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Allomermis solenopsi n. sp. (Nematoda: Mermithidae) parasitising the fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in Argentina

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Abstract *Allomermis solenopsi* n. sp. (Mermithidae: Nematoda) is described from the fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in Argentina. Diagnostic characters of the new species include stiff and erect processes on the surface of the mature egg, small female amphids, extension of the latero-medial rows of male genital papillae beyond the middle rows, an obliquely truncate spicule tip and a ventrally swollen male terminus. This is the first record of *Allomermis* Steiner, 1924 from South America and the first host record for members of this genus. Previous records of mermithids from *Solenopsis* spp. are summarised. The placement in *Allomermis* was confirmed by molecular analyses based on nuclear 18S ribosomal DNA sequences, the first such molecular framework for the Mermithidae. The possible life-cycle of the parasite is discussed, with the aim of using *A. solenopsi* as a biological control agent for fire ants in the United States.

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Introduction

Ant parasitism by mermithid nematodes has been ongoing for some 40 million years (Poinar, 2002) and these associations occur throughout the world today. Mermithids have been reported from at least 17 species of Neotropical ants; however, only four species have been described and, of these, only the description of *Meximermis ectatommi* Poinar et al. (2006) included the adults of both sexes.

Mermithids have also been reported from various species of *Solenopsis* Westwood (Table 1); however, none were identified or described. Thus it was of considerable interest when, during a study of native parasites of Argentinean populations of fire ants with the objective of appraising potential biological control agents for release in the United States, populations of *Solenopsis invicta* Buren were discovered to be parasitised by an undescribed mermithid species. Since mermithids are known to kill their hosts and have been used previously in the biological control of insect pests (Petersen & Willis, 1972), the present

Table 1 Mermithid parasitism reported for ants of the genus *Solenopsis*

Ant species	Mermithid	Locality	Reference
S. fugax Mayr	Undetermined	Europe	Gösswald, 1930
S. geminata (Forel)	Undetermined	Florida	Mitchell & Jouvenaz, 1985
S. geminata (Forel)	Undetermined	Florida	McInnes & Tschinkel, 1996
S. invicta Buren	Undetermined	Florida	McInnes & Tschinkel, 1996
S. invicta Buren	*Allomermis solenopsi* n. sp.	Argentina	Present study
S. pergandei Forel	Undetermined	Florida	McInnes & Tschinkel, 1996
S. richteri Forel	Undetermined	Argentina	Jouvenaz & Wojcik, 1990
Ant species	Mermithid	Locality	Reference
S. fugax Mayr	Undetermined	Europe	Gösswald, 1930

species was considered worthy of description and further investigation.

Little is known of phylogenetic affinities among mermithid nematodes; the only available hypothesis is a morphology-based phylogeny that divides the Mermithidae Braun, 1883 into seven subfamilies (Gafurov, 1996). However, because limited information was provided as to how character states were coded in the generation of this phylogeny, an independent test for relationships among mermithid nematodes was desired. To this end, a molecular framework for the Mermithidae based on nuclear 18S ribosomal DNA sequences was developed and the position of the fire ant nematode mapped onto this tree. This phylogenetic approach indicated the subfamily into which the fire ant nematode should be placed.

Materials and methods

In late December and early January, 2006, mermithid nematodes were discovered parasitising fire ant workers in five of seven *Solenopsis invicta* colonies collected within several kilometres of the Corrientes Biological Station (Estación Biológica Corrientes, 27°33′S, 58°41′W), near the town of San Caytano, approximately 18 km SE of Corrientes. The same nematodes were also found in four of five colonies collected at the National Center for Aquiculture Development (Centro Nacional de Desarrollo Acuícola, 27°23′S, 58°41′W) about 17 km NE of Corrientes. Infested ant colonies were kept at room temperatures of about 22–26°C. Nematodes that emerged from ants were maintained in small cups or culture wells containing water. For descriptive studies, adult and postparasitic juvenile mermithids were heat-killed (70°C), fixed in 70% ethanol and processed to glycerine for observations and measurements. Type-material of *Allomermis trichotopson* Steiner, 1924 for comparative studies was obtained from Dr Zafar A. Handoo, USDA, ARS, Nematology Laboratory, Beltsville, Md, USA.

Total cellular DNA used as template in PCR amplification reactions was isolated from individual J3 or adult nematodes using the method of Powers & Hyman (1986). In all cases, template DNA was prepared from individual nematodes.

The primer pair:

18S-5F: 5′-GCGAAAGCATTTGCCAAGAA-3′
18S-9R: 5′-GATCCTTCCGCAGGTTCACCT-3′

(Vandergast & Roderick, 2003) was used to amplify an 18S small subunit (SSU) rDNA PCR product of *c*.800 base pairs from the taxa listed in Table 2. PCR thermocycling conditions included an initial strand denaturation step of 94°C for one minute followed by 35 cycles that included denaturation at 94°C, 30 seconds; primer annealing at 50°C, 40 seconds; and an elongation step at 72°C for one minute. A final elongation step was then conducted at 72°C. for 10 minutes. Agarose gel electrophoresis was used to confirm successful amplifications. PCR products were purified using the QIAQuick PCR Purification Kit (Qiagen, Valencia, CA) and nucleotide sequence of both strands determined by direct sequencing of the 18S rDNA PCR products at the University of California–Riverside Institute for Integrated Genome Biology core facility using an Applied Biosystems (Foster City, CA) 3730xl automated DNA sequencer.

Table 2 Nematodes used in phylogenetic analysis

Family	Mermithid subfamily[a]	Host[b]	Locality	Source	GenBank accession no.
Mermithidae					
Agamermis changshaensis	Agamermithinae	Not reported	Hubei, China	G. Wang, Central China Normal University	DQ628908
Gastromermis sp.	Gastromermithinae	*Prosimulium* sp.	Ontario, Canada	A. Sharp, Brock University, Canada	DQ533954
Heleidomermis magnapapula	Heleidomermithinae	*Culicoides variipennis*	Chino, CA, USA	B. Mullens, Univ. of California-Riverside	DQ533955
Hexamermis agrotis	Agamermithinae	Not reported	Henan, China	G. Wang, Central China Normal University	DQ530350
Mermis nigrescens	Mermithinae	Not reported	British Columbia, Canada	J. Burr, Simon Fraser University, Canada	DQ518905
***Allomermis solenopsi* n. sp.**	Mermithinae	*Solenopsis invicta*	Corrientes, Argentina	S. Porter, USDA-ARS	DQ533953
Mononchidae					
Clarkus sp.	n/a[c]	Free-living	Not known	GenBank	AY284748
Mylonchulus arenicolus	n/a	Free-living	St Albans, UK	GenBank	AF036596

[a] After Gafurov (1996). [b] If identified during collection. [c] Not applicable

All DNA sequences are available in GenBank with accession numbers listed in Table 2.

Clustal X was used to align 18S rDNA sequences. Phylogenetic analysis was performed in PAUP version 4.0b10 (Swofford, 1998) using Maximum Likelihood analysis employing a General Time Reversible Model (GTR) with an estimated shape parameter and proportion of invariable sites, and six substitution rate parameters whose values were also estimated. Because mermithid lineages are ancient and likely to have undergone considerable divergence, the GTR model was used as it accommodates differences in nucleotide frequencies and substitution rates among taxa under comparison.

Sources of nematode taxa employed in the phylogenetic component of this study are described in Table 2.

Mermithidae Braun, 1883

Allomermis Steiner, 1924

Diagnosis (amended)

Adult and postparasitic juvenile cuticles without cross-fibres; four cephalic (head) papillae, no lip papillae; amphids located far forward, near or at tip of head and anterior to circle of cephalic papillae; mouth opening ventral, located posterior to level of cephalic papillae; retractor muscle extends from top of pharyngeal tube to opposite body wall; six hypodermal chords at mid-body; vagina short, modified S- shaped, with bends perpendicular to body plane; spicules paired, separate, slightly curved to straight, shorter than body width at cloaca; genital papillae arranged in three longitudinal series, one middle (ventromedial) and two latero-medial (submedial); each series composed of two or more columns of small papillae; eggs small, bearing flexible or stiff processes; post-parasitic juvenile without tail appendage. Type-species: *A. trichotopson* Steiner, 1924.

Comments

While Steiner (1924) originally stated that *A. trichotopson* possessed cross-fibres in the adult cuticle, this character could not be confirmed in the type-specimens. The absence of cross-fibres in both the adult and post-parasitic juvenile cuticles, as occurs in *Allomermis* Steiner, 1924, is rare in mermithids parasitising terrestrial insects, being more typical of aquatic mermithids. Even those terrestrial mermithid genera that lack cuticular cross-fibres in the adult stage, such as members of the spider parasite genus *Aranimermis*

Poinar & Early, 1990, often possess them in the post-parasitic juveniles. Steiner also thought that there were eight hypodermal chords; however, there are only six at mid-body in the present specimens.

Allomermis can be separated from other mermithid genera by the following characters: (1) a vagina that curves perpendicular to the body plane; (2) A mouth opening that is located posterior to the circle of cephalic papillae; (3) amphids located anterior to the cephalic papillae at or just beneath the head tip; and (4) the presence of processes on the mature eggs. *Camponotimermis* Ipatieva, Pimenova & Much-amedzianova (1990), whose members parasitise ants in temperate climates, is similar to *Allomermis* in possessing four cephalic papillae, small eggs and flask-shaped amphids, but the mouth opening is anterior to the cephalic papillae and the vagina, with a single curve in the distal portion, is not perpendicular to the plane of the body.

Pheromermis Poinar, Lane & Thomas, 1976, which contains the European ant mermithid *P. villosa* Kaiser, 1986, also has four cephalic papillae, anteriorly positioned amphids and a ventrally displaced mouth, but the mouth is anterior to the cephalic papillae, the vagina is not bent in a transverse plane to the body and the eggs do not contain processes. *Pseudomermis* de Man, 1903 also has four cephalic papillae, but the vagina is straight and barrel-shaped, the mouth is terminal and the amphids are located posterior to the cephalic papillae.

The only described Neotropical species of mermithids infecting ants is *Meximermis ectatommi* Poinar et al., 2006, which parasitises *Ectatomma ruidum* Roger in southern Mexico. However, this species has a terminal mouth or one that is shifted only slightly ventrally, six cephalic papillae, cup-shaped amphids located posterior to the level of the cephalic papillae and a pair of J-shaped spicules 2.7 times as long as the tail width.

Allomermis solenopsi n. sp.

Type-host: The fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae).

Type-locality: Corrientes Biological Station (Estación Biológica Corrientes, 27°33′S, 58°41′W), near the town of San Caytano, *c*.18 km SE of Corrientes in Corrientes Provence, northern Argentina.

Type-material: Holotype (male) no. 5651 and allotype (female) no. 5622 deposited in the Facultad de Ciencias Naturales y Museo, Universidad de La Plata, Paseo del Bosque s/n, B1900FWA, La Plata, Buenos Aires Province, Argentina; paratypes T-5434p (male) and T-5435p (female) deposited in the USDA Nematode Laboratory, Beltsville, Maryland and the authors' collections.

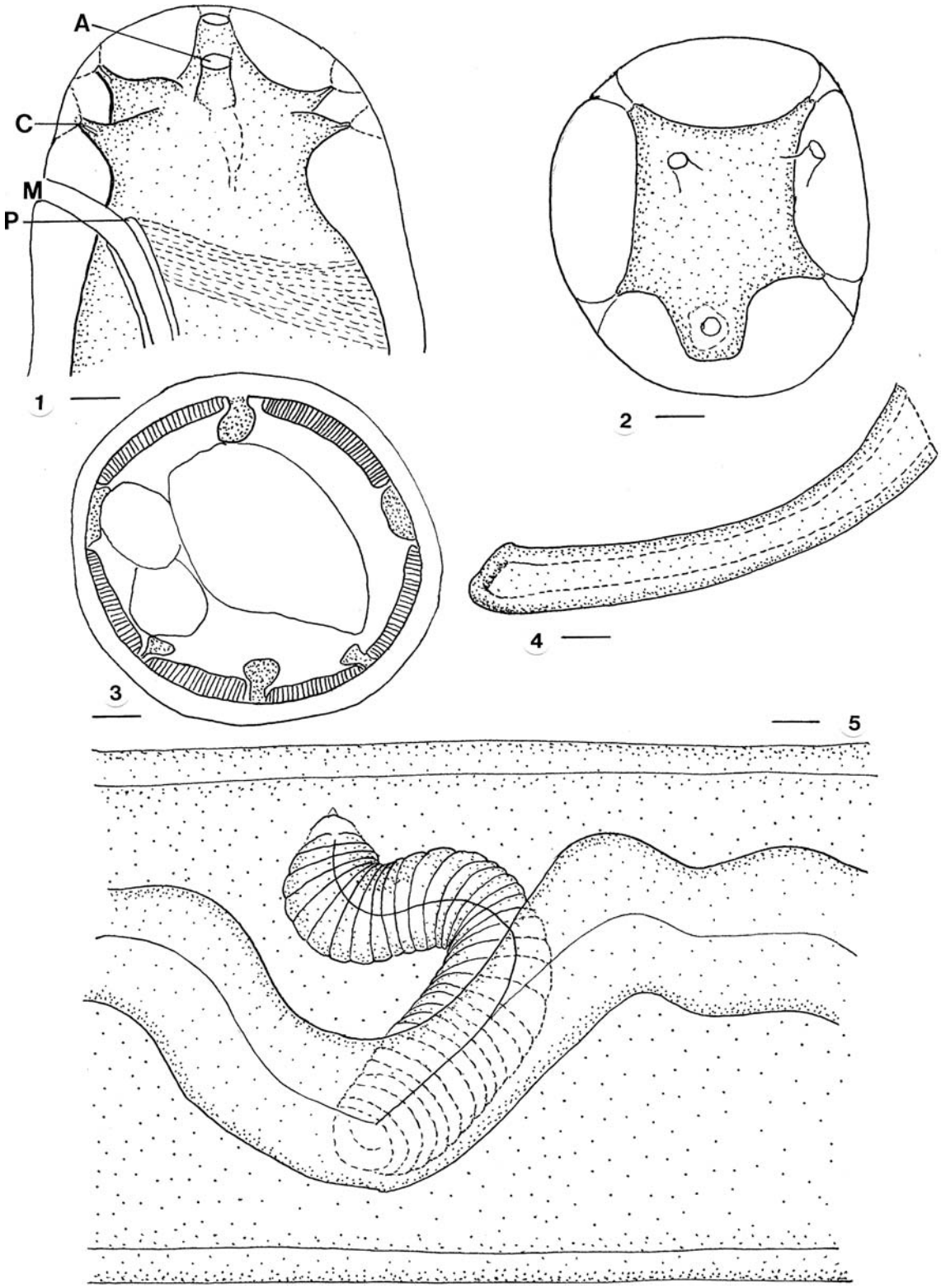
Description (Figs. 1–13, 15–19)

Adults. With characters listed under amended generic diagnosis; size small; colour white; body-cavity contains circular to elliptical protein platelets.

Female (n = 10). Length 20 (13–27) mm; greatest width 239 (183–284); cephalic papillae 4; amphids flask-shaped, located near tip of head; diameter of amphidial apertures 5 (4–8); anterior to circle of cephalic papillae, with oval apertures; distance cephalic extremity to amphidial apertures 7 (5–8), to nerve-ring 250 (190–311), to end of pharyngeal tube 35 (27–45), to mouth opening 27 (21–32), to apertures of cephalic papillae on cuticle 15 (11–19); vagina muscular, length in lateral view 136 (111–159), bent perpendicular to plane of body, modified S-shaped (curved twice), with second curve slight, just anterior to junction with vulva; vulva at 51 (48–52) % of body length; tail bluntly rounded; vestigial cloaca rarely present, located 149 from tip of tail; eggs mostly spherical when mature, covered with elongate, stiff, spiny processes (Figs. 13, 18), with diameter excluding processes 34 (32–37) and including processes 48 (43–51).

Male (n = 11). Length 12 (10–13) mm; greatest width 174 (139–183); diameter of amphidial apertures 4 (3–5); distance cephalic extremity to amphidial apertures 7 (5–8), to nerve-ring 259 (200–317), to end of pharyngeal tube 33 (27–46), to mouth opening 26 (24–32), to apertures of cephalic papillae on cuticle 14 (13–16); spicules paired, separate but closely appressed, length 98 (79–120) and less than tail width

Figs. 1–5 *Allomermis solenopsi* n. sp. 1. Lateral view of female head showing points of measurements in the description. 2. *En face* view of male. 3. Cross-section of male at mid-body showing six hypodermal chords and six muscle fields. 4. Spicule showing obliquely truncate tip. 5. Ventral-lateral view of vagina. *Abbreviations*: A, amphidial openings on cuticle; P, end of pharyngeal tube; M, mouth opening; C, aperture of cephalic papillae on cuticle. *Scale-bars*: 1, 8 μm; 2, 10 μm; 3, 23 μm; 4, 9 μm; 5, 17 μm ▶


A
C
M
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1
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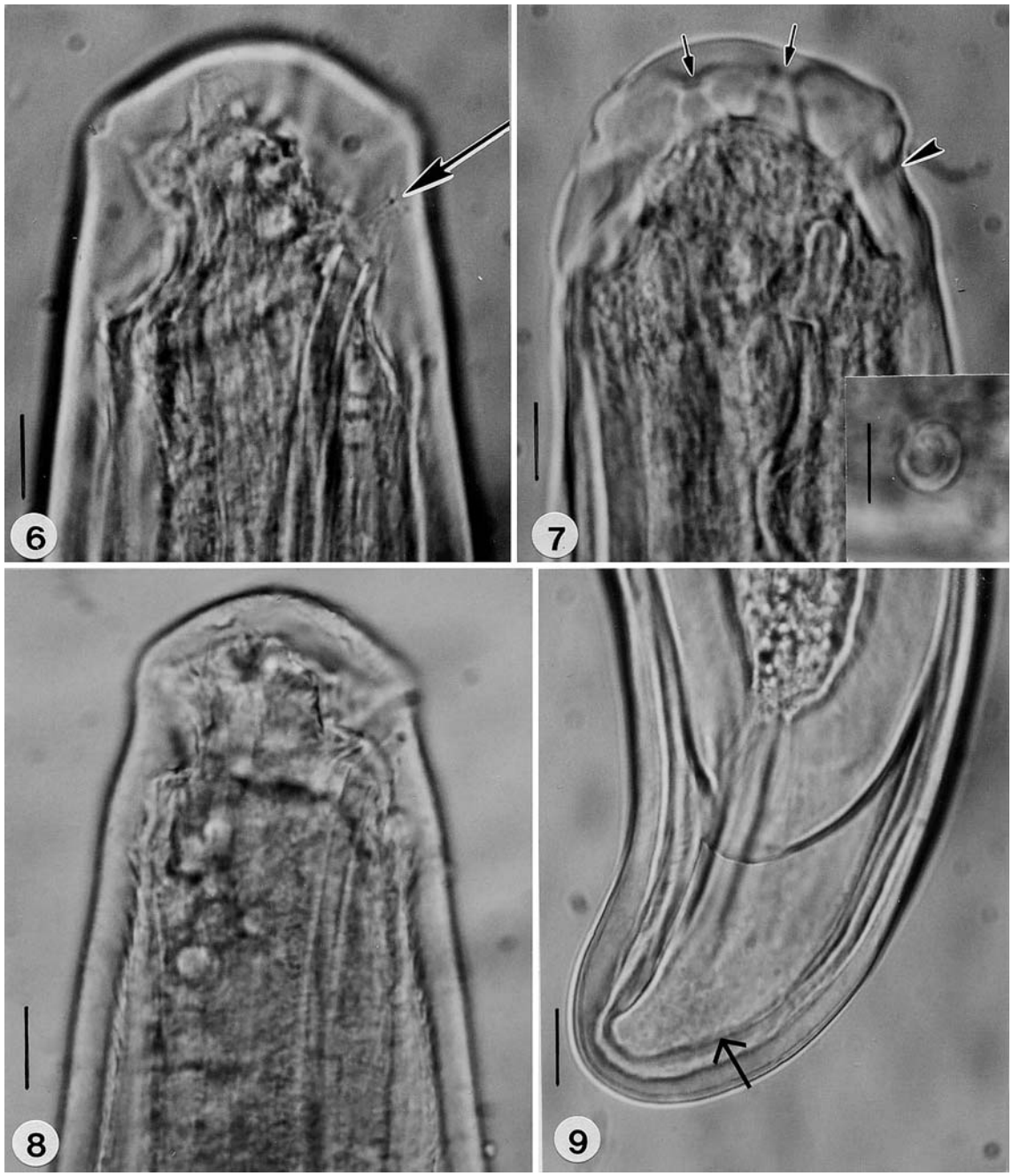


Figs. 6–9 *Allomermis* species. 6. Lateral view of female head of *A. solenopsi* n. sp. Arrow shows mouth opening. 7. Ventral lateral view of female head of *A. solenopsi*. Arrows show apertures of anteriorly placed amphids. Arrowhead shows mouth opening. Insert shows ventral view of mouth opening. 8. Lateral view of female head of *A. trichotopson* (from type-slide T-4692p, Nematology Laboratory, Beltsville, Md). 9. Double moult of post-parasitic juvenile female of *A. solenopsi*. Arrow shows fine inner fourth stage cuticle within outer, thicker, third-stage cuticle. *Scale-bars*: 21 μm

at cloaca, width at distal end 14 (13–16); spicule tips obliquely truncate; 3 double or multiple rows of genital papillae, with middle row ending *c*.2–3 tail lengths from cloaca and latero-medial rows extending almost quarter of body length, terminating opposite constriction separating proximal glandular and distal

muscular portion of vas deferens; genital papillae small and numerous, numbering hundreds in latero-medial rows; tail bluntly rounded, with swelling at tip, length 172 (127–172); body width at cloaca 134 (127–190).

Post-parasitic juveniles (n = 5). The size of the post - parasitic juveniles is similar to that of the adults, although the cephalic papillae and amphids are reduced in size. Post-parasitic juveniles moult twice to reach the adult stage and the second moult is represented by a delicate cuticle that remains inside the thicker third-stage cuticle. There is no appendage on the tail and the cuticle lacks cross-fibres.

Remarks

In his classification of the Mermithidae, Rubstov (1978) listed two additional species in *Allomermis*, namely *A. lasiusi* Rubstov, 1970 and *A. myrmecophila* Crawley & Baylis, 1921. These were placed in *Allomermis* due to their association with ants and because they shared some common characters, such as the ventral mouth opening and an S- shaped vagina. However, in his analysis of the mermithid parasites of terrestrial insects, Artyukhovsky (1990) removed these two species from *Allomermis* and placed them in *Camponotimermis* Ipateva, Pimenova & Mukhamedzyanova, 1990. He considered *A. trichotopson* Steiner, 1924 the only species in the genus, thereby recognising its uniqueness. Artyukhovsky's treatment is followed here and thus *A. solenopsi* n. sp. represents the second valid species of *Allomermis*. The two species of *Allomermis* are quite similar, but can be separated by the following characters: (i) the nature of the processes on the surface of the mature egg, as these tend to be stiff and erect in *A. solenopsi*, whereas most are bent and curved in *A. trichotopson*; (ii) the amphids are much smaller in the females than in the males of *A. trichotopson*, but are approximately the same size in both sexes of *A. solenopsi*; (iii) the lateral-medial rows of male genital papillae extend much further anteriorly than the middle rows in *A. solenopsi*, whereas in *A. trichtopson* the middle rows extend further anterior than the latero-medial rows; (iv) the tip of the spicule is rounded in *A. trichotopson* but obliquely truncate in *A. solenopsi*; and (v) the male terminus is swollen on the ventral side in *A. solenopsi* but symmetrical in *A. trichotopson*. The similarity between *A. trichotopson* and *A. solenopsi* suggests that the host for the former species may also be an ant, possibly *Solenopsis germinata* (Fab.), which occurs naturally in Jamaica, the type-locality of *A. trichotopson* (see Trager, 1991).

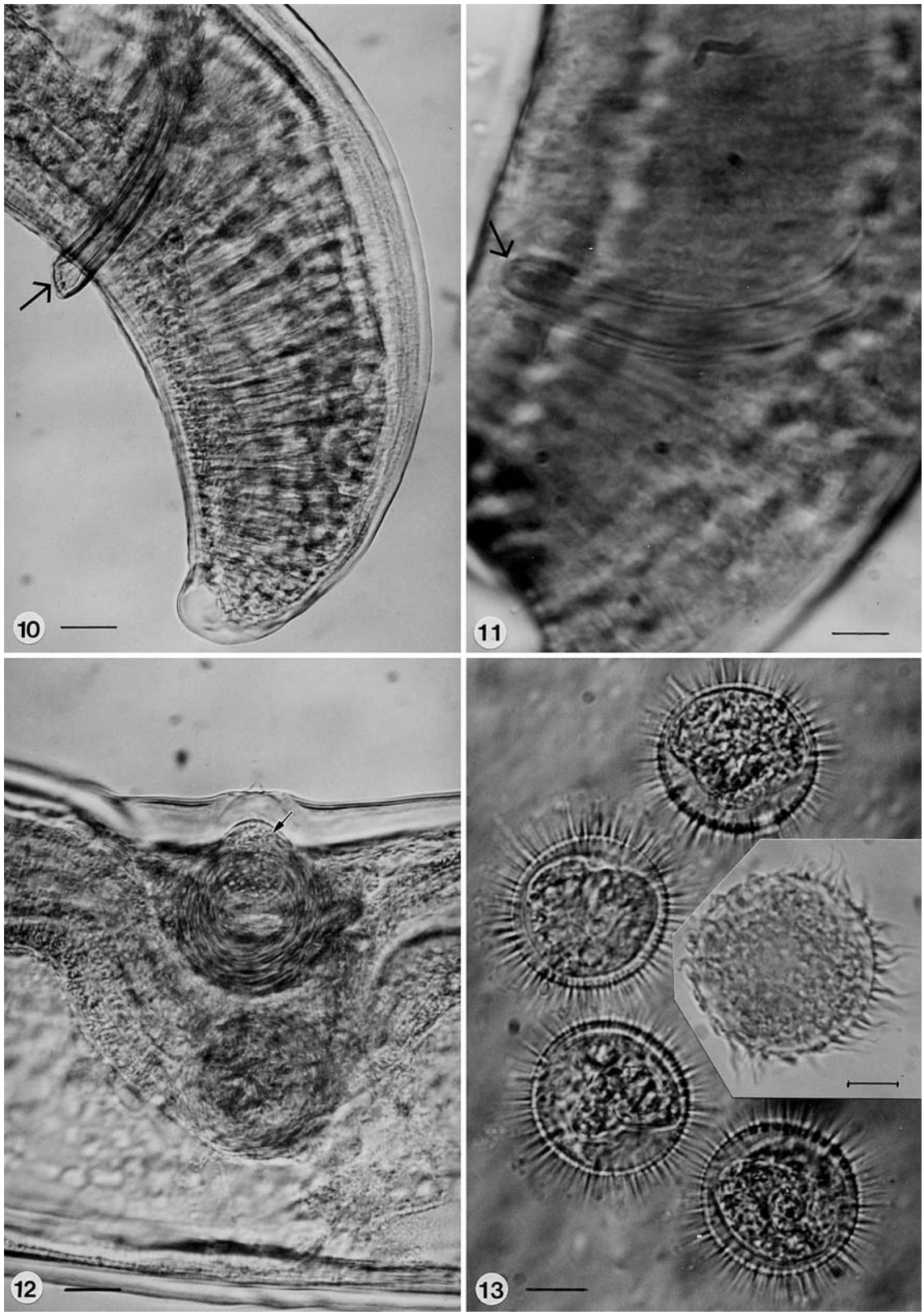
None of the mermithid parasites of *Solenopsis* spp. listed in Table 1 has been identified, so it is not possible to determine whether some of these belong to *Allomermis*. The extremely long size of the parasites reported by McInnes & Tschinkel (1996) from *Solenopsis* spp. in Florida (length emerging from gynes averaged 114 mm and from males 79 mm) clearly separate that taxon from *A. solenopsi*.

Molecular analysis

The molecular phylogenetic analysis presented here is guided by a morphology-based phylogeny that divides the nematode family Mermithidae into seven subfamilies (Gafurov, 1996). Nucleotide sequences of PCR-amplified 18S rDNA were obtained from six mermithid genera representing five of the seven subfamilies (Table 2); taxa representing the subfamilies Pheromermithinae Artyukhovsky, 1990 and Neomermithinae Artyukhovsky, 1990 were not available for molecular analysis. In addition, 18S rDNA sequence data already available in GenBank was obtained from material of two representative genera of the family Mononchidae Chitwood, 1937 *Clarkus* sp. and *Mylochulus* sp. In the popular 18S rDNA phylogeny that provided a molecular framework for the entire phylum Nematoda (see Blaxter et al., 1998), the Monochidae was the closest sister taxon to the Mermithidae. As such, these species provided useful outgroups with which to root the mermithid tree.

Clustal X produced an alignment of 772 nucleotides. A molecular phylogenetic analysis using Maximum Likelihood consistently yielded a single tree (Fig. 20) after a series of full heuristic searches. Based on this limited sampling, the family Mermithidae was resolved as monophyletic, consistent with the conception of Gafurov (1996) and with early immunological data which also supports a monophyletic taxon (Kaiser, 1983).

The 18S data unambiguously places the tested fire ant mermithid as a sister taxon to *Mermis* Dujardin, 1842, a representative of the subfamily Mermithinae. The fire ant mermithid nematode and *M. nigrescens* Dujardin, 1842 have a GTR genetic distance of 3.25%. A separate GTR bootstrap analysis of the tree


10
11
12
13

(100 simulations) shows that the test fire ant memithid and *M. nigrescens* cluster together, to the exclusion of other taxa, 100% of the time. The present data could not enable a more specific placement within the Mermithinae or alternatively, within a sister taxon, the subfamily Pheromermithinae (see Gafurov, 1996). However, based on this analysis, nematode genera found in the subfamilies Agamermithinae Artyukhovsky, 1990, Heleidomermithinae Gafurov, 1996, Gastromermithinae Gafurov, 1996 and Mesomermithinae Gafurov, 1996 could likely be eliminated from further consideration.

Biological observations

Fire ant workers parasitised with *A. solenopsis* n. sp. were easily distinguished by their unusually large gasters (Fig. 14). About 0.5% of workers were parasitised (n = 170) in a fire ant colony collected near the biological station. About 2.5% of workers were parasitised (> 800) in a colony collected around ponds adjacent to the aquaculture centre. The sex ratio of nematodes was about equal in the first colony (35 males to 37 females), but somewhat male biased in the second colony (147 males to 89 females). A few parasitised workers were also found in four additional colonies collected around the biological station and three additional colonies collected at the aquaculture centre.

Parasitised workers were removed from their colonies and placed in separate rearing boxes with sugar water, nest tubes and plaster moisture blocks like those used to hold fire ants when rearing phorid decapitating flies (Porter, 2000). Several of the workers were parasitised by mermithids in their gasters and phorid decapitating flies in their heads.

Parasitised workers contained an average of 1.4 nematodes. In a sample of 102 parasitised workers, 69% harboured single worm infections, 27% contained 2 worms and 4% contained three or more worms. About half of the nematodes came from hosts with multiple infections and up to nine have been found in a single ant. Both male and female nematodes often occurred in a single host. All parasitised workers died within two months of being field collected.

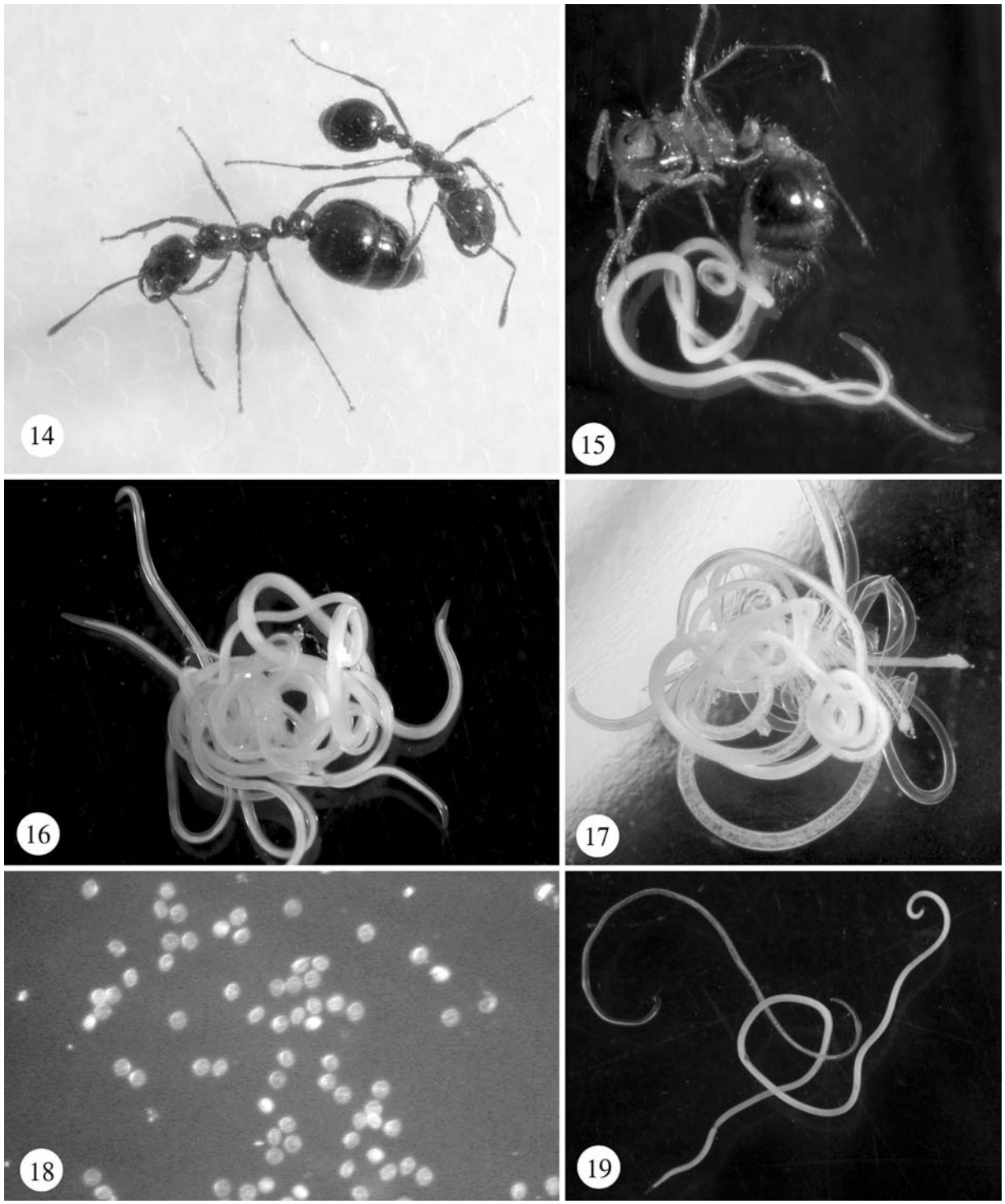
Post-parasitic juvenile mermithids rarely emerged from workers that died in plastic holding boxes, even when the humidity was maintained at 80% or higher. Approximately 200 dead parasitised workers were placed on moist plaster under conditions approaching 100% humidity (an environment previously used to rear phorid decapitating flies - see Porter, 2000), but nematodes emerged from only about 5% of the workers and most died within a few millimetres of their host. The remaining 95% died within their hosts. In contrast, when dead parasitised ants were placed in water, the nematodes readily emerged, often within minutes. Crawley & Baylis (1921) reported that *Mermis* parasitizing *Lasius* ants in England also quickly emerged when dead parasitised ants were placed on a film of water.

Most nematodes emerged from their ant hosts within 6–12 hours of being placed in water but some took 24 hours or longer to emerge. Upon emerging, the nematodes appeared to imbibe a considerable amount of water, because the volume of emerged nematodes usually appeared much greater than the original volume of the host gaster from which they emerged. Most nematodes emerged via the anus of their host (Fig. 15); however, some also escaped through ruptures in the intersegmental membranes of the host gaster.

When post-parasitic juveniles that emerged from their ant host were placed on wet sand or moist vermiculite, little locomotion was observed and most died after several weeks. In contrast, nematodes left in water quickly formed mating clusters (Fig. 16), moulting in 4–5 days and ovipositing in 6–12 days. Eggs were deposited in a single layer on the bottom of the plastic holding cup and adhered to the bottom, possibly as result of an adhesive associated with their unique surface ornamentation (Fig. 18). A single female was capable of laying about 3,000–8,000 eggs over a period of several days to a week. There was no evidence of autotoky, since unmated females held alone underwent a moult but did not produce eggs.

During and after oviposition, the nematodes remained in mating clusters intertwined with cast cuticles and eventually with moribund members of

◀ **Figs. 10–13** *Allomermis* species. 10. Male tail of *A. solenopsi* n. sp. Arrow shows obliquely truncate spicule tip. 11. Male tail of *A. trichotopson* (from type-slide T-4688p, Nematology Laboratory, Beltsville, Md). Note uniformly rounded spicule tip (arrow). 12. Vagina of *A. solenopsi*. Arrow shows an egg passing out through the vulva. 13. Eggs of *A. solenopsi*. Insert shows an egg of *A. trichotopson* (from type-slide T- 4683p, Nematology Laboratory, Beltsville, Md). *Scale-bars*: 10, 22 µm; 11, 12 µml 12, 20 µm; 13, 13 µm (insert 11 µm)



Figs. 14–19 *Allomermis* life stages. 14. Parasitised *Solenopsis invicta* fire ant worker (left) with a greatly enlarged gaster or abdomen compared to a similar sized unparasitised worker (right). 15. Two nematodes partly emerged from fire ant worker. 16. Pre-ovipositional mating cluster of *A. solenopsi* n. sp. 17. Post-ovipositional mating cluster of *A. solenopsi;* note empty moulted skins on right and a loop of an egg-depleted female on bottom. 18. Eggs of *A. solenopsi* attached to bottom of holding cup. 19. Male (left) and female (right) *A. solenopsi*

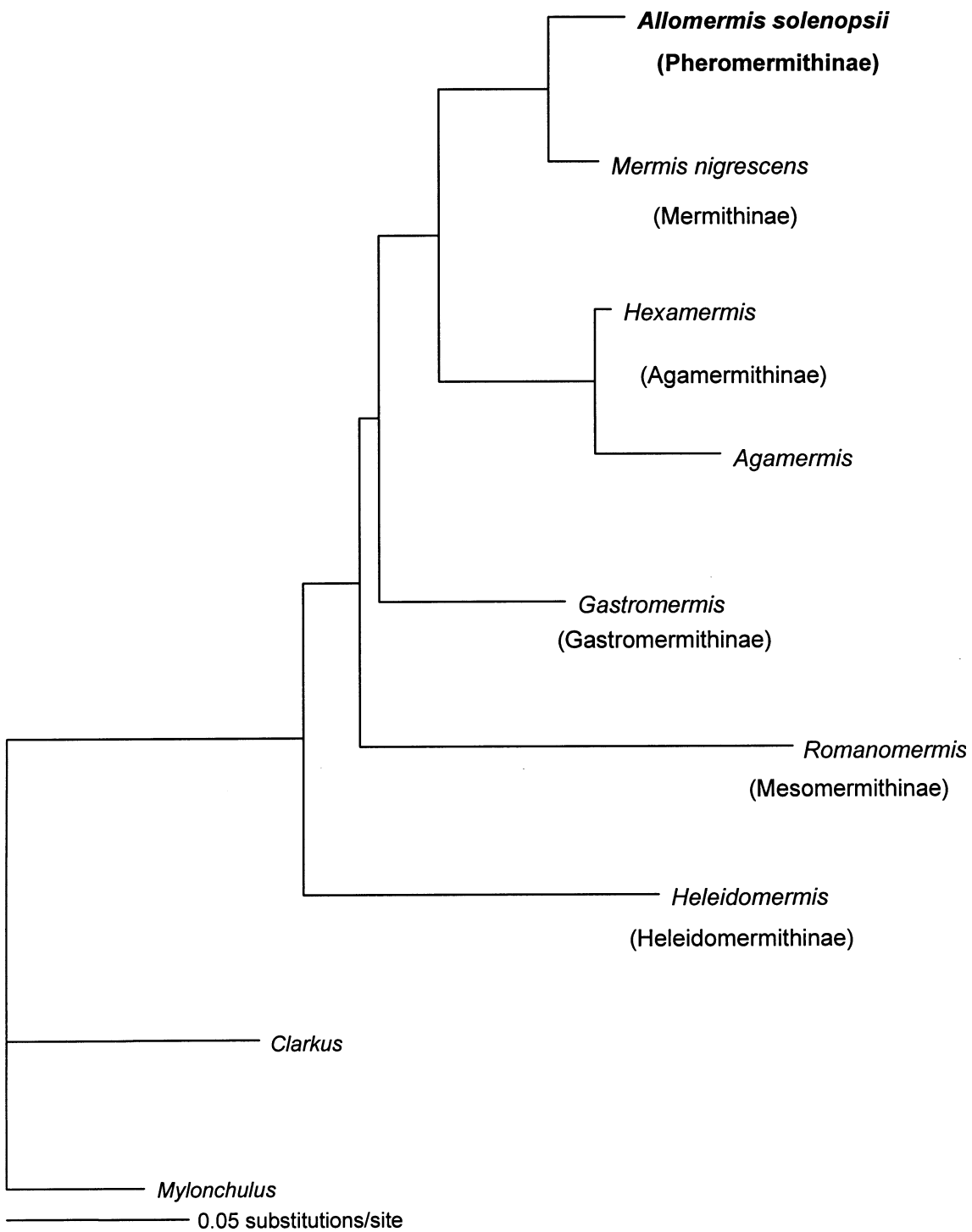


Fig. 20 Maximum likelood tree obtained from a heuristic search of SSU rDNA (18S) sequence data. The model was a General Time Reversible Model (GTR) with an estimated shape parameter and proportion of invariable sites and six substitution rate parameters whose values were also estimated. Branch lengths are scaled to the expected number of substitutions per site. A series of full heuristic searches consistently yielded a single tree with the ML score of (-Ln likelihood = 2733.24c756)

their cohort (Fig. 17). At room temperature, unmated nematodes survived in water cups for two or three months; however, those in mating clusters tended to lose vigour and die several weeks to a month after oviposition. The conditions for egg-hatching are unknown.

Discussion

Biological observations

Based on these findings, it is apparent that *Allomermis solenopsi* n. sp. requires standing water to emerge from its host, mate and oviposit. This observation raises two intriguing questions about its life-history. The first is, how do nematodes reach water from their ant hosts? The second is, how do the nematodes infect ants? At present, both of these questions remain unanswered. Mermithids in the genus *Pheromermis*, as well as some species of *Mermis*, apparently cause their ant hosts to seek open water when they are ready to emerge (Kaiser, 1986a, 1986b; Maeyama et al., 1994), and it is possible that *A. solenopsi* may induce similar behaviour in its host. An alternate possibility is that dead ants are washed into water by heavy rains. However, the latter appears less likely, because unemerged nematodes live only a few days once their host dies and even less if exposed to dryness and high daytime temperatures. Based on the very limited motility of newly emerged nematodes on moist sand and plaster, it does not seem that they could migrate appreciable distances in moist soil. Another possibility is that *Solenopsis invicta* colonies have tunnels leading down below the water table. If this is the case, then perhaps mating and oviposition occur within the ant nest.

With regard to how newly hatched nematodes in water infect ants, if oviposition occurs within the nest, then newly hatched preparasitic juveniles would have easy access to their hosts. However, the ornamentation on the surface of the eggs, apparently resulting from a glandular deposit originating in the distal portion of the oviduct, is an unusual feature of *Allomermis* eggs and could play an important role in the infection process. This may serve to attach the eggs to worker ants, which could carry them back to the nest and possibly contaminate food given to the developing larvae. This would be the scenario in a direct life-cycle. However, if the life-cycle is indirect, involving a paratenic host, then the eggs may adhere to the paratenic host, which is carried back to the nest as food for the ant larvae. If *A. solenopsi* requires a paratenic host, then fire ant workers are probably infected as larvae since the latter receive all solid food collected by colony foragers. Adult fire ant workers only consume liquids and have a very effective filter system that usually removes particles even as small as bacteria. An indirect type of life-cycle occurs in the European mermithid ant parasite *Pheromermis villosa* Kaiser, 1986 (see Kaiser, 1986a, 1986b).

Paratenic hosts are usually small aquatic organisms that ingest mermithid eggs while feeding on the bottom debris. After hatching in the gut of the paratenic host, the preparasitic mermithids penetrate the gut wall, enter the body cavity and initiate a quiescent stage. Infection of the primary host occurs when the mermithid juveniles are ingested along with tissues of the paratenic host. The mermithids would then enter the body cavity of the primary host and initiate development. An indirect life cycle of this type involving oligocete worms as the paratenic host and *Lasius* ants as the primary host occurs in the European mermithid *Pheromermis villosa* (see Kaiser, 1986a, 1986b).

A. solenopsi may be a useful self-sustaining biocontrol agent for imported fire ant colonies located near ponds or other sources of open water in the United States. However, before determining its potential as a classical biocontrol agent, more information regarding the life-cycle, distribution, seasonality and rates of parasitism are required. It also must be determined whether *A. solenopsi* is host-specific to fire ants of the genus *Solenopsis* and how the parasite is dispersed among fire ant colonies. If winged adult fire ants are parasitised, it could ensure rapid rates of natural dispersal.

Studies on other *Solenopsis*-mermithid infections (not involving *Allomermis* species) showed that the nematodes caused a reduction of the reproductive structures of winged queens and males, and the ants died shortly after the nematodes emerged (McInnes & Tschinkel, 1996). Once the life-cycle of *A. solenopsi* is elucidated, it could possibly be used in an integrated control programme against imported fire ant populations in North America. Experimental trials would probably depend on the development of a mass

rearing programme, similar to that developed for the mosquito mermithid *Romanomermis culicivorax* Ross & Smith, 1976 (see Petersen & Willis, 1972).

Molecular phylogeny

The Mermithidae was initially considered to be polyphyletic, with two subfamilies based on the infection process (Artyukovsky, 1971). Parasitism by the Mermithinae, represented by the single genus, *Mermis*, occurred passively by oral ingestion of unhatched eggs. All other mermithid genera were placed in the Paramermithinae Artyukhovsky, 1990 based on observed and presumed direct penetration of their hosts by infective juveniles. Monophyly has been proposed in later revisions of the family (Kaiser, 1983; Gafurov, 1996) and the 18S rDNA molecular tree (Fig. 20) supports this hypothesis.

At the terminal branches of the molecular phylogeny, affinities identified by Gafurov (1996) can be observed. These include assigning *Hexamermis* Steiner, 1924 and *Agamermis* Cobb, Steiner & Christie, 1923 as sister genera within the Agamermithinae Artyukhovsky, 1990 a sensible juxtaposition in that both taxa share the unusual behavioural trait of transverse phototaxis (Robinson et al., 1990). Importantly, Gafurov (1996) positioned the Pheromermithinae and Mermithinae as sharing a most recent common ancestor. Associating these two subfamilies is supported by the observation that both genera infect their hosts by passive oral ingestion of unhatched nematode eggs (*Pheromermis* and *Mermis*, respectively), a life-history trait that occurs infrequently. While a representative of the Pheromermithinae was not available for molecular analysis, *Allomermis* clustered with *Mermis* 100% of the time, again showing the close relationship of these two subfamilies.

The topologies of the Gafurov (1996) and the 18S rDNA trees are not congruent at more primitive nodes. Gafurov depicted the Pheromermithinae and Mermithinae as sharing a common ancestor near the base of the mermithid tree, whereas these same subfamilies are positioned as the most derived sister groups in the present SSU 18S tree. Moreover, Gafurov (1996) considered the Gastromermithinae Gafurov, 1996 and Heleidomermithinae Gafurov, 1996 as sister subfamilies with recent divergence; however, the SSU 18S rDNA data reveal a very different topology with the Heleidomermithinae occupying the most basal position in the molecular tree.

The validity of these competing schemes is difficult to evaluate because character-state assignments were not included in Gafurov's treatment. However, the lack of congruence between molecular trees and those generated by more traditional treatments is not without precedence. One recent example can be found in a description of the nematode Order Cephalobina, where morphological classifications and molecular phylogenies have resulted in competing hypotheses (Nadler et al., 2006). Resolving the different hypotheses for relationships within the Mermithidae could be achieved by including a larger number of taxa within the molecular tree.

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