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Molecular rDNA phylogeny of Telotylenchidae Siddiqi, 1960 and evaluation of tail termini

L. K. Carta, A. M. Skantar, Z. A. Handoo

Abstract: Three stunt nematode species, *Tylenchorhynchus leviterminalis*, *T. dubius* and *T. claytoni* were characterized with segments of small subunit 18S and large subunit 28S rDNA sequence and placed in molecular phylogenetic context with other polyphyletic taxa of Telotylenchidae. Based upon comparably sized phylogenetic breadth of outgroups and ingroups, the 28S rDNA contained three times the number of phylogenetically informative alignment characters relative to the alignment total compared to the larger 18S dataset even though there were fewer than half the number of taxa represented. Tail shapes and hyaline termini were characterized for taxa within these subfamily trees, and variability discussed for some related species. In 18S trees, similar terminal tail thickness was found in a well-supported clade of three *Tylenchorhynchus*: broad-tailed *T. leviterminalis* branched outside relatively narrow-tailed *T. claytoni* and *T. nudus*. Terminal tail thickness within Merliniinae, Telotylenchinae and related taxa showed a mosaic distribution. Thick-tailed *Trophurus, Macrotrophurus* and putative *Paratrophurus* did not group together in the 18S tree. Extremely thickened tail termini arose at least once in *Amplimerlinius* and *Pratylenchoides* among ten species of Merliniinae plus three *Pratylenchoides*, and three times within twelve taxa of Telotylenchinae and Trophurinae. Conflicting generic and family nomenclature based on characters such as pharyngeal overlap are discussed in light of current molecular phylogeny. Contrary to some expectations from current taxonomy, *Telotylenchus* and *Tylenchorhynchus* cf. *robustus* did not cluster with three *Tylenchorhynchus* spp. Two putative species of *Neodolichorhynchus* failed to group together, and two populations of *Scutylenchus quadrifer* demonstrated as much or greater genetic distance between them than among three related species of *Merlinius*.
Key words: character analysis, evolutionary convergence, morphology, nomenclature, phylogeny, stunt nematode, systematics, tail, taxonomy, *Tylenchorhynchus*.

Stunt nematodes (*Tylenchorhynchus sensu lato*) and relatives within the Telotylenchidae Siddiqi, 1960 are extremely common in the rhizosphere of native and cultivated plants. Because of the large number of stunt nematode species, taxonomists have been motivated to simplify identification into more manageable generic units, but an unusual number of confusing and competing systems based on different character priorities now exist. Since an earlier comprehensive review of *Tylenchorhynchus sensu lato* (Allen, 1955), various taxonomic designations for stunt nematode genera have been proposed, from *Merlinius*, *Quinisulcius* and *Uliginotylenchus* listed in the compendium of Tarjan, 1973 through a current assemblage of five genera within Merliniinae Siddiqi, 1971, twelve genera in Telotylenchinae Siddiqi, 1960, and six junior synonyms under *Tylenchorhynchus* itself (Siddiqi, 2000). These taxa were based on different hierarchies of characters such as pharyngeal gland overlap, number of lines in the lateral field, male genitalia, and major differences in female terminal tail features (Jairajpuri and Hunt, 1984; Gomez-Barcina et al., 1992). However, some of the newer generic names of stunt nematodes and relatives have been ignored in recent compendia for ease of practical identification (Fortuner and Luc, 1987; Brzeski and Dolinski, 1998; Handoo, 2000), and the morphological characters to distinguish them are often not discrete. Molecular phylogenetic analyses are needed to evaluate competing taxonomic schemes and the characters on which they are based before any new names can be readily accepted. These phylogenies can provide an independent means to understand character distribution that impacts stability of higher taxonomic categories. Molecular sequences also provide important information on genetic variation of populations within morpho-species, and evaluating whether similar species that lack males should be synonymized with species that have them. Tied to morphology, similarity searches of sequences are especially useful when competing generic and subfamily names are used in the literature, as is currently the case for Telotylenchidae.

An isolate of *Tylenchorhynchus leviterminalis* (Siddiqi, Mukherjee and Dasgupta, 1982) Siddiqi, 1986 was identified by us from a foreign plant interception, and there are no records of this species' existence in North America. ITS rDNA sequences are available in GenBank for *T. leviterminalis* (Chen et al., 2006), but comparable sequences from other relatives are lacking. Among species of *Tylenchorhynchus*, it has a relatively thick tail, but this character has not previously been examined in relation to the tails of other family members in a morphology-independent molecular phylogenetic context. A limited number of telotylenchine taxa were included in recent small subunit (SSU) 18S (Holterman et al., 2006; Meldal et al., 2007; Holterman et al., 2009; van Megen et al., 2009) and large subunit (LSU) 28S trees (Subbotin et al., 2006), demonstrating that Telotylenchinae and Merliniinae are polyphyletic. They also demonstrated support for the Merliniidae (Siddiqi, 1971) Ryss, 1993, an amended family generally possessing deirids (except in *Scutylenchus*) that includes Merliniinae and *Pratylenchoides*. They also supported the Telotylenchidae Siddiqi, 1960/syn. Tylenchorhynchidae Eliava,

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1964 that includes members of Tylenchorhynchinae Eliava, 1964, Trophurinae Paramonov, 1967, Macrotrophurinae Fotedar and Handoo, 1978, and Telotylenchinae Siddiqi, 1960 as reviewed in Siddiqi (2000). However, information from these broad molecular trees, each with relatively few taxa, was insufficient for inferences about morphological characters within the subfamilies to be made. Therefore, we generated both LSU and SSU rDNA sequences for *T. leviterminalis* and two other common species, *T. claytoni* Steiner, 1937 and *Bitylenchus dubius* (Bütschli, 1873) Filipjev, 1934 [= *T. dubius* (Bütschli, 1873) Filipjev, 1936] and constructed phylogenetic trees in order to investigate their genetic relationships and especially for tail character analysis. Thickened female tail termini are immediately noticeable traits among tylenchid nematodes, so tail termini measurements based on specimens and literature were mapped onto a tree for an initial look at character distribution and reliability as they relate to taxonomy and nomenclature.

Materials and Methods

Specimens: Tylenchorhynchus leviterminalis is found in Asia and was collected in late 2001 from soil originating in Vietnam and relayed via APHIS in February 2002 to the USDA Nematology Laboratory for species identification. *Tylenchorhynchus claytoni* was found in soil from sorghum in Trenton, SC in April 2005. *Bitylenchus dubius* originated from soil under a cool season perennial bunch grass (probable orchard grass, *Dactylis glomerata*) at the base of a sycamore (*Platanus occidentalis*) tree in Beltsville, MD. Specimens were identified and imaged with high-power light microscopy before processing for PCR.

Microscopy: Tail images were taken with a Zeiss Ultraphot III (Carl Zeiss, Inc., Jena, Germany, and Baltimore Instrument Company, Baltimore, MD, USA) using Differential Interference Contrast (DIC) optics, and recorded with a Toshiba IKTU CCD camera (Toshiba Corp., Japan) (Fig. 1). Tail drawings representing taxa used in 18S trees (Fig. 2) were made from original and other descriptions (Allen, 1955; Caveness, 1958; Loof, 1958; Loof, 1956, Loof, 1959, Loof, 1963, Loof, 1978; Thorne, 1949; *Tylenchorhynchus* cf. *robustus, Paratrophurus* sp., and *Sauertylenchus maximus* measures made from web vouchers at http://nematode.unl.edu/). Images were scanned and uniformly sized using HyperSnap-DX ver. 5.60.00 (Hyperionics, Inc., Murrysville, PA) and PhotoShop ver. CS (Adobe Systems Inc., San Jose, CA). The percent of hyaline tail terminus length to total tail length was calculated from these drawings and/or literature and coded as moderately thick (+) ≥ 4%, thick + ≥ 9%, or very thick ++ (≥ 20%) (Fig. 2) and assigned to tree branches in tree Figures 3 through 5. Taxonomic categories and synonyms with the nomenclature of Siddiqi (2000) used in this work are also given in the tables.

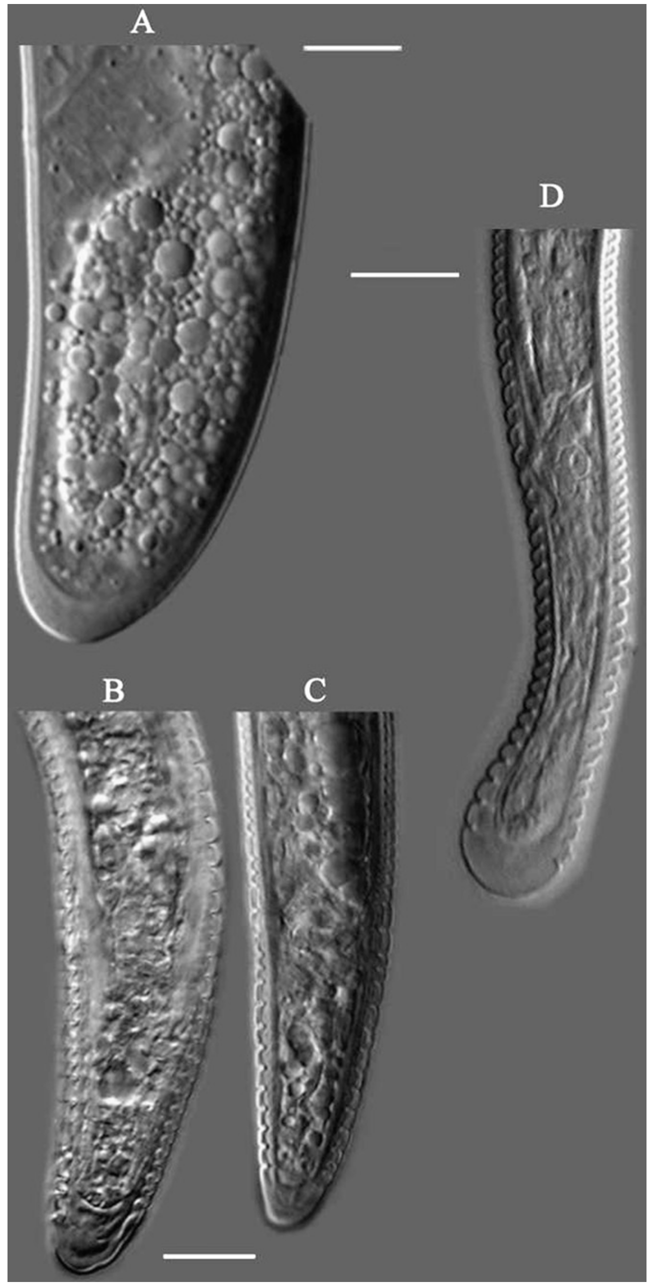

Fig. 1. *Bitylenchus* and *Tylenchorhynchus* female tails, lateral view A) *B. dubius.* B, C) *T. claytoni.* D) *T. leviterminalis.* Scale Bar = 10 μm.

PCR and sequencing: Multiple adults were collected and identified for *B. dubius* and *T. claytoni,* and a single female was used for *T. leviterminalis.* Nematodes were mechanically disrupted in 20 μl of extraction buffer as described by Thomas et al. (1997), and then stored in PCR tubes at –80°C until needed. Extracts were prepared by incubating the tubes at 60°C for 60 min, followed by 95°C for 15 min to deactivate the proteinase K and centrifuged briefly prior to use in PCR. For 28S, each 25 μl reaction contained 1 unit Platinum Taq (Invitrogen, Carlsbad, CA), 1X reaction buffer [20 mM Tris-HCl pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$], 0.2 mM dNTP mix, 0.8 μM primers D2A (5'-ACAAGTACCGTG

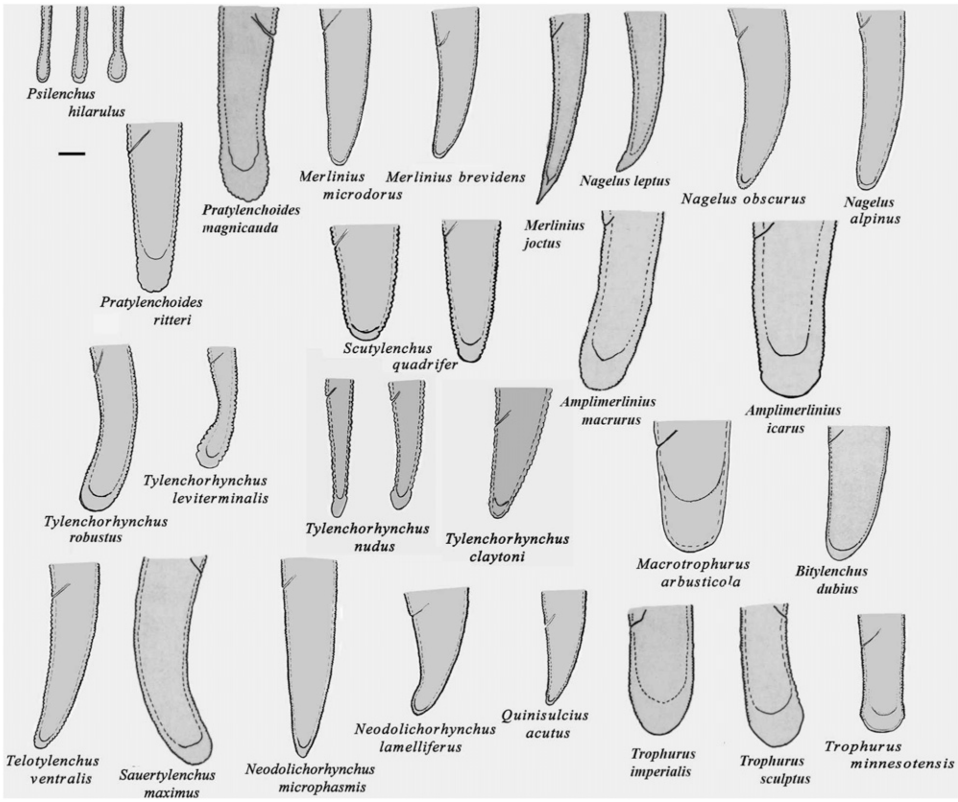

Fig. 2. Tail terminal drawings of Merliniidae *sensu* Ryss, 1993 and Telotylenchidae *sensu* Siddiqi, 2000 (syn. Tylenchorhynchidae Eliava, 1964). Drawings based on literature, web vouchers of the Powers lab at University of Nebraska, USDANC slides and Fig. 1. Coding of terminal tail thickening for very thick (≥ 20%) ++, for thick + (≥ 9%), and (+) for moderately thick terminal tail (≥ 4%) are given for the % ratio of hyaline tail length/tail length: *Psilenchus hilarulus* < 1% - (Thorne, 1949), *Pratylenchoides ritteri* 22.5% ++ (Sher, 1970), *Pratylenchoides magnicauda* 19% +(+) (Baldwin et al., 1983), *Merlinius microdorus* 2.6% (+) (Geraert, 1966), *Merlinius brevidens* 3% (+) (Allen, 1955), *Merlinius joctus* 16% + (Thorne and Malek, 1968), *Nagelus leptus* 11 - 16 % + (Thorne, 1949, http://nematode.unl.edu/nagle4.jpg, Powers et al., 1983), *Nagelus obscurus* 4.3% (+)(Allen, 1955), *Nagelus alpinus* 3.7% (+) (Allen, 1955), *Scutylenchus quadrifer* 9.3 – 13.7% + (Loof, 1978), *Amplimerlinius macrurus* 20% ++ (USDANC slide G-3022), *Amplimerlinius icarus* 27% ++ (USDANC slide G3024), *Tylenchorhynchus robustus* 11.3% + (Thorne and Malek, 1968), *Tylenchorhynchus leviterminalis* 9.4 - 16% +, *Tylenchorhynchus nudus* 10.4 - 16.2% + (Loof, 1959), and *Tylenchorhynchus claytoni* 12.5 - 25% +(+), *Macrotrophurus arbusticola* 46.7% ++ (Loof, 1958), *Bitylenchus dubius* 4.4% (+), *Telotylenchus ventralis* 6% (+) (Loof, 1963), *Sauertylenchus maximus* 11 - 14% + (http://nematode.unl.edu/tymax15.jpg, Allen, 1955), *Neodolichorhynchus microphasmis* 7.7% (+) (Loof, 1959), *Neodolichorhynchus lamelliferus* 3.1% (+) (Allen, 1955), *Quinisulcius acutus* 4.5 - 17% + (Allen, 1955, http://nematode.unl.edu/quina9.jpg), *Trophurus imperialis* 30% ++ (Loof, 1956), *Trophurus minnesotensis* 21% ++ (Caveness, 1958), *Trophurus sculptus* 25% ++ (Loof, 1956), *Tylenchorhynchus* cf. *robustus* 7% (+) (http://nematode.unl.edu/tylerob3.jpg), *Paratrophurus* sp. 13% + (http://nematode.unl.edu/patrop.htm). Scale Bar = 10 μm.

AGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCA GCTACTA-3'), and 5 μl nematode extract. Cycling was performed as described in De Ley et al. (2005). Partial 18S sequence was amplified in two overlapping segments, using the primers SSU-550F (5'-GGCAAGTCT GGTGCCAGCAGCC-3') with eukR(10) (5'-TGATCCT CCTGCAGGTTCACCTAC-3'), and SSU-385F (5'-CGG TGGTTATAACGGGTAACGGAG-3') with 18S-R-1108R (5'-CCACTCCTGGTGGTGCCCTTCC-3') (more information available at http://nematol.unh.edu/protocols.php). Reactions were assembled in 25 μl and included 1 unit DyNAzyme polymerase (MJ Research, Waltham, MA), 1X reaction buffer including 1.5 mM $MgCl_2$, 0.63 μM each primer, 0.2 mM dNTP mix, and 2.5 μl template DNA. Cycling conditions for 18S consisted of 1 cycle of 94°C for 2 min, followed by 40 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 2 min, and finishing with 1 cycle of 72°C for 10 min.

PCR products were visualized with UV illumination after ethidium bromide staining. DNA was excised from the gels and purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). Clean PCR products were sequenced directly at the University of Maryland Center for Biosystems Research. DNA sequences were assembled using Sequencher 4.7 (Genecodes, Ann Arbor, MI). DNA sequences were analyzed using the BLASTN megablast program optimized for highly similar sequences, http://www.ncbi.nlm.nih.gov/blast/Blast.cgi. Sequences were submitted to GenBank under accession numbers *T. leviterminalis* 18S (EU368585), *Bitylenchus dubius* 18S (EU368586), *T. claytoni* 18S (EU368587), *T. claytoni* 28S D2-D3 (EU368589), *B. dubius* 28S D2-D3 (EU368590), and *T. leviterminalis* 28S D2-D3 (EU368591).

Phylogenetic Reconstruction: To construct 18S trees, GenBank SSU rDNA sequences were collected for various genera and species of Telotylenchinae and outgroups

TABLE 1. SSU 18S rDNA GenBank Sequences for Merliniinae, Telotylenchinae, Macrotrophurinae, Pratylenchidae (Radopholinae) and Outgroups in Fig. 3, 4 Trees.

Accession #	Nematode Species and Strain	Bp #	Sequence Reference
AJ966511	*Tylenchulus semipenetrans*[e]	1740	Meldal et al., 2007
AJ966471	*Hemicycliophora conida*[e]	1764	Meldal et al., 2007
EF025336	*Dolichodorus* sp.[e]	1723	Ye et al., 2007
DQ912919	*Belonolaimus longicaudatus*[e]	1725	Zeng et al., 2007
AY284593	*Psilenchus* cf. *hilarulus*[e]	1710	Holterman et al., 2006
AY919271	*Psilenchus hilarulus*[e]	634	Powers et al., 2005
AJ966494	*Nacobbus aberrans*[e]	1765	Meldal et al., 2007
FJ969114	*Amplimerlinius macrurus*[a]	1731	van Megen et al., 2009
EU306351	*Amplimerlinius icarus*[a]	1764	Bert et al., 2008
EU306352	*Bitylenchus dubius*[b] (T)	1746	Bert et al., 2008
AY284595	*Macrotrophurus arbusticola* [c]	1714	Holterman et al., 2006
AY284597	*Merlinius brevidens*[a]	1709	Holterman et al., 2006
FJ969128	*Merlinius joctus*[a]	1731	van Megen et al., 2009
AY919184	*Merlinius* cf. *microdorus*[a]	634	Powers et al., 2005
AY146449	*Nagelus alpinus*[a, g] (M)	634	Mullin, 2004
EU306350	*Nagelus obscurus*[a]	1760	Bert et al., 2008
AY919217	*Nagelus leptus*[a]	634	Powers et al., 2005
AY284598	*Neodolichorhynchus lamelliferus*[a]	1598	Holterman et al., 2006
AY593903	*Neodolichorhynchus microphasmis*[a] (T)	837	Holterman et al., 2006
AY919229	*Paratrophurus* sp.[b]	635	Powers et al., 2005
FJ969137	*Pratylenchoides* sp.[d]	1732	van Megen et al., 2009
AF202157	*Pratylenchoides magnicauda*[d]	1643	Félix et al., 2000
AJ966497	*Pratylenchoides ritteri* [d]	1831	Meldal et al., 2007
DQ080517	*Quinisulcius*[h] *acutus*	634	Powers et al., 2005
AY993979	*Sauertylenchus maximus*[b] (T)	1766	Meldal et al., 2007
AY284599	*Scutylenchus*[i] *quadrifer*[a]	1598	Holterman et al., 2006
AY993977	*Scutylenchus*[i] *quadrifer* (G)[a]	1765	Meldal et al., 2007
AY593905	*Telotylenchus*[h] *ventralis*[b]	1743	Holterman et al., 2006
FJ969144	*Trophurus imperialis*[b]	1743	van Megen et al., 2009
AY146555	*Trophurus minnesotensis*[b]	635	Mullin, 2004
DQ080547	*Tylenchorhynchus* cf. *robustus*[b]	1695	Powers et al., 2005
EU368587	*Tylenchorhynchus claytoni* [b, f]	1338	Skantar, Carta, Handoo
EU368585	*Tylenchorhynchus leviterminalis*[b, f]	1407	Skantar, Carta, Handoo
DQ080546	*Tylenchorhynchus nudus*[b]	634	Powers et al., 2005

Synonyms given with these accessions: (G) = *Geocenamus,* (M) = *Merlinius* (T) = *Tylenchorhynchus.*
Bp = base pair or nucleotide.
[a]Merliniinae.
[b]Telotylenchinae.
[c]Macrotrophurinae.
[d]Pratylenchidae (Radopholinae).
[e]Outgroups.
[f]Original sequences.
[g]Synonym *Merlinius alpinus* (Powers et al., 1983).
[h]Junior synonyms of *Tylenchorhynchus* (Fortuner and Luc, 1987).
[i]G isolate of *Scutylenchus quadrifer* listed as *Geocenamus quadrifer* (Andrássy, 1954) Brzeski, 1991 in agreement with Brzeski (1991) who considered *Scutylenchus* a junior synonym, but this species was not included within a recent 12-species key of *Geocenamus* (Chitambar and Ferris, 2005).

(Table 1.). Outgroups included *Tylenchulus semipenetrans* Cobb, 1913, *Hemicycliophora conida* Thorne, 1955, *Dolichodorus* sp. Cobb, 1914, *Belonolaimus longicaudatus* Rau, 1958, and *Psilenchus hilarulus* de Man, 1921. Ingroup taxa included *Amplimerlinius macrurus* (Goodey, 1932) Siddiqi 1976, *Amplimerlinius icarus* (Wallace and Greet, 1964) Siddiqi 1976, *Bitylenchus dubius*, *Macrotrophurus arbusticola* Loof, 1958, *Merlinius brevidens* (Allen, 1955) Siddiqi, 1970, *Merlinius joctus* (Thorne, 1949) Sher 1974, *Merlinius* cf. *microdorus* (Geraert, 1966) Siddiqi, 1970, *Nagelus alpinus* (Allen, 1955) Siddiqi, 1979, *Nagelus obscurus* (Allen, 1955) Powers, Baldwin and Bell, 1983, *Nagelus leptus* (Allen, 1955) Siddiqi, 1979, *Neodolichorhynchus(Mulkorhynchus) lamelliferus* (de Man, 1880) Volkova, 1993, *Neodolichorhynchus (Neodolichorhynchus) microphasmis* (Loof, 1959) Jairajpuri and Hunt, 1984, *Paratrophurus* sp. Arias, 1970, *Pratylenchoides magnicauda* (Thorne, 1935) Baldwin, Luc and Bell 1983, *Pratylenchoides ritteri* Sher, 1970, *Pratylenchoides* sp. (Thorne, 1935) Baldwin, Luc and Bell 1983, *Quinisulcius acutus* (Allen, 1955) Siddiqi, 1971, *Sauertylenchus maximus* (Allen, 1955) Siddiqi, 2000, *Scutylenchus quadrifer* (Andrassy, 1954) Siddiqi, 1979, *Telotylenchus ventralis* Loof, 1963, *Trophurus imperialis* Loof, 1956, *Trophurus minnesotensis* (Caveness, 1958) Caveness, 1959, *Tylenchorhynchus nudus* Allen, 1955, and *Tylenchorhynchus* cf. *robustus* Thorne and Malek, 1968. Cf. was used to designate a population as similar to a valid species but not identified as such with certainty.

To construct a 28S tree, *Tylenchorhynchus leviterminalis, T. claytoni*, and *Bitylenchus dubius* sequences were assembled with the following taxa having LSU rDNA D2-D3

TABLE 2. LSU 28S rDNA Genbank Sequences for Merliniinae, Telotylenchinae, Macrotrophurinae, Pratylenchidae (Radopholinae) and Outgroups in Fig. 5 Tree.

Accession #	Nematode Species and Strain	Bp #	Sequence Source
AY780972	*Tylenchulus semipenetrans*[e]	547	Subbotin et al., 2005
AY780973	*Hemicycliophora typica*[e]	542	Subbotin et al., 2005
DQ915803	*Belonolaimus longicaudatus*[e]	723	Zen et al., 2007
DQ838803	*Dolichodorus mediterraneus*[e]	755	Jimenez Guirado et al., 2007
DQ328716	*Psilenchus* sp.[e]	655	Subbotin et al, 2006
AM412741	*Nacobbus aberrans*[e,d]	316	Vovlas et al., 2007
DQ328714	*Amplimerlinius icarus*[a]	653	Subbotin et al, 2006
EU368590	*Bitylenchus dubius* (T) [b,f]	654	Skantar, Handoo, Carta
DQ328708	*Macrotrophurus arbusticola*[c]	662	Subbotin et al, 2006
DQ328715	*Nagelus leptus*[a]	652	Subbotin et al, 2006
DQ328709	*Trophurus sculptus*[b]	671	Subbotin et al, 2006
EU368589	*Tylenchorhynchus claytoni*[b,f]	661	Skantar, Carta, Handoo
EU368591	*Tylenchorhynchus leviterminalis*[b,f]	660	Skantar, Carta, Handoo

Synonyms given with these accessions: (G) = *Geocenamus*, (T) = *Tylenchorhynchus*.
Bp = base pair or nucleotide.
[a]Merliniinae.
[b]Telotylenchinae.
[c]Macrotrophurinae.
[d]Pratylenchidae (Nacobbinae).
[e]Outgroups.
[f]Original sequences.

sequences from GenBank in Table 2: *Tylenchulus semipenetrans*, *Hemicycliophora typica* de Man, 1921, *Belonolaimus longicaudatus*, *Dolichodorus mediterraneus* Jiménez Guirado et al., 2006, *Nacobbus aberrans* (Thorne, 1935) Thorne and Allen, 1944, *Amplimerlinius icarus, Trophurus sculptus* Loof, 1956, *Macrotrophurus arbusticola*, and *Nagelus leptus.*

Alignments were made with ClustalW2 (Larkin et al., 2007) checked by eye for consistency of conserved positions, and edited in GeneDoc (Nicholas et al., 1997). Initially the alignment was run through PAUP*4b10 (Swofford, 2002). Heuristic simple and bootstrapped Maximum Parsimony (MP) searches were conducted employing tree bisection-reconnection (TBR) branch swapping, and accelerated transformation (ACCTRAN) character-state optimization. The computationally-intensive, probabilistic Maximum likelihood (ML) method is less affected by sampling error and infers better trees than distance or parsimony methods (Swofford et al., 1996), so ML trees are presented in figures. Alignments were subjected to ModelTest ver. 3.7 (Posada and Crandall, 2001) as implemented in Geneious Pro ver. 4.7 (Biomatters, Auckland, New Zealand; Drummond et al., 2009). The Akaike information criterion (AIC) for model selection was used rather than that of the likelihood ratio test (LRT) due to demonstrated superiority (Posada and Buckley, 2004) and because it is the standard within the Geneious module. Alignments in phylip format were run in web-based RAxML (Stamatakis et al., 2008) with 100 bootstrap runs and maximum likelihood estimate of 25 per site rate categories. The alignment was also subjected to Bayesian inference (BI) analysis with the MrBayes (Huelsenbeck and Ronquist, 2001) plugin for Geneious. ModelTest parameters were used for input into the MrBayes plugin which ran 1.1 million chains with Burnin = 110,000. Tree structures in Figs. 3–5 are based on the RAxML phylogeny, with branch support values above 50% given for ML followed by those for BI, and ML parameters given in figure legends. Bootstrap proportions (BP) that represent 'true' clades with 95% confidence intervals occur above 70%, a level considered robust support, with moderate support between 50-70%. Maximum likelihood BPs are mostly lower than Bayesian Posterior probability (BPP) scores that use all data rather than subsamples (reviewed in Zander, 2004).

RESULTS AND DISCUSSION

The morphology of male and female heads and tails of *B. dubius* (Fig. 1A), *T. claytoni* (Fig. 1 B, C), and *T. leviterminalis* (Fig. 1 D) were consistent with original descriptions and revisions (Steiner, 1937; Thorne, 1949; Siddiqi et al., 1982; Golden et al., 1987; Vovlas and Cheng, 1988).

Bitylenchus dubius had a crenate tail tip that was not always easy to see at certain planes of focus (Fig. 1A). The hyaline tail ranged from 8 to 12%, n = 6 of the tail length in this population of *B. dubius.* While the tail was not figured in the original description of *Tylenchus dubius* females (Bütschli, 1873), it was drawn later (Goodey, 1931) with a hyaline region 12% of the tail length.

Tylenchorhynchus claytoni had a smooth tail tip, with the hyaline tail region ranging from 12 to 25%, n = 6, of which the terminal cuticle represented 4 to 7% of the tail length; hyaline deposits of one (Fig. 1 B) or two layers (Fig. 1 C) can also be seen. Most of the tail variation already described in other populations of *T. claytoni* involved the shape, number of annules and

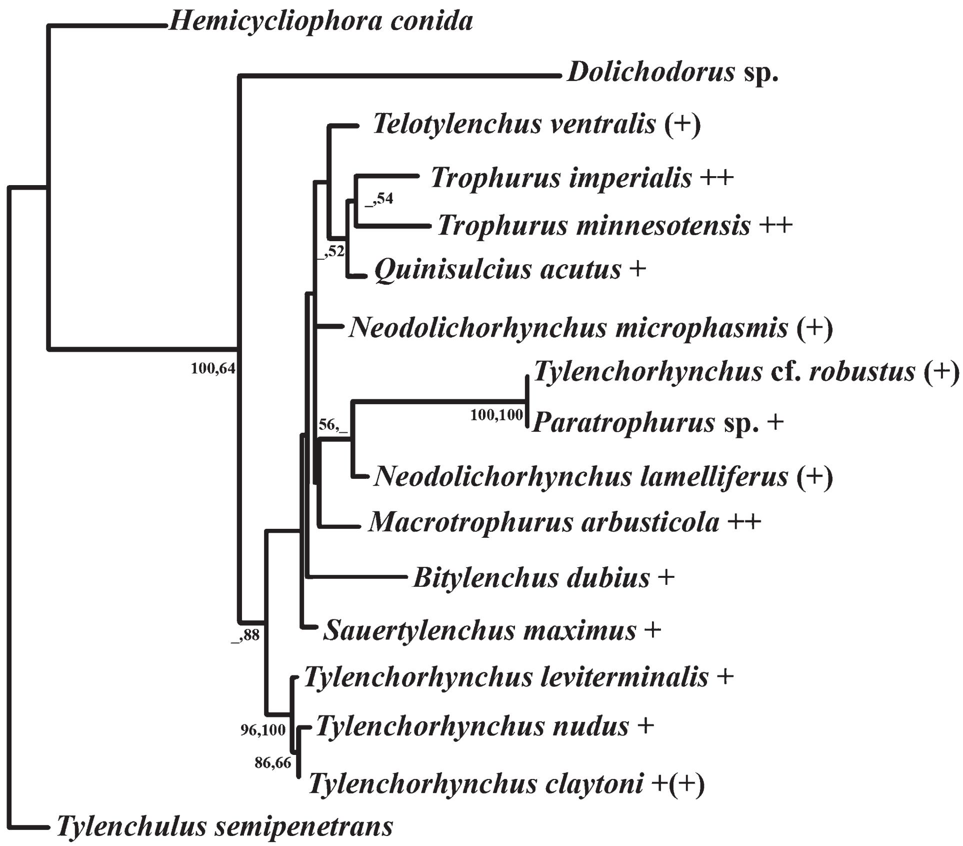

FIG. 3. Maximum Likelihood (ML) best SSU 18S tree of Telotylenchinae and Macrotrophurinae as implemented in RAxML, including Bayesian Inference (BI) clade support. Clade support percentages for ML are followed by BI near nodes, with __ representing the absence of a corresponding value. The 1731 position ClustalW alignment had 346 distinct alignment patterns. Likelihood of final tree evaluated and optimized under GAMMA, Final ML Optimization Likelihood: -4777.415574, Model Information: alpha: 0.178052, Tree-Length: 0.738249. The percentage of hyaline tail terminus length to total tail length coded as moderately thick (+) ≥ 4%, thick + ≥ 9%, or very thick ++ (≥ 20%).

their proximity to the tip (Golden et al., 1987), and not the quality of internal tissue as viewed with DIC. The currently studied population of *T. claytoni* had relatively thicker and more variable hyaline tail dimensions compared to other related taxa with tail variation drawn in the literature, e.g. *Tylenchorhynchus tritici* Golden et al., 1987 (13.5 to 18%, n = 5) (Golden et al., 1987), *T. nudus* (10.5 to 15.5%, n = 3) (Loof, 1959), *T. areoterminalis* Siddiqi, 2008 (17 to 21%, n = 2) (Siddiqi, 2008), *Scutylenchus* (= *Merlinius*) *quadrifer* (11.4 to 14.3%, n = 3) and *Merlinius rugosus* (15.6 to 18.4%, n = 3) (Loof, 1978).

For *T. leviterminalis,* the hyaline tail region represented 11.6 to 16%, n = 6 of the tail. A *T. leviterminalis* population from China had about a 15% hyaline tail /tail proportion based on median values (Vovlas and Cheng, 1988), and a population from Japan had a 23 to 24% hyaline tail region based on derived average measurements and a drawing (Talavera et al., 2002).

Drawings plus relative measurements and codes of tail termini for the other taxa represented in molecular phylogenetic trees are given in Fig. 2.

18S Trees for Telotylenchinae and Macrotrophurinae (Fig. 3): The three types of trees (MP, ML, and BI) had slightly different topologies and only the ML tree is shown in Fig. 3. MP analysis detected 209/1731 parsimony informative characters, yielding eight trees from a heuristic search. ModelTest found Model Tamura-Nei (TrN) + I + G, with nst = 6, gamma shape = 0.591, and proportion of invariant sites (pinvar) = 0.516. BI resulted in Log likelihood (LnL) mean = -5883.53, TL mean = 0.744, and alpha shape parameter of gamma distribution = 0.196.

Among the ingroups within the three outgroups in the Fig. 3 ML tree there was a basal clade with 100% ML/96% BI support for (*Tylenchorhynchus nudus* + *T. claytoni*) and *T. leviterminalis* branching just outside. This group had a sister group composed of the other telotylenchid taxa: *Sauertylenchus maximus* and *Bitylenchus dubius* outside a polytomy of three groups of (*Telotylenchus ventralis, Quinisulcius acutus,* two species of *Trophurus*), (*Neodolichorhynchus microphasmis*), and (*Macrotrophurus arbusticola, N. lamelliferus, Tylenchorhynchus robustus/ Paratrophurus* sp.). These last two morphologically-identified genera had identical sequence for populations with different hyaline tail dimensions. The BI tree (not shown) was somewhat different from the ML tree in having *Trophurus* branching outside the three *Tylenchorhynchus* species (72% clade support), and *Tylenchorhynchus* cf. *robustus/Paratrophurus* outside the entire remaining ingroup (74% clade support). For all

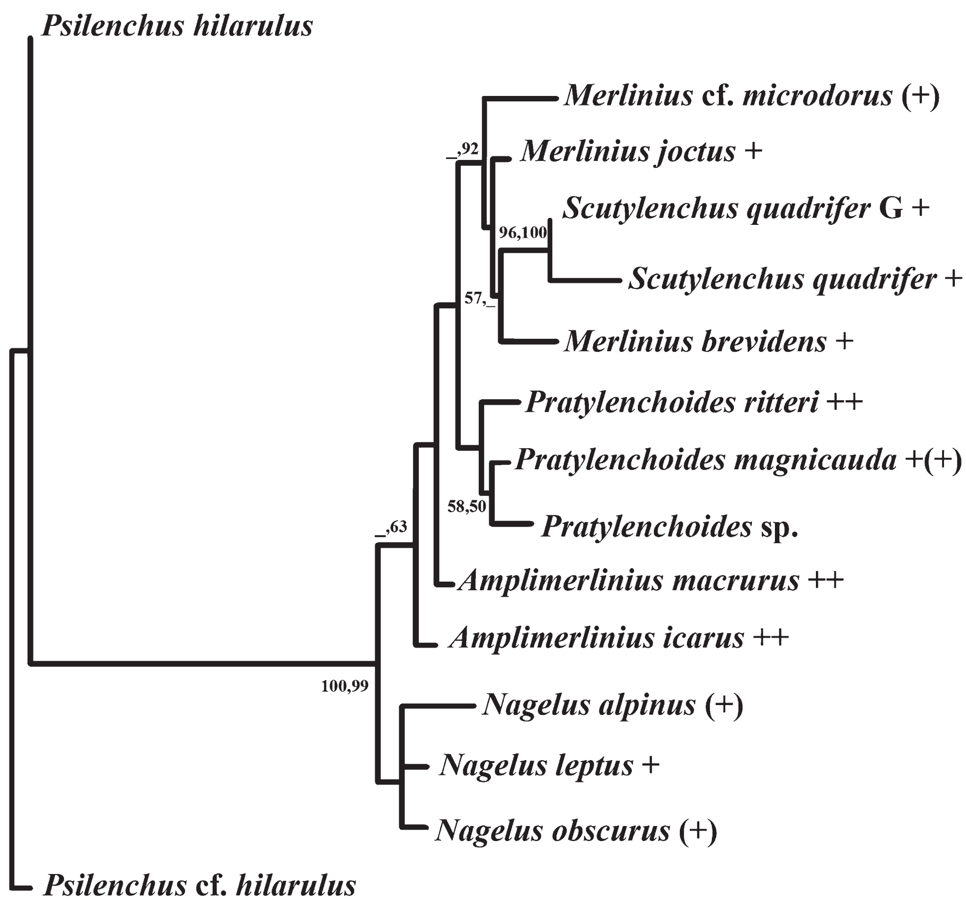

FIG. 4. Maximum Likelihood best SSU 18S tree for Merliniinae and *Pratylenchoides* spp. as implemented in RAxML, including Bayesian Inference (BI) clade support. Clade support percentages for ML are followed by BI near nodes, with __ representing the absence of a corresponding value. The 1777 position Clustal W alignment had 98 distinct alignment patterns. Likelihood of final tree evaluated and optimized under GAMMA+P-Invar, ML Optimization Likelihood: -2580.137694, alpha: 0.020013, pinvar: 0.000117, Tree-Length: 0.105073. The percentage of hyaline tail terminus length to total tail length coded as moderately thick (+) ≥ 4%, thick + ≥ 9%, or very thick ++ (≥ 20%).

trees, *Macrotrophurus* did not group with *Trophurus* into a clade of taxa with long hyaline tails, nor did *Telotylenchus* group with *Tylenchorhynchus*, a prediction from proposed synonymy of *Telotylenchus* (Fortuner and Luc, 1983). Both species of *Neodolichorhynchus* failed to group together. *Neodolichorhynchus* is a genus characterized by longitudinal ridges outside the lateral field. One subgenus *Neodolichorhynchus (Neodolichorhynchus) microphasmis* was defined without a bursal notch and lacking lateral vulval membranes, while *Neodolichorhynchus (Mulkorhynchus) lamelliferus* had these features (Jairajpuri and Hunt, 1984). In light of their diverged position relative to one another on the Fig. 3 tree, subgenus *Mulkorhynchus lamelliferus* might change rank in the future.

In the 18S alignments, there were only 2 nucleotide differences (0.3% of sequence) between *T. claytoni* and *T. nudus* and between *T. claytoni* and *T. leviterminalis*. They all had similar tail thickness, but the *T. leviterminalis* tail was wider. There were about 40 nucleotide differences between *T. leviterminalis* and the population similar to *T. robustus*.

Tylenchorhynchus leviterminalis, T. nudus and *T. robustus* but not *T. claytoni* were included in a proposed new genus carved from *Tylenchorhynchus* called *Macrorhynchus* Sultan, Singh and Sakhuja, 1991, based on coarse body annulations and continuous lip region (Sultan et al., 1991). This scheme is not congruent with the current tree topology since *T. nudus* makes a more likely and well supported clade with *T. claytoni* rather than with *T. leviterminalis,* and putative *T. robustus* is far removed from this clade in Fig. 3. One discrete difference among these *Tylenchorhynchus* spp. is that *T. robustus* has more than twice the number of tail annules (40 – 50) compared to the other three *Tylenchorhynchus* (10 – 21) (in Handoo, 2000).

Bitylenchus Filipjev, 1934 and *Sauertylenchus* Sher, 1974 were located on adjacent branches (Fig. 3), consistent with their lack of a gubernaculum crest that is present in *Tylenchorhynchus* and *Paratrophurus* (Gomez-Barcina et al., 1992; Siddiqi, 2000).

Trophurus Loof, 1956 was the first genus among Telotylenchidae to be defined by its enlarged hyaline tail region plus a single gonad. *Paratrophurus* Arias, 1970 had two female gonads, and its thick tail was loosely defined by "cuticle strongly swollen on tail terminus." Various degrees of ovary regression are often associated with tail regression in *Paratrophurus* (Luc et al., 1987;

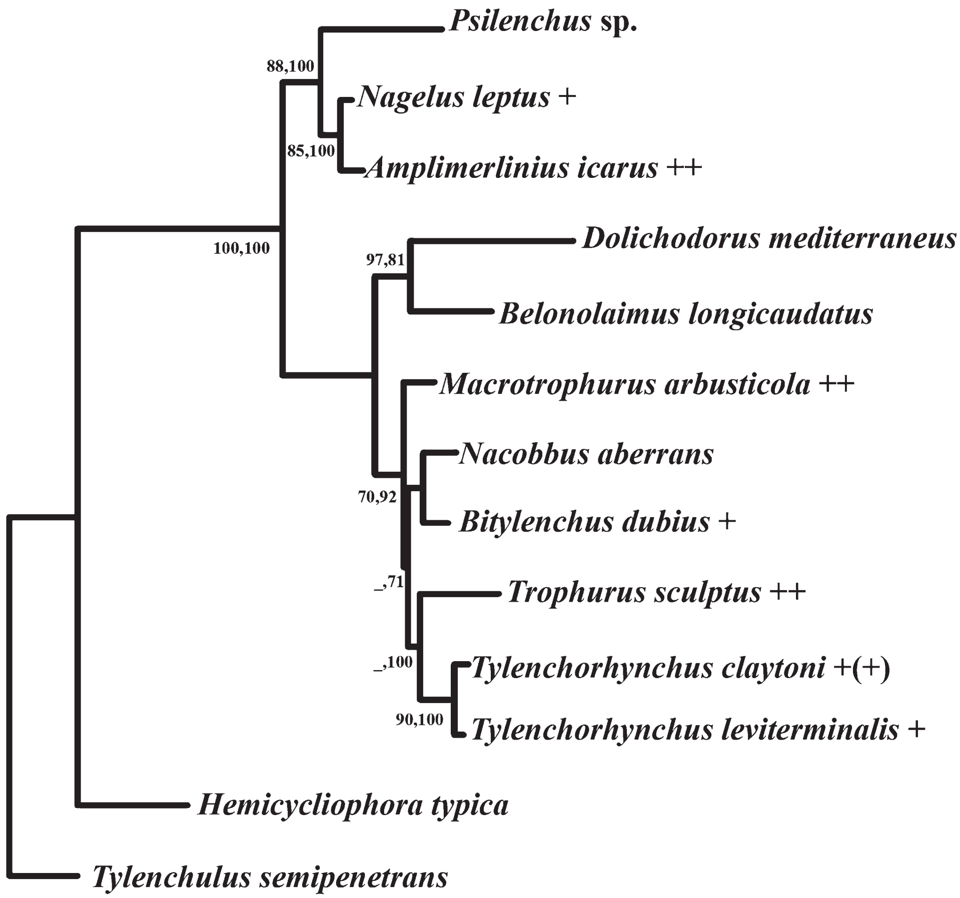

FIG. 5. Maximum Likelihood (ML) best LSU 28S tree for Telotylenchidae as implemented in RAxML, including Bayesian Inference (BI) clade support. Clade support percentages for ML are followed by BI near nodes, with __ representing the absence of a corresponding value. The 780 position ClustalW alignment had 412 distinct alignment patterns. GAMMA+P-Invar Model parameters, Final ML Optimization Likelihood: -4993.382411, alpha: 1.317998, invar: 0.323274, Tree-Length: 4.438284. The percentage of hyaline tail terminus length to total tail length coded as moderately thick (+) ≥ 4%, thick + ≥ 9%, or very thick ++ (≥ 20%).

Kleynhans, 1992). *Paratrophurus loofi* Arias, 1970 from wheat in Sevilla, Spain, had a hyaline tail region representing 33% of the tail (Arias, 1970). This type species had a much shorter postanal intestinal sac than that in a second species, *Paratrophurus acristylus* Siddiqi and Siddiqui, 1983. The latter was described as having the hyaline portion of the female tail terminus equaling 21 to 28% of tail length as well as a prominent postanal intestinal sac, a feature also characteristic of *Bitylenchus* spp. (Gomez-Barcina et al., 1992). *Bitylenchus dubius* in particular has very similar morphometrics to *P. acristylus* from Libya and Morocco (Castillo et al., 1989), but *P. acristylus* has a somewhat shorter, thicker tail and terminus (*B. dubius vs. P. acristylus*: c = 12 to 17 *vs.* 16 to 19, c' = 2.2 to 3.7 *vs.* 2.3 to 2.7 in Brzeski and Dolinski, 1998; Castillo et al., 1989), and lips not annulated or offset. Except for the tail terminus, *Paratrophurus* spp. are very similar to *Tylenchorhynchus* spp. (Castillo et al., 1989; Siddiqi, 2000). An even more forceful argument was made for *Paratrophurus* synonymy on the basis of intermediate length tails of *Paratrophurus bursifer* populations extending into the range of those for *Tylenchorhynchus* spp. (Sturhan and Lišková, 2004). The original description of *Tylenchorhynchus bursifer* (pre-*Paratrophurus* synonymy) showed a 60% hyaline tail proportion (Loof, 1959). Tail termini measurements of other species assigned to the genus *Paratrophurus* (Arias, 1970) ranged from 20 to 40% (Castillo et al., 1989; Kleynhans, 1992). These proportions overlap those of *T. leviterminalis,* the current population of *T. claytoni,* and other *Tylenchorhynchus* species with long hyaline tails such as *T. clavicaudatus* Seinhorst, 1963 (34.5%) (Seinhorst, 1963). Voucher images of female *Paratrophurus* spp. and *Tylenchorhynchus* cf. *robustus* (Mullin, 2000a; Mullin, 2000b) revealed differences in thickness of hyaline tail termini (13% vs. 7%) despite having identical 18S sequences. This situation illustrates the difficulty in assigning genera to species or populations with tail retraction, and no firm phylogenetic conclusions can be made until sequences from defined species and type populations of *Paratrophurus* are compared.

18S trees for Merliniidae (Fig. 4): Three types of trees had slightly different topologies and only the ML tree is shown. MP analysis detected 39/1777 parsimony informative characters yielding 28 trees from a heuristic search employing TBR branch swapping. ModelTest selected GTR + I + G, gamma shape = 0.806, and pinvar = 0.812. BI gave Log Likelihood (LnL) mean = -3279.557, Tree length (TL) mean = 0.117, and alpha shape parameter of gamma distribution = 0.038.

A clade of *Merlinius* and *Scutylenchus* (92% support) with thick tails formed a sister clade to *Pratylenchoides* spp., and these three genera plus *Amplimerlinius* (very thick tails) formed a sister group to *Nagelus* (moderately thick to thick tails). This could be interpreted as very thick tails arising first in *Amplimerlinius*, continuing in *Pratylenchoides* and reverting to merely thick tails in *Merlinius* and *Scutylenchus*. The two populations of *Scutylenchus quadrifer* demonstrated as much or greater genetic distance between them as among the three related species of *Merlinius,* possibly due to cryptic speciation, different haplotypes or misidentification.

Nagelus alpinus (Siddiqi, 1979; Siddiqi, 2000) is considered *Merlinius* by some, so it is significant that *N. alpinus* did not appear within the well-supported *Merlinius* and *Scutylenchus* clade. Relatively long hyaline tail regions were noted in *Nagelus leptus* and related species (sequences not available), having deirids at the part of the lateral field where there are six incisures, as opposed to the other *Nagelus* spp. with deirids at the junction of four incisures (Powers et al., 1983). *Amplimerlinius* spp., characterized by thickened female tail terminal cuticle and extended hyaline tail regions, also had deirids and six lines in the lateral field (Siddiqi, 1976). Consistent with similar morphology (Powers et al., 1983) *Amplimerlinius* spp. branched just outside *Nagelus* spp.

28S trees of Merliniidae and Telotylenchidae (Fig. 5): Three types of trees had slightly different topologies and only the ML tree is shown. MP analysis detected 302/780 parsimony informative characters yielding a single tree of length 1020, and CI = 0.63. ModelTest gave the General time reversible model (GTR) + I + G, nst = 6, gamma shape = 1.2967, and pinvar = 0.3307. BI gave LnL mean = -5017.331, TL = 3.262, and alpha shape parameter of gamma distribution = 0.435.

In terms of information content measured by absolute numbers of parsimony informative characters in these tree alignments, the 28S tree had 31% more parsimony informative characters than the 18S alignment for Telotylenchinae (Fig. 3) and ten times more than that in the Merliniinae alignment (Fig. 4). The parsimony informative characters divided by the total alignment characters were 39% for the 28S Fig. 5 alignment, 12% for the 18S Fig. 3 alignment, and 2.3% for the Fig. 4 alignment. Therefore the 28S rDNA alignment contained at least three times the number of phylogenetically informative alignment characters relative to the alignment total compared to the larger 18S dataset. There is also broader taxon sampling for the 18S molecule which is better for revealing deeper phylogenetic relationships than for these genus and species level comparisons.

In this 28S tree, *Macrotrophurus* was basal to *Bitylenchus dubius*, both of which formed a sister group with *Tylenchorhynchus claytoni* and *T. leviterminalis*. *Belonolaimus* and *Dolichodorus* were positioned between these Telotylenchinae/Tylenchorhynchidae, dividing them from *Nagelus, Amplimerlinius* (Merliniinae) and *Psilenchus*. *Nacobbus* was included based on its appearance outside Telotylenchinae and Macrotrophurinae in a recent Bayesian tree (Holterman, 2009), so the appearance in this tree of *Macrotrophurus* in the expected position of *Nacobbus* outside the other Telotylenchinae may be an artifact of insufficient taxon sampling. The topology of this tree was otherwise congruent with those from the 18S trees, although the sparse taxon representation does not provide much information for taxonomic evaluation.

Thick-tailed *Trophurus* and *Macrotrophurus* did not group together in any 18S tree or in the 28S tree. Regardless of the variation in tree topologies, thick and thin tail termini alternated within tree clades and at the species and genus level in these trees. From hyaline tail measurements (Fig. 2), and tail termini designations on the trees, it appears that very thick tail termini have arisen at least three times within this assemblage of Telotylenchinae with *Trophurus, Macrotrophurus* and *Tylenchorhynchus claytoni* (Fig. 3) and once for *Amplimerlinius* and *Pratylenchoides* within this group of Merliniinae/Merliniidae (Fig. 4).

Arguments over which morphological characters will be most reliable over time underlie conflicting higher taxonomic categories. The original character of greatest historical concern to stunt nematode taxonomy was the degree of overlap, if any, of the pharyngeal glands relative to the intestine (Thorne, 1949). A number of taxonomists have argued against the use of this character at the family and even genus level (Fortuner and Luc, 1987; Loof, 1987). It is interesting that *Telotylenchus* with overlapping glands and a *Tylenchorhynchus*-like face pattern (Sher and Bell, 1975) was far removed from the clade in 18S trees containing *Tylenchorhynchus*, a genus composed of species either lacking or possessing a slight overlap (e.g. *T. clarus* Allen, 1955). Also *Pratylenchoides ritteri* had a long gland overlap (Fortuner and Luc, 1987) unlike the taxa that surrounded it in the tree.

In summary, populations of thick-tailed *Trophurus, Macrotrophurus* and putative *Paratrophurus* did not group together in any molecular tree, and tail thickness was mosaically distributed among species within Merliniinae and Telotylenchinae and related taxa, with extreme thickness arising at least once in Merliniinae and three times in Telotylenchinae. However, more taxa and molecular characters are needed to better delineate and support various groups represented by this data set. Although it is currently a major character for differentiating genera of *Trophurus, Paratrophurus, Telotylenchoides* Siddiqi, 1971, *Meiodorus* Siddiqi, 1976, and *Amplimerlinius* (Siddiqi, 2000), the striking character of a thickened, retracted female tail terminus should be considered a highly convergent, species-level feature. Otherwise, insufficient or inappropriate keys may be erroneously consulted for borderline populations similar to *Paratrophurus* or thick-tailed *Bitylenchus* or *Tylenchorhynchus.* It is important to initially identify stunt nematodes within a broad framework. Newer component genera or possibly subgenera might earn wider usage once their relatives fill in the not-always obvious gaps within the spectrum of current sequences. If paraphyly continues to be confirmed with more taxa for ribosomal genes and for key taxa using other genes, usage of Telotylenchidae will be inappropriate if taxonomy is to reflect monophyletic groups. Whether Telotylenchidae persists should have little effect on alpha taxonomy though. The basic tension between practical identification with stable names and more theoretical phylogenetics for refining taxon limits (de Pinna, 1999) contributes to competing names for stunt nematodes. Agreement on one system is not likely in the near future.

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