

Chapter 3

Phylogeny and Evolution of Nematodes

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3.1 Introduction

A phylogenetic framework is needed to underpin meaningful comparisons across taxa and to generate hypotheses on the evolutionary origins of important properties and processes. In this chapter we will outline the backbone of nematode phylogeny and focus on the phylogeny and evolution of plant-parasitic Tylenchomorpha. We will conclude with some recent insights into the relationships within and between two highly successful representatives of the Tylenchomorpha; the genera *Pratylenchus* and *Meloidogyne*.

3.2 Backbone of Nematode Phylogeny

The phylum Nematoda can be seen as a success story. Nematodes are speciose and are present in huge numbers in virtually all marine, freshwater and terrestrial environments. Analysis of large EST data sets recently reconfirmed the placement of the phylum Nematoda within the superphylum Ecdysozoa (Dunn et al. 2008), a major animal clade proposed by Aguinaldo et al. (1997) that unites all moulting animals.

Blaxter et al. (1998) (53 taxa) and Aleshin et al. (1998) (19 taxa) were among the first to exploit the potential of small subunit ribosomal DNA (SSU rDNA) sequence data to resolve phylogenetic relationships among nematodes. Holterman et al. (2006) presented a subdivision of the phylum Nematoda into 12 clades based on a series of mostly well-supported bifurcations in the backbone of the tree (339 taxa) (Fig. 3.1). The under-representation of marine nematodes in these phylogenetic overviews was, to some extent, lifted by SSU rDNA-based papers from Meldal et al. (2007) and Holterman et al. (2008a). Recently, a phylogenetic tree based on

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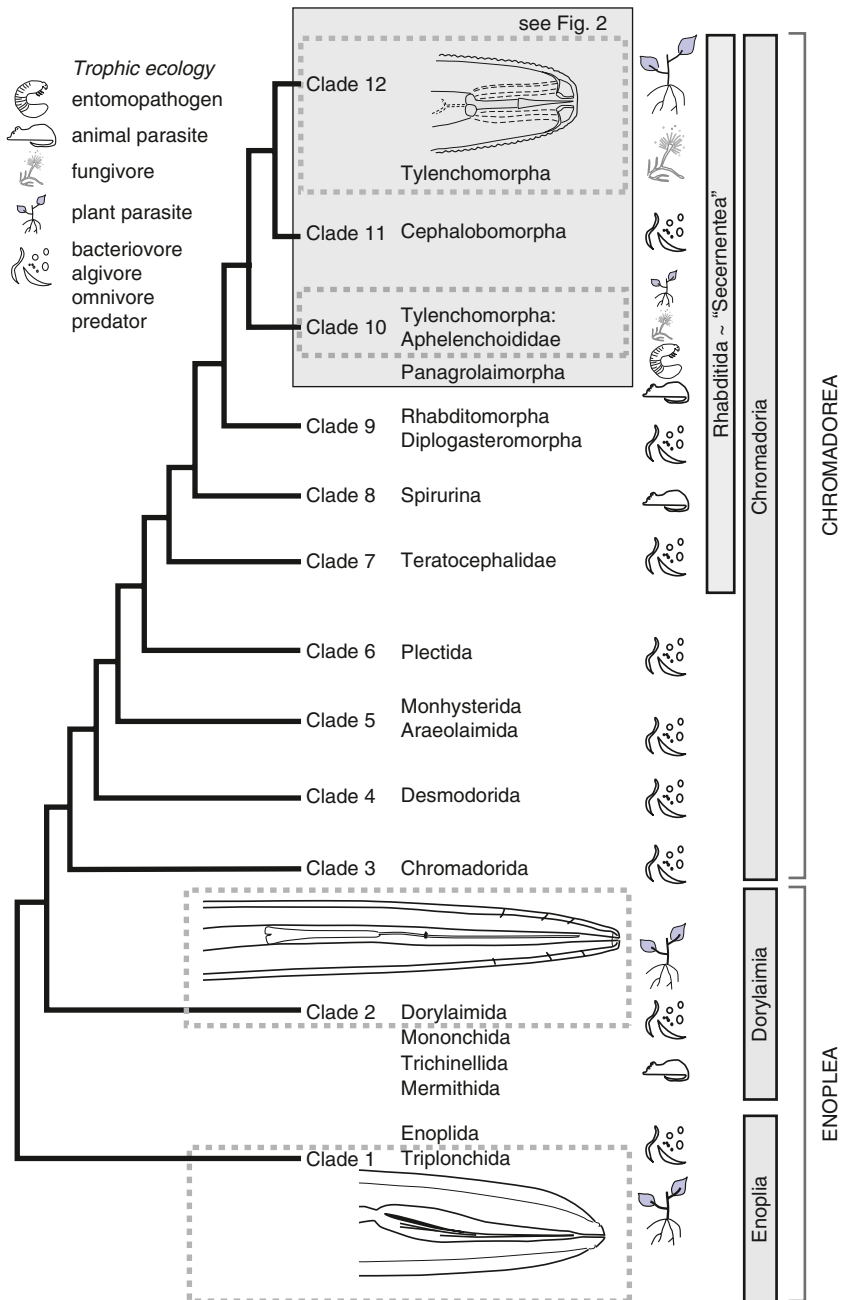


Fig. 3.1 Schematic overview of the evolution of the phylum Nematoda derived from SSU rDNA sequence data (based on Holterman et al. 2006). Major lineages of plant-parasites are indicated by dotted boxes (Tylenchomorpha, Dorylaimida and Triplonchida). It is noted that the infraorder Tylenchomorpha is possible a polyphyletic group; it includes the members of Clade 12 and the Aphelenchoididae, a family within Clade 10

1,215 small subunit ribosomal DNA sequences covering a wide range of nematode taxa was presented by van Megen et al. (2009). The overall topology of this phylogenetic tree resembles that of Holterman et al. (2006). However, the support values for the backbone tend to be lower. The deep subdivision of the phylum Nematoda should be regarded as a ‘work in progress’, and a multi loci approach will be required for a more definitive framework.

The extensiveness of convergent evolution is one of the most striking phenomena observed in the phylogenetic tree of nematodes—it is hard to find a morphological, ecological or biological characteristic that has not arisen at least twice during nematode evolution. Convergent evolution appears to be an important additional explanation for the seemingly persistent volatility of nematode systematics. One of the peculiarities of the phylum Nematoda is the multitude of times that (animal- or plant-) parasitic lifestyles have arisen. Understanding the phylogenetic history of the acquisition of particular phenotypes associated with successful parasitism permits fuller appreciation of the evolutionary constraints experienced by organisms adapting to new hosts. Plant-parasitism has evolved independently in each of three major clades in the phylum Nematoda (Fig. 3.1). The plant-parasitic Tylenchomorpha, Dorylaimida, Triplonchida have acquired their ability to parasitize plants independently. The infraorder Tylenchomorpha comprises manifestly the economically most relevant plant-parasites and within this chapter we will mainly focus on this particular group.

Apart from the scientific merits of studying of the phylogeny of the Nematoda, the underlying molecular framework can be used for DNA barcode-based nematode detection and community analysis. It is (in most cases) possible to define species-specific sequence signatures and to design simple and cheap PCR primers that allow real-time PCR-based detection and quantification of pathogenic nematodes in complex DNA backgrounds. At the same time, the SSU rDNA alignment has been used to design many family-specific PCR primers (see for example Holterman et al. 2008b) and quantitative DNA barcode-based nematode community analyses under field conditions are currently being tested.

3.3 Phylogeny of Tylenchomorpha

3.3.1 Overview

The Tylenchomorpha, the most intensively investigated infraorder within the Tylenchina, comprises the largest and most economically important group of plant-parasitic nematodes. Although there are examples of nematodes that exploit all plant organs including flowers and seeds, they mostly attack roots. The evolution of plant-parasitic Tylenchomorpha is of particular interest because associations range from transitory grazing by root-hair feeders to the highly complex host-pathogen interactions of gall-inducing nematodes and their hosts. Non plant-parasitic Tylenchomorpha feed on fungi, algae, lichens, mosses, insects, mites, leeches or frogs

(Siddiqi 2000). However, the evolution of this diversity of complex feeding traits is not yet fully understood. In recent years LSU and SSU rDNA sequences have been used to infer relationships among Tylenchomorpha (Subbotin et al. 2006; Bert et al. 2008; Holterman et al. 2009). A multiple gene approach derived from an EST mining strategy has been used to characterise the relationships between the plant-parasitic genera *Meloidogyne*, *Heterodera* and *Globodera* (Scholl and Bird 2005). Other phylogenetic studies within Tylenchomorpha, mainly based on rDNA sequences or the internal transcribed spacers, have been restricted to individual (super) families or genera. Recent studies include analyses of Heteroderidae (Subbotin et al. 2001), Anguinidae (Subbotin et al. 2004), Criconelematoidea (Subbotin et al. 2005), Hoplolaimidae (Subbotin et al. 2007), *Meloidogyne* (Tandingan De Ley et al. 2002; Tenente et al. 2003; Tiganò et al. 2005) and *Pratylenchus* (Subbotin et al. 2008).

In Fig. 3.2, a schematic phylogenetic framework of the Tylenchomorpha based on Bert et al. (2008) and Holterman et al. (2009) is shown. The families Hoplolaimidae (including Heteroderinae), Pratylenchidae (except *Pratylenchoidea*) and Meloidogynidae, which comprises the economically most important plant-parasites, plus the genera *Tylenchorhynchus* and *Macrotrophurus* form a well supported clade. A robust sister relationship between *Meloidogyne* (root-knot nematodes) and representatives of the migratory endoparasitic Pratylenchidae (*Pratylenchus*, *Zygotylenchus* and *Hirschmanniella*) can be observed. The Hoplolaimidae, which include the Heteroderinae, Hoplolaiminae, Rotylenchoidinae and Rotylenchulinae according to the classification of de Ley and Blaxter (2002), appear as a monophyletic group. Remarkably, the migratory endoparasitic *Radopholus*, a notorious pest in banana and citrus, has a well-supported sister relationship with the Hoplolaimidae. Thus, the cyst-forming (Heteroderinae) and root-knot nematodes (*Meloidogyne*) are likely to have arisen independently and the migratory endoparasitic Pratylenchidae appears to be polyphyletic.

Between the earliest divergences within the Tylenchomorpha (Aphelenchoidea) and the top parts of the tree, a number of branching points remain unresolved. Although we cannot define the relationship between the suborder Criconelematoidea and other Tylenchomorpha, its members clearly constitute a separate and well supported clade. Also, the tylenchid nematodes with supposedly ancestral morphological characters, including Tylenchidae and Sphaerularioidea, do not have an established phylogenetic relationship. Nevertheless, the tylenchid nematodes—those Tylenchomorpha that are characterized by a tylenchid stylet (Tylenchomorpha without Aphelenchoidea)—appear to be clearly monophyletic. The Aphelenchoidea or “aphelenchs” comprising the mainly fungal-feeding Aphelenchidae and Aphelenchoidea are appointed as polyphyletic in all molecular analyses to date. However, the morphology based hypothesis of their monophyly could not be significantly rejected based on statistical analysis of molecular data (Bert et al. 2008). Several studies have confirmed the sister relationship of the predominantly plant-parasitic Tylenchomorpha (without Aphelenchoidea) with the bacterivorous Cephalobidae (Blaxter et al. 1998). However, this was not unequivocally supported by van Meegen et al. (2009).

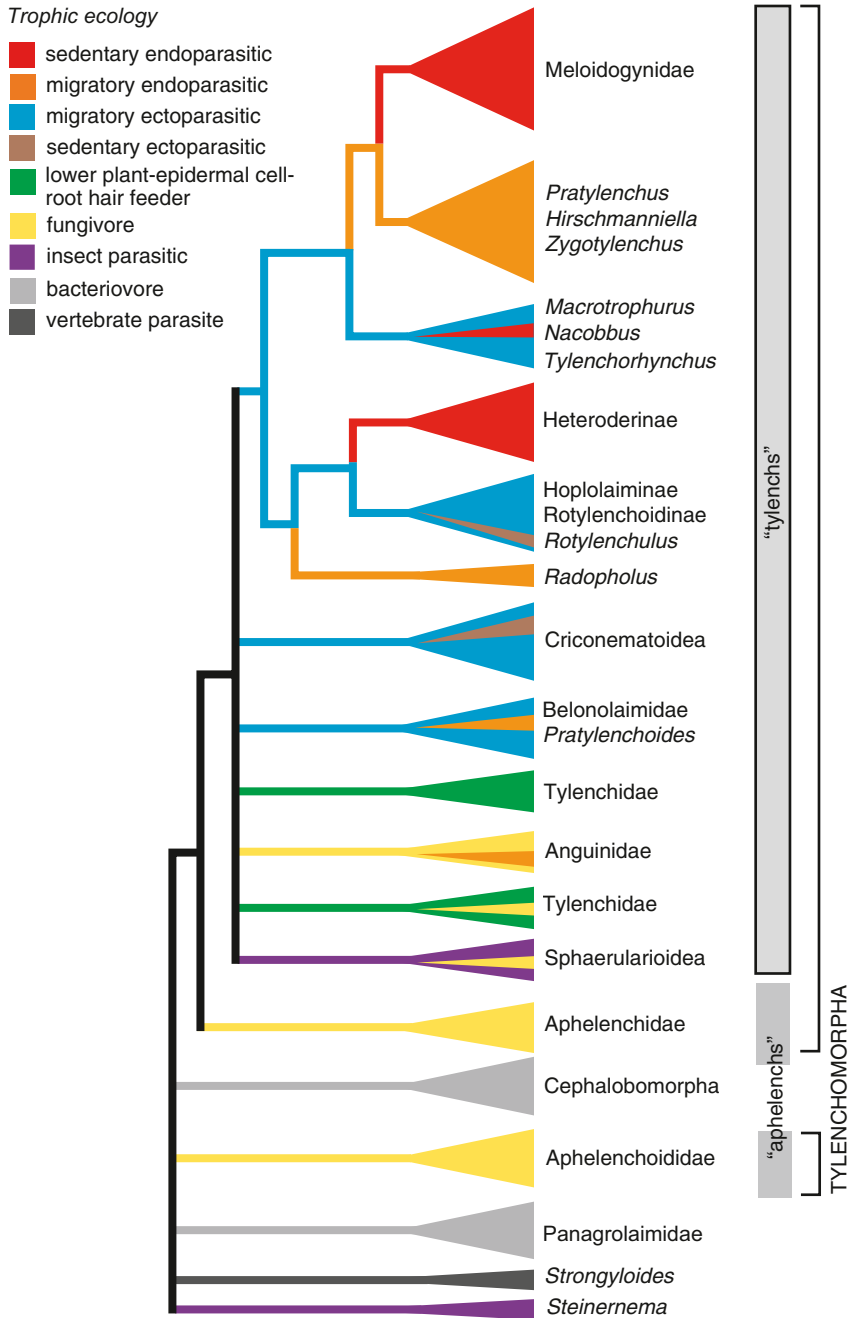


Fig. 3.2 The evolution of feeding among Tylenchomorpha super posed on a ribosomal DNA-based phylogenetic backbone. (Based on Bert et al. 2008; Holterman et al. 2009)

3.3.2 Evolution of Plant-Parasitism in Tylenchomorpha

The development of plant-parasitism in the Tylenchomorpha 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 (Luc et al. 1987). To investigate this hypothesis, ancestral feeding types were reconstructed among Tylenchomorpha using three different methods: unordered parsimony, parsimony using a stepmatrix, and likelihood (Bert et al. 2008; Holterman et al. 2009). Here we present only a schematic overview of these results (Fig. 3.2).

The feeding type analysis supported the classical hypothesis of the gradual evolution of simple forms of plant-parasitism, such as root hair and epidermal feeding and ectoparasitism towards more complex forms of endoparasitism. However, the ancestral feeding state of the Tylenchomorpha (without Aphelenchoididae) is still ambiguous; depending on the reconstruction method this is fungal-feeding, lower plant/root-hair feeding or bacteriovore feeding. Conclusions on this hypothesis await a better resolution in the basal part of the Tylenchomorpha tree and additional information on the feeding behavior of the basal Tylenchomorpha such as the Tylenchidae and Anguinidae. Within the tylenchid nematodes, migratory ectoparasitic feeding is ancestral for all major clades of nematodes that exclusively parasitize higher plants (Fig. 3.2). Migratory endoparasitism has evolved independently several times within Anguinidae and four times, always from migratory ectoparasitic ancestors, in the polyphyletic Pratylenchidae. Sedentary endoparasitism has also evolved three times independently; *Nacobbus* (false root-knot nematodes) and the cyst-forming nematodes most likely evolved from migratory ectoparasitic nematodes, while root-knot nematodes appear to have evolved from migratory endoparasitic nematodes. The number of independent developments is higher than expected mainly due to the polyphyly 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 including ectoparasites (giving rise to *Heteroderinae*, Pratylenchidae, *Pratylenchoides* and *Tylenchulus*) and epidermal and root hair feeders (from which the Anguinidae evolved).

Although the parasitic biology of certain plant-parasitic Tylenchomorpha is now relatively well documented, we should achieve a broader understanding of the evolution of the mode and direction of plant-parasitism from intermediate groups, including economically less important plant-parasites. Within Clade 12 the family Aphelenchidae (fungivores) appears in a sister position to all “tylenchs” (Holterman et al. 2006; Bert et al. 2008). However, it should be noted that in a more recent, maximum likelihood analysis this positioning could not be robustly confirmed (van Megen et al. 2009). For the plant-parasitic Tylenchomorpha families positioned at the base of clade 12 such as the Tylenchidae, the Psilenchidae and the Belonolaimidae (the ‘economically less important plant-parasites’), the number of (ribosomal)

DNA sequences available is very limited. It is expected that molecular characterisation of specimens from these families and improved insight in their feeding habits will give us more insight in the transition from fungivorous lifestyles, via facultative plant-parasitism towards obligatory plant-parasites.

3.4 Tylenchomorpha—Top End Plant-Parasites

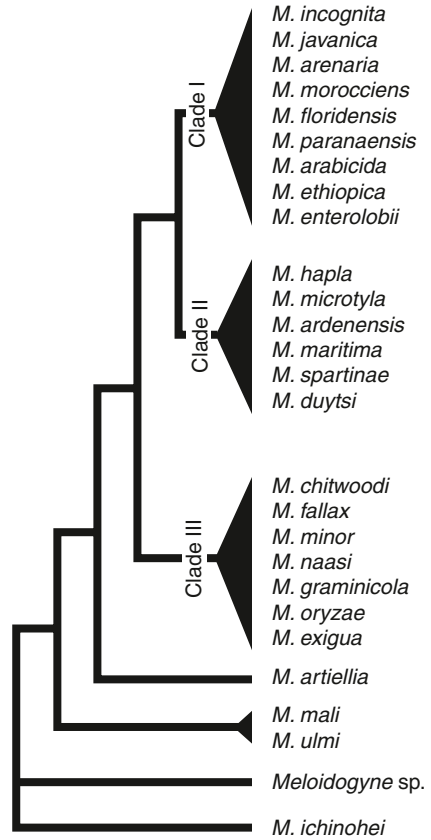
3.4.1 *Root-Knot Nematodes—Towards the Origin(s) of This Highly Successful Genus*

3.4.1.1 Introduction

Root-knot nematodes—members of the genus *Meloidogyne*—can be said without too much exaggeration to represent the ultimate success story among plant-parasitic nematodes. The more distal members of this genus have host ranges encompassing more than a thousand plant species, including numerous major crops, and are spread all over the temperate and (sub)tropical regions of the world. On the basis of SSU rDNA sequences Tandingan de Ley et al. (2002) defined three distal clades within the genus *Meloidogyne*. Clade I comprises a number of species often referred to as the tropical root-knot nematodes (e.g. *M. incognita*, *M. javanica* and *M. arenaria*), the most well known representative of Clade II is *M. hapla*, while Clade III harbors a number of species with EPPO A2 quarantine status such as *M. chitwoodi* and *M. fallax* (EPPO=European and Mediterranean Plant Protection Organisation) (Fig. 3.3). Analysis of a larger molecular data set and more *Meloidogyne* species resulted in a confirmation of these distal clades. Ribosomal DNA sequence data point at *Meloidogyne artiellia*—a polyphagous root-knot nematode typically inducing very small galls (host plant range includes both mono and dicotyledons)—as being sister to the distal *Meloidogyne* clades I, II and III (III being basal to Clades I and II) (Holterman et al. 2009).

One of the elements that could explain the success of this genus is the ability of these endoparasites to migrate between plant cells. Cell wall-degrading enzymes and expansins are likely to be widespread throughout *Meloidogyne*, although they have so far only been characterized for some of the more derived members of this genus. These enzymes and proteins make it possible for these plant-parasites to move towards the most nutritional part of the plant root, namely the vascular cylinder (stele). Migrating between cells, rather than through cells as is the case for cyst nematodes, may help the pre-parasitic second-stage juveniles to outpace and/or avoid the host plant defense response. The question why lesion (*Pratylenchus* spp.) and cyst nematodes (*Globodera* spp. and *Heterodera* spp.) do not migrate intercellularly while infective life stages are producing and secreting various cell wall degrading enzymes and proteins remains to be answered.

Fig. 3.3 Schematic overview of the phylogeny of the Meloidogynidae derived from SSU rDNA sequence data. (Based on Holterman et al. 2009)



3.4.1.2 Modes of Reproduction Among Root-Knot Nematodes

Another fascinating aspect of root-knot nematode biology is the wide range of modes of reproduction present with the group. *Meloidogyne incognita* multiplies by mitotic parthenogenesis. Mothers that produce genetically identical daughters could be considered as a dead end road in evolutionary terms—such a genetic constitution would make it impossible for an individual to cope with changing environmental conditions. Nevertheless, in terms of distribution and host plant range this is probably the most successful member of the genus *Meloidogyne*. *M. incognita* is one of the members of Clade I (as defined above). It is notable that Clade I is not homogenous in its mode of replication. Although mitotic parthenogenesis dominates this clade, one of its members (*M. floridensis*) multiplies by meiotic parthenogenesis. The same holds true for Clades II and III; although meiotic parthenogenesis is most frequently found there are a number of exceptions. Most remarkable is the case of *Meloidogyne hapla* for which two races are defined. Race A reproduces by facultative meiotic parthenogenesis, whereas the polyploid Race B multiplies by mitotic

parthenogenesis. It would be useful to examine more basal root-knot nematode species when seeking further insight into the extreme diversity in modes of reproduction of root-knot nematodes.

According to McCarter (2008), most root-knot species are “sexually reproducing diploids, a status thought to reflect the root-knot nematode ancestral state”. A careful examination of *M. artiellia*, which is sister to the distal *Meloidogyne* Clades I—III, suggests this could be correct; the frequent occurrence of males suggests this species could multiply sexually. However, based on the analysis of rDNA sequence data at least four *Meloidogyne* species are placed in more basal positions, viz. *M. ulmi*, *M. mali*, an undescribed *Meloidogyne* species found on *Sansevieria*, and *M. ichinohei* (Holterman et al. 2009).

Meloidogyne ichinohei is an unusual root-knot nematode species both in its morphology and in its extreme host specificity. Unlike most RKN females, adult females of *M. ichinohei* show a prominent posterior protuberance, a laterally located neck and a perineal pattern showing remarkably faint and broken striae (Araki 1992). Previously the first two characters were considered to be rather atypical, and they had been the characteristics defining the genus *Hypsoperine*, a genus synonymized with *Meloidogyne* by Jepson (1987). *M. ichinohei* males are very rare according to the original description by Araki (1992). This observation has been confirmed by the Dutch National Plant Protection Organisation (NPPO); not a single male was found in *M. ichinohei* population C2312 (G. Karssen, unpublished data).

3.4.1.3 On the Former Genus *Hypsoperine*

Araki remarked that *M. ichinohei* would have been a typical member of the genus *Hypsoperine* if it still existed (Araki 1992). Sledge and Golden (1964) suggested that the genus *Hypsoperine* actually occupied a position in between *Heterodera* and *Meloidogyne* (though showing closer resemblance to the root-knot nematodes). This information prompted us to check a number of other *Hypsoperine*-like root-knot nematodes: (1) *Hypsoperine acronea* (now *Meloidogyne acronea*); (2) *H. graminis* (*M. graminis*); (3) *H. mersa* (*M. mersa*); (4) *H. ottersoni* (*M. ottersoni*); (5) *H. propora* (*M. propora*); (6) *H. spartinae* (*M. spartinae*) and (7) *H. megriensis* (*M. megriensis*). As very little is published about the members of this genus, information about host range and mode of reproduction (if known) is summarized here.

1. *Meloidogyne acronea* is a root-knot nematode-like parasite found on roots of sorghum (*Sorghum vulgare*) by Coetzee in 1956. The author reported that this particular isolate could also parasitize and multiply on beans and tomatoes. In the original description morphological characteristics of both males and females are given. *M. acronea* seems to have a very restricted geographical distribution as it has been reported from southern Africa only.
2. *Meloidogyne graminis* was found in 1964 by Sledge and Golden as a parasite on St. Augustine grass (*Stenotaphrum secundatum*). When few males were present, *M. graminis* reproduces by meiotic parthenogenesis. However, in the presence of

- males reproduction was by amphimixis (Triantaphyllou 1973). Based on cytological data, Triantaphyllou (1973) suggested a close phylogenetic relationship of *M. graminis*, *M. ottersoni*, *M. graminicola* (Clade III) and *M. naasi* (Clade III).
3. *Meloidogyne mersa* is an unusual root-knot nematode found in a marine habitat (mangrove swamps in Brunei) parasitizing roots of the mangrove apple (*Sonneratia alba*). The original paper describes both males (100 paratypes) and females (50 paratypes) and males are not rare (Siddiqi and Booth 1991). The morphology of *M. mersa* resembles that of *M. spartinae*, another root-knot nematode species living in marine or brackish habitats.
 4. Thorne (1969) described *Meloidogyne ottersoni* as a parasite of canary grass (*Phalaris arundinacea*). As for *M. graminis*, *M. ottersoni* was reproduced by meiotic parthenogenesis when males are absent but in the presence of males reproduction was by amphimixis (Triantaphyllou 1973). Based on cytological data, Triantaphyllou suggested for a close phylogenetic relationship of *M. ottersoni*, *M. graminis*, *M. graminicola* (Clade III) and *M. naasi* (Clade III).
 5. *Meloidogyne propora* was first described by Spaul (1977) as a parasite on the roots of *Cyperus obtusiflorus* and *Solanum nigrum* from an atoll in the Indian Ocean (Aldabra). Males were reported to be common in soil around infested roots and near ovigerous females.
 6. *Meloidogyne spartinae* is a root root-knot nematode producing galls on cord-grass (*Spartina alterniflora*) which is found in intertidal wetlands, especially estuarine salt marshes. On the basis of SSU rDNA sequences Plantard et al. (2007) clearly showed this species to be related to *M. maritima*, and as such residing in *Meloidogyne* Clade II.
 7. *Meloidogyne megriensis*—rather incomplete description published in Russian by Poghossian (1971)—was collected from roots of *Mentha longifolia*. Only known from the type locality, an orchard in Megri, and a nearby village named Vagravar, Armenia Karssen and van Hoenselaar 1998.

This overview, based on various data, indicates that members of the former genus *Hypsoperine* are scattered all over the *Meloidogyne* phylogenetic tree, and—other than the observation that their morphology is different from what is considered to be typical for root-knot nematodes—they have little in common.

Another *Meloidogyne* species sharing a number of morphological characteristics with *M. ichinohei* (but never classified as member of the genus *Hypsoperine*) is *Meloidogyne kralli*. *M. kralli* was first found in 1968 by Dr. E. Krall on the roots of sedge (*Carex acuta*) (Jepson 1983). Adult females share a number of characteristics with *M. ichinohei*; they have a distinct neck (contrary to *M. ichinohei*, the neck was not set off) and the vulva was positioned on a posterior protuberance. Although males have been described for *M. kralli* (Jepson 1983), they are seldom found under natural conditions (Karssen 2002). *Meloidogyne kralli* seems to have a relatively small host range as it is found only on four *Carex* species, namely *C. acuta*, *C. vesicaria*, *C. riparia* and *C. pseudocyperus*, and on *Scirpus sylvaticus*. Under laboratory conditions *M. kralli* populations were reported to reproduce well on barley (Jepson 1983). It is noted that both *M. ichinohei* and *M. kralli* exclusively parasitize a small number of monocotyledons. However, SSU rDNA sequence analysis revealed that

M. ichinohei and *M. kralli* are rather distinct, with *M. kralli* robustly positioned in *Meloidogyne* Clade III close to *M. oryzae* (Helder and Karssen, unpublished results).

Based on the information discussed above, *M. acronea* would be worthwhile investigating in more detail, as it resembles *M. ichinohei*. Cytological and scattered molecular data place most of the former members of the genus *Hypsoperine* in the distal *Meloidogyne* Clades II or III, except for *M. acronea* and *M. propora*. It is concluded that molecular data from particularly these two species could possibly contribute to a better understanding of the origin of the genus *Meloidogyne*. However, both species seem to have a restricted distribution (southern Africa, and an atoll in the Indian Ocean) and this complicates the collection of DNA from these highly interesting root-knot nematode species. Currently, *M. ichinohei*—together with a so far non-described *Meloidogyne* species from Kenya found on *Sansevieria* (Holterman et al. 2009)—seem to reside genuinely at the very base of the *Meloidogyne* tree of life. Both species have a very limited host range, and for both species males are very rare. In our eyes, this justifies a hypothesis stating that (at least facultative) asexual reproduction is the root-knot nematode ancestral state.

3.4.2 *Lesion Nematodes—A Stenomorphic Genus Closely Related to Root-Knot Nematodes*

Lesion nematodes—members of the genus *Pratylenchus*—can be recognized easily. Lesion nematodes are relatively small (usually around 500 μm), have a short and stout stylet (11–22 μm) with strong basal knobs, a low lip region with a well-developed sclerotized framework, and glands forming a rather short lobe which ventrally overlaps the intestine (see e.g. Castillo and Vovlas 2007). However, recognizing individual species is difficult as the number of diagnostic characters at this particular taxonomic level is small. The identification of lesion nematodes is further complicated by intra-specific variation in some of these characters. *Pratylenchus* identification to species level is usually done on the basis of the morphology of adult females. They have more informative characters than males, and—at least equally important—for a number of species males are extremely rare or even non-existent. Despite these difficulties (or possibly as a consequence of them), this genus comprises approximately 70 nominal species (Castillo and Vovlas 2007).

Using the labial region as a distinguishing character, Corbett and Clark (1983) distinguished three groups of *Pratylenchus* species. Group I includes (among others) *P. brachyurus*, *P. coffeae*, *P. crenatus*, *P. loofi*, and *P. zaeae*; group 2 includes *P. neglectus* and *P. thornei*; and *P. penetrans*, *P. pratensis*, *P. scribneri* and *P. vulnus* belong to group 3. Combined analysis of D2–D3 of 28S (= LSU rDNA) and partial 18S (= SSU rDNA) alignments revealed a subdivision of the genus *Pratylenchus* into six groups (Subbotin et al. 2008). Members of group 1 *sensu* Corbett and Clarke (1983) are scattered over major clades a, b and d. Group 2 (Corbett and Clarke 1983) corresponds to group c (Subbotin et al. 2008), and Group 3 (Corbett and Clarke 1983) members all reside in group a according to Subbotin et al.

(2008) except for *P. scribneri* which resides in clade b. No representatives of the genus *Meloidogyne* were included in this analysis. Holterman et al. (2009) presented a phylogenetic tree based on a full length SSU rDNA data set including nine *Pratylenchus* species. However, poor backbone support in this particular part of the tree did not allow for the identification of a *Pratylenchus* (or at least Pratylenchidae) candidate that could be a likely living representative close to the common ancestor of all root-knot nematodes.

3.4.3 *Phylogenetic Relationship Between the Genera Meloidogyne and Pratylenchus*

On the basis of shared morphological characteristics of the labial region and pharyngeal structures Ryss (1988) and Geraert (1997) proposed a common ancestry between the genera *Pratylenchus* and *Meloidogyne*. Analysis of SSU and LSU rDNA sequences from a considerable range of lesion and root-knot nematodes supports a close phylogenetic relationship between the two genera. Subbotin et al. (2006) found “evidence for a *Pratylenchus*, *Hirschmanniella* and *Meloidogyne* clade” (based on D2-D3 sequence data). Two years later Bert et al. (2008) concluded “... root-knot nematodes are most closely related to *Pratylenchus spp.* and appear to have evolved from *migratory endoparasitic* nematodes” (based on SSU rDNA data). In 2009 Holterman et al. concluded “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” (based on more extensive SSU rDNA data). All in all the following can be concluded:

1. Ribosomal DNA sequences suggest that the genus *Pratylenchus* is paraphyletic as all *Meloidogyne* species are nested in it.
2. If this (1.) is correct, the ultimate consequence would be the abolishment of the genus *Pratylenchus* (*Meloidogyne* has priority following the rules of the International Commission on Zoological Nomenclature). This could be undesirable for numerous practical reasons, but in scientific research and especially in our thinking about root-knot nematode evolution this could be useful.
3. The discussion about ancient (a)sexuality as the ancestral state of root-knot nematodes should be replaced by a discussion about the ancestral mode of reproduction of a clade encompassing (at least) all members of the genera *Pratylenchus* and *Meloidogyne*.

3.5 Concluding Remarks

The steep increase in the amount of molecular data over the last decade has allowed for the establishment of more and more robust and versatile phylogenetic frameworks for the phylum Nematoda (Blaxter et al. 1998; Holterman et al. 2006, Van

Megen et al. 2009). It is noted that current frameworks are based on a single cistron (SSU and/or LSU ribosomal DNA sequences), and marine taxa (including numerous plant-parasites) are strongly underrepresented. Current data suggest at least three independent lineages of plant-parasites. This number will probably increase over time; analysis of a 5' region of LSU rDNA pointed at multiple plant-parasite lineages among the Dorylaimida (Holterman et al. 2008b).

The infraorder Tylenchomorpha (equivalent to Clade 12 (Fig. 3.1) with the addition of the family Aphelenchoididae) comprises the largest group of plant-parasitic nematodes. Analysis of rDNA sequences resulted in a good resolution in the more distal parts of this clade only. Basal Tylenchomorpha—mostly plant-parasites with virtually no economic importance—are currently underrepresented, and this might co-explain why the relationships within this group are poorly resolved (Subbotin et al. 2006; Bert et al. 2008; Holterman et al. 2009). Additional sequencing of basal Tylenchomorpha probably will improve our understanding of the evolution of the more advanced (and successful) members of this infraorder. The relationships between and within distal taxa in Clade 12 including cyst, lesion and root-knot nematodes are far better resolved. Within this chapter the relatedness of the two latter genera—*Pratylenchus* and *Meloidogyne*—was discussed in more detail, and current data suggest that root-knot nematode are in fact nested in (and derived from) the *Pratylenchus* branch.

At this moment, several, so-called next generation DNA sequencing technologies are becoming widely available. This will make it possible to generate sequence data from hundreds if not thousands of genes from individual nematodes at a reasonable cost price. Given that we still have nematode taxonomists around to ensure the link between classical systematics and multigene phylogenetics, we foresee that within a few years from now the notoriously volatile nematode systematics will be turned into a single, more or less robust and widely accepted framework.

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