

## Trends in the Evolution of Insect Parasitism by Nematodes as Inferred from Fossil Evidence<sup>1</sup>

GEORGE POINAR, JR.<sup>2</sup>

**Abstract:** Fossil evidence can reveal a wealth of information regarding entomogenous nematodes. Amber is an excellent medium for the preservation of such fossils because it protects delicate organisms like nematodes, often along with their insect hosts. Such fossils establish a baseline for the appearance and continuation of parasite lineages as well as parasite-host associations. Thus far, fossil records of insect parasites have been discovered in the following nematode groups: terrestrial and aquatic Mermithidae, Tetradonematidae, Iotonchidae, Diplogasteridae, Allantonematidae, Sphaerulariidae, and Rhabditiidae. Records date from 15 to 130 million years, demonstrating that these associations have existed for a considerable length of time.

**Key words:** amber, evolution, fossils, paleonematology, parasites.

To best understand the evolutionary history of any group, we use information acquired from several different sources. These include comparative morphology, DNA analysis, geographical distribution, and behavior of extant members and fossils. Because each of these methods is incomplete, they all should be taken into consideration to elucidate how and when various groups evolved.

Fossils provide the only direct evidence of the past history of a group, and a single fossil has the potential to provide an age for the origin of multiple evolutionary lineages. Often fossils challenge phylogenetic histories based on living organisms and result in significant modifications. One of the difficulties in attempting to analyze the phylogeny of nematodes is the rarity of fossils, especially for endoparasites.

Fossils establish baselines for the appearance of various nematode lineages. They may not tell us from which stem group a particular lineage emerged, but they can give us minimum dates when various clades adapted a parasitic mode of life. To assist in an understanding of the fossils, I have relied on the principle of behavior fixity, which states that the behavior and ecology, including climatic preferences of fossil organisms, will be similar to those of their present-day descendants at the generic and often family level (Boucot, 1990). Behavior includes host selection, which is an important feature in many of the obligate parasitic nematode groups. Theoretically, we can expect to see similar host-parasite relationships in the past as occur today, even

extending back tens of millions of years, depending on the particular lineage involved.

Several types of fossilization processes preserve nematodes or their activities. These include sedimentary deposits, cherts, and amber. Fine-grained sedimentary deposits may reveal trace fossils such as nematode tracks, which have been reported from the Green River Eocene beds (Moussa, 1969). However, they can be used only to establish the presence of free-living representatives of the phylum Nematoda.

Nematode fossils occur in amber, with records extending back some 130 million years. I have been searching for such fossils during the past 30 years. The advantage of amber fossils is that the medium preserves soft-bodied organisms like nematodes and the host is usually also preserved, thus facilitating identification and establishing host-parasite records.

A mermithid emerging from its planthopper host in 40-million-year-old Baltic amber establishes a base time for terrestrial mermithids of the *Agamermis*–*Hexamermis* clade (Fig. 1) (Poinar, 2001b). Today, parasitic associations between mermithids and planthoppers exist in North America and Asia; however, records from northern Europe, where this fossil was found, are lacking. Other terrestrial mermithid clades are represented by forms parasitizing ants (Formicidae: Hymenoptera) (Poinar, 2002) and spiders (Thomsidae: Araneae) (Poinar, 2000) in Baltic amber.

Records of aquatic mermithid lineages extend back some 130 million years and are represented by *Cretacimermis libani* parasitizing adult midges (Chironomidae: Diptera) in 135-million-year-old Lebanese amber (Poinar et al., 1994) and *Heydenius dominicus* attacking mosquitoes (Culicidae: Diptera) in 15- to 45-million-year-old Dominican amber (Poinar, 1984b). Mermithid clades attacking chironomid midges also occur in Baltic amber (Fig. 2). Placing mermithid fossils in extant genera is usually not feasible because generic identification

Received for publication 9 July 2002.

<sup>1</sup> Paper delivered in a symposium on Evolution of Parasitism in Nematoda at the 40th Annual Meeting of The Society of Nematologists, 24–29 August 2001, Salt Lake City, UT.

<sup>2</sup> Professor, Department of Entomology, Oregon State University, Corvallis, OR 97331.

E-mail: poinarg@bcc.orst.edu

This paper was edited by B. C. Hyman.



FIGS. 1–3. A mermithid emerging from a planthopper (Fulgoroidea: Hemiptera) in 40-million-year-old Baltic amber provides evidence of terrestrial mermithids, especially the *Hexameris*-*Agameris* clade, in the Eocene (accession # N-3-51 in the Poinar amber collection maintained at Oregon State University). Fig. 2. A mermithid that emerged from its adjacent midge host (Chironomidae: Diptera) in 40-million-year-old Baltic amber indicates evidence of aquatic mermithids, possibly of the *Hydromermis* clade, in the Eocene. Note also the presence of an ectoparasitic mite of the family Oxiidae (arrow) that detached from its midge host. Such cases of double parasitism in the fossil record are rare (accession # N-3-35B in the Poinar amber collection maintained at Oregon State University). Fig. 3. Dauer and postdauer juveniles of *Parasitodiplogaster* sp. that emerged from the body cavity of a fig wasp (Agaonidae: Hymenoptera) in 15- to 45-million-year-old Dominican amber (accession # N-3-24A in the Poinar amber collection maintained at Oregon State University).

is based on characters of the adult forms and, with few exceptions, mermithids emerge from their hosts as juveniles. Taylor (1935) erected a collective fossil genus *Heydonius* for all fossil mermithids. His generic name was based on the 19th-century biologist Carl von Heyden; however, Taylor misspelled von Heyden's name as von Heydon. This "lapsus calami" was corrected by Doll-

fus (1950) to *Heydenius*. It is proposed here to restrict *Heydenius* to fossil mermithids from the Tertiary and to use *Cretacimermis*, a generic name proposed by Poinar (2001a), for the Cretaceous mermithid parasitizing a chironomid midge in Lebanese amber (Poinar et al., 1994) as a collective genus for Cretaceous mermithids. These fossil genera have the equivalent standing to the genus *Agameromermis* Stiles, which was proposed for extant immature mermithids that could not be assigned to previously described genera (Poinar and Welch, 1981).

The family Tetradonematidae also was established by the Eocene as represented by nematode parasites of a sciarid fly (Sciaridae: Diptera) in Baltic amber and a psychodid fly (Psychodidae: Diptera) in Dominican amber. The mermithids—at least the terrestrial and freshwater forms—and tetradonematids appear to have evolved from stem groups in the Dorylaimida. If we wish to use the old dictum that ontogeny recapitulates phylogeny, then it can be shown that the pre-parasitic stages of mermithids possess an onchiostylet, a complete digestive system without modifications, and a normal cuticle—very much like the dorylaims. Later in the insect host, the mermithids lose their stylet, the intestine separates from the pharynx and becomes highly modified, and the cuticle becomes porous for food uptake.

Fossil representatives of the Tylenchida and Aphelenchida also exist. One of the most interesting (because of worldwide presence of this host-parasite association today) is a member of the nematode family Allantonematidae parasitizing a drosophilid fly (Poinar, 1984a). The juveniles in this case are well-enough preserved that it probably will be possible to assign the parasite to one of the two extant genera known to attack fruit flies throughout the world. Other fossil allantonematid clades include forms that parasitize members of the family Staphylinidae (Coleoptera) (Poinar and Brodzinsky, 1985).

Fossil members of the family Sphaerulariidae include forms attacking flies (Diptera) of the families Scatopsidae and Sciaridae in Dominican and Baltic amber, respectively. These are possibly early lineages of *Scatonema* and *Tripisus*, respectively.

Current theory is that the Tylenchida arose from free-living Rhabditida stem groups feeding on microbes (Blaxter et al., 1998; Poinar, 1993). The walls of the stoma gradually closed and became a stylet, which allowed these forms to feed on fungi in the same habitat. Such forms were continuously in contact with invertebrates and gradually invaded the body cavity of these potential hosts. In some present-day forms, the fungal-feeding cycle has been continued and alternates with an insect-parasitic cycle (Poinar, 1991). A fossil representative of one of these groups is a member of the family Iotonchidae, which has been recovered from a dark-winged fungus gnat (Mycetophilidae: Diptera) in

Dominican amber (Poinar, 1991). Only two genera of nematodes are known to parasitize mycetophilid fungus gnats in nature—*Steinernema* and *Iotonchium*. The insect parasitic juveniles of *Iotonchium* (which include the first, second, and third stages) range in length from 130 to 570 microns (Poinar, 1991). The dauer stages of the fungus gnat strain of *Steinernema feltiae* range from 617 to 857 microns in length (Poinar, 1992). On the basis of size alone, it would appear that the fossils are immature *Iotonchium*.

Fossils also exist of plant-feeding nematodes being carried by insects. Two of the classic examples of this condition today are representatives of the nematode genera *Parasitodiplogaster* and *Schistonchus* transported by fig wasps (Agaonidae: Hymenoptera) to fig flowers. It appears that internal phoresis occurs with *Schistonchus* where only the adults, which range from 354 to 800 microns in length, occur in the wasp host. The dauer of *Parasitodiplogaster*, which average 257 microns, enter the body of the fig wasp and develop to the adult or pre-adult stages. The fossil nematodes emerging from the fig wasp shown here in Dominican amber average 306 (261–360) microns in length. Based on these size ranges and the shape (Fig. 3), it appears that the nematodes are the dauer and post-dauer juveniles of a *Parasitodiplogaster* sp.

Discovering fossil entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) is difficult. First, because most infections by entomopathogenic nematodes occur in terrestrial larvae of holometabolous insects, they rarely would turn up in the fossil record because larvae, especially if they are already diseased, would rarely fossilize. Infected adult insects could be found; however, naturally occurring infections, even in extant adult insects, are rare and thus far limited to a strain of *Steinernema feltiae* that attacks only adult mycetophilid flies (Poinar, 1992). While actual fossils of *Steinernema* and *Heterorhabditis* are lacking, there are records of closely related insect parasitic rhabditoid nematodes in amber. Developing rhabditoid nematodes closely resembling the genus *Rhabditophanes* have been recovered from the hemocoel of nematoceran flies in 22- to 26-million-year-old Mexican amber. These nematodes do not appear to be associated with bacteria and may develop only to the adult or pre-adult stage in the insect. It has been hypothesized that the stem group that gave rise to *Rhabditophanes* also gave rise to the *Steinernema* lineage (Poinar, 1993). Over the course of evolution, a *Rhabditophanes*-like ancestor acquire a bacterial symbiont (such as *Xenorhabdus*) and introduced it into the body cavity of an insect host. In this new location, the bacteria multiplied and established favorable conditions for nematode development. The bacteria associated with *Steinernema* probably originated from members of the Enterobacteriaceae, like the ubiquitous *Proteus*. In contrast to *Xenorhabdus*, which is associated with members of *Steinernema*, *Photo-*

*rhabdus* is associated with *Heterorhabditis* species. This nematode genus, while possessing a similar life cycle to that of *Steinernema*, represents a separate clade and appears to have evolved from a *Pellioidites* stem group in a coastal environment (Blaxter et al., 1998; Poinar, 1993). Their associated bacteria have many similarities with the luminescent marine genus *Lucibacterium*. The bacteria associated with both nematode genera co-evolved with the nematodes, losing their ability to form resting stages that would enable them to survive, even for a short period, in the environment because they were protected inside a nematode or insect. Morphological and physiological modifications of both nematodes and bacteria occurred to stabilize the association.

Some of the parasitic associations that appear in the fossil record have not been documented in today's world. Whether they have not yet been discovered or have simply disappeared over time is an interesting question. Examples that I have found include an elongate coiled nematode associated with a small phorid fly (which appears different from the *Howardula* that attacks the extant *Megaselia*) in Dominican amber and some nematodes, possibly parasitic, associated with a trichogrammatid wasp in Mexican amber.

Aside from parasitic and internal phoretic associations, some fossils indicate a phoretic association between insects and nematodes. These are represented by dauer juveniles associated with worker ants, stingless bees, platypodid beetles, and bark beetles.

The fossils discussed here provide minimum ages not only for origins and differentiation of nematode clades but also of co-evolution involving phoresis and parasitism. We can conclude that many of the current insect-nematode relationships were established tens of millions of years ago—long before humans entered the scene—and that many will continue to persist long after we have exited the arena.

#### LITERATURE CITED

- Blaxter, M. L., P. De Ley, J. R. Garey, L. X. Liu, P. Scheldeman, A. Vierstraete, J. R. Vanfleteren, L. Y. Mackey, M. Dorris, L. M. Frisse, J. T. Vida, and W. K. Thomas. 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature* 392:71–74.
- Boucot, A. 1990. *Evolutionary Paleobiology of behavior and coevolution*. Amsterdam: Elsevier.
- Dollfus, R. Ph. 1950. Liste des Nematelminthes connus a l'etage fossil. *Compte Rendu Sommaire de la Société Géologique de France* 5:82–85.
- Moussa, M. T. 1969. Nematode fossil tracks of Eocene age from Utah. *Nematologica* 15:376–380.
- Poinar, Jr., G. O. 1984a. First fossil record of parasitism by insect parasitic Tylenchida (Allantonematidae: Nematoda). *Journal of Parasitology* 70:306–308.
- Poinar, Jr., G. O. 1984b. *Heydenius dominicus* sp. n. (Nematoda: Mermithidae), a fossil parasite from the Dominican Republic. *Journal of Nematology* 16:371–375.
- Poinar, Jr., G. O. 1991. The mycetophagous and entomophagous stages of *Iotonchium californicum* n. sp. (Iotonchidae: Tylenchida). *Revue de Nématologie* 14:565–580.

Poinar, Jr., G. O. 1992. *Steinernema feltiae* (Steinernematidae: Rhabditida) parasitizing adult fungus gnats (Mycetophilidae: Diptera) in California. *Fundamental and Applied Nematology* 16:333–338.

Poinar, Jr., G. O. 1993. Origins and phylogenetic relationships of the entomophilic rhabditids, *Heterorhabditis* and *Steinernema*. *Fundamental and Applied Nematology* 16:333–338.

Poinar, Jr., G. O. 2000. *Heydenius araneus* n. sp. (Nematoda: Mermithidae), a parasite of a fossil spider, with an examination of helminthes from extant spiders (Arachnida: Araneae). *Invertebrate Biology* 119:388–393.

Poinar, Jr., G. O. 2001a. *Heydenius brownii* sp. n. (Nematoda: Mermithidae) parasitizing a planthopper (Homoptera: Achilidae) in Baltic amber. *Nematology* 3:753–757.

Poinar, Jr., G. O. 2001b. Nematoda and Nematomorpha. Pp. 255–295 in J. H. Thorp and A. P. Covich, eds. *Ecology and classification of*

*North American freshwater invertebrates*, 2nd ed. New York: Academic Press.

Poinar, Jr., G. O. 2002. First fossil record of nematode parasitism of ants: A 40-million-year tale. *Parasitology* 125:1–3.

Poinar, Jr., G. O., A. Acra, and F. Acra. 1994. Earliest fossil nematode (Mermithidae) in Cretaceous Lebanese amber. *Fundamental and Applied Nematology* 17:475–477.

Poinar, Jr., G. O., and J. Brodzinsky. 1985. Fossil evidence of nematode (Tylenchida) parasitism in Staphylinidae (Coleoptera). *Nematologica* 31:353–355.

Poinar, Jr., G. O., and H. E. Welch. 1981. Parasites of invertebrates in the terrestrial environment. Pp. 947–954 in W. Slusarski, ed. *Review of advances in parasitology*. Warsaw: Polish Scientific Publications.

Taylor, A. L. 1935. A review of the fossil nematodes. *Proceedings of the Helminthological Society of Washington* 2:47–49.