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

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ABSTRACT

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Cyst (Heteroderidae), root knot (*Meloidogyne* spp.), and lesion (*Pratylenchus* spp.) nematodes all belong to a single nematode order, Tylenchida. However, the relationships between and within these economically highly relevant groups, and their relatedness to other parasitic Tylenchida is unclear. We constructed a phylogeny of 116 Tylenchida taxa based on full length small subunit ribosomal DNA (small subunit [SSU] rDNA) sequences. Ancestral state reconstruction points at a gradual development of simple to more complex forms of plant parasitism. Good resolution was observed in distal clades that include cyst, root knot, and lesion nematodes, and monophyly of most families was confirmed. Our data suggest that root knot nematodes have evolved from an ancestral member of the genus *Pratylenchus*, but it remains unclear which species is closest to this branching point. Contrary to the notoriously polyphagous distal representatives, basal members of the genus *Meloidogyne* (and probably, their common ancestor) have narrow host ranges. Our analysis also shows that mitotic parthenogeny has arisen at least two times independently among root knot nematodes. In many cases resolution till species was observed, suggesting that SSU rDNA sequences have a potential for DNA barcode-based species identification with, due to the overall conserved nature of this gene, limited intra-species variation.

Nematodes are one of the most successful groups of animals; they are abundant, diverse, and live in virtually all soil, freshwater, and marine habitats. These small vermiform organisms occupy several trophic levels and play an important role in the soil ecosystem (6). Certain groups of nematodes also cause large economic losses as parasites of animals (including humans) or parasites of plants (2,32). Total losses caused by plant-parasitic nematodes are estimated at $80 billion annually (1), and most of these—including the cyst (family Heteroderidae), lesion (*Pratylenchus* spp.) and root knot nematodes (*Meloidogyne* spp.)—belong to the order Tylenchida.

Because of the economic importance of this group, the Tylenchida have always received ample taxonomic attention. Nematodes are colorless, unsegmented, wormlike animals, round in cross section. As such, nematodes show a paucity of clearly distinguishable, informative morphological characters. Moreover, the polarity of morphological characters (ancestral or derived) is often hard to determine. This has led to several different classifications and constant taxonomic changes (9,24,32). Molecular data can provide a virtually unlimited number of characters, and therefore can help to create more clarity in the phylogenetic relationships within this group. Such an analysis could also show whether or not a selected DNA segment has a potential for DNA-based pathogen identification.

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*The *e*-Xtra logo stands for "electronic extra" and indicates that there are two additional tables showing GenBank accession numbers and feeding types and two figures showing maximum likelihood tree of Tylenchida and maximum likelihood trees of Pratylenchidae and Meloidogynidae.

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The Tylenchida contain mainly plant parasites, but it is hypothesized that this plant parasitism evolved from fungal feeding ancestors that acquired the ability to feed on algae, mosses or root hair, and epidermal cells (24). These simple forms of plant parasitism are thought to have evolved gradually, via intermediary forms, towards more complex forms of plant parasitism that can be found among sedentary endoparasites such as cyst and root knot nematodes. The availability of a robust molecular phylogeny would allow us to test this hypothesis. Remarkably, the Tylenchida also harbor a number of insect parasites (members of the suborder Hexatylina). These represent the only case in the phylum Nematoda in which insect parasites are closely related to (and probably evolved from) fungal and plant feeding nematodes.

In recent years much research has been done on the molecular interactions between plant-parasitic nematodes (mainly endoparasites) and plants. One of the remarkable things discovered was that several endoparasitic species—*Globodera*, *Heterodera* (33), *Meloidogyne* (28), *Pratylenchus* (41), and *Bursaphelenchus* (21)—produce and secrete cell wall-degrading enzymes, something which had never been recorded before in animals (33). If we would like to understand the genesis of plant parasitism, it is essential to have a robust phylogenetic framework based on (a) neutral (pathogenicity unrelated) gene(s). The phylogeny presented here is based on the small subunit ribosomal DNA (small subunit [SSU] rDNA) gene, which has proven to be useful for reconstructing nematode phylogeny. Accelerated evolution in this clade (Clade 12 [16]), a phenomenon probably related to the plant-parasitic nature of virtually all its members, resulted in a high resolution among the Tylenchina in most cases even up to species level. Apart from establishing the relationships between some notorious plant parasites, the number of autapomorphies for

many plant-parasitic nematode species (the tropical root knot nematodes constitute a major exception) suggests that the SSU rDNA gene could be exploited for DNA-based species identification.

MATERIALS AND METHODS

Taxon sampling and systematics. Nematodes were collected from various habitats throughout the Netherlands. They were extracted from the soil using standard techniques and identified under a light microscope. Prior to DNA extraction, pictures were taken using a CCD camera (CoolSnap, RS Photometrics, Tucson, AZ). SSU rDNA sequences from collected species were supplemented with sequences taken from GenBank. For the systematics throughout this paper, we adhere to the classification of Siddiqi (32). For the confirmation of the identity of some *Meloidogyne* species, isozyme electrophoresis patterns were used. The young adult female was separated from the egg mass. Isozyme electrophoresis was done on the young female (19). The egg mass was subsequently used for DNA extraction.

DNA extraction and SSU rDNA sequencing. Single nematodes were transferred to 0.2 ml polymerase chain reaction (PCR) tube containing 25 µl of sterile water. An equal volume of lysis buffer containing 0.2 M NaCl, 0.2 M Tris-HCl (pH 8.0), 1% (vol/vol) β-mercaptoethanol, and 800 µg/ml of proteinase K was added. Lysis took place in a Thermomixer (Eppendorf, Hamburg, Germany) at 65°C and 750 rpm for 2 h followed by a 5 min incubation at 100°C. Lysate was immediately used or stored at –20°C. SSU rDNA was amplified as two partially overlapping fragments using three universal and one nematode-specific primer (1912R). For the first fragments, either the primer 988F (5′-ctcaaagattaagccatgc-3′) or the primer 1096F (5′-ggtaattctggagctaatac-3′) was used in combination with the primer 1912R (5′-tttacggtcagaactaggg-3′). The second fragment was amplified with primers 1813F (5′-ctgcgtgagaggtgaaat-3′) and 2646R (5′-gctaccttgttacgactttt-3′). PCR was performed in a final volume of 25 µl containing 3 µl of 100× diluted crude DNA extract, 0.1 µM of each PCR primer and a Ready-to-go PCR bead (GE Healthcare, Little Chalfont, UK). The following PCR program was used: 94°C for 5 min; 5× (94°C, 30 s; 45°C, 30 s; 72°C, 70 s); 35× (94°C, 30 s; 54°C, 30 s; 72°C, 70 s); 72°C for 5 min. Gel-purified amplification products (Marligen, Ijamsville, MD) were cloned into a TOPO-TA vector (Invitrogen, Carlsbad, CA) and sent off for sequencing using standard procedures. Some of these sequences have been used in a previous study (16), sequences newly generated for this study were deposited at GenBank with accession numbers EU669909 to EU669959 and EU682391 to EU682392.

Sequence alignment and phylogenetic analysis. The sequences were aligned using the ClustalW algorithm as implemented in the program BioEdit 7.0.1 (13). The alignment was then manually corrected using arthropod secondary structure information (42). The final Tylenchida alignment included 116 sequences with a total length of 1,916 bases, including gaps.

The Tylenchida SSU rDNA tree was constructed using Bayesian inference and a fast maximum likelihood model. Modeltest 3.06 (26) selected the GTR model with invariable sites and a gamma substitution as the best fitting nucleotide substitution model using both the likelihood ratio test and the Akaike information criterion. The Bayesian phylogeny was constructed with the program MrBayes 3.1.2 (27) using the GTR model with invariable sites and a gamma-shaped distribution of substitution rates. On the basis of the secondary structure, the overall alignment was subdivided into stem and loop partitions. Parameters were unlinked between the partitions and the rate prior was set to variable. The default flat priors were used. Four independent runs were made with 4 Markov chains per run. The program was run for 5 million generations. Stabilization of the likelihood and parameters was checked with the program Tracer 1.2.1 (http://evolve.zoo.ox.ac.uk/) and the burnin was set at 700,000 generations.

The second phylogenetic tree was constructed with a fast maximum likelihood method. The SSU rDNA alignment was analyzed at a distant server running the program, RAxML-VI-HPC v.4.0.0 (Randomized Axelerated Maximum Likelihood for High Performance Computing [34]). A GTR model with invariable sites and gamma distribution was used, and the dataset was divided in a stem and loop partition. One hundred bootstraps were performed.

For the Pratylenchidae and *Meloidogyne* tree, six additional *Pratylenchus*, seven additional *Hirschmanniella*, and 48 additional *Meloidogyne* sequences were included. The alignment consisted of 81 sequences with a total alignment length of 1,815 bases including gaps. Bayesian and fast maximum likelihood trees were constructed using the same programs and settings as used for the Tylenchida trees. Burnin for the Bayesian tree was 100,000 generations.

Alternative topologies for the position of *Radopholus similis* were compared with the Shimodaira-Hasegawa (SH) test (31) using the program phylogenetic analysis using parsimony (PAUP*) v.4.0b10 (36). The parameter values calculated by Modeltest for the GTR+I+G model were used as the likelihood settings.

Reconstruction of ancestral feeding types. Feeding types of each species were according to Yeates et al. (44). When more than one feeding type was listed for a genus, we referred to Siddiqi (32) or Bongers (5) to infer the correct feeding type for each species. In a few cases other literature had to be used and for one species, *Ditylenchus adasi*, the correct feeding type (hyphal feeding, migratory endoparasite, or both) could not be determined.

Ancestral feeding types were reconstructed using the program Mesquite 1.12 (http://mesquiteproject.org). Ancestral character states can be reconstructed in several different ways. We chose to reconstruct ancestral feeding types using both the parsimony and likelihood model. Both an unordered parsimony analysis and a parsimony analysis using a step matrix (Table 1) were performed. For the likelihood analysis no polymorphic character states could be included, so two character matrices were used, one including the most ancestral character state (usually hyphal feeding) for polymorphic species and the second matrix containing the most derived character states. Branches in the tree with a posterior probability of <0.95 were collapsed for the analyses.

RESULTS AND DISCUSSION

Relationships between major plant-parasitic nematode families. Two independent algorithms (Bayesian inference and maximum likelihood) were used to reconstruct relationships within the nematode order Tylenchida on the basis of 116 full-length small subunit ribosomal DNA sequences (≈1.700 bp each). The Bayesian tree (Fig. 1) and maximum likelihood tree were virtually identical in terms of their resolution. In the top part of the tree, a robust sister relationship between members of the Hoplolaimidae and the Heteroderidae (A) and representatives of the Meloidogynidae, Pratylenchidae, and most Telotylenchidae (B) is observed. Together with the genus *Pratylenchoides* and a few representatives of the Telotylenchidae, whose SSU rDNA sequences defined a separate subclade (C), these major subclades constitute the suborder Hoplolaimina. In between the most basal (Aphelenchidae and Paraphelenchidae) and the top parts of the tree, a number of branching points are poorly supported and there are several polytomies. Although we cannot define the relationship between the suborder Criconematina and other Tylenchida suborders, its members clearly constitute a separate and well-supported subclade (Fig. 1D). On the other hand, SSU rDNA sequences from representatives of the suborders Tylenchina and

Hexatylina do not cluster as monophyletic groups. The fungivorous families Aphelenchidae (harboring a single genus; *Aphelenchus*) and the Paraphelenchidae (harboring a single genus; *Paraphelenchus*) are robustly positioned at the base of this phylogenetic tree.

At family level, SSU rDNA-based systematics corresponds closely to current morphology-based systematics. Only 6 out of the 22 families included are demonstrably para- (Hoplolaimidae, Criconematidae) or polyphyletic (Pratylenchidae, Telotylenchidae, Ecphyadophoridae, and Neotylenchidae). Here, we will focus further on the Hoplolaimina, since this is the region of the tree that is best supported and it contains most of the economically important plant parasites.

Heteroderidae and Hoplolaimidae (A). Except for the odd placement of *Rotylenchus* (*Pararotylenchus*) sp., a member of the subgenus *Pararotylenchus*, as the sister taxon to the Heteroderidae, the Heteroderidae, and the Hoplolaimidae appear as valid (=monophyletic), closely related sister families. Additional sequences from *Pararotylenchus* will be needed to establish the relationship between this subgenus and the Heteroderidae.

The placement of burrowing nematode *Radopholus similis* (member of the Pratylenchidae) as sister group of the Hoplolaimidae (Fig. 1) is consistent with earlier analyses by Bert et al. (4), and Subbotin et al. (35) based on partial large subunit (LSU) rDNA sequences. The remarkable long branch length prompted us to test two alternative positions of *R. similis* (alt. 2) at the base of Clade B, and (alt. 3) basal in the Pratylenchidae and Meloidogynidae subclade. Using the SH test both alternatives were discarded ($P = 0.011$ and $P = 0.000$). There are a number of morphological characters that support its placement with the Hoplolaimidae. First of all, its strong sexual dimorphism in the cephalic region is shared by the Hoplolaimidae and Heteroderidae, and absent in the Pratylenchinae, Hirschmanniellinae, and Telotylenchidae (32). *Radopholus* (and *Radopholoides*, not included) is also the only member of the Pratylenchidae to have a protrusible gubernaculum, a trait shared by part of the Hoplolaimidae (Hoplolaiminae, *Rotylenchus*, *Aphasmatylenchus* [32]). Given the results of the current and previous (35) molecular phylogenetic analyses and the morphological heterogeneity of the Pratylenchidae (23), a revision of the family and especially the subfamily Radopholinae seems necessary.

Meloidogynidae, Pratylenchidae, and Telotylenchidae (B). The current phylogenetic analysis places *Meloidogyne* within the Pratylenchidae. This is consistent with earlier analyses (16,35). Remarkably, the false root knot nematode *Nacobbus aberrans* clusters with the Telotylenchidae in the Bayesian tree. Because the support for this position is weak, no further attention will be paid to this observation.

A more extensive Pratylenchidae and *Meloidogyne* tree (Fig. 2) was constructed to see whether the Meloidogynidae really originate from within the Pratylenchidae, and to verify whether there is indeed an evolutionary trend among *Pratylenchus* and *Meloidogyne* species from amphimixis towards multiplication through parthenogenesis as suggested by Ryss (30), and Trudgill and Blok (40).

The SSU rDNA Pratylenchidae tree is well able to separate different Pratylenchus species, but the support for the Pratylenchidae backbone remained poor (Fig. 2). The inclusion of additional *Hirschmanniella* representatives resulted in a separation between *Hirschmanniella* cf. *belli* and *H. loofi* and the other *Hirschmanniella* species (38). The relationship between the Hirschmanniellinae and the Pratylenchinae, and the relatedness between members of the two Pratylenchinae genera—*Zygotylenchus* and *Pratylenchus*—remain to be resolved. Nevertheless, our analysis provided ample support for three *Pratylenchus* clusters: cluster a including *P. thornei* and *P. neglectus*, cluster b with *P. vulnus* and *P. pratensis*, and cluster c comprising *P. crenatus*, *P. penetrans*, and *P. convallariae* (according to this analysis equivalent to *P. penetrans*). Our analysis suggests that the genus *Pratylenchus* as currently defined is not monophyletic, and as such the nomenclature of this important group of plant parasites requires reconsideration. It is realized that only a fraction (≈13%) of the 68 valid *Pratylenchus* species (8) was included in this study. Nevertheless, the molecular diversity within this genus is remarkably high, and, most likely, species identification on the basis of SSU rDNA-based sequence signatures will be possible.

Among root knot nematodes, the addition of new SSU rDNA sequences from so far uncharacterized *Meloidogyne* species did not change the overall topology as proposed by Tandingan de Ley et al. (37). Three well supported major clades can be distinguished within *Meloidogyne* (Fig. 2). Clade I contains polyphagous tropical nematodes such as *M. incognita*, *M. javanica*, and *M. arenaria*, Clades II and III contain polyphagous nematodes from more temperate regions. Clade III can be divided into two sister groups: group A containing *M. chitwoodi*, *M. fallax*, and *M. minor*, and group B containing *M. naasi*, *M. oryzae*, and *M. graminicola*. *M. exigua* occupies a basal position in clade III. The basal *Meloidogyne* species consist of the polyphagous *M. artiellia* and the oligophagous *M. mali*, *M. ulmi*, and *M. ichinohei* and a new as yet undescribed species found on *Sansevieria* (G. Karssen, *unpublished data*). As far as root knot nematodes are concerned, the topology of the SSU rDNA tree reveals a tendency towards polyphagy from the basal towards the more distally positioned *Meloidogyne* species.

Many *Meloidogyne* species are parthenogenetic and the occurrence of parthenogenesis has been correlated with an increasing importance as crop parasites (7). Clade I consists almost exclusively of mitotic parthenogenetic species (except *M. floridensis* which is meiotic parthenogenetic; Fig. 2). Clade III is almost exclusively meiotic parthenogenetic (with the exception of the mitotic parthenogenetic *M. oryzae*). Clade II contains both amphimictic (*M. microtyla* and *M. spartinae*) and meiotic par-

TABLE 1. Step matrix used in parsimony reconstruction of ancestral feeding types[a]

Feeding type	Bacterial feeding	Hyphal feeding	Algal, lichen, and moss feeding	Root hair and epidermal feeding	Ectoparasite	Semi-endoparasite	Migratory endoparasite	Sedentary endoparasite	Insect parasite
Bacterial feeding	x	3	4	5	6	7	8	9	3
Hyphal feeding	3	x	1	2	3	4	5	6	3
Algal, lichen, and moss feeding	4	1	x	1	2	3	4	5	3
Root hair and epidermal feeding	5	2	1	x	1	2	3	4	3
Ectoparasite	6	3	2	1	x	1	2	3	3
Semi-endoparasite	7	4	3	2	1	x	1	2	3
Migratory endoparasite	8	5	4	3	2	1	x	1	3
Sedentary endoparasite	9	6	5	4	3	2	1	x	3
Insect parasite	3	3	3	3	3	3	3	3	x

[a] The different feeding types were put on a gradual scale ranging from bacterial feeding at the one end to sedentary endoparasitism on the other end. The change from bacterial feeding to hyphal feeding was considered to be greater, because of the morphological changes required. Insect parasitism did not fit in this gradual scale so every change to or from insect parasitism was considered equally likely.

thenogenetic (*M. hapla* race A) species. The current phylogeny also shows that mitotic parthenogeny has arisen at least two times independently among root knot nematodes (in Clades I and III).

It is generally assumed that the first *Meloidogyne* species were amphimictic and that facultative meiotic parthenogenetic species arose from sexually reproducing ancestors (40). The mitotic parthenogenetic species in turn then evolved from these meiotic

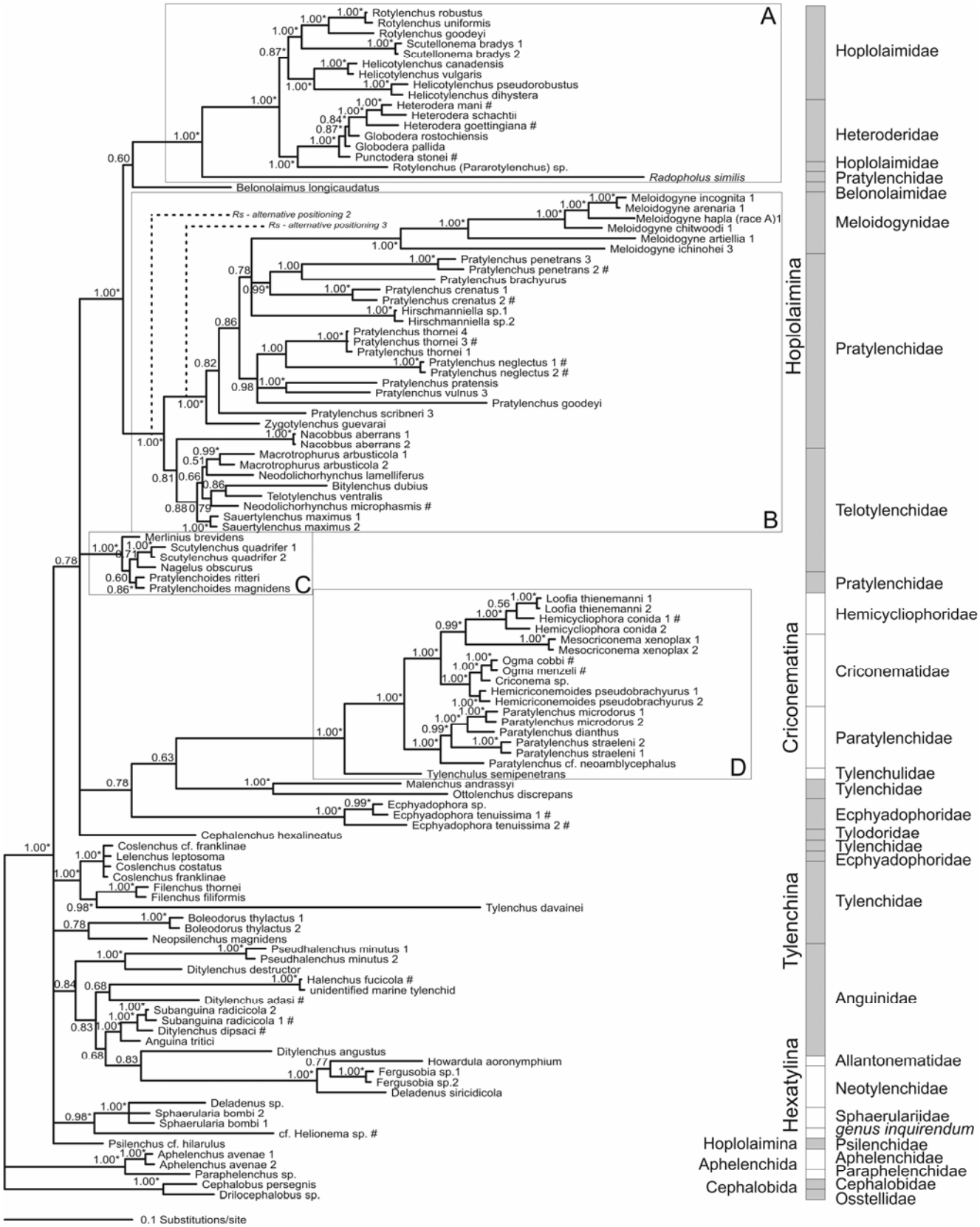

Fig. 1. Bayesian small subunit (SSU) rDNA tree of the Tylenchida. Numbers near nodes indicate posterior probabilities. An asterisk (*) indicates if the nodes is also supported by the maximum likelihood tree (bootstrap value >70%). Newly generated SSU rDNA sequences are labeled with a #. Vertical bar indicates families and suborders.

parthenogenetic species after polyploidization or interspecific hybridization events (7). SSU rDNA sequence analyses revealed five *Meloidogyne* species that are robustly positioned at the base of Clades I, II, and III, namely *M. ichinohei*, a so far undescribed species from *Sansevieria* (G. Karssen, *personal communication*), *M. ulmi*, *M. mali*, and *M. artiellia.* In the case of the two most basal species, males are very rare ([3] and G. Karssen, *personal communication*). On the other hand, males are commonly found

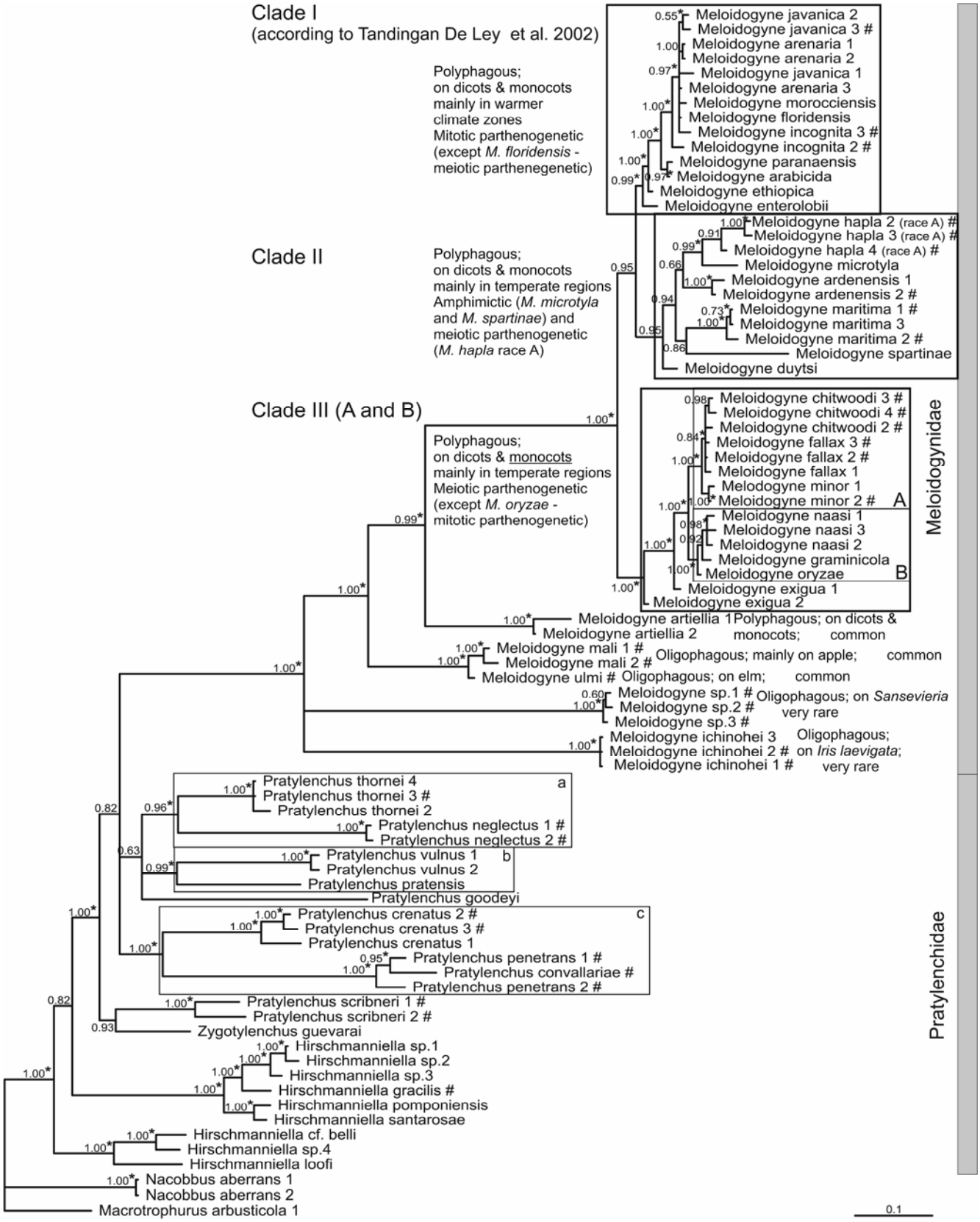

Fig. 2. Bayesian small subunit (SSU) rDNA tree of Pratylenchidae and Meloidogynidae. Numbers near nodes indicate posterior probabilities. An asterisk (*) indicates the nodes is also supported by the maximum likelihood tree (bootstrap value >70%). Newly generated SSU rDNA sequences are labeled with a '#'.Vertical bar indicates families and subfamilies.

for three species phylogenetically positioned between the most basal taxa and Clades I, II, and III. Hence, the current data support the sexual nature of the common ancestor of clades I, II, and III. On the other hand, the very low frequency of males among the most basal *Meloidogyne* species could be at odds with the supposed amphimictic nature of the common ancestor of the genus *Meloidogyne*.

Although only 26 of the ≈89 known nominal *Meloidogyne* species (18) are included, the SSU rDNA seems to contain sufficient variation to distinguish *Meloidogyne* species from each other. However, this does not hold for most of the tropical *Meloidogyne* species represented in Clade I. A possible explanation for this was given by Hugall et al. (17). On the basis of internal transcribed spacer sequences they suggested that some of the apomictic Clade I members such as *M. arenaria*, *M. javanica*, and *M. incognita* could be the result of reticulate evolution.

Pratylenchoides and Merliniinae (C). Representatives of the genus *Pratylenchoides*, obligate migratory endoparasites of plants so far considered as basal members of the family *Pratylenchidae* (39) are placed in the Merliniinae (Telotylenchidae) in the phylogeny (Fig. 1). The Merliniinae are distinguished from the other Telotylenchidae by (amongst other characters) the presence of deirids (sensory organs located in the lateral field) and having six incisures in the lateral field. These characters are shared by *Pratylenchoides* (though some species only have four incisures). Similarity in the tail region was the reason for Ryss (29) to transfer *Pratylenchoides* to the Merliniinae and recognize them as a separate family. He was not followed in this by other authors however. This phylogeny confirms the close relationship between *Pratylenchoides* and the Merliniinae.

Evolution of plant parasitism. The development of plant parasitism in the Tylenchida has traditionally been seen as a gradual evolution from fungal feeding to facultative parasitism of root hairs and epidermal cells into more complex forms of plant parasitism, culminating in the development of sedentary endoparasitism (24). To investigate this hypothesis, ancestral feeding types were reconstructed among Tylenchida using three different methods: unordered parsimony (Fig. 3A), parsimony using a step-matrix (Fig. 3B), and likelihood (Fig. 3C and D). It seems reasonable to assume that the transition from root hair feeding to migratory endoparasitism requires more changes than the evolution from semi-endoparasitism to migratory endoparasitism. Therefore, next to the unordered parsimony analysis where every change is considered equally likely, we designed a step matrix for the second parsimony-based analysis (Table 1). Likelihood methods do not accept multiple states, and—as some Tylenchids feed on multiple kinds of food sources (e.g., on fungi and on plant roots [44])—two input matrices were used. Figure 3C is based on the (supposed) most derived trophic states, whereas (supposed) ancestral trophic states were used in Figure 3D. Analysis of the basal part of the Tylenchida tree is complicated by the fact that the feeding type(s) of several Tylenchidae and Anguinidae are not known with certainty. They are often considered as obligate plant parasites, while more close investigation could reveal that they can feed on fungi or oomycetes as well. This point is illustrated by the genus *Filenchus*, known as epidermal cell and root hair feeders (44), while some *Filenchus* species were recently shown to feed on fungi as well (25).

Our analysis confirmed Luc's hypothesis on the gradual evolution of simple forms of plant parasitism, such as root hair and epidermal feeding and ectoparasitism towards more complex forms of endoparasitism (Fig. 3; Table 2). First of all, the number of forward changes far exceeds the number of reverse changes, especially in the likelihood reconstructions, confirming the trend of development towards more complex feeding types. Second, the average magnitude of state changes in plant parasitism is about 1.8 to 1.9 in the forward changes and about 1 in the reverse changes, confirming this development is gradual.

Remarkably, endoparasites evolved at least ten times within the order Tylenchida; migratory endoparasitism developed no less than six times, and sedentary endoparasitism four times. Only in one instance (*Meloidogyne*) sedentary endoparasitism evolved directly from migratory endoparasitism (a confirmation of the results presented by Bert et al. [4]). The number of independent developments is higher than expected mainly due to the paraphyly of the Pratylenchidae. Although the development of plant parasitism is usually gradual, endoparasitism seems to have developed directly from several simple forms of plant parasitism such as ectoparasites (giving rise to *Meloidogyne*, Pratylenchidae, *Pratylenchoides*, and *Tylenchulus*), epidermal and root hair feeders (from which the Anguinidae evolved) and possibly even insect parasitism (*Fergusobia*, although it is very well possible that its ancestor, just like *Deladenus*, fed on insects and fungi). On the basis of these findings we hypothesize that the potential to evolve into endoparasites is present in most if not all the members of the Tylenchida.

Only the parsimony step matrix analysis reconstructed the common ancestor of the Aphelenchida and Tylenchida as a fungal feeder. In the other three analyses, we could neither confirm nor discard the hypothesis saying that plant-parasitic Tylenchida arose from fungivorous ancestors. Firm conclusions on this hypothesis await a better resolution in the basal part of the Tylenchida tree, and additional information on the feeding behavior of the basal Tylenchida such as the Tylenchidae and Anguinidae.

The origin of cell wall-degrading enzymes. Genes encoding cell wall-degrading have been found in five nematode genera (*Globodera*, *Heterodera* [33], *Meloidogyne* [28], *Pratylenchus* [41], and recently *Bursaphelenchus* [22]). It has been proposed that these genes may have been acquired through horizontal gene transfer (20,43). Cellulases (EC 3.2.1.4) are found in fourteen glycoside hydrolase families (GHF 5, 6, 7, 8, 9, 10, 12, 26, 44, 45, 48, 51, 61, and 74), and remarkably, all Tylenchid cellulases isolated so far belong to a single GH family, namely GHF 5. It is noted that cellulases present in plant parasitic and mycetophagous *Bursaphelenchus* (order Aphelenchida) species belong to GHF 45 [21]). The question arises whether these cellulases have a common origin (cellulases were present/acquired before separation of subclades A and B; Fig. 1), or a separate origin (cellulases were present/acquired after the separation of subclades A and B). The current analysis identifies the genera *Hirschmanniella*, *Zygotylenchus*, and *Nacobbus*, and *R. similis* as being highly relevant in addressing the question about the origin of Tylenchida cellulases. Apart from this, it would be highly interesting to investigate the two Telotylenchidae branches (basal part of subclade B and C) for the presence/absence of cellulases and their nature (Telotylenchidae are ectoparasites, but members of the genus *Pratylenchoides* are endoparasites).

DNA barcode-based plant pathogen diagnostics; one for all? Data presented here shows that SSU rDNA sequences harbor, in most cases, sufficient phylogenetic signal to be potentially useful for species identification of plant pathogenic nematodes till species level. Currently ≈1,500 full length SSU rDNA sequences are available including representatives from all major terrestrial nematode groups in the temperate climate regions (14–16), and this makes it possible to identify DNA sequence signatures that are unique among most if not all terrestrial nematode taxa. Would similar results have been obtained if we would have used a 650-base fragment from the 5′ end of the mitochondrial gene cytochrome *c* oxidase I (COI), a fragment frequently used among animals (12)? This seems unlikely; COI was successfully used for population genetics of nonparasitic nematode species (10,11). Bearing in mind that a plant-parasitic lifestyle is associated with elevated rates of evolution (16), COI could be too variable for barcode-based pathogen identification. Would it be possible to identify a global standard for DNA barcode-based diagnostics of plant-pathogenic bacteria, oomycetes, fungi, and nematodes?

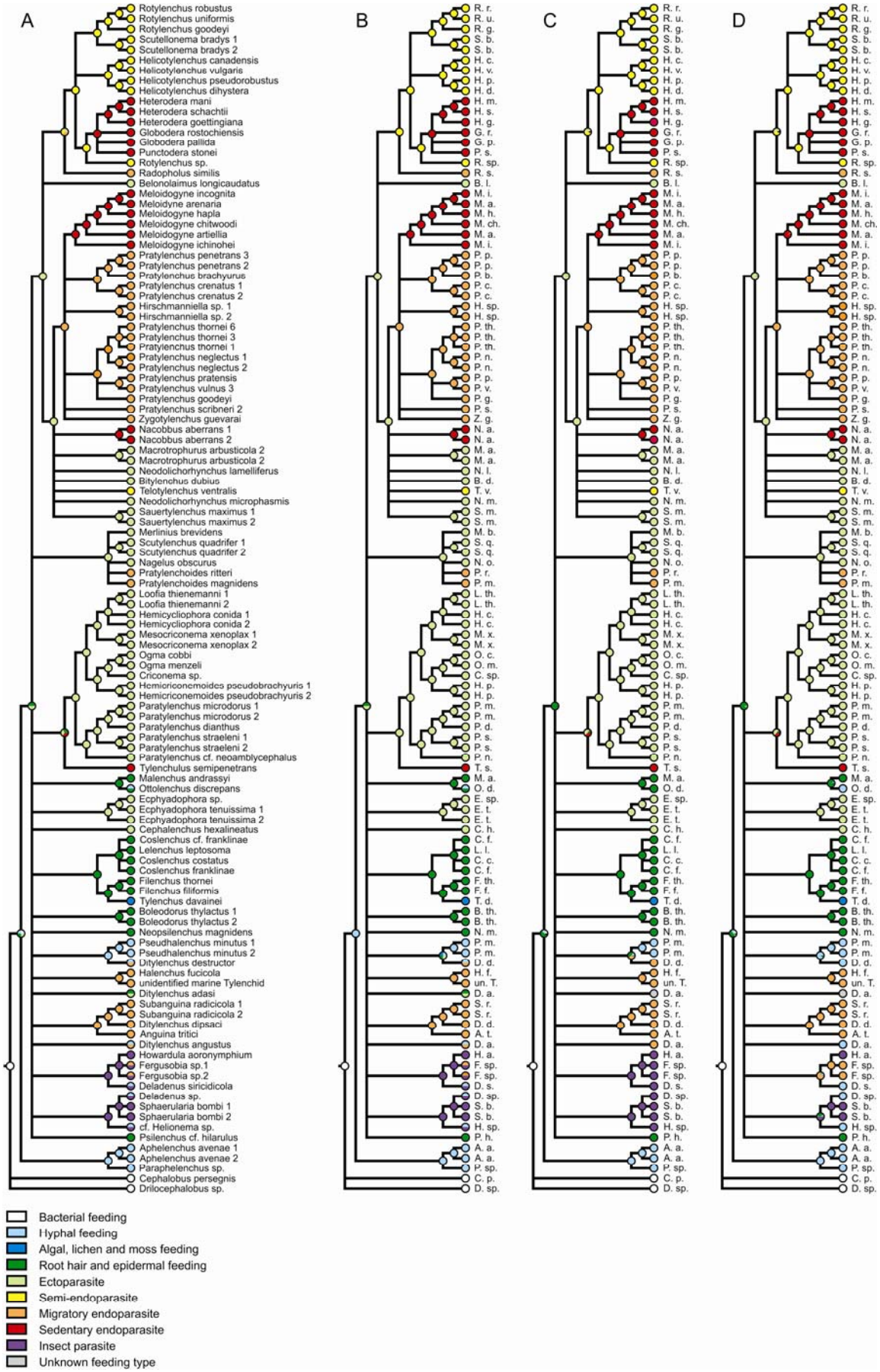

Fig. 3. Ancestral state reconstructions of feeding types. Nodes with a posterior probability lower than 0.95 have been collapsed. **A,** Unordered parsimony; **B,** parsimony with step matrix; **C,** likelihood with "derived" character states for species with two feeding types; and **D,** likelihood with "ancestral" character states for species with two feeding types.

TABLE 2. Total number of forward and reverse changes in feeding type and average magnitude of state changes in plant parasitism[a]

Nature and magnitude of change	Unordered parsimony	Parsimony step matrix	Likelihood derived characters	Likelihood ancestral characters
Forward changes	19.7	20.5	22	21
Reverse changes	12.3	12.6	3	8
Average magnitude forward changes plant parasitism	1.9	1.87	1.89	1.83
Average magnitude reverse changes plant parasitism	1.08	1.03	1	1

[a] For the parsimony analyses, the changes were counted according to the proportion they occupied in each of the most parsimonious solutions. For the likelihood analyses, the state with the best likelihood was assumed for each node.

Probably not, currently the most promising gene regions are 16S rDNA for bacteria, COI for oomycetes, ITS regions for fungi and SSU and/or LSU rDNA for nematodes.

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