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## An 18S rDNA Perspective on the Classification of Criconematoidea

THOMAS POWERS, TIMOTHY HARRIS, REBECCA HIGGINS, PETER MULLIN, AND KIRSTEN POWERS

**Abstract:** In the nematode family Criconematidae, a taxonomy primarily based on cuticle characters has created classifications that are notoriously volatile. Molecular characters may lead to their stabilization. A phylogenetic tree of Criconematoidea was constructed using 166 new near full-length 18S rDNA sequences and 58 sequences from GenBank. Bayesian and maximum likelihood (ML) analyses produced trees with similar topologies. Major features include a strongly supported clade that includes Criconematidae and Hemicycliophoridae, excluding Paratylenchidae and Tylenchulidae. Another well-supported clade groups *Criconema*, *Ogma*, *Crossonema*, and *Hemicriconemoides* plus *Xenocriconemella*, combining nematodes with cuticular scales with those without scales at any life stage. *Mesocriconema*, *Discocriconemella limitanea*, *Hemicallosia*, and *Lobocriconema* are recognized as monophyletic groups, but *Criconemoides* is paraphyletic. Both trees support an unexpected sister relationship between *Bakerinema* and *Hemicycliophora*. The 18S rDNA dataset was insufficient for distinguishing genus boundaries between *Criconema*, *Ogma*, and *Crossonema*. The relationships depicted by the 18S rDNA phylogeny suggest that key morphological characters used in the classification of Criconematidae are not homologous.

**Key words:** 18S rDNA, classification, criconematidae, phylogeny, systematics, taxonomy.

In the molecular-based, 18S rDNA nematode classifications of De Ley and Blaxter (2004) and Meldal et al. (2007), Criconematoidea is recognized as a superfamily of plant-parasitic nematodes in the suborder Tylenchina and the infraorder, Tylenchomorpha. As a globally distributed group, Criconematoidea has been widely considered a monophyletic group based on the structure of the pharynx, sexual dimorphism, a monopodelphic ovary without a post vulva sac, and loss of phasmids and deirids (Siddiqi, 2000; Subbotin et al., 2005; Andrassy, 2007). There is virtual unanimity regarding the monophyly of the group as a whole (Holterman et al., 2009; Bert et al., 2011; Cid Del Prado Vera and Talavera, 2012), with some variations in the taxonomic level applied to classify the group (suborder Criconematina vs. superfamily Criconematoidea). In contrast to the consensus of opinion regarding monophyly of the superfamily are the disagreements concerning families, subfamily, and genus level groupings. Some of the disagreements are merely nomenclatural in nature. For example, the economically important criconematid species known for its association with Peach Tree Shortlife (Nyczepir et al., 1983) and global distribution, has been referred to as *Mesocriconema xenoplax* (Raski, 1952) Loof, 1989 in the classifications of Brzeski et al. (2002b), Subbotin et al. (2005), Andrassy (2007), and Geraert (2010). The same species has also been called *Macroposthonia xenoplax* by Siddiqi (2000) and Wouts (2006), *Criconemella xenoplax* by Xiang et al. (2010) and Mitchum et al. (2013), and *Criconemoides xenoplax* by Decraemer and Geraert (2006), Decraemer and Hunt (2006), and Cid Del Prado Vera and Talavera (2012). Nomenclatural issues aside, a larger systematic issue is the phylogenetic evidence that may, or may not exist, in

support of subfamily, genus, and subgenus groupings. In the classifications of Decraemer and Hunt (2006) and Van den Berg et al. (2017), a single group, *Criconemoides* Taylor, 1936 is proposed to represent the aforementioned genera, whereas the classifications of Andrassy (2007), Brzeski et al. (2002a, 2002b), Geraert (2010), Siddiqi (2000), and Wouts (2006) recognize two separate genera, *Mesocriconema* (*Macroposthonia*) and *Criconemoides* (Table 1). Maggenti et al. (1988) considered both *Macroposthonia* and *Criconemoides* genera *dubia*, preferring instead the single genus *Criconemella* De Grisse and Loof, 1965. The validity of phylogenetic groupings within Criconematina was addressed by Subbotin et al. (2005) using the D2/D3 region of 28S rDNA. The results of their analyses highlighted several taxonomic questions such as the distant relationship of *Mesocriconema sphaerocephalum* (Taylor, 1936) Loof and De Grisse, 1989 to other species of *Mesocriconema* Andrassy, 1965 and the relatively distant relationship between the two sheath-forming genera, *Hemicriconemoides* Chitwood and Birchfield, 1957 and *Hemicycliophora* de Man, 1921, but otherwise found little “resolution of relationships between main lineages” (Subbotin et al., 2005). An ML D2/D3 tree from that study modified by collapsing nodes unsupported by bootstrap values of at least 50% is presented in Fig. 1. A lack of taxonomic resolution among criconematid genera was similarly noted by Zeng et al. (2015) in their 18S rDNA phylogenetic analysis.

In the present study, we have constructed phylogenetic trees of Criconematoidea specimens from 224 18S rDNA sequences which include 58 sequences from GenBank. We compare the relationship of the groups formed in the 18S trees to existing classifications and generic definitions. Taxonomic implications and recommendations for future research are drawn from 18S rDNA and cytochrome oxidase subunit I(COI) DNA sequences.

### MATERIALS AND METHODS

**Nematode collection:** Nematodes used in this analysis were part of an ecoregion survey of criconematid nematodes of North America collected from 2010 to 2016. Soil samples

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TABLE 1. Classifications of Criconematidae.

Classification of Criconematidae according to Geraert, 2010 (5 subfamilies, 18 genera)	Classification of Criconematidae according to Maggenti et al., 1988 (2 subfamilies, 11 genera)
Family: Criconematidae Taylor, 1936 (1914) (Thorne, 1949) <b>Subfamily: Hemicriconemoidinae Andrassy, 1979</b> <ul style="list-style-type: none"> <li>Genus: <i>Hemicriconemoides</i> Chitwood &amp; Birchfield, 1957</li> </ul> <b>Subfamily: Discocriconemellinae Geraert, 2010</b> <ul style="list-style-type: none"> <li>Genus: <i>Discocriconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Xenocriconemella</i> De Grisse &amp; Loof, 1965</li> </ul> <b>Subfamily: Macroposthoniinae Skarbilovich, 1959</b> <ul style="list-style-type: none"> <li>Genus: <i>Mesocriconema</i> Andrassy, 1979</li> <li>Genus: <i>Bakernema</i> Wu, 1964</li> <li>Genus: <i>Criconemoides</i> Taylor, 1936</li> <li>Genus: <i>Neobakernema</i> Ebsary, 1981</li> <li>Genus: <i>Nothocriconemoides</i> Maas, Loof &amp; De Grisse, 1971</li> </ul> <b>Subfamily: Criconematinae Taylor, 1936</b> <ul style="list-style-type: none"> <li>Genus: <i>Criconema</i> Hofmanner &amp; Menzel, 1914</li> <li>Genus: <i>Croserinema</i> Khan, Chawla, &amp; Saha, 1976</li> <li>Genus: <i>Crossonema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Lobocriconema</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Neolobocriconema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Pateracephalanema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Ogma</i> Southern, 1914</li> <li>Genus: <i>Orphreyus</i> Siddiqi, 2000</li> </ul> <b>Subfamily: Blandicephalanematinae Geraert, 2010</b> <ul style="list-style-type: none"> <li>Genus: <i>Blandicephalanema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Amphisbaenema</i> Orton Williams, 1982</li> </ul>	Family: Criconematidae Taylor, 1936 (1914) (Thorne, 1949) <b>Subfamily: Criconematinae Taylor, 1936</b> <ul style="list-style-type: none"> <li>Genus: <i>Criconema</i> Hofmanner &amp; Menzel, 1914</li> <li>Genus: <i>Ogma</i> Southern, 1914</li> <li>Genus: <i>Hemicriconemoides</i> Chitwood &amp; Birchfield, 1957</li> <li>Genus: <i>Bakernema</i> Wu, 1964</li> <li>Genus: <i>Criconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Discocriconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Nothocriconemoides</i> Maas, Loof &amp; De Grisse, 1971</li> <li>Genus: <i>Blandicephalanema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Pateracephalanema</i> Mehta &amp; Raska, 1971</li> <li>[Genus <i>dubium</i>: <i>Macroposthonia</i> de Man, 1921]</li> <li>[Genus <i>dubium</i>: <i>Criconemoides</i> Taylor, 1936]</li> </ul> <b>Subfamily: Hemicycliophorinae Skarbilovich, 1959</b> <ul style="list-style-type: none"> <li>Genus: <i>Hemicycliophora</i> de Man, 1921</li> <li>Genus: <i>Caloosia</i> Siddiqi &amp; Goodey, 1964</li> </ul>
Classification of Criconematidae according to Siddiqi, 2000 (3 subfamilies, 13 genera, 12 subgenera)	Classification of Criconematidae according to Decraemer & Hunt, 2006 (3 subfamilies, 9 genera)
Family: Criconematidae Taylor, 1936 (1914) (Thorne, 1949) <b>Subfamily: Criconematinae Taylor, 1936</b> <ul style="list-style-type: none"> <li>Genus: <i>Criconema</i> Homanner &amp; Menzel, 1914</li> <li>Subgenus: <i>Criconema</i> Hofmanner &amp; Menzel, 1914</li> <li>Subgenus: <i>Amphisbaenema</i> Orton Williams, 1982</li> <li>Subgenus: <i>Nothocriconemella</i> Ebsary, 1981</li> <li>Subgenus: <i>Notholetus</i> Ebsary, 1981</li> <li>Genus: <i>Bakernema</i> Wu, 1964</li> <li>Genus: <i>Blandicephalanema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Crossonema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Lobocriconema</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Neolobocriconema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Ogma</i> Southern, 1914</li> <li>Subgenus: <i>Ogma</i> Southern, 1914</li> <li>Subgenus: <i>Croserinema</i> Khan, Chawla, &amp; Saha, 1976</li> <li>Subgenus: <i>Macrocriconema</i> Minagawa, 1986</li> <li>Subgenus: <i>Orphreyus</i> Siddiqi, 2000</li> <li>Subgenus: <i>Pateracephalanema</i> Mehta &amp; Raski, 1971</li> <li>Subgenus: <i>Seriespinula</i> Mehta &amp; Raski, 1971</li> </ul> <b>Subfamily: Macroposthoniinae Skarbilovich, 1959</b> <ul style="list-style-type: none"> <li>Genus: <i>Macroposthonia</i> de Man, 1880</li> <li>Genus: <i>Criconemoides</i> Taylor, 1936</li> <li>Subgenus: <i>Criconemoides</i> Taylor, 1936</li> <li>Subgenus: <i>Criconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Discocriconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Nothocriconemoides</i> Maas, Loof &amp; De Grisse, 1971</li> <li>Genus: <i>Xenocriconemella</i> De Grisse &amp; Loof, 1965</li> </ul> <b>Subfamily: Hemicriconemoidinae Andrassy, 1979</b> <ul style="list-style-type: none"> <li>Genus: <i>Hemicriconemoides</i> Chitwood &amp; Birchfield, 1957</li> </ul>	Family: Criconematidae Taylor, 1936 (1914) (Thorne, 1949) <b>Subfamily: Criconematinae Taylor, 1936</b> <ul style="list-style-type: none"> <li>Genus: <i>Bakernema</i> Wu, 1964</li> <li>Genus: <i>Criconema</i> Hofmanner &amp; Menzel, 1914</li> <li>Genus: <i>Lobocriconema</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Neolobocriconema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Ogma</i> Southern, 1914</li> </ul> <b>Subfamily: Macroposthoniinae Skarbilovich, 1959</b> <ul style="list-style-type: none"> <li>Genus: <i>Criconemoides</i> Taylor, 1936</li> <li>Genus: <i>Discocriconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Xenocriconemella</i> De Grisse &amp; Loof, 1965</li> </ul> <b>Subfamily: Hemicriconemoidinae Andrassy, 1979</b> <ul style="list-style-type: none"> <li>Genus: <i>Hemicriconemoides</i> Chitwood &amp; Birchfield, 1957</li> </ul>
Classification of Criconematidae according to Andrassy, 2007 (3 subfamilies, 16 genera)	Classification of Criconematidae according to Andrassy, 2007 (3 subfamilies, 16 genera)
Family: Criconematidae Taylor, 1936 (1914) (Thorne, 1949) <b>Subfamily: Criconematinae Taylor, 1936</b> <ul style="list-style-type: none"> <li>Genus: <i>Criconema</i> Homanner &amp; Menzel, 1914</li> <li>Subgenus: <i>Criconema</i> Hofmanner &amp; Menzel, 1914</li> <li>Subgenus: <i>Amphisbaenema</i> Orton Williams, 1982</li> <li>Subgenus: <i>Nothocriconemella</i> Ebsary, 1981</li> <li>Subgenus: <i>Notholetus</i> Ebsary, 1981</li> <li>Genus: <i>Bakernema</i> Wu, 1964</li> <li>Genus: <i>Blandicephalanema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Crossonema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Lobocriconema</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Neolobocriconema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Ogma</i> Southern, 1914</li> <li>Subgenus: <i>Ogma</i> Southern, 1914</li> <li>Subgenus: <i>Croserinema</i> Khan, Chawla, &amp; Saha, 1976</li> <li>Subgenus: <i>Macrocriconema</i> Minagawa, 1986</li> <li>Subgenus: <i>Orphreyus</i> Siddiqi, 2000</li> <li>Subgenus: <i>Pateracephalanema</i> Mehta &amp; Raski, 1971</li> <li>Subgenus: <i>Seriespinula</i> Mehta &amp; Raski, 1971</li> </ul> <b>Subfamily: Macroposthoniinae Skarbilovich, 1959</b> <ul style="list-style-type: none"> <li>Genus: <i>Macroposthonia</i> de Man, 1880</li> <li>Genus: <i>Criconemoides</i> Taylor, 1936</li> <li>Subgenus: <i>Criconemoides</i> Taylor, 1936</li> <li>Subgenus: <i>Criconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Discocriconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Nothocriconemoides</i> Maas, Loof &amp; De Grisse, 1971</li> <li>Genus: <i>Xenocriconemella</i> De Grisse &amp; Loof, 1965</li> </ul> <b>Subfamily: Hemicriconemoidinae Andrassy, 1979</b> <ul style="list-style-type: none"> <li>Genus: <i>Hemicriconemoides</i> Chitwood &amp; Birchfield, 1957</li> </ul>	Family: Criconematidae Taylor, 1936 (1914) (Thorne, 1949) <b>Subfamily: Criconematinae Taylor, 1936</b> <ul style="list-style-type: none"> <li>Genus: <i>Bakernema</i> Wu, 1964</li> <li>Genus: <i>Criconema</i> Hofmanner &amp; Menzel, 1914</li> <li>Genus: <i>Lobocriconema</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Neolobocriconema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Blandicephalanema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Crossonema</i> Mehta &amp; Raski, 1971</li> <li>Genus: <i>Croserinema</i> Khan, Chawla, &amp; Saha, 1976</li> <li>Genus: <i>Ogma</i> Southern, 1914</li> <li>Genus: <i>Orphreyus</i> Siddiqi, 2000</li> <li>Genus: <i>Pateracephalanema</i> Mehta &amp; Raski, 1971</li> </ul> <b>Subfamily: Macroposthoniinae Skarbilovich, 1959</b> <ul style="list-style-type: none"> <li>Genus: <i>Criconemoides</i> Taylor, 1936</li> <li>Genus: <i>Mesocriconema</i> Andrassy, 1979</li> <li>Genus: <i>Nothocriconemoides</i> Maas, Loof &amp; De Grisse, 1971</li> <li>Genus: <i>Discocriconemella</i> De Grisse &amp; Loof, 1965</li> <li>Genus: <i>Xenocriconemella</i> De Grisse &amp; Loof, 1965</li> </ul> <b>Subfamily: Hemicriconemoidinae Andrassy, 1979</b> <ul style="list-style-type: none"> <li>Genus: <i>Hemicriconemoides</i> Chitwood &amp; Birchfield, 1957</li> </ul>

were collected using a standardized collection procedure to facilitate consistent and optimal recovery between sampling sites (Neher et al., 1995). Soils were processed from a 200-mL subsample using a modified flotation-sieving and centrifugation method (Jenkins, 1964). The nematodes collected in this survey were digitally

photographed, measured, and PCR amplified by multiple primer sets for systematic studies to provide a linked set of analyses derived from a single individual specimen (Supplementary Table 1). Ongoing efforts are underway to store metadata associated with each specimen in the Barcode of Life Database (<http://v4.boldsystems.org/>).

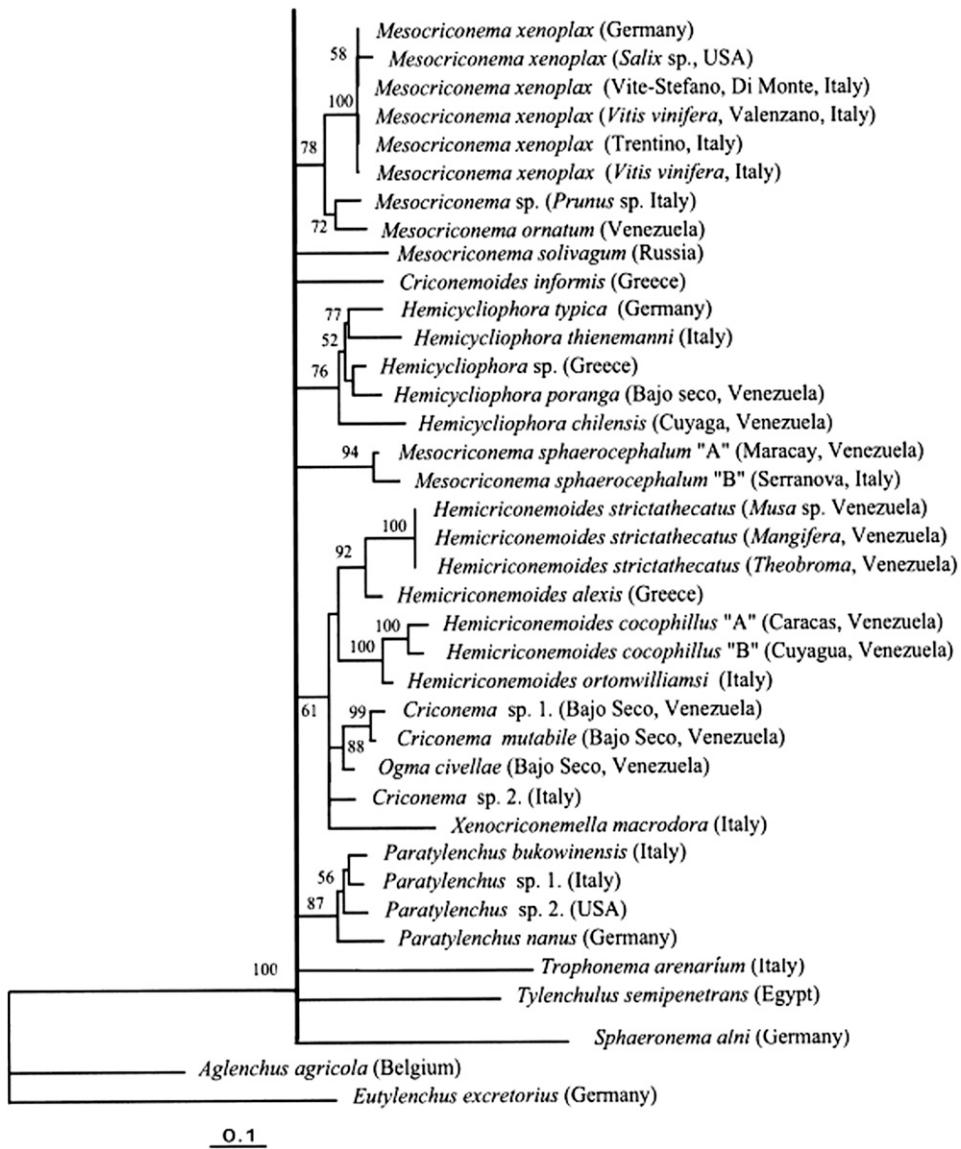


FIG. 1. A D2–D3 28S rDNA phylogenetic tree of Criconematina from Subbotin et al. (2005). Nodes with less than 50% bootstrap support have been collapsed to emphasize regions of uncertainty in the tree.

**18S rDNA amplification and DNA sequencing:** DNA was amplified by PCR and sequenced as described in Powers et al. (2014). Near-complete 18s ribosomal DNA sequence was obtained for 166 specimens using the following two primers sets: 18s39F – 5'-AAAGATTAAGCCATGCATG-3' and 18s977R – 5'-TTTACCGTTAGAACTAGGGCGG-3' produce a 0.97-kb amplification product which is reduced to 951 bp when primers are trimmed off. The second set, 18s900F – 5'-AAGACGGACTACAGCGAAAG-3' and 18s1713R – 5'-TCACCTACAGCTACCTTGTACG-3' produce a 0.85-kb amplification product: 818 bp when trimmed of primer sequences. Together, the sets usually produce a final near-complete 18S product of 1,706 bp with a 63-bp overlap between sets. Cleaned DNA was sent to UCDNA Sequencing Facility, UC Davis. Fifty-eight specimens from GenBank were added to the dataset for a 224 specimen total.

**Phylogenetic analysis:** Phylogenetic trees were constructed by ML and neighbor joining in MEGA version 6, and Bayesian tree estimation by MrBayes in TOPALi V2.5 (Milne et al., 2004). Sequences were edited using CodonCode Aligner version 4.2 (<http://www.codoncode.com/>) and aligned using Muscle within MEGA version 6 (Tamura et al., 2013). Gap opening penalty was set at –400 with a gap extension penalty of 0. The General Time Reversible Model with Gamma distributed rates plus invariant sites (GTR + G + I) was determined to be the best substitution model by Bayesian Information Criterion using the Best Fit Substitution Model tool in MEGA 6.0. ML trees used a partial deletion option for gaps and 200 bootstrap replications to assess clade support. Bayesian inference used two independent MCMC chains for two million generations sampled every 1,000 generations, with a burnin of 25%.

**Nomenclatural points:** Each nematode specimen in this study receives a Nematode Identification Number (NID) and a tentative species identification based on morphological analysis at the time of microscopic examination. When species identifications were uncertain, the genus name plus species was appended to the NID number. In most cases where species names are applied, additional DNA information from COI and the internal transcribed spacer 1 (ITS1) was available. On these grounds, *Criconema warrenense* Cordero et al., 2012 is transferred to *Lobocriconema warrenense* (Cordero et al., 2012) n. comb. Based on ITS1 and COI sequence of specimens obtained from the type locality, the placement of these specimens within haplotype group 6 of *Lobocriconema* (Powers et al., 2016), and an 18S sequence that positioned the topotype specimens within the *Lobocriconema* clade and not *Criconema*, this species belongs to the genus *Lobocriconema*.

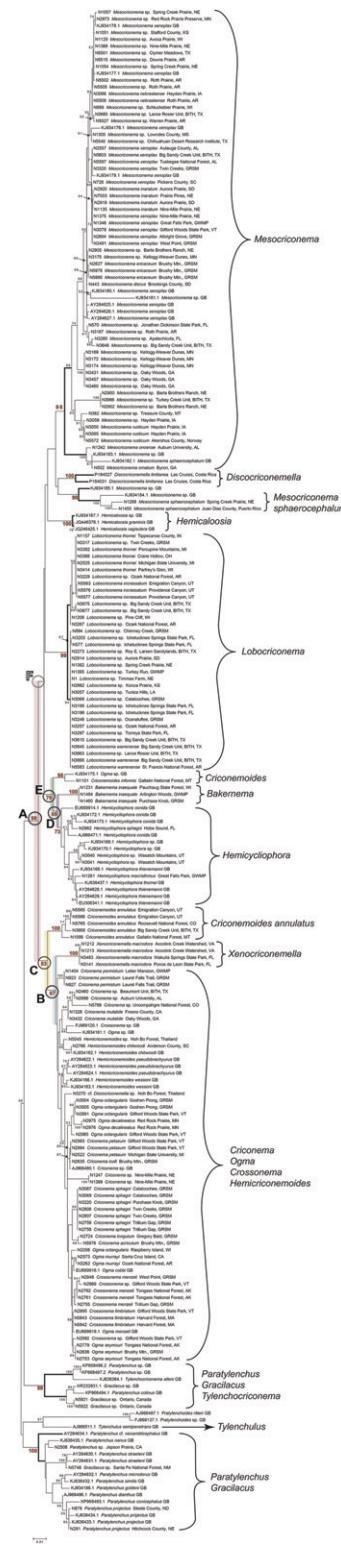
GenBank specimens added to the tree were unaltered with regard to species name.

## RESULTS

The ML tree for the complete 224 specimen dataset is presented in Fig. 2. A Bayesian tree of the same sequences with the redundant sequences removed resulting in a tree of 166 sequences is shown in Fig. 3. Major nodes that denote five taxonomically significant clades are labeled A to E on both trees. All five of these clades include equivalent taxa in both trees. The posterior probability values of Bayesian trees tend to give stronger support than the bootstrap values in ML trees. One difference between the trees is the recognition of clade F in the Bayesian tree. This clade was not identified by bootstrap values above 50% in ML trees. Within these clades are lineages represented by high bootstrap values (in bold) on the ML tree (Fig. 2) and posterior probability values of 1.0 in the Bayesian analysis (Fig. 3).

## DISCUSSION

In the introduction to Geraert's (2010) *The Criconematidae of the World – Identification of the Family Criconematidae (Nematoda)*, he states that "there is a chaotic situation in the taxonomy of the Criconematidae." This immensely useful resource was an effort to assemble descriptions of all of the recognized species of Criconematidae. It is not a revision of the family, although two new subfamilies are proposed and suggested synonymies are included in the text. Subfamily structure is a convenient point of departure for an examination of supporting molecular evidence for each of the classifications presented in Table 1. Because no specimens of *Amphisbaenema* Orton Williams, 1982 or *Blandicephalanema* Mehta and Raski, 1971 were available for this 18S analysis, examination of the subfamily Blandicephalanematiniae Geraert, 2010 was not possible. However, with the increased



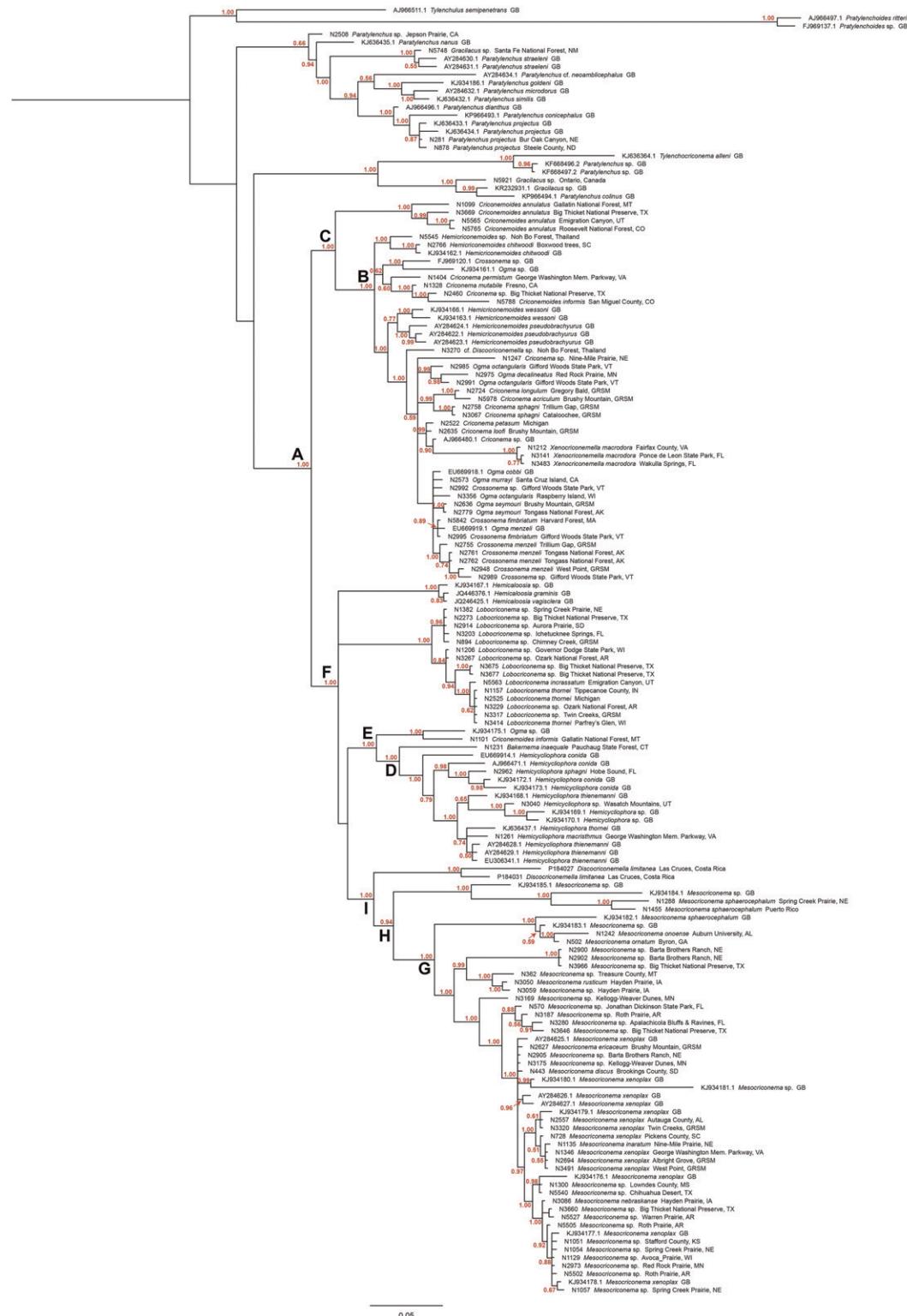


FIG. 3. Bayesian tree of Criconematoidea 18S rDNA of 159 unique sequences reduced by removing redundant sequences from the 224 sequence dataset. Posterior probability values are in red and major nodes discussed in the text are labeled A to I.

taxonomic coverage in the current analysis, it is possible to evaluate relationships among the majority of existing genera. The first surprising observation is how little support exists for deeper nodes in the 18S ML phylogenetic tree (Fig. 2). Consistent strong support is

found in the deepest node (A) that excludes the two *Paratylenchus* Micoletzky, 1922 groups, *Tylechulus* Cobb, 1913, and *Tylenchocriconema* Raski and Siddiqui, 1975, but includes the family Criconematidae Taylor, 1936 and all the *Hemicycliophora* specimens in the

dataset. This grouping is equivalent to the combination of the families of Criconematidae and Hemicycliophoridae in the systems of Decraemer and Hunt (2006) and Andrassy (2007), and the superfamilies of Criconematoidea and Hemicycliophoroidea in the system of Siddiqi (2000). Within this strongly supported clade is one major clade B denoted by a node that groups five genera (Fig. 2). This clade includes *Ogma* Southern, 1914, *Crossonema* Mehta and Raski, 1971, *Criconema* Hofmanner and Menzel, 1914, *Hemicriconemoides*, and *Xenocriconemella* De Grisse and Loof, 1965. There is no analogue to this grouping in any of the published classifications. *Ogma*, *Crossonema*, and *Criconema* have always been classified within the subfamily Criconematinae largely based on the presence of scales in the juvenile stages, and except for *Criconema*, scales on the female cuticle. Two other genera have scales in the juvenile stages, *Lobocriconema* De Grisse and Loof, 1965 and *Hemicriconemoides*. In each of the four modern (21st century) classifications in Table 1, *Hemicriconemoides* is always presented as a separate monotypic subfamily because of the presence of a sheath in the adult stage. *Lobocriconema*, is typically considered a member of Criconematinae, but in both ML and Bayesian 18S trees, it is a separate and distinct lineage with no clearly identified sister group. *Xenocriconemella* does not have scales on either juvenile or adult stages, and is most often included in the subfamily Macroposthoniinae. Geraert (2010) created a new subfamily, Discocriconemellinae to accommodate *Xenocriconemella* and *Discocriconemella* De Grisse and Loof, 1965 on the basis of a labial structure which lacks submedian lobes or pseudolobes, the absence of scales, and the relatively numerous, narrow body annuli. The 18S trees do not provide support for this new subfamily. Although no existing classification recognizes a grouping consistent with Clade B, the D2/D3 trees of Subbotin et al. (2005) produce an equivalent grouping (Fig. 1). In the ML tree, weak bootstrap support (53%) links Clade B to a single species, *Criconemoides annulatus* Cobb in Taylor, 1936. The Bayesian tree posterior probability of this relationship is 1.0. This species, with its type locality in Utah, is distributed throughout the Rocky Mountains in western North America. It does not form a group with other *Criconemoides* specimens in the 18S dataset.

Another clade that groups genera in the 18S tree is designated by node E, and includes *Hemicycliophora*, *Bakernema* Wu, 1964, and *Criconemoides*. *Hemicycliophora* is recognized as a separate family in all modern criconematid classifications, with the cuticular sheath in both adult and juvenile stages interpreted as a taxonomically important character (Siddiqi, 2000). At first glance, the sister taxa relationship between *Bakernema* and *Hemicycliophora* (node D) may appear unusual in a morphological context. However, similarities between the membranous scales of *Bakernema* and the sheath in *Hemicycliophora*, and SEM face views that feature a prominent oral disc with proportionally large amphid

apertures surrounded by a smooth, continuous annulus, suggest that homologous features may be revealed with an in-depth morphological analysis (Fig. 4; and Subbotin et al., 2014 for SEM images of *Hemicycliophora* species). Within this near-full length 18S analysis, *Criconemoides informis* (Micoletzky, 1922) Taylor, 1936 serves as a representative of a second species group in the genus *Criconemoides*. A second phylogenetic analysis using only a 592-bp 3' region of 18s (Powers et al., 2011) adds to this group with the inclusion of type locality specimens of *C. inusitatus* Hoffmann, 1975 (GB acc. FJ489532, FJ489533, FJ489535), and an undescribed *Criconemoides* species from Xalatlaco, Mexico (GB acc. FJ489591, FJ489592). The distant relationship between these *Criconemoides* species and *C. annulatus* indicate that *Criconemoides* is a paraphyletic taxon.

Other genera in the 18S tree are strongly supported by bootstrap values as distinct lineages, but in ML analyses, they lack any clear evidence of sister-group relations within Criconematidae. *Mesocriconema* is represented by eight described species in the dataset, several species yet to be described, and 11 GenBank sequences. Some sequences highlight potential misidentified species in GenBank and an annotated list of these species is provided in Supplementary Table 2. *M. sphaerocephalum* is not included in the *Mesocriconema* clade, nor is it included in a clade with *Criconemoides* species. This placement was also observed in the D2/D3 trees of Subbotin et al. (2005) although a more recent analysis positions it with moderate support as a sister taxon to a clade that includes *Criconemoides*, *Caloosia* Siddiqi and Goodey, 1964, and *Hemicycliophora* (Van den Berg et al., 2011). Two other genera, *Discocriconemella* and *Hemicaloosia*, although represented by a limited number of specimens in this 18S dataset, do not exhibit a close relationship with any other criconematid lineage. Bayesian analysis, however, recognizes a strong hierarchical relationship between *Discocriconemella limitanea* and a clade of *M. sphaerocephalum* and *Mesocriconema* (Fig. 3). Node G in Fig. 3 identifies a clade that coincides with the genus *Mesocriconema*. Node H recognizes a sister relationship between *Mesocriconema* and a lineage that includes *M. sphaerocephalum*, and node I supports a sister relationship between *Discocriconemella limitanea* and all specimens denoted by node H.

In addition to the lack of strong support for current subfamily classifications, the generic boundaries are not well defined for clade B, the grouping that includes *Ogma*, *Crossonema*, *Criconema*, and *Hemicriconemoides*. Sequence from populations within a species tends to group together. For example, the seven named species of *Criconema* in the dataset, *C. acriculum* (Raski and Pinochet, 1976) Raski and Luc, 1985, *C. longulum* Gunhold, 1953, *C. loofi* (De Grisse, 1967) Raski and Luc, 1985, *C. mutabile* (Taylor, 1936) Raski and Luc, 1985, *C. permistum* (Raski and Golden, 1966) Raski and Luc, 1985, *C. petasum* (Wu, 1965) Raski and Luc, 1985, and

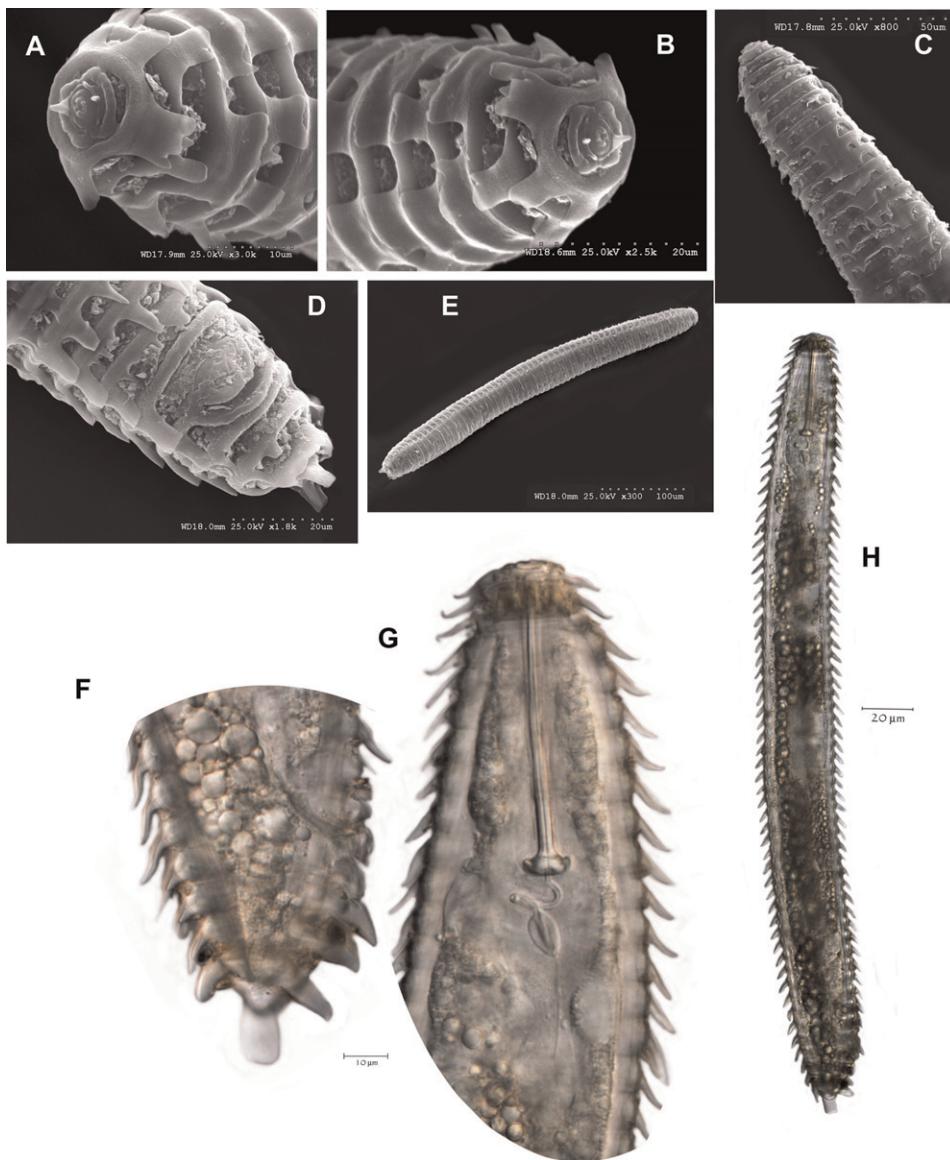


FIG. 4. Images of *Bakernema inaequale* specimens. A to E. SEM micrographs. F to H. Light micrographs. A, B. Face view, NID 4545 from Purchase Knob, Great Smoky Mountains National Park. C. Anterior region showing irregular pattern of membranous scales, NID 4539 from Purchase Knob, GRSM. D. Tail and vulva, NID 4542 from Purchase Knob, GRSM. E. Entire body, NID 4543, from Purchase Knob, GRSM. F. Tail, NID 723 from Chimney Creek, GRSM. G. Head, NID 723 from Chimney Creek, GRSM. H. Entire body, NID 1484 from Arlington Woods, George Washington Memorial Parkway, VA.

*C. sphagni* Micoletzky, 1925 are generally identified as distinct entities in the phylogenetic tree. However, the 18S sequence data do not provide evidence that these seven species form a monophyletic group. Similarly *Ogma* and *Crossonema* are two genera primarily differentiated by the arrangement of scales on the adult female. In *Crossonema*, the scales form a continuous fringe along the annulus margin, whereas in *Ogma*, the scales are arranged in discrete longitudinal rows. The classifications of Maggenti et al. (1988), Decraemer and Hunt (2006), and Wouts (2006) do not recognize the validity of *Crossonema*. The 18S dataset does not support the monophyly of either genus.

It is clear that in 18S analyses, the divisions between *Ogma*, *Crossonema*, *Criconema*, and *Hemicriconemoides*

sequences are not of the same magnitude as differences between *Mesocriconema*, *Lobocriconema*, *Hemicaloosia*, and *Discocriconemella limitanea*. In all phylogenetic trees, long branch lengths characterize the latter taxa, and relatively short branch lengths characterize the taxa in clade B. Assuming roughly constant rates of 18S evolution within Criconematidae, it appears that the morphological differentiation that has occurred among members of clade B is relatively recent in evolutionary time compared with the differentiation among lineages such as *Mesocriconema*, *Discocriconemella limitanea*, and *Lobocriconema*. Much of the differentiation of genera like *Ogma*, *Crossonema*, and *Criconema* reside in characters of the cuticle. Geraert (2010) has expressed concern for the over-reliance on characteristics of the

cuticle in subfamily classification of Criconematidae. This concern could also be applied to the determination of genus boundaries.

One goal of a phylogenetic classification is to create a classification that is logically consistent with its hypothesized phylogeny (Wiley and Lieberman, 2011). Unfortunately, unresolved polytomies in the 18S tree do not allow for a fully resolved classification based on relatedness. Given the level of uncertainty in the 18S dataset, we provide a conservative list of taxonomic recommendations for classification within Criconematoidea.

- i) The recognition of a group that unites the taxa in clade A exclusive of *Paratylenchus*, *Gracilacetus*, *Tylenchocriconema*, and *Tylenchulus*. This grouping is similar in content to the family Criconematidae in the classification of Maggenti et al. (1988) in that *Hemicyclophora* and *Caloosia* are in-group members with other Clade A taxa.
- ii) *Hemicriconemoides* should not be accorded separate subfamily status apart from other members of clade B.
- iii) An integrated morphological and DNA-based analysis should explore the evolutionary relationship between *Hemicyclophora* and *Bakernema*. Both Bayesian and ML analysis support this grouping.
- iv) *Criconemoides* should be recognized as a paraphyletic taxon separate from *Mesocriconema*. Characters other than closed vulva and the lack of true submedian lobes are necessary to morphologically differentiate among *Criconemoides* lineages.
- v) *Mesocriconema*, *Discocriconemella limitanea*, *M. sphaerocephalum*, *Hemicaloosia*, and *Lobocriconema* are all distinct taxa. *M. sphaerocephalum*, after an additional study, should be accorded separate genus status.
- vi) Bayesian analysis supports a grouping of (*Discocriconemella limitanea* [*M. sphaerocephalum* + *Mesocriconema*]).
- vii) Genus boundaries between *Ogma*, *Criconema*, and *Crossonema* need to be redefined. DNA evidence does not support a distinction between *Ogma* and *Crossonema*.
- viii) *Xenocriconemella* groups together with *Ogma* + *Criconema* + *Hemicriconemoides*. It does not form a group with *Discocriconemella limitanea*.

#### LITERATURE CITED

- Andrássy, I. 2007. Free-living nematodes of Hungary. *Pedozoologica Hungarica*, No 4. Budapest, Hungary: Hungarian Natural History Museum.
- Bert, W., Karssen, G., and Helder, J. 2011. Phylogeny and evolution of nematodes. Pp. 45–59 in Genomics and molecular genetics of plant-nematode interactions. The Netherlands: Springer.
- Brzeski, M. W., Choi, Y. E., and Loof, P. A. 2002a. Compendium of the genus *Criconemoides* Taylor, 1936 (Nematoda: Criconematidae). *Nematology* 4:325–339.
- Brzeski, M. W., Choi, Y. E., and Loof, P. A. 2002b. Compendium of the genus *Mesocriconema* Andrásy, 1965 (Nematoda: Criconematidae). *Nematology* 4:341–360.
- Cid Del Prado Vera, I., and Talavera, M. 2012. Criconematoidea. Pp. 479–519 in R. H. Manzanilla-López and N. Marbán-Mendoza, eds. Practical plant nematology. Estado de México, México: Biblioteca Básica de Agricultura.
- Decraemer, W., and Geraert, E. 2006. Ectoparasitic nematodes. Pp. 153–184 in R. N. Perry, and M. Moens, eds. Plant nematology. Wallingford, UK: CABI.
- Decraemer, W., and Hunt, D. 2006. Structure and classification. Pp. 3–32 in R. N. Perry, and M. Moens, eds. Plant nematology. Wallingford, UK: CABI.
- De Ley, P., and Blaxter, M. L. 2004. A new system for Nematoda. in R. Cook, and D. J. Hunt, eds. Proceedings of the Fourth International Congress of Nematology, 8–13 June 2002, Tenerife, Spain. Nematology Monographs and Perspectives 2.
- Geraert, E. 2010. The Criconematidae of the world. Identification of the family Criconematidae (Nematoda). Gent, Belgium: Academia Press.
- Holterman, M., Karssen, G., Van Den Elsen, S., Van Megen, H., Bakker, J., and Helder, J. 2009. Small subunit rDNA-based phylogeny of the *Tylenchida* sheds light on relationships among some high-impact plant-parasitic nematodes and the evolution of plant feeding. *Phytopathology* 99:227–235.
- Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.
- Maggenti, A. R., Luc, M., Raski, D. J., Fortuner, R., and Geraert, É. 1988. A reappraisal of Tylenchina (Nemata). 11. List of generic and supra-generic taxa, with their junior synonyms. *Revue Nématol* 51: 177–188.
- Meldal, B. H., Debenham, N. J., De Ley, P., De Ley, I. T., Vanfleteren, J. R., Vierstraete, A. R., Bert, W., Borgonie, G., Moens, T., Tyler, P. A., and Austen, M. C. 2007. An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. *Molecular Phylogenetics and Evolution* 42:622–636.
- Milne, I., Wright, F., Rowe, G., Marshal, D. F., Husmeier, D., and McGuire, G. 2004. TOPALI: Software for automatic identification of recombinant sequences within DNA multiple alignments. *Bioinformatics* 20:1806–1807.
- Mitchum, M. G., Hussey, R. S., Baum, T. J., Wang, X., Elling, A. A., Wubben, M., and Davis, E. L. 2013. Nematode effector proteins: An emerging paradigm of parasitism. *New Phytologist* 199:879–894.
- Neher, D. A., Peck, S. L., Rawlings, J. O., and Campbell, C. L. 1995. Measures of nematode community structure and sources of variability among and within agricultural fields. *Plant and Soil* 170:167–181.
- Nyczepir, A. P., Zehr, E. I., Lewis, S. A., and Harshman, D. C. 1983. Short life of peach trees induced by *Criconemella xenoplax*. *Plant Disease* 67:507–508.
- Powers, T. O., Bernard, E. C., Harris, T., Higgins, R., Olson, M., Lodema, M., Mullin, P., Sutton, L., and Powers, K. S. 2014. COI haplotype groups in *Mesocriconema* (Nematoda: Criconematidae) and their morphospecies associations. *Zootaxa* 3827:101–146.
- Powers, T., Bernard, E., Harris, T., Higgins, R., Olson, M., Olson, S., Lodema, M., Matczyszyn, J., Mullin, P., Sutton, L., and Powers, K. 2016. Species discovery and diversity in *Lobocriconema* (Criconematidae: Nematoda) and related plant-parasitic nematodes from North American ecoregions. *Zootaxa* 4085:301–344.
- Powers, T., Harris, T., Higgins, R., Mullin, P., Sutton, L., and Powers, K. 2011. MOTUs, morphology and biodiversity estimation: A case study using nematodes of the suborder Criconematina and a conserved 18S DNA barcode. *Journal of Nematology* 43:35–48.
- Siddiqi, M. R. 2000. Tylenchida: Parasites of plants and insects. 2nd ed. Wallingford, UK: CABI Publishing.
- Subbotin, S. A., Chitambar, J. J., Chizhov, V. N., Stanley, J. D., Inserra, R. N., Doucet, M. E., McClure, M., Ye, W., Yeates, G. W., Molloy, D. S., and Cantalapiedra-Navarrete, C. 2014. Molecular

- phylogeny, diagnostics, and diversity of plant-parasitic nematodes of the genus *Hemicycliophora* (Nematoda: Hemicycliophoridae). *Zoological Journal of the Linnean Society* 171:475–506.
- Subbotin, S. A., Vovlas, N., Crozzoli, R., Sturhan, D., Lamberti, F., Moens, M., and Baldwin, J. G. 2005. Phylogeny of Criconematina Siddiqi, 1980 (Nematoda: Tylenchida) based on morphology and D2–D3 expansion segments of the 28S-rRNA gene sequences with application of a secondary structure model. *Nematology* 7:927–944.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729.
- Van den Berg, E., Marais, M., and Swart, A. 2017. Nematode morphology and classification. Pp. 33–71 in *Nematology in South Africa: A view from the 21st century*. Switzerland: Springer International Publishing.
- Van den Berg, E., Tiedt, L. R., and Subbotin, S. A. 2011. Morphological and molecular characterisation of *Caloosia longicaudata* (Loos, 1948) Siddiqi and Goodey, 1963 (Nematoda: Caloosiidae) from Maui, the Hawaiian Islands with notes on some species of the genus. *Nematology* 13:381–393.
- Wiley, E. O., and Lieberman, B. S. 2011. *Phylogenetics: Theory and practice of phylogenetic systematics*. Hoboken, NJ: John Wiley and Sons.
- Wouts, W. M. 2006. Criconematina (Nematoda: Tylenchida). *Fauna of New Zealand* 55:1–228.
- Xiang, M. C., Xiang, P. A., Jiang, X. Z., Duan, W. J., and Liu, X. Z. 2010. Detection and quantification of the nematophagous fungus *Hirsutella minnesotensis* in soil with real-time PCR. *Applied Soil Ecology* 44:170–175.
- Zeng, Y., Ye, W., Kerns, J., Tredway, L., Martin, S., and Martin, M. 2015. Molecular characterization and phylogenetic relationships of plant-parasitic nematodes associated with turfgrasses in North Carolina and South Carolina, United States. *Plant Disease* 99:982–993.