

***Myrmeconema neotropicum* n. g., n. sp., a new tetradonematid nematode parasitising South American populations of *Cephalotes atratus* (Hymenoptera: Formicidae), with the discovery of an apparent parasite-induced host morph**

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Abstract A new genus and species of tetradonematid nematode, *Myrmeconema neotropicum* n. g., n. sp., is described from larval, pupal and adult stages of *Cephalotes atratus* L. (Hymenoptera: Formicidae) in Peru and Panama. Diagnostic characters of the new genus include: males and females subequal in size; cuticle with minute annulations; six cephalic papillae; stylet present in all stages; stichocytes absent; trophosome degenerate; three penetration glands; gonads paired and opposite; vulva in mid-body region; single spicule; genital papillae absent; adult tails rounded; infective juveniles moult once in egg; and adults of both sexes remain in the host throughout their development. As the female nematodes mature inside the worker ants, the host gasters change colour from black to red.

Introduction

A wide range of nematodes parasitise ants, including members of the Mermithidae, Tetradonematidae, Allantonematidae, Rhabditidae, Seuratidae and Diplogasteridae (see Poinar, 2003). The present investigation, together with previous reports, suggests that representatives of the Mermithoidea parasitise a wide range of Neotropical ants (Nickle & Jouvenaz, 1987; Poinar et al., 2006, 2007). While most of the published reports refer to members of the family Mermithidae, the present paper describes a new genus of tetradonematids parasitising the larvae, pupae and adults of a Neotropical ant, *Cephalotes atratus* (L.), in Peru and Panama. A gross diagnostic feature of late infections is the change of colour of the ant gasters from black to red.

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Materials and methods

Colonies of *Cephalotes atratus* were studied on Barro Colorado Island (BCI), Panama (9.16°N, 79.85°W) in May–June and November of both 2005 and 2006. In addition, *C. atratus* colonies were examined on an occasional basis from late 2005 through to early 2007 at the following four lowland locations in the Peruvian Amazon: (1) in the city of Iquitos and surrounding rural communities (3.75°S, 73.25°W); (2) within 2 km on either side of the paved road connecting the cities of Iquitos and Nauta (4.33°S,

73.53°W); (3) in the general vicinity of the Amazon Conservatory for Tropical Studies (ACTS) and Explornapo field stations, c.65 km NE of Iquitos (3.25°S, 72.91°W); and (4) at the Amazon Conservation Association's Los Amigos (CICRA) field station 100 km W of Puerto Maldonado (12.57°S, 70.10°W).

Infection rates were estimated in parasitised colonies by counting the number of red-gastered (an indication of infection) and black-gastered workers returning to the nest via well-established trails, which were generally restricted to woody vines and thus easily monitored. In addition, bait (turkey or tuna mixed with honey) were placed at several points near active trails and the number of red and black-gastered workers noted. The nematodes were removed from parasitised hosts after opening the body-cavity of the ant in a 1% saline solution.

For descriptive purposes, the nematodes were fixed in 70% or 95% ethanol and processed to glycerine. Observations and photographs were made with a Nikon Optiphot Microscope.

Results

The description is based on nematodes removed from larval, pupal and adult ants from the locality in Peru. A comparison of the two populations did not show any morphological differences and the Panamanian populations are considered to be conspecific with those from Peru.

Mermithida Hyman, 1951
Mermithoidea Braun, 1883
Tetradonematidae Cobb, 1919

Myrmeconema n. g.

Diagnosis

Adult males and females subequal in size; six cephalic papillae; cuticle with minute annulations; adult males and females occur only in host; stylet present in infective stages of male and female; stichocytes absent; intestine degenerate; three penetration glands in infective juveniles carried over into adults; gonads paired, opposite, reflexed or outstretched; vulva in mid-body area; single spicule;

bursa and genital papillae absent; adult tails rounded; eggs remain in mature female; infective juveniles moult in egg. Type species *M. neotropicum* n. sp.

Myrmeconema neotropicum n. sp.

Type-host: *Cephalotes atratus* (L.) (Hymenoptera: Formicidae).

Type-locality: Peru: Madre de Dios, CICRA field station, 100 km W of Puerto Maldonado, 12.57°S, 70.10°W, 250 m elev.

Other locality: Panama: Canal Zone, Barro Colorado Island, 9.16°N, 79.85°W.

Type-material: Holotype male (registration number T-561t) deposited in the USDA Nematode Collection, Beltsville, Maryland. Paratypes deposited in both the authors collections and at the Museo de Historia Natural Javier Prado, Lima, Peru and the Fairchild Museum, University of Panama.

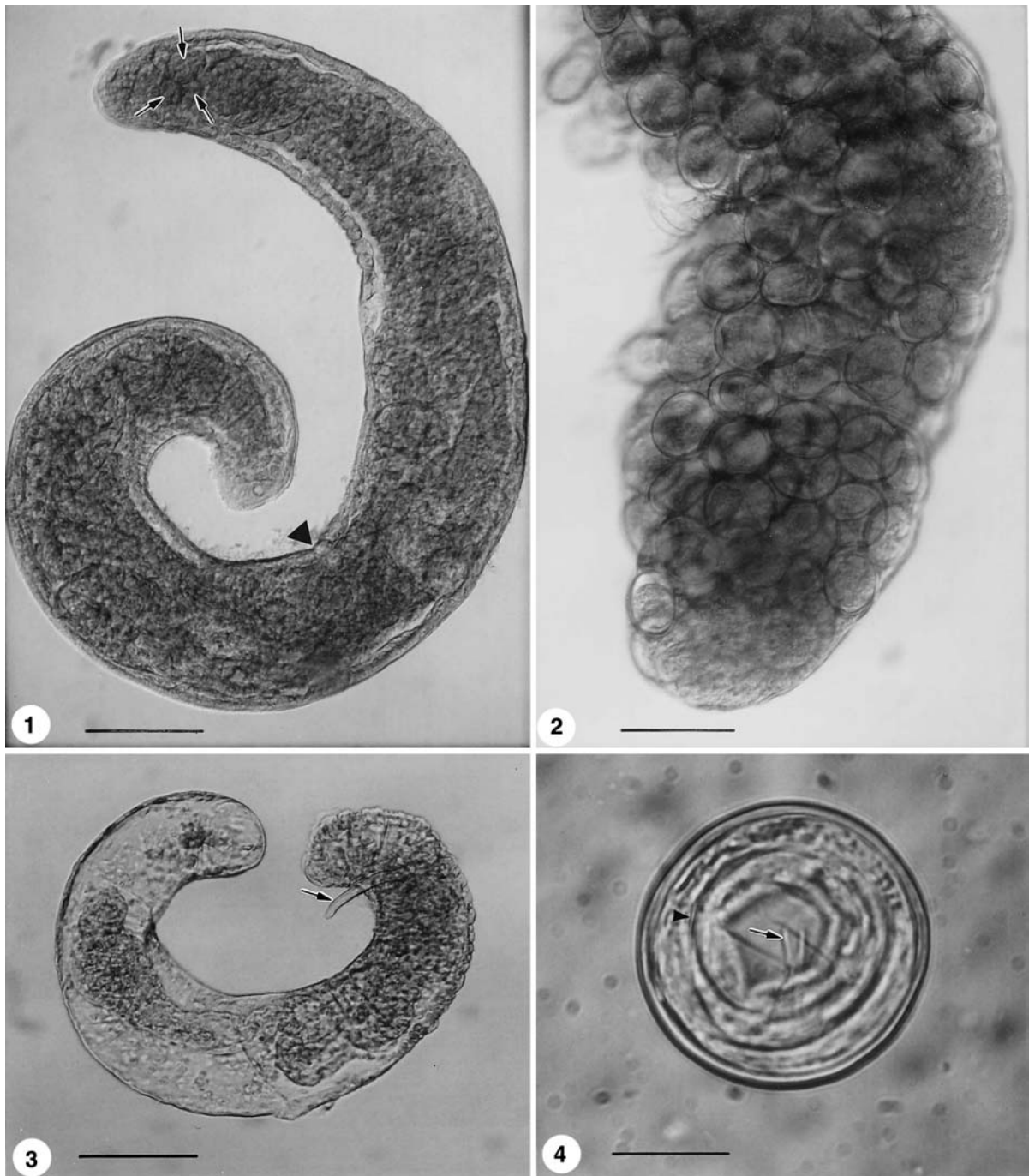
Description (Figs. 1–7)

With characters of genus.

Female (n = 15). Stout, mostly curved ventrally at death; stylet present, often located sub-terminally; nerve-ring area with 8–10 spherical ganglionic cells bordering fibrous portion; pharynx greatly reduced; pharyngeal tube ends shortly after nerve-ring area; three penetration gland-cells located posterior to nerve-ring area; intestine greatly reduced, represented by some loosely connected cells continuing through body to faint anal opening; hypodermal cells well developed; vulva centrally located; vagina very short, barrel-shaped; ovaries opposed, filling body cavity with at least 1 and often 2 loops; tips usually reflexed but sometimes outstretched; tail bluntly rounded.

Male (n = 15). Stout, tightly coiled at death; stylet present, often located sub-terminally; nerve-ring area reduced; pharynx and intestine degenerate, rarely detected past mid-body; gonads paired, opposite, outstretched or occasionally reflexed at tip; spicule single, large, from nearly straight to noticeably curved; genital papillae absent; tail tip rounded, tightly coiled around female mid-body during mating.

Infective stage juveniles (n = 15). Development completed inside egg in uterus of mother; body



Figs. 1–4 *Myrmeconema neotropicum* n. sp. 1. Lateral view of mated developing female removed from a pupa of *Cephaotes atratus*. Arrows show reduced penetration glands. Arrowhead shows location of vulva. 2. Eggs filling body of a female removed from a black-gastered worker *C. atratus*. 3. Dying, spent male removed from a black-gastered worker *C. atratus*. Arrow shows spicule. 4. Fully-developed egg removed from a red-gastered worker *C. atratus*. Arrow shows cast skin of first moult. Arrowhead shows stylet. Scale-bars: 1, 80 μm ; 2, 93 μm ; 3, 90 μm ; 4, 70 μm

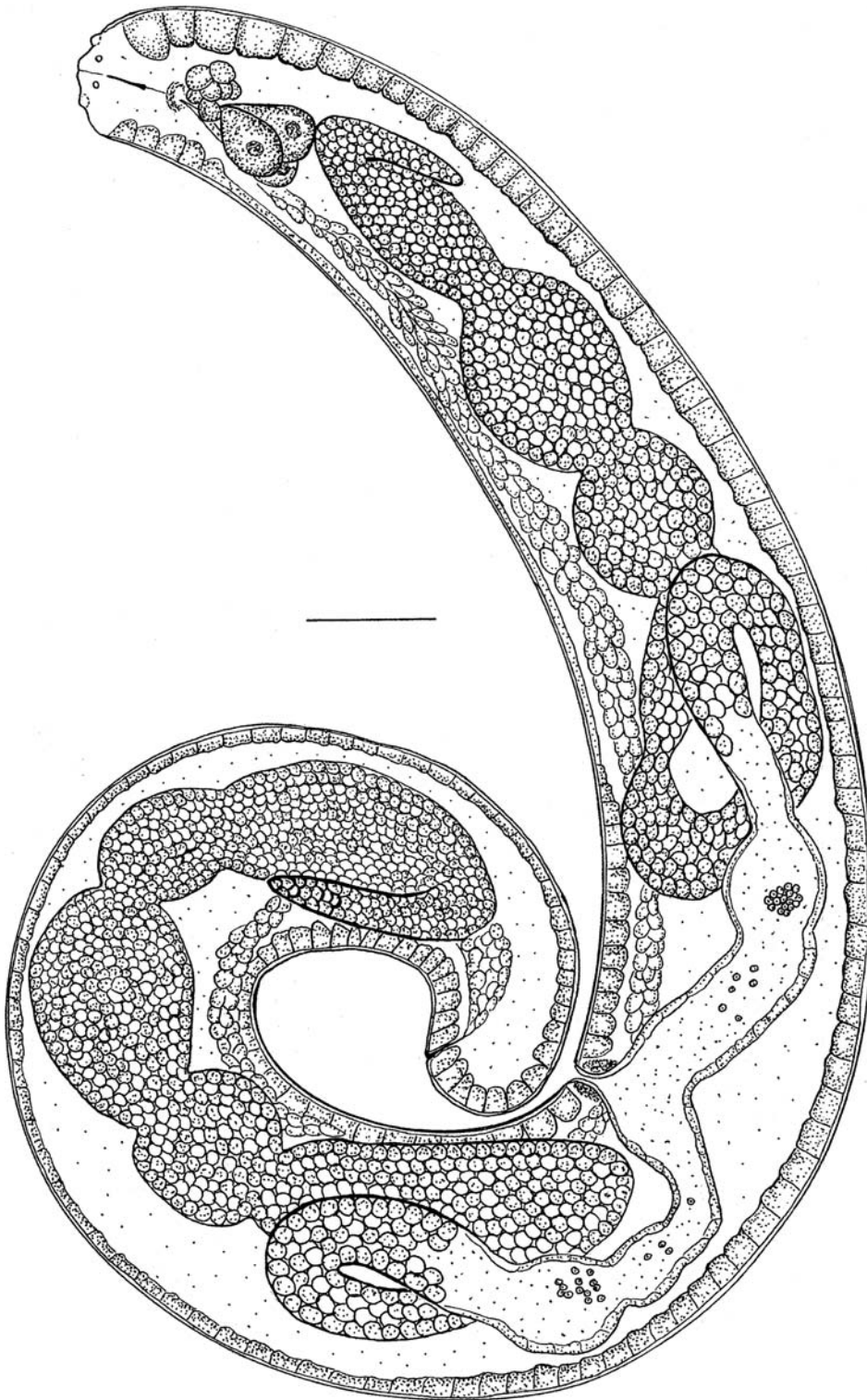


Fig. 5 Fertilised female *Myrmeconema neotropicum* n. sp. removed from a pupa of *Cephalotes atratus*. Scale-bar: 80 μ m

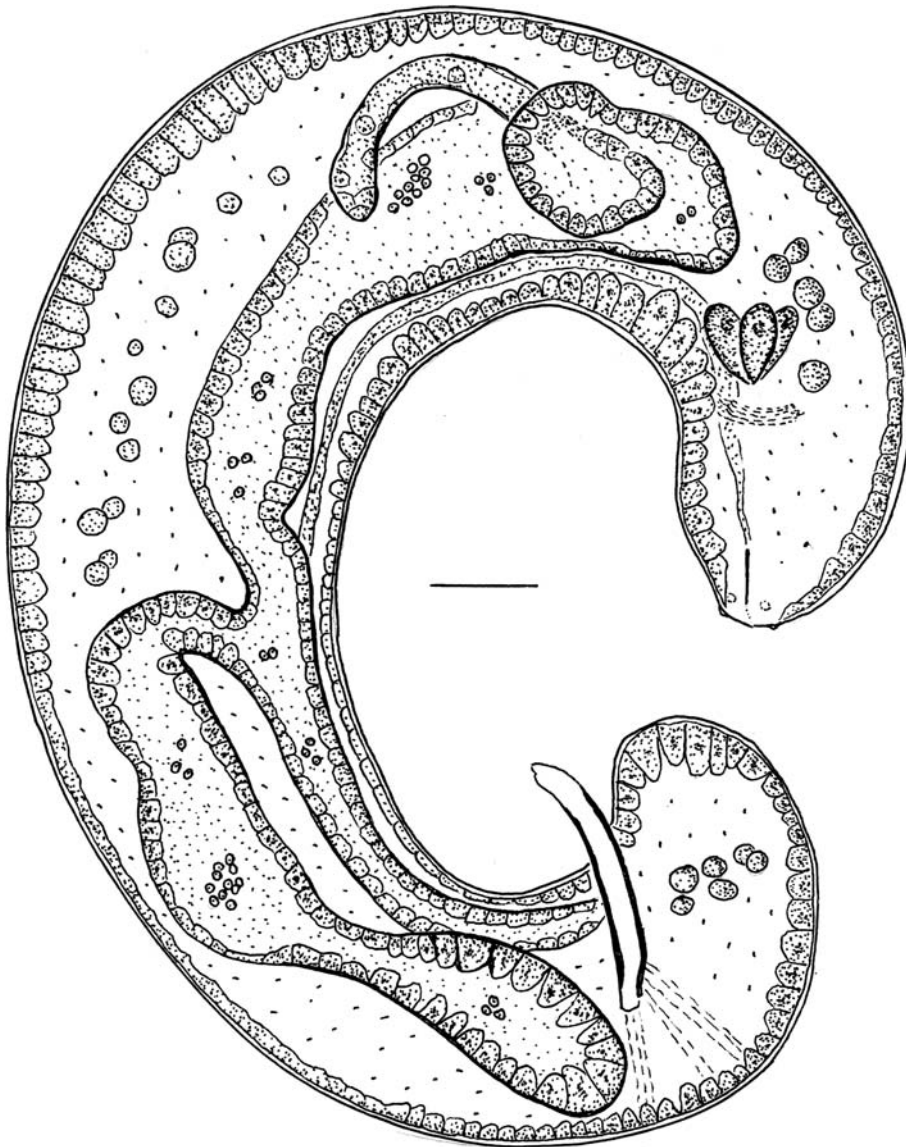


Fig. 6 Male *Myrmeconema neotropicum* n. sp. (after mating) removed from a pupa of *Cephalotes atratus*. Scale-bar: 30 μ m

narrow, elongate; stylet present; with 3 penetration glands; pharynx and intestine connected; stichocytes not observed; intestinal lumen open, terminating in anus; gonad anlagen near base of pharynx; first moult occurs in egg.

Measurements

Female (n = 15). Length 1.00 (0.8–1.2) mm; greatest width 112 (95–133) μ m; stylet length 13 (11–16) μ m;

distance head to nerve-ring 40 (22–63) μ m; length of largest penetration gland 24 (16–32) μ m; V = 56 (50–68)%; tail length 31 (16–40) μ m; egg diameter 57 (53–60) μ m.

Male (n = 15). Length 680 (590–780) μ m; greatest width 97 (63–111) μ m; stylet length 13 (11–16) μ m; distance head to nerve-ring 38 (32–48) μ m; length of largest penetration gland 16 (11–21) μ m; tail length

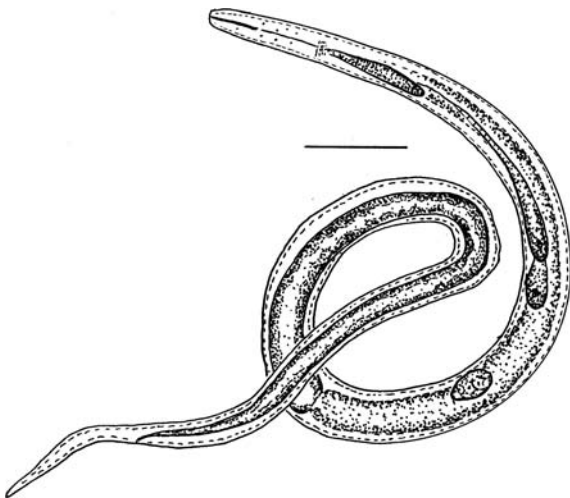


Fig. 7 Second stage infective juvenile of *Myrmeconema neotropicum* n. sp. removed from egg. Scale-bar: 30 μ m

46 (40–56) μ m; spicule length 69 (59–80) μ m; spicule greatest width 9 (7–13) μ m; diameter of spherical sperm 2.5 (2–3) μ m.

Infective stage juveniles (n = 15) (removed from mature eggs which contained cast skins of first moult). Length 446 (351–588) μ m; greatest width 14 (12–18) μ m; stylet length 14 (12–17) μ m; distance head to nerve-ring, 41 (34–47) μ m; distance head to tip of penetration glands 102 (90–115) μ m; length of gonad anlagen 21 (17–28) μ m; tail length 41 (38–44) μ m.

Remarks

Myrmeconema n. g. is distinguished from the following genera of tetradonematids: from *Tetradonema* Cobb, 1919, *Aproctonema* Keilin, 1917, *Heterogonema* van Waerebeke & Remillet, 1973, *Didilia* Tang et al., 1993 and *Bispiculum* Zervos, 1980 by the absence of stichocytes and the degenerate intestine; from *Bispiculum*, *Corethrellonema* Nickle, 1969 and *Mermithonema* Goodey, 1941 by the absence of genital papillae; and from *Aproctonema* by the absence of an intestine occupying nearly the entire breadth of the body-cavity. The intestine of *Aproctonema* also contains a diverticulum, which is absent in *Myrmeconema*. While the description of *Tetradonema solenopsis* Nickle & Jouvenaz, 1987 from *Solenopsis* ants in Brazil is incomplete, the nematode

has some characters of *Myrmeconema* (single spicule, absence of genital papillae, three penetration glands, reduced pharynx) and may belong in this genus. However, *T. solenopsis* differs from *M. neotropicum* n. sp. in having smaller males (292–440 μ m in length vs 590–780 μ m), larger females (1.2–1.6 mm in length vs 0.9–1.2 mm), smaller eggs (27–28 μ m in diameter vs 53–60 μ m), smaller spicules (length 25–48 μ m vs 59–80 μ m) and smaller infective stage juveniles (203–229 μ m vs 351–588 μ m).

Biological observations

In Panama, two of five different colonies of *Cephalotes atratus* monitored at BCI were found to be parasitised. An average of 3.9% and 2.3% of the workers observed outside the respective nests were infected. The colonies were not monitored all year-round, but the presence of infected workers in both May and November suggest that infections persist throughout the year. In Peru, infected (red-gastered) *C. atratus* were only found in one of c.80 colonies examined, which was located at the CICRA field station. Dissections of that colony's brood revealed that 6.4% of the pupae (n = 110) contained nematodes.

Developing juvenile nematodes occurred mostly in ant pupae, while callow adults contained mostly mating nematodes, black adult ants contained fertilized female nematodes with developing ovaries and males, and ants with red gasters contained mature female nematodes filled with mature eggs.

Parasitised ants are 10% smaller (average head width of infected ants = 3.00 ± 0.052 mm) than non-parasitised ants (3.35 ± 0.039 mm; $t = 5.29$, $df = 133$, $P < 0.0001$), but are on average 40% heavier (range: 18–84%) than similar-sized uninfected ants. They are also sluggish, clumsy and generally less aggressive than non-parasitised workers. They do not bite or grab skin when handled, and their alarm/defence pheromone production apparently is significantly reduced or absent. However, the parasites do not seem to influence the gliding behaviour of *C. atratus* (see Yanoviak et al., 2005). Despite the dramatic increase in gaster mass, infected workers were still able to direct their aerial descent back to the tree trunk when dropped from branches.

Discussion

This study solves the mystery of the taxon *Cephalotes atratus* var. *rufiventris* of Emery (1894), a variety erected solely on the basis of its red abdomen (see de Andrade & Baroni Urbani, 1999). When the females of *M. neotropicum* n. sp. have completed their development within the host, the abdomen (gaster) of the infected worker ants turns from black to red, a unique marker which makes them easy to detect. This colour morph occurs in Colombia and Brazil as well as in the closely related species *C. marginatus* (Fab.) (de Andrade & Baroni Urbani, 1999). It is highly likely that all of these colour changes were due to infections by *Myrmeconema* n. g. and, based on their geographical locations, it would appear that the parasite is widely distributed throughout populations of *Cephalotes atratus* in the American tropics.

The Tetradonematidae comprises a small group of mermithoid nematodes which have been little studied with regard their anatomical features or life-histories. An examination of the infective stage of *M. neotropicum* revealed a number of characters shared by both the Tetradonematidae and the Mermithidae. This resulted in a re-evaluation of several features previously considered unique to the Tetradonematidae. These characters are discussed below and their presence in members of both families is indicated.

The infective stages of *M. neotropicum* possess a well-developed stylet, penetration glands for entering the host, a complete digestive tract with the pharynx connected to the intestine, a gonad anlagen and an anus. These characters are very similar to those found in the infective stages of mermithid nematodes (Poinar & Hess, 1974). The moult of *Myrmeconema* within the egg is also typical of mermithids and may occur in at least one other tetradonematid (van Waerebeke & Remillet, 1973).

Penetration glands are a basic feature of mermithid infective juveniles and are often carried into the early parasitic stages (Poinar, 1975). Cells referred to as companion cells in some tetradonematids are considered homologous to the penetration glands of mermithids. Whether they serve a function during the early stages of parasitic development is unknown. One dorsal and two subventral penetration glands is the basic number for nematodes (Bird, 1971), and the infective stages of *Agamermis decaudata* Cobb,

Steiner & Christie, 1923 contains this number (Christie, 1936). There is a tendency for the two subventral glands to fuse or for one to degenerate (Bird, 1971) thus resulting in only two functional penetration glands, as occurs in the infective stages of the mermithids *Octomyomermis troglodytes* Poinar & Sanders, 1974, *Isomermis lairdii* Mondet, Poinar & Bernadou, 1977, *Filipjevimermis leipsandra* Poinar & Welch, 1968 and *Heleidomermis magnapapula* Poinar & Mullens, 1987. In tetradonematids, two (in *Tetradonema*) or three (in *Corethrellonema*, *Aproctonema* and *Myrmeconema*) penetration glands may be present (see *inter alia* Poinar & Hess, 1974; Poinar & Sanders, 1974; Mondet, Poinar & Bernadou, 1977; Poinar & Mullens, 1987).

A pharynx with cells containing prominent nuclei (stichocytes) is a common feature of mermithids. This character is lacking in *Myrmeconema*, but may be absent or difficult to discern in some mermithids as well, as with *Pheromermis pachysoma* Poinar, Lane & Thomas, 1976 (see Poinar, Lane & Thomas, 1976). When present, mermithids normally have 16 stichocytes, but some species, such as *Mermis nigrescens* Dujardin, 1842 and *H. magnapapula* only contain eight (Christie, 1937; Poinar & Hess, 1974; Poinar & Mullens, 1987). In tetradonematids, there is a tendency for the reduction or complete elimination of the stichocytes. Eight have been reported in *Mermithonema*, four (the tetrad) in *Tetradonema*, *Heterogonema*, *Bispiculum* and *Didilia*, but they appear to be absent or greatly reduced in *Corethrellonema*, *Aproctonema* and *Myrmeconema*.

All mermithids have a prominent intestine, which becomes separated from the remaining alimentary tract and functions as a food storage organ (the trophosome). This food storage organ provides nutrients to the non-feeding postparasites and adults during their free-living existence. Those tetradonematids that remain inside the host have a reduced or degenerate intestine, as in *Myrmeconema*, since, by obtaining nourishment directly from the host, there is no need for a food storage organ. However, those species whose females leave the host to oviposit (*Aproctonema*, *Corethrellonema* and some strains of *Tetradonema*) have a distinct intestine that may serve as a trophosome. It is quite likely that tetradonematids absorb nutrients through their cuticle, similar to the pattern of digestion in mermithids (Poinar & Hess, 1977; Rutherford & Webster, 1974).

Paired gonads are a feature of all mermithids and tetradonematids, except *Didilia*, where the gonads are single in both sexes. Mermithids have one or two spicules or partly fused spicules, while all tetradonematids have a single spicule except *Bispiculum*, which has paired spicules. All mermithids have genital papillae; however, only the tetradonematids *Mermithonema*, *Bispiculum* and *Corethrellonema* possess genital papillae. A gubernaculum and bursa are lacking in both mermithids and tetradonematids.

While an anus is sometimes evident in the infective stages of mermithids and tetradonematids, it is often degenerate in the adults. *Myrmeconema* is rather unique in having a faint anus in the adult females.

Mermithids parasitise a wide range of insect hosts (as well as other arthropods and even molluscs) (Poinar, 1975). Thus far, tetradonematids have been reported only in three holometabolous insect orders (Diptera, Coleoptera and Hymenoptera).

It is obvious that the infective stage of *Myrmeconema* n. g. has many characters found in mermithids and *M. neotropicum* n. sp. is probably an early offshoot from one of the mermithid lineages. How ants become infected with *M. neotropicum* is unknown. However, *Myrmeconema* is the only member of the Mermithoidea that causes its host to radically change colour (from black to red). Studies are now underway to determine whether this colour change plays a role in the life-cycle of the parasite.

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