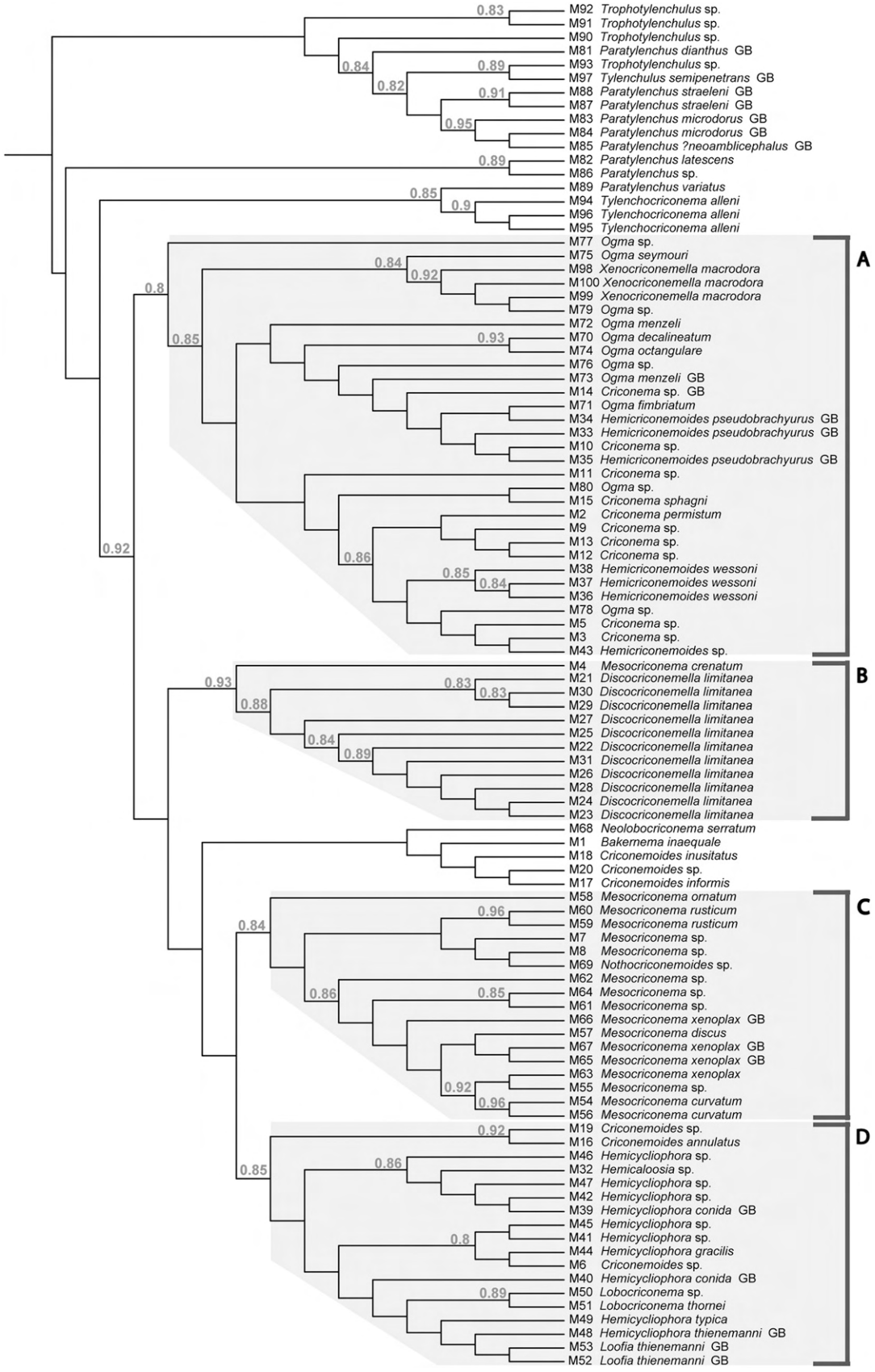

Fig. 1. Maximum likelihood tree of Criconematina 18S 3' MOTUs. Shaded clades A-D were analyzed separately by character-based barcode analysis. Species binomials followed by GB were sequences added to the analyses from GenBank. Images and measurements of terminal taxa can be seen at http://nematode.unl.edu/CriconematidProject_Trees.htm Approximate likelihood-ratio test support values above 0.80 identify nodes of relatively strong support.

TABLE 4. Clade C polymorphic and diagnostic nucleotide positions with diagnostic characters. Diagnostic nucleotides are those shared by all individuals of a species and not found in any other species. The diagnostic nucleotide characters are shaded with a bold outline. Synapomorphic characters are shaded without a bold outline. Numbers after taxon labels refer to the number of specimens examined. GB refers to sequences obtained from GenBank. Numbering of the nucleotide position starts with 1 which is the first nucleotide following primer 18S1.2a.

MOTU	Taxa	Position 10 31 34 36 37 38 43 46 47 48 49 53 56 59 75 109 197 212 214 322 341 349 351 353 360 365 367 369 384 398 400 408 472 476 483 487 488 491 496 503 504 544 561 564 574 579 581 582 592 599 600 601
M7	*Mesocriconema* sp. (1)	T C C G G T A C A A G C G G C C C G G C G G C T C G A T G G C G G G T C G T A C G T A G T A T G A G T T
M8	*Mesocriconema* sp. (1)	T C C G G T A C A A G C G G C C C G G C G G C C C G A T G G C G G G T C G T A C A T A G T A T G A G T T
M54	*Mesocriconema curvatum* (13)	T T G G G T A C A A G C C A C C C A G C A G C C T T A T A G T G G A T C G T A C G T A G T A T G A G T T
M55	*Mesocriconema* sp. (2)	T C C G G T A C C A G C G G C C C A A C A G C C T T A T A G T G G A T T G T A C G T A G T A T G A G T T
M56	*Mesocriconema curvatum* (1)	T T G G G T A C A A G C C A C C C A G C A G C C T T A T A G T G G A T C G T A C G A G C G A T C C G T T
M57	*Mesocriconema discus* (4)	T C C G G T A C C A G C G G C C C G G C A G C C T G A T G G C G G A T C G T A C G T A G T A T G A G T T
M58	*Mesocriconema ornatum* (2)	T C C G G C C T A C G C G G C C C G G C G G C C C G A T G G C G G G T C G T A G G T A G T A T G A G T T
M59	*Mesocriconema rusticum* (6)	T C C G G T C T A A G C G G C C C G G C G G C T C G A T G G C G A G T C T T A G G T A G T A T G A G T T
M60	*Mesocriconema rusticum* (1)	C C C G G T C T A A G C G G C C C G G C G G C T C G A T G G C G A G T C T T A G G T A G T A T G A G T T
M61	*Mesocriconema* sp. (1)	T C C G G C T C C A G C G G C C C A G C A A C C T G A T G G C G G A T C G T A C G T A G T A T G A G T T
M62	*Mesocriconema* sp. (4)	T C C G G T T C C A A C G G C C C A G T A G T C T T A T G G C A G G T C G T A C G T A G T A T G A G T T
M63	*Mesocriconema xenoplax* (18)	T C C G G T A C C A G C G G C C C A G C A A C C T G A T A G T G G A T C G T A C G T A G T A T G A G T T
M64	*Mesocriconema* sp. (1)	T C C G G C A C C A G C G G C C C A G C A G C C T G A T G G C G G A T C G T A C G T A G T A T G A G T T
M65	*Mesocriconema xenoplax* GB (1)	T C C G G T A C C A G C G G T C C A G C A G C C T T G C G G C G G A T C G T A C G T A G T A T G A G T T
M66	*Mesocriconema xenoplax* GB (1)	T C C G G T A C C A G C A G C T C A G C A A C C T G A T G G C G G A T C G T G C G T A G T A T G A G T T
M67	*Mesocriconema xenoplax* GB (1)	T C C G G T A C C A G C G G C C A A G C A A C C T G A T G G C G G A C C G T A C G T A G T A T G A G T T
M69	*Nothocriconemoides* sp. (2)	T C C A C T C C C A A T G G C C C G G C G G C C C G A T G A C G G G T C G G A C G T A G T G C G A A C C

represented by 6 specimens and two MOTUs collected from 5 U.S. states. Four nucleotide sites, 31, 34, 56, and 59 diagnose *M. curvatum*. MOTU M63 was represented by 18 specimens and includes two morphologically identifiable species *M. xenoplax* and *Discocriconemella inarata*. A previous paper has addressed the more detailed taxonomy of these two species (Powers et al., 2010). There are no diagnosable characters in this 18S barcode for discrimination between *M. xenoplax* and *D. inarata*. Three additional MOTUs, M65, M66 and M67 from GenBank have been identified as *M. xenoplax* from Europe. *Mesocriconema discus* (M57) was collected at its type locality in South Dakota, however there were no discrete nucleotide characters that could be considered as diagnosable nucleotide sites.

Clade D was largely comprised of *Hemicycliophora* species, the two related sheath genera *Hemicaloosia* and *Loofia*, *Lobocriconema*, and *Criconemoides* species (Table 5). *Criconemoides annulatus* (M16), represented by two specimens from the Rocky Mountains in Colorado, possessed three diagnostic nucleotide sites. *Lobocriconema thornei* (M51) and a closely related *Lobocriconema* species (M50) had four synapomorphic sites, and each was diagnosable by a single autapomorphic site. Among the sheath genera, two synapomorphic nucleotide sites at 43 and 47 united all specimens. *Hemicycliophora gracilis*, represented by a single MOTU collected in Colorado and Nebraska, possessed five autapomorphic diagnostic sites. *Hemicycliophora typica* and the two species from GenBank in this dataset did not possess diagnosable nucleotides in the 18S barcode.

Five notable, diagnosable species in the 100-MOTU dataset did not fall within clades A-D (Table 6). *Bakernema inaequale* is a species endemic to North America and immediately recognizable by its irregularly arranged, membranous cuticular scales. Seven specimens from Tennessee and Connecticut shared a single MOTU (M1) and were diagnosable in the full dataset by an A at nucleotide position 63. *Criconemoides informis* (M17) had two diagnostic nucleotides: an A and T at positions 343 and 357, respectively. *Criconemoides inusitatus* (M18), collected from the type locality in Ames, IA and from Delaware, had a single diagnostic nucleotide site at position 365. A species morphologically conforming to *Neolobocriconema serratum* (M68) collected from Missouri and Nebraska, had a single diagnostic site at position 360. Three MOTUs (M94, M95, M96) represented the unusual criconematid nematode *Tylenchocriconema alleni*, a species known solely from epiphytic bromeliads in the new world tropics (Raski and Siddiqui, 1975). Two nucleotide sites at 347 and 348 were diagnostic for the three MOTUs.

Discussion

The small number of phylogenetically informative nucleotide sites (76) and the relatively few well-supported clades observed in the maximum likelihood tree indicate that limited phylogenetic inference can be derived from this 3' region of 18S. None of the well-supported clades could be interpreted as support for the existing morphologically-based classification of Criconematina sensu Siddiqi (2000). Conversely, there is not strong support for alternative groupings of MOTUs. Simply there are not enough phylogenetically informative sites in this 18S barcode to construct a robust phylogeny. Subbotin et al., (2005, 2006) arrived at a similar conclusion with analysis of the D2/D3 region of 28S rDNA. Those studies included 23 nominal taxa from 11 genera. The 38 samples analyzed exhibited a geographic coverage that included two specimens from North America, 12 from Venezuela, and the remaining specimens from Europe. According to the authors, "none of the phylogenetic analyses of the D2-D3 dataset allowed resolution of the relationships between main lineages."

Lack of phylogenetic resolution does not mean that the 3'-18S barcode does not have value as a measure of biodiversity or as an aid in diagnostics. A major advantage of the primer set is that PCR amplification is consistent and reliable across the entire nematode phylum. That consistency allows for an unbiased comparison of nematode community composition. Within the suborder Criconematina, barcode discrimination is at multiple taxonomic levels. In some cases a single MOTU clearly identified a complex of species. MOTU 76, for example, corresponded to a group of *Ogma* species that have scales arranged singularly in longitudinal rows along the length of the body, or arranged in rows consisting of clusters of 4-6 scales, or with scales densely packed on the annules forming a continuous elongated fringe. Similarly MOTU 63 consists of geographically wide-spread North American isolates that conform to *Mesocriconema xenoplax* and *Discocriconemella inarata*, a grassland species that appears to have secondarily lost the submedian lobes (Powers et al., 2010). In other cases, multiple MOTUs seem to correspond to a morphologically conserved species complex. *Discocriconemella limitanea* is comprised of multiple MOTUs with no indication of corresponding morphological change. The nucleotide variability within the barcode identifies subgroups that may suggest the existence of cryptic species. Here the barcode analysis has provided initial evidence in the species discovery process and should be followed by a complete taxonomic analysis to resolve the taxonomic status of the subgroups.

The absence of a direct correspondence between MOTUs as defined in this study (1 bp cutoff) and morphologically identified species suggest that the MOTUs generated by the 3'-18S barcode should not be uncritically considered as proxies for species. The relationship between MOTUs and species can be evaluated by character-based DNA barcode analysis, which is a method to discover diagnostic characters in species where the delimitation step has already been established

TABLE 5. Clade D polymorphic and diagnostic nucleotide positions with diagnostic characters. Diagnostic nucleotides are those shared by all individuals of a species and not found in any other species. The diagnostic nucleotide characters are shaded with a bold outline. Synapomorphic characters are shaded without a bold outline. Numbers after taxon labels refer to the number of specimens examined. GB refers to sequences obtained from GenBank. Numbering of the nucleotide position starts with 1 which is the first nucleotide following primer 18S1.2a.

		Position																																						
MOTU	Taxa	25	38	42	43	46	47	48	89	151	152	158	212	333	339	340	341	351	352	353	359	360	361	362	363	364	365	366	476	488	489	496	503	507	513	519	541	571	572
M6	*Criconemoides* sp. (1)	A	T	T	T	C	T	A	T	G	T	T	G	C	C	A	G	T	T	C	C	C	T	T	T	C	G	C	A	G	T	G	C	A	T	G	G	A	C
M16	*Criconemoides annulatus* (2)	A	T	T	T	C	T	T	T	G	A	G	A	A	T	G	G	C	T	C	C	T	T	C	—	—	T	C	G	T	T	A	G	A	T	A	A	T	C
M19	*Criconemoides* sp. (1)	A	T	T	T	C	T	A	T	G	A	G	A	A	C	G	G	C	T	C	C	T	T	G	—	—	T	C	G	G	T	A	C	A	T	A	A	T	C
M32	*Hemicaloosia* sp. (2)	A	T	T	C	T	A	C	T	G	A	G	G	A	C	T	G	T	T	C	C	T	A	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M39	*Hemicycliophora conida* GB (1)	A	C	T	C	C	A	A	T	G	A	G	G	A	C	G	G	C	T	C	C	C	A	T	—	—	G	T	G	G	T	A	C	A	T	A	A	T	C
M40	*Hemicycliophora conida* GB (1)	A	T	T	C	C	A	A	C	G	A	G	G	A	C	G	G	T	A	C	C	C	A	G	—	—	G	C	G	G	T	A	C	A	T	A	A	T	T
M41	*Hemicycliophora* sp. (1)	A	T	T	C	T	A	C	T	G	A	G	G	A	C	A	G	T	A	C	T	T	T	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M42	*Hemicycliophora* sp. (1)	A	C	T	C	C	A	A	T	G	A	G	G	A	C	G	G	T	A	T	C	C	A	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M44	*Hemicycliophora gracilis* (3)	A	T	C	C	T	A	T	T	A	G	G	G	A	C	T	A	T	A	C	C	C	A	T	—	—	G	C	A	G	T	A	C	A	T	A	A	C	T
M45	*Hemicycliophora* sp. (2)	A	C	T	C	T	A	C	T	G	A	G	G	A	C	A	G	T	A	C	C	A	T	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M46	*Hemicycliophora* sp. (2)	A	C	T	C	C	A	A	T	G	A	G	A	A	C	G	G	C	A	T	C	C	A	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M47	*Hemicycliophora* sp. (2)	A	C	T	C	C	A	A	T	G	A	G	G	A	C	G	G	C	A	T	C	C	A	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M48	*Hemicycliophora thienemanni* GB (1)	A	T	T	C	T	A	C	T	G	A	G	G	A	C	G	G	T	A	T	C	T	A	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M49	*Hemicycliophora typica* (2)	A	T	T	C	T	A	A	T	G	A	G	G	A	C	G	G	T	A	C	C	C	A	G	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C
M50	*Lobocriconema* sp. (1)	A	T	T	A	T	C	T	T	G	A	G	G	A	C	G	G	T	T	C	C	C	T	C	—	—	T	C	G	A	T	A	T	A	C	A	A	T	C
M51	*Lobocriconema thornei* (5)	A	T	T	A	T	C	T	T	G	A	G	G	A	C	G	G	T	T	C	C	C	T	C	—	—	T	C	G	A	C	A	T	A	T	A	A	T	C
M52	*Loofia thienemanni* GB (1)	A	T	T	C	T	A	C	T	G	A	G	G	A	C	G	G	T	A	T	C	T	A	T	—	—	G	C	G	G	T	A	C	G	T	A	A	T	C
M53	*Loofia thienemanni* GB (1)	G	T	T	C	T	A	C	T	G	A	G	G	A	C	G	G	T	A	T	C	T	A	T	—	—	G	C	G	G	T	A	C	A	T	A	A	T	C

TABLE 6. Diagnostic nucleotide positions for species not included in clades A-D. These species compared against the entire Criconematina data set. Numbers after taxon labels refer to number of specimens examined. Numbering of the nucleotide position starts with 1 which is the first nucleotide following primer 18S1.2a.

MOTU	Taxa	At position	Has	All others have
M1	*Bakernema inaequale* (7)	63	A	G
M17	*Criconemoides informis* (2)	343	A	C
		357	T	G
M18	*Criconemoides inusitatus* (3)	365	A	G/T
M68	*Neolobocriconema serratum* (4)	360	G	C/T
M94	*Tylenchocriconema alleni* (1)	347	T	—
		348	C	—
M95	*Tylenchocriconema alleni* (1)	347	T	—
		348	C	—
M96	*Tylenchocriconema alleni* (1)	347	T	—
		348	C	—

(DeSalle et al., 2005; DeSalle 2006; Kelly et al., 2007; Rach et al., 2008; Wong et al., 2009; Naro-Maciel et al., 2010). As a character-based approach it is compatible with traditional morphological identification systems in its recognition of diagnostic characteristics based on the assumption that members of established taxonomic groups share attributes that are absent from comparable groups (Sarkar et al., 2002; Rach et al. 2008). *Bakernema inaequale*, for example, is diagnosable by the presence of irregularly spaced membranous scales on the cuticle and an A at nucleotide position 63 in the 3'-18S barcode. *Xenocriconemella macrodora* is diagnosable by an approximately 100 *u*m flexible stylet, an A and G substitution at positions 349 and 352 respectively, plus a TC insertion at position 363-364. In the 100-MOTU Criconematina dataset, 14/25 *a priori* identified species had at least one diagnostic character. Moreover, in several cases, while no diagnosable nucleotide characters were recognized at the species level, a synapomorphic character was present that indicated grouping at a higher taxonomic level (e.g. *Ogma decalineatum*, *O. octangulare*, *O. seymouri)*. Given the evolutionarily conserved nature of the 3' portion of the 18S gene, it is surprising that over 50% of the known species would possess putative diagnostic nucleotides. Alternative explanations for the apparent diagnostic signal could be attributed to sequencing error, insufficient sampling of species and populations, or misidentification of the nominal species. The validation of these results will require increased sampling of species throughout their known range. These caveats notwithstanding, from a biodiversity and biogeographic perspective the application of this barcode to a comparison of nematode communities could hasten the effort to describe the pattern of nematode diversity as it currently exists at the landscape scale. Also the characterization of new MOTUs will identify gaps in the taxonomic knowledge and lead to species discovery. Furthermore, it is important to emphasize that barcode approaches, whether they target individual specimens or an entire community of specimens, are still dependent on reference databases to convey meaningful taxonomic information, with the recognition that sequences alone, apart from their biological context, are limited in their systematic value (Hajibabaei et al., 2007; Stevens et al., 2011).

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