Review Article

Nematode Parasites and Associates of Ants: Past and Present

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Ants can serve as developmental, definitive, intermediate, or carrier hosts of a variety of nematodes. Parasitic ant nematodes include members of the families Mermithidae, Tetradonematidae, Allantonematidae, Seuratidae, Physalopteridae, Steinernematidae, and Heterorhabditidae. Those nematodes that are phoretically associated with ants, internally or externally, are represented by the Rhabditidae, Diplogastridae, and Panagrolaimidae. Fossils of mermithids, tetradonematids, allantonematids, and diplogastrids associated with ants show the evolutionary history of these relationships, some of which date back to the Eocene (40 mya).

1. Introduction

Nematodes are one of the most abundant groups of animals known. Studies on their evolutionary history suggest that they probably arose in the Precambrian, which explains their wide abundance today in the terrestrial and marine environments. While only some 20,000 have been described, their species diversity has been estimated to be as high as 10 million [1].

One would assume that with their strict housekeeping habits, ants would not tolerate nematodes in or around their nests and would quickly dispose of any nest mates that might have become infected. However nematodes have been able to use some astonishingly sophisticated tactics to successfully parasitize these social insects. The present work covers the systematics, life history, pathology, and records of all described extant and fossil nematodes associated with formicids. This includes representatives of the nematode families Mermithidae, Tetradonematidae, Allantonematidae, Seuratidae, Physalopteridae, Steinernematidae, Heterorhabditidae, Rhabditidae, Diplogastridae, and Panagrolaimidae. Fossil records of mermithids, tetradonematids, allantonematids, and diplogastrids associated with ants reveal the evolutionary history of these associations, some of which date back 40 million years.

2. Mermithidae

The family Mermithidae includes parasites of invertebrates, especially insects. Because of their large size, mermithids are easily detected in ants upon dissection (Figure 1) or as they leave their hosts (Figure 2). Most mermithid species, including those that attack ants, parasitize only a specific host species, genus, or family while others can infect representatives of several insect orders. Mermithids that attack aquatic insects, such as midges (Chironomidae, Ceratopogonidae) and mosquitoes (Culicidae), have a direct life cycle. Direct life cycles occur when, after growth and development is completed in the host, the mermithid emerges, molts to the adult stage, mates, and oviposits in the host's environment. The infective stage mermithid emerges from the egg, actively locates and enters a host, and initiates development in the hemocoel.

Some mermithids have an indirect life cycle, which is more complicated but allows hosts to be parasitized in environments hostile to nematodes. In an indirect cycle, the mermithid emerges from the host, molts, mates, and oviposits in the environment. But instead of emerging from the egg to search for a developmental host, the infective stage remains in the egg, waiting to be ingested by an invertebrate that serves as a paratenic host. When mermithid eggs are ingested by a paratenic host, the hatching infective stage penetrates the gut wall and enters the body cavity. But instead of developing, the mermithid encysts and enters a diapause. The encysted nematode can be carried through the different stages of host metamorphosis, but for its cycle to be completed, the paratenic host must be captured and fed to the brood of the developmental host. At the completion of the mermithids growth phase in the development host (like an ant), the latter is attracted to an aquatic or semiaquatic habitat favorable

Figure 1: Mermithid exposed in the gaster of *Camponotus* sp. from the Sierra Nevada, California.
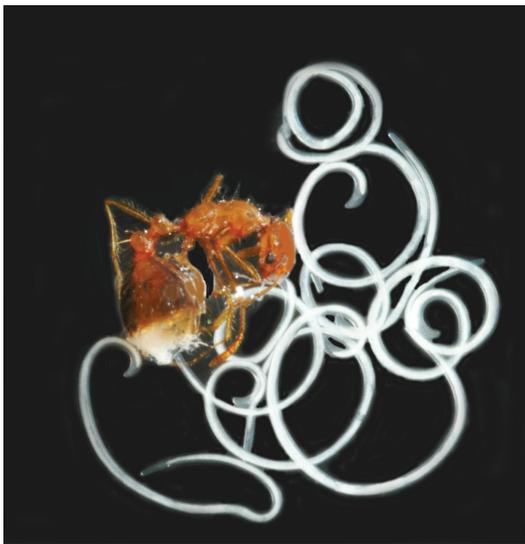

Figure 2: Parasitic juveniles of *Allomermis solenopsi* removed from the gaster of a fire ant worker. Photo courtesy of S. D. Porter, USDA-ARS.

Table 1: Section from Gould [2] referring to the first reported instance of mermithid parasitism of ants.

Amongft other Incidents that tend to leffen and deftroy Ant-Flies, it is obfervable that abundance of them are demolifhed by a white and long Kind of Worm, which is often met with in their Bodies. You may frequently take three from the Infides of the large, but feldom more than one from a fmall Ant-Fly. Thefe Worms lie in a fpiral Form, and fome of them may be extended Half an Inch.

Table 2: Mermithid nematodes described from ants.

Mermithid	Host	Reference
Agamomermis cephaloti	*Cephalotes minutus*	[11]
Agamomermis costaricensis	*Odontomachus hastatus*	[11]
Agamomermis ecitoni	*Eciton burchellii*	[11]
Allomermis solenopsi	*Solenopsis invicta*	[12]
Camponotimermis bifidus	*Camponotus aethiops*	[13]
Comanimermis clujensis	*Formica fusca*	[14]
	Camponotus aethiops	
Heydenius formicinus	*Prenolepis henschei*	[15]
Heydenius myrmecophila	*Linepithema* sp.	[11]
Meximermis ectatommi	*Ectatomma ruidum*	[11]
Pheromermis lasiusi	*Lasius niger*	[16]
Pheromermis myrmecophila	*Lasius* spp.	[5]
Pheromermis villosa	*Lasius flavus, L. niger*	[17]

* fossil.

to the nematode. This is when the mermithid exits, leaving the dying host behind. The developmental host is usually not only larger, but usually in a completely different taxonomic category and environment from the paratenic host. While the developmental host can live in a relatively dry habitat, the paratenic host usually inhabits an aquatic, semiaquatic, or damp biome. Also, both hosts can be widely separated taxonomically and may not even belong to the same phylum.

The first written account of a nematode parasite of ants was made by the Reverend William Gould in his 1747 book *An account of English Ants* (Table 1) [2].

The "white and long kind of worm, which is often met within their bodies" certainly refers to mermithid nematodes. For a number of years, mermithids were listed under "*Filaria*," "*Gordius*," or "*Mermis*," and that is why mermithid systematics can be confusing and why early names for Gould's ant mermithid included *Gordius formicarum* Diesing [3] and *Filaria formicarum* von Siebold [4].

The first described ant mermithid was *Pheromermis myrmecophila* from *Lasius* spp. [5]. However it was originally described in the genus "*Mermis*", then assigned to the genus

Pheromermis [6], then moved to the genus *Allomermis* [7] and lastly to the genus *Camponotimermis* [8]. Its position in the genus *Pheromermis* was recently confirmed by Kaiser, who showed its similarity with the European ant mermithid, *Pheromermis villosa* [9]. Over the years, a large number of ant species have been reported parasitized by mermithids. A list of Holarctic parasitized ants was presented by Passera [10] and Neotropical parasitized ants by Poinar et al. [11]. A compilation of all described mermithids from ants is presented in Table 2.

Fossils, such as the postparasitic juvenile of *Heydenius formicinus* emerging from a male *Prenolepis henschei* (Figure 3) [15], as well as from a worker ant (Figure 4) in Baltic amber [1] show that ants have been parasitized by mermithids for at least 40 million years and probably much longer. The fossil record of Neotropical mermithid parasites of ants is represented by a parasitic juvenile of *Heydenius myrmecophila* adjacent to its ant host, *Linepithema* sp. in 20–30-million-year-old Dominican amber (Figure 5) [11]. It is assumed that the traumatic events of the ant host entering the resin caused the mermithid to emerge prematurely from an opening in the gaster of the ant.

FIGURE 3: The fossil nematode, *Heydenius formicinus*, emerging from a male *Prenolepis henschei* in Baltic amber.

FIGURE 4: *Heydenius formicinus* adjacent to its worker ant host in Baltic amber.

FIGURE 5: *Heydenius myrmecophila* adjacent to its *Linepithema* ant host in Dominican amber.
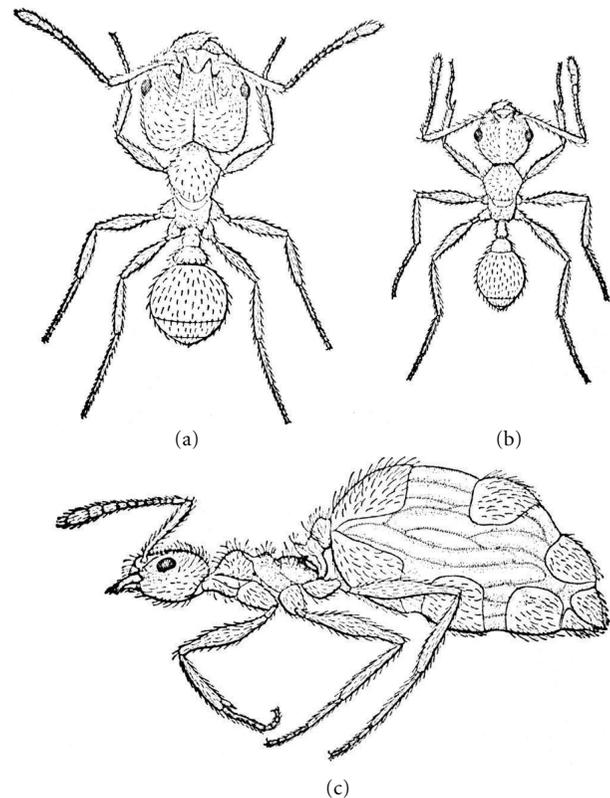

(a)   (b)

(c)

FIGURE 6: Plate (modified) of *Pheidole dentata* (referred to as *P. commutata*) from [18] showing the first evidence that mermithid nematodes could cause intercastes of ants. (a) Normal soldier; (b) normal worker; (c) parasitized worker (mermithergate).

Depending on the caste and length of time the mermithid is associated with its host, various degrees of host intercastes and abnormalities appear. Wheeler [18] was the first to provide an explanation for these phenomena by correlating the unusual morphological conditions with mermithid infections (Figure 6). Parasitized queen ants (mermithogynes) are shorter, have a smaller thorax (stenothoracy), reduced wings (brachyptery), enlarged abdomen (physogastry), and smaller head (microcephaly) than their uninfected counterparts. Parasitized worker ants (mermithergates or macroergates) often develop morphological features characteristic of queens and soldiers. Attacked male ants (mermithaners) have shorter wings but enlarged heads, eyes, and gasters. Infected soldiers (mermithostratiotes) have reduced heads, an ocellus, and changes in pilosity (Figure 7) [19–24].

The life cycle of most ant mermithids remains a mystery. Crawley and Baylis [5] assumed that *P. myrmecophila* has a direct cycle, where infection is brought about by the eclosing preparasitic mermithid entering the ant host. When development is completed, the postparasitic juvenile emerges, molts to the adult stage in the ant's habitat, mates, oviposits and the cycle continues. However, no one has demonstrated a direct cycle for any mermithid parasite of ants. In 1934, Vandel [25] studied a mermithid parasite of *Pheidole pallidula* and realized that the infection must be initiated in the ant larva. He assumed that the nematodes were in the soil surrounding the ant colony so the infective stages could penetrate directly into

FIGURE 7: Two mermithid-infected soldiers (mermithostratiotes) (arrows) of *Pheidole pallidula* adjacent to smaller workers and an uninfected soldier (with large head). Note smaller heads on infected soldiers. Photo courtesy of Luc Passera.
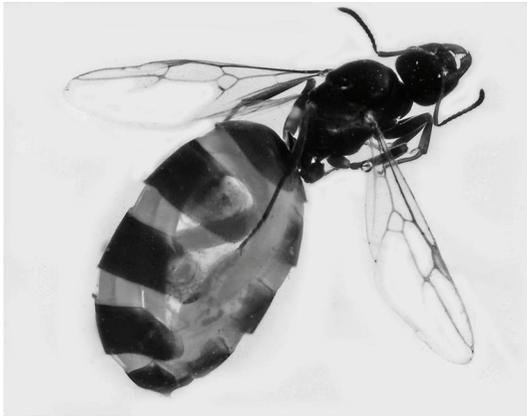

FIGURE 8: *Pheromermis villosa* in a carpenter ant from Holland.

the ant larva; however he was unable to confirm the infection process.

The first life cycle of an ant mermithid was achieved by Kaiser with the European *Pheromermis villosa* [17, 26] (Figure 8). Kaiser showed that *P. villosa* had an indirect cycle involving oligochaetes as paratenic hosts. Workers of *Lasius* spp. collecting protein for the brood capture oligochaetes containing the infective stages of *P. villosa* and, unknowingly, feed them to the developing larvae. At this point, the nematode becomes active, penetrates into the ant larva's hemocoel, and initiates development. It was a significant discovery and raises the question whether all mermithid infections of ants have indirect life cycles. Other possible paratenic hosts for *Pheromermis* could be small aquatic insects that ingest mermithid eggs from the bottom debris of seepage areas or the edges of other water sources. The wasp parasite, *Pheromermis pachysoma* [6], also has an indirect cycle and uses caddis flies as paratenic hosts, which the eusocial wasps (Vespidae) feed to their brood [27].

Thus far, seven genera of mermithids are known to infect ants, namely, *Agamomermis, Allomermis, Camponotimermis, Comanimermis, Heydenius, Meximermis,* and *Pheromermis* (Table 2). All of the ant hosts of these mermithids feed their brood animal protein (in contrast to other genera, such as

the leaf cutting ants), and this behavior suggests they have an indirect life cycle involving a paratenic host. The two genera, *Agamomermis* and *Heydenius,* are collective group genera for immature extant and fossil mermithids, respectively [1].

There are some morphological and behavioral patterns that characterize mermithids with indirect cycles. They normally have smaller eggs with thicker shells than the eggs of direct development soil or freshwater mermithids. Also their eggs are completely embryonated when laid. Finally, the deposited eggs will not hatch in the environment even though the enclosed parasitic juvenile is fully developed. Hatching only occurs when a potential invertebrate paratenic host ingests the eggs. The eggs of *Pheromermis* spp. are small, numerous, fully embryonated when laid and do not hatch in the environment. Fully embryonated eggs ensure that the infective stages are ready to enter paratenic hosts as soon as they are ingested [6, 17].

The ant mermithid, *Allomermis solenopsi* [12], possess an unusual morphological feature on the mature eggs that could play a crucial role in its life cycle. The surface of the eggs is covered with elongate, erect, spiny adhesive processes. How these function in the life cycle is unknown, but the related species, *A. trichotopson*, possesses similar structures [28]. Since *A. solenopsi* parasitizes the fire ant, *Solenopsis invicta* in Brazil (Figure 2), the related *A. trichotopson*, whose host is unknown, may infect *Solenopsis geminata* in Jamaica. Could these egg processes somehow be connected with parasitism of *Solenopsis* spp.?

Can mermithids be manipulated to control ants? Aside from killing the ant host upon emergence, mermithids drain the host of food, reduce the flight muscles and fat body, and cause morphological modifications as mentioned above [9, 17, 24, 26]. Since mermithid-infected *Solenopsis* has reduced reproductive organs and die shortly after the nematodes emerge [11, 12, 29], it has potential as a biological control agent. However, if the cycle is always indirect as shown for *P. villosa*, it would be very difficult to artificially infect the ant brood. It would be necessary to first infect the paratenic host and then supply large numbers of these infected invertebrates to worker ants for transport back to the nest. Working with a mermithid that has a direct cycle would be easier; however there is still the problem of raising and disseminating the nematodes.

3. Tetradonematidae

The tetradonematids are a diverse group of nematodes that have traditionally been aligned with the Mermithidae. However, aside from some distinctive morphological characters, female tetradonematids normally mature, mate, and produce eggs within the host, which does not occur with mermithids. Two tetradonematids have been described from extant ants. *Tetradonema solenopsis* is a parasite of the red imported fire ant, *Solenopsis invicta*, in Brazil [30, 31]. Very little is known about this nematode aside from the scant description showing that females contained eggs and worker infection levels reached 12.5%. Parasitized ants that succumbed to the infections could be recognized by their slightly enlarged gaster with scallop-appearing dorsal sclerites.
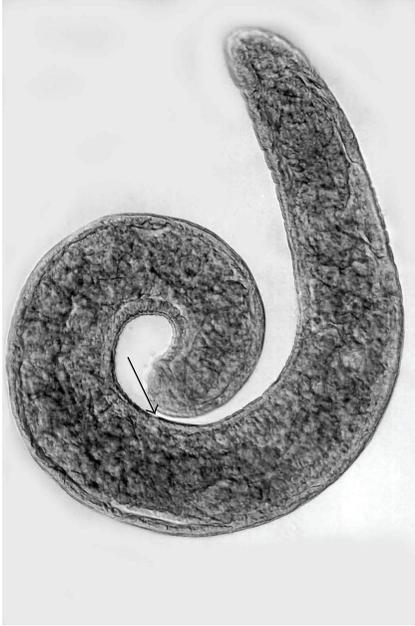
FIGURE 9: Mature female of *Myrmeconema neotropicum* in the early stages of egg production removed from a pupa of *Cephalotes atratus*. Arrow shows position of vulva.

FIGURE 11: Eggs of *Myrmeconema neotropicum* released from the gaster of an infected *Cephalotes atratus* worker in Peru.

FIGURE 10: Mature females of *Myrmeconema neotropicum* packed with eggs in the gaster of a *Cephalotes atratus* worker in Peru.

FIGURE 12: Worker of *Cephalotes atratus* infected with *Myrmeconema neotropicum*. The raised, red abdomen occurs when the nematode eggs are infective and ready for transport by birds. Photo courtesy of Stephen P. Yanoviak.

The second tetradonematid from extant ants is *Myrmeconema neotropicum* from *Cephalotes atratus* in Peru and Panama [32]. *Myrmeconema* is the only nematode that causes its ant host to radically change color (from black to red), which is crucial for completion of its life cycle [33]. This color change was a mystery for early taxonomists and the variety *Cephalotes atratus* var. *rufiventris* was erected solely on the basis of its red abdomen, which was later shown to be the result of *Myrmeconema* infections [32].

Developing females of *M. neotropicum* occur in ant pupae (Figure 9) but do not produce masses of eggs until they are carried into the adult ant (Figure 10). As the females deteriorate, eggs are released into the ant's hemocoel (Figure 11). At this stage of development, the gasters of the infected worker ants turn from black to red and are held high in the air (Figure 12) [33]. Birds mistake the red gasters for fruits and the nematode eggs are passed through the birds' digestive system and end up in the droppings, which are deposited on leaves and branches. *Cephalotes* workers collect and feed the infested excreta to their brood, which is how the larvae become infected [33].

Aside from their red gasters, parasitized ants are smaller with reduced head widths. They are sluggish, clumsy, generally less aggressive, and about 40% heavier than nonparasitized workers. They do not bite when handled, and their alarm/defense pheromone supply is significantly reduced or absent.

Myrmeconema is probably widely distributed throughout the Neotropics since this association has been in existence for some 20–30 million years. The fossil worker ant, *Cephalotes serratus* in Dominican amber, is surrounded by the eggs of *Myrmeconema antiqua* (Figure 13) [1]. The ant has a hole in its abdomen that quite possibly was made by a bird. Many of the eggs, which closely resemble those of *M. neotropicum* in size and shape, contain fully developed juveniles (Figure 14). All indications suggest that *M. antiqua* had a similar life history to the extant *M. neotropicum* and involved bird carriers.
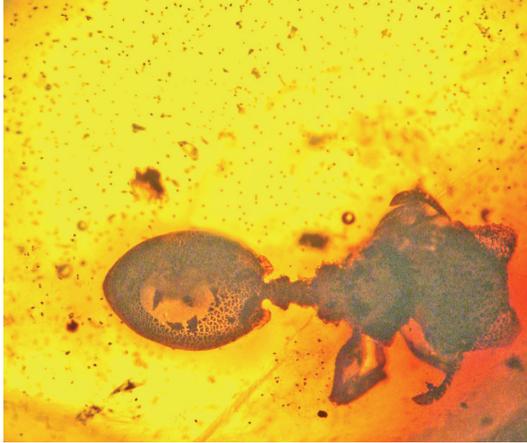
FIGURE 13: Worker of *Cephalotes serratus* infected with *Myrmeconema antiqua* in Dominican amber. Note microscopic eggs widely distributed in the amber that were released from a hole in the ant's gaster.
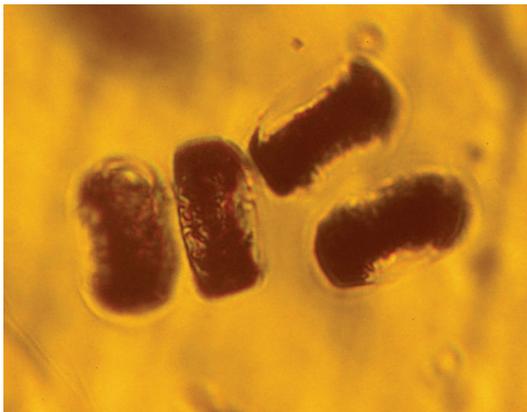

FIGURE 15: Female of the allantonematid *Formicitylenchus oregonensis* removed from the body cavity of *Camponotus vicinus* in Oregon, USA.

FIGURE 14: Detail of eggs of *Myrmeconema antiqua* in various stages of development in Dominican amber.

4. Allantonematidae

It is curious why so few cases of allantonematid infections have been reported in ants. Since ants are probably one of the most investigated insect groups, is the absence of tylenchid parasitism due to a lack of observations or its rarity? The first and only described allantonematid parasite of extant ants is *Formicitylenchus oregonensis* that was parasitizing a queen *Camponotus* ant in Western Oregon, USA [34]. The queen had already chewed off her wings and appeared to be searching for a nesting site. There was a single large parasitic female (Figure 15) and 120 third-stage juvenile nematodes in the ant's gaster. The third-stage juveniles exited through the ants reproductive and digestive tracts and molted twice to reach the adult stage. The enlarged pharyngeal glands in the free-living females suggest that they penetrate the cuticle to enter the body cavity of the host, probably ant larvae. Although the complete life cycle is unknown, the nematodes are clearly distributed by infected queen ants. The gonads of the infected ant were greatly reduced, and her eggs were

abnormal. Since carpenter ants can be damaging to structures, *F. oregonensis* can be considered as a potential biological control agent.

Since the original report of this parasite, the present author recovered a worker carpenter ant also infected with *F. oregonensis,* thus indicating that *Formicitylenchus* is probably restricted to ant hosts, especially members of the genus *Camponotus*. *Formicitylenchus* shows a close relationship with the allantonematid beetle parasite, *Metaparasitylenchus* [34]. It is possible that their last common ancestor parasitized beetles and the host shift from arboreal beetles to arboreal ants occurred during the anagenesis of *Formicitylenchus*. The close physical association between wood-boring beetles and *Camponotus* ants may be significant. Rogers [35] commented that "…the potential parasite would be expected to find its hosts in organisms which occupy the same niche largely independent of their phylogenetic position. In fact the specificity of many parasites is based on the ecological relationship of the hosts, especially in groups which have only recently become parasitic."

Another reason that allantonematid parasitism of ants may be more widespread than presumed is the discovery of juveniles of a fossil allantonematid, *Palaeoallantonema cephalotae,* in the ant, *Cephalotes serratus*, in Dominican amber [1] (Figure 16). Just before this fossil was discovered, Steven Yanoviak submitted an extant worker of *Cephalotes christopherseni* from Peru that was also infected with an allatonematid. The parasitic female (Figure 17) of this still undescribed species and the developing juveniles inside her body (Figure 18) show features typical of the family.

5. Seuratidae

The discovery of adults of *Rabbium paradoxus* [36] inside the gaster of worker *Camponotus castaneus* in Florida (Figure 19) was a surprise since all known nematodes of the Seuratidae are heteroxenous and develop to the adult stage in the digestive tract of vertebrates [37]. However, in *R. paradoxus*, the
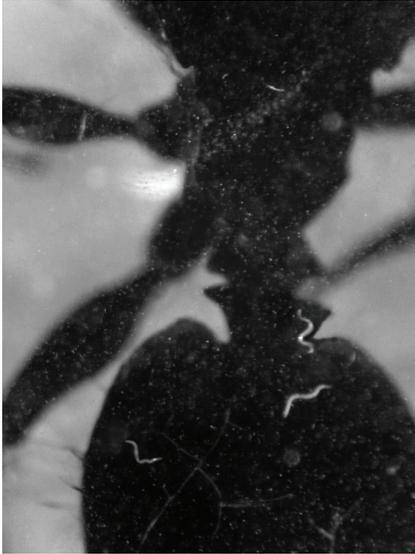
FIGURE 16: Three juveniles of the allantonematid, *Palaeoallantonema cephalotae,* that emerged from a worker *Cephalotes* ant in Dominican amber.

FIGURE 19: Adults of *Rabbium paradoxus* adjacent to their ant host, *Camponotus castaneus,* in Florida.

FIGURE 17: Parasitic female of an undescribed allantonematid from workers of *Cephalotes christopherseni* in Peru.

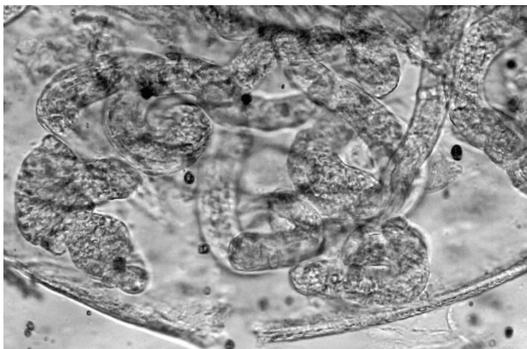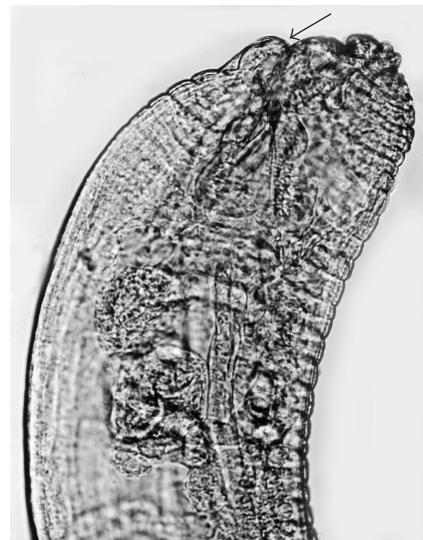FIGURE 20: Head of a female of *Rabbium paradoxus.* Arrow shows anteriorly located vulva.

FIGURE 18: Juveniles developing inside the body of the female allantonematid shown in Figure 17.

vertebrate host is obviously not required for adult development. The females of *R. paradoxus* have an anteriorly placed vulva (Figure 20), and the eggs embryonate inside the uterus (Figure 21). Since the other member of the genus, *R. caballeroi,* occurs in the gut of lizards in the Bahamas [38], it is likely that *R. paradoxus* originally had (or still has) a lizard definitive host. If the complete life cycle occurs just in ants, then *C. castaneus* would serve as both intermediate and definitive hosts. *C. castaneus* is a generalist feeder and will ingest vertebrate feces so it could acquire nematode eggs from lizard droppings. Parasitized worker ants had swollen gasters and showed unusual behavior by foraging during the day instead at night. This would make them easily captured by vertebrate predators.

FIGURE 21: Embryonated eggs in the uterus of *Rabbium paradoxus*.

The original life cycle of *R. paradoxus* may have been similar to that of the seuratoid *Skrjabinelazia galliardi*, a parasite of sphaerodactyline lizards in Brazil [37]. The female nematodes living in the gut of the lizard produce eggs that are passed out and ingested by insects. These eggs hatch in the insect gut and the juveniles enter the body cavity without further development. Growth is resumed when the insect intermediate hosts are eaten by lizards [38]. Unfortunately, the complete life cycle of *R. paradoxus* remains a mystery, but its precocious development is quite interesting.

6. Physalopteridae

There are few reports of heteroxenous nematodes utilizing ants as intermediate hosts, that is, hosts where the nematodes develop only to the third-stage infective juveniles. Maturity to the adult stages occurs when the intermediate host is eaten by a vertebrate definitive host. One such nematode is the physalopterid, *Skrjabinoptera phrynosoma* that lives in the stomach of the Texas horned lizard, *Phrynosoma cornutum*, and uses the harvester ant, *Pogonomyrmex barbatus*, as an intermediate hosts [39]. However instead of depositing isolated eggs that would pass from the lizard, the gravid nematodes die with the retained eggs enclosed in thick walled capsules. The females with their enclosed eggs pass out of the lizard and are collected by worker ants that feed them to their brood. The nematode eggs hatch in the gut of the ant larvae and the juveniles enter the fat body, where they develop only to the third stage. These juveniles are carried through the pupal and into the adult stage of the ant, where they eventually reside in membranous capsules. The nematodes complete their development to the adult stage when infected ants are eaten by the lizards. Worker ants with more than 10 nematodes were still active but had enlarged, lighter colored gasters. The interesting, pivotal stage in this life cycle is the attractiveness of the dead, egg-laden female nematodes to worker ants.

7. Rhabditidae, Diplogastridae, and Panagrolaimidae

This category includes juvenile nematodes living in the postpharyngeal glands of ants (internal phoresis) or being carried on the outside surface of ants (external phoresis) (Table 3). While these might not be considered parasites, in some instances where the association has been examined critically [40] damage has been inflicted on the ant's postpharyngeal glands and some of the nematodes increased in size during their stay in this location. Thus at most, they could be considered weak parasites. If they break through the glands and introduce microbes into the body cavity of the ant, they could even be regarded as pathogenic. However the latter scenario has not been documented.

Most of the nematodes in the postpharyngeal glands are dauer juveniles of free-living microbotrophs living in the ant's environment. The dauers enter the glands when external conditions become unsuitable (low humidity or diminished food supply). These resistant dauer juveniles can survive for relatively long periods. The nematodes may leave the glands when the environment is more suitable (moist with associated microbes), if the ant dies and the dauer initiates development within the decomposing ant, or when the nematodes are transferred from ant to ant during trophallaxis.

Janet [43] was the first to discover postpharyngeal rhabditids (*Oscheius dolichurus*) in *Lasius flavus* and *Formica rufa* in France. Wahab [42] was the first to systematically study these associations in the ant genera *Lasius, Formica, Tetramorium,* and *Myrmica* in Germany (Table 3). More recently Köhler [41] examined nematodes in the heads of ants collected from sap fluxes and rotten wood on trees in Germany. The most common ant that visited these fluxes was *Lasius brunneus* and, from a total of 262 workers collected, 43.5% carried nematodes, with *Koerneria histophora* being the most common associate. While most ants carried a single nematode, numbers occasionally reached up to 85 dauers per ant. Köhler [41] also found diplogastrid dauers in 4 males and a queen of *L. brunneus*. The infection rate of ants associated with *L. brunneus* workers varied depending on the weather cycle. There were more nematodes in ants during the dry period in August than during the rainy months of April and May. Also important in determining the rate of nematodes being carried by the ants was the location of the nests. Rates of infestation by nematodes in *L. brunneus* were much higher when the ants were collected from sap fluxes and rotten wood [41], than were collected from under stones and leaf litter [42].

Köhler [41] was able to infest ants by placing them in a Petri dish with rotten wood containing waving dauer stages. Both Wahab [42] and Köhler [41] provided evidence that the dauers can be transmitted from ant to ant via trophallaxis, which was supported in part by the experiments of Naarmann [53] showing that *Formica* ants mix food with secretions from the postpharyngeal gland before regurgitating it to nest mates. These ant-dauer associations probably occur worldwide since Markin and McCoy [40] reported *Diploscapter lycostoma* in the postpharyngeal glands of the Argentine Ant, *Linepithema humile* in California and Nickle & Ayre

TABLE 3: Juvenile nematodes of Rhabditida and Tylenchina associated with ants.

| Nematode	Family	Host	Reference
Diplogasteroides spengelii	Diplogastridae	*Lasius brunneus*	[41]
Diploscapter lycostoma	Rhabditidae	*Formica* spp., *Lasius* spp.	[42]
"	"	*Myrmica rugulosa*	[42]
"	"	*Linepithema humile*	[40]
Formicodiplogaster myrmenema	Diplogastridae	*Azteca alpha*	[1]
Halicephalobus similigaster	Panagrolaimidae	*Lasius brunneus*	[41]
Koerneria histophora	Diplogastridae	*Lasius* spp.	[42]
"	"	*Lasius brunneus*	[41]
Oscheius dolichurus	Rhabditidae	*Formica rufa,*	[42, 43]
"	"	*Lasius flavus*	[42, 43]
"	"	*Tetramorium caespitum*	[42]
"	"	*Camponotus herculeanus*	[44]
"	"	*Lasius claviger*	[44]
"	"	*Lasius brunneus*	[41]
Pristionchus lheritieri	Diplogastridae	*Formica rufa, Lasius* spp.	[42]
*Unknown	"	*Azteca* spp.	[1]
Unknown	"	*Formica obscuriventris*	Present work (Figure 22)

*Fossil.
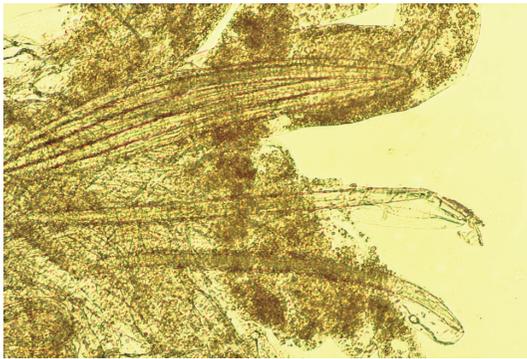
FIGURE 22: Dauer juveniles of a diplogastrid in the postpharyngeal glands of *Formica obscuriventris clivia* from Oregon.

[44] found *Oscheius dolichurus* in the head glands of *Camponotus herculeanus* and *Lasius claviger* in Ontario, Canada. The author has also found dauer diplogastrids in the postpharyngeal glands of workers of *Formica obscuriventris* in Oregon (Figure 22).

The association between dauer nematodes and ants is at least 20–30 million years old. Evidence for this is the discovery of dauer juveniles of the fossil diplogastrid, *Formicodiplogaster myrmenema*, carried by *Azteca alpha* workers in Dominican amber [1] (Figures 23 and 24). The dauer stages appear to be associated with the abdomen of the ants, suggesting that they were being carried in the segmental membranes of the gaster (external phoresis). None of the fossil stages occurred around the mouthparts of the ants. Also, developing stages of *F. myrmenema* were associated with nest material adjacent to worker *Azteca* ants in Dominican amber

FIGURE 23: Three dauer juveniles of *Formicodiplogaster myrmenema* adjacent to a worker of *Azteca alpha* in Dominican amber.

[1]. This indicates that *F. myrmenema* was developing in the nests of *A. alpha*, which is probably the case with extant nematodes in the head glands of ants. Whether the dauers of *F. myrmenema* were also in the postpharyngeal glands of the fossil ants is unknown.

TABLE 4: Ants infected by entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora)* under laboratory and/or field conditions.

Ant	Nematode	System	Reference
Acromyrmex octospinosus	*S. carpocapsae*	Aqueous	[45]
Camponotus sp.	*S. carpocapsae*	Sucrose	[46]
Camponotus sp.	*S. carpocapsae*	Aqueous	[47]
Myrmica sp.	*S. carpocapsae*	Aqueous	[47]
Pogonomyrmex sp.	*S. carpocapsae*	Sucrose	[48]
Solenopsis spp.	*S. carpocapsae*	Alginate capsules	[46]
Solenopsis geminata	*S. carpocapsae*	Aqueous	[49]
Solenopsis invicta	*S. carpocapsae*	Aqueous	[50, 51]
Solenopsis invicta	*H. bacteriophora*	Aqueous	[52]
Solenopsis richteri	*H. bacteriophora*	Aqueous	[52]
Solenopsis richteri	*S. carpocapsae*	Aqueous	[50]

FIGURE 24: Detail of a dauer juvenile of *Formicodiplogaster myrmenema* adjacent to a worker of *Azteca alpha* in Dominican amber.

FIGURE 25: Developing stages of *Steinernema carpocapsae* removed from the body of an infected queen of *Solenopsis invicta*.

8. Steinernematidae and Heterorhabditidae

Included in this section are the so-called entomopathogenic nematodes belonging to the genera *Steinernema* and *Heterorhabditis*. It is quite likely that entomopathogenic nematodes infect ants under natural conditions, but no reports are known. Infection is initiated by a third-stage infective juvenile that enters the host's body cavity, apparently per os [50]. After reaching the hemocoel, the infective stage initiates development and, in so doing, releases a symbiotic bacterium (*Xenorhabdus* spp. in *Steinernema* nematodes and *Photorhabdus* spp. in *Heterorhabditis* nematodes) that is carried in the infective stage's gut lumen. The bacterium kills the insect soon after it is released in the body cavity. The nematodes feed on the mixture of bacteria and insect hemolymph and develop to the adult stage in the body cavity. With adequate nourishment, the nematodes undergo a second generation but when nourishment is limited, the juveniles form third-stage infective juveniles. By introducing the bacteria that quickly kill the hosts, these nematodes avoid specific defense responses and have a wide host range, attacking representatives of many insect orders and even other arthropods [54].

Laboratory experiments have shown that these nematodes can infect a number of ant species (Table 4) and they also have been used in the field against pest ants [50, 52, 54–56]. Poole [50] attempted to control field populations of ants (*Solenopsis richteri* and *S. invicta*) with *Steinernema carpocapsae*. Using a dose of 1 million infective stages per mound for *S. invicta*, the nematodes caused 35% mortality in the fall and 80% mortality in the spring. With *S. richteri*, the death rate was 80% in the spring and 36% in the fall. Poole [50] noted that workers were infected less than other stages, possibly because of their greater activity and grooming behavior. However, workers regurgitated infective stages to the alates and larvae. Queen ants were more susceptible and up to 3,000 infective stage juveniles could be produced in some infections (Figure 25).

Further field trials of *S. carpocapsae* and *Heterorhabditis bacteriophora* against the red imported fire ant, *S. invicta*,

TABLE 5

(1)	Nematodes represented as dauer or postdauer juveniles in the pharyngeal glands of ants	Rhabditidae, Diplogastridae and Panagrolaimidae
	Nematodes developing in the body cavity of ants	(2)
(2)	Only juvenile nematodes present	(3)
	Adult nematodes with or without juveniles	(4)
(3)	Elongate nematodes normally over 15 mm in length at completion of development; not enclosed in membranous capsules	Mermithidae
	Nematodes under 10 mm in length; enclosed in membranous capsules	Physalopteridae
(4)	Nematodes reproducing in dead ants; infective juveniles produced	(5)
	Nematode adults, eggs and/or juveniles in living ants; infective juveniles absent	(6)
(5)	Males with a bursa; females with a pointed tail	Heterorhabditidae
	Males without a bursa; females with a bluntly rounded tail, often bearing a small point at tip	Steinernematidae
(6)	Eggs and juveniles present	Allantonematidae
	Eggs, but no juveniles present	(7)
(7)	Vulva positioned at middle or lower half of body	Tetradonematidae
	Vulva positioned in upper fourth of body	Seuratidae

gave control rates of 37.5% with *S. carpocapsae* but less with *H. bacteriophora* [52]. In field trials comparing applications of *Steinernema carpocapsae* and amidinohydrazone against *S. invicta*, Morris et al. [55] estimated that nematode applications at a rate of 2 million per gallon per mound resulted in 47% mortality.

Controlling fire ants in the field is difficult because of the small mound opening through which the nematodes are introduced. Also, it is desirable to have recycling of the nematodes in the nests, but healthy ants appear to remove infected individuals before the cycle is completed. Since the number of nematodes needed to overwhelm a colony of ants is quite high using inundative methods, consideration was given to the development of baits or other more efficient delivery systems [46, 48, 52, 55, 56]. These other methods are still under investigation.

9. Unknown Nematodes

Gösswald [57] reported the presence of several encysted nematodes in the flight muscles of a queen *Teleutomyrmex schneideri* in Germany. The cysts were quite small, being only 25 μm in diameter. Except for their small size, the cysts are similar in appearance to those of the vespid mermithid, *Pheromermis pachysoma,* formed in the body wall of Trichoptera paratenic hosts [27] and the ant parasite, *P. villosa,* in the body of oligochete paratenic hosts [26]. However, the *Pheromermis* cysts are 60–100 μm and 80 μm in diameter, respectively. It is possible that juvenile nematodes of a mermithid parasite were acquired after the queen was fully formed and the nematodes preferred to encyst rather than initiate development. The other likelihood is that the nematodes were the infective stages of a heteroxenous nematode parasite and were waiting for transfer to a vertebrate definitive host. However, the only cysts of heteroxenous nematodes known from ants are those of the physalopterid, *S. phryn-*

osoma, the smallest of which measures 633 μm in diameter [39].

In 1907, Janet [58] found nematodes 7-8 mm in length developing in the head cavities and emerging from the labial region of workers of *Formica fusca*. Just before the nematodes emerged, the infected ants began trembling and eventually died. The head cavities of infected ants were empty upon nematode exit. This behavior of developing in the head of ants is known for some phorid flies but not for nematodes. Whether this was a mermithid with an unusual developmental location or a heteroxenous nematode using the ant as an intermediate host is unknown.

10. Identification Key to Nematode Families Associated with Ants

See Table 5.

11. Conclusions

Representatives of most invertebrate parasitic nematode families attack ants, with the exception of sphaerulariids, entaphelenchids, and oxyurids. While mermithids are the most commonly encountered nematode parasites of ants, the complete life cycle of only a single species is known. The life cycle of ant mermithids can be quite complicated when it involves paratenic hosts living in completely different habitats. Even less is known about the life cycles of other ant parasitic nematodes, certainly not enough to consider using them as biological control agents. While the inundative application of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) can control ants in isolated colonies, establishing nematodes for the sustained control of ant populations has not been achieved.

There are probably many additional nematode parasites of vertebrates utilizing ants as intermediate hosts. Reptiles,

mammals, and amphibians eat ants, and it follows that nematodes other than *Skrjabinoptera phrynosoma* would have devised methods of cycling themselves through ants to reach their definitive hosts. In the mysterious case involving *Rabbium paradoxus*, the presence of adults of a heteroxenous nematode in an ant raises the question of whether formicids can serve as sole hosts or this is just a case of precocity.

Fossils show that mermithids were infecting ants over 40 million years ago and tetradonematids and allantonematids had established parasitic associations with ants some 20–30 million years ago. Such fossils, which can be used to calibrate molecular clocks, provide minimum dates for the occurrence of nematode lineages and show the antiquity of nematode-ant relationships.
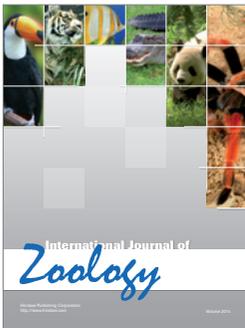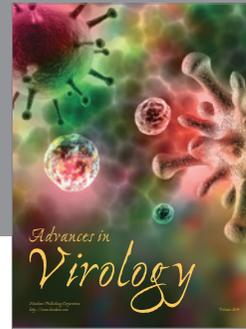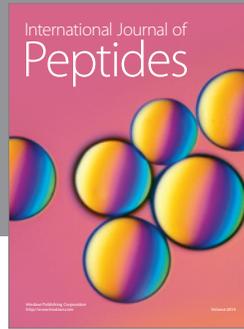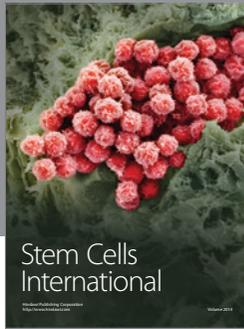
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