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Parasitylenchus myiophagus n. sp. (Nematoda: Parasitylenchidae), a tylenchid nematode parasite of long-legged flies (Diptera: Dolichopodidae)

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Abstract

A tylenchid nematode parasite of a male long-legged fly, *Tachytrechus sanus* Osten Sacken (Diptera: Dolichopodidae) from Montana, USA is described as *Parasitylenchus myiophagus* **n. sp.** (Nematoda: Parasitylenchidae). The new species is characterized by the presence of an extremely long first generation female and numerous short and wide second generation males and females produced in "enclosed" clusters in the host's body cavity. Both generation female nematodes are ovoviviparous, with short stylets lacking knobs and simple tails lacking spikes, palps or mucrons. The second generation males have paired, separate spicules, short stylets, and a bursa but no visible gubernaculum. The fly host shows evidence of demasculinization, which is attributed to nematode parasitism. The gonads of the second generation adults are infected with a microsporidium (Microsporidia), which is a new host record for tylenchid nematodes. A tylenchid-infected Baltic amber dolichopodid shows that associations between these two organisms extend back at least to the Eocene.

Key words: Baltic amber, demasculinization, Microsporidia, new species, *Tachytrechus*

Introduction

Long-legged flies (Diptera: Dolichopodidae) are small flies, usually with metallic coloration and long legs in proportion to their bodies. The family is cosmopolitan with some 230 genera and 7,500 described species (Bickel, 2009). Adults and larvae occur in various field and garden sites and are often abundant in areas with heavy plant growth under damp conditions, especially near water. The larvae and adults of most species are predators of other small invertebrates (Robinson & Vockeroth, 1981; Ulrich, 2004). Very little is known about nematode parasites of dolichopodids, however mermithid nematodes have been found in adults in Scandinavia (Kahanpää, 2008; Hedström, 1976). There appear to be no extant records of tylenchid nematodes from this family of flies, however, the recent discovery of a tylenchid-infected Baltic amber dolichopodid shows that associations between these two lineages extend back at least to the Eocene (Poinar & Brown, 2021).

Recently we discovered males of *Tachytrechus sanus* Osten Sacken (Diptera: Dolichopodidae: Dolichopodinae) from Montana, USA, that were attacked by nematodes. The present study describes this nematode in the family Parasitylenchidae (Nematoda: Tylenchida). This is the first description of an extant tylenchid parasite of a dolichopodid. During the present investigation, microsporidia were observed developing in the gonads of second generation female and male nematodes. The possible effects that these pathogens could have on the reproductive success of the nematodes are discussed.

Materials and methods

The presence of nematodes in males of *T. sanus* Osten Sacken could be determined by the modified male genital capsule and weak development of other male sex-specific characteristics (Kahanpää, 2008). Infected male flies were

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placed in 70% ethanol and kept under refrigeration until dissection, when the nematodes were processed to glycerin (Poinar, 1975). The nematodes were then mounted on a microscope slide and examined and photographed (Nikon Optiphot Microscope). Helicon Focus Pro X64 was used to stack photos for better clarity and depth of field. In some figures portions of the background were removed for better viewing of the selected features (Adobe Photoshop). Systematic treatment of the insect parasitic Tylenchida follows Siddiqi (2000).

Results

Tylenchida Thorne, 1949

Sphaerularioidea Lubbock, 1861

Parasitylenchidae Siddiqi, 1986

Parasitylenchinae Siddiqi, 1986

Parasitylenchus Micoletzky, 1922

A list of species included in *Parasitylenchus* and their known hosts are provided in Table 1.

TABLE 1. Species in the genus *Parasitylenchus* Micoletzky, 1922 (modified from Sidiqui, 2000) and known hosts and distribution.

Species	Known hosts	Known distribution
P. aculeatus Slankis, 1972	Coleoptera: Curculionidae: Scolytinae	Palearctic (central Europe)
P. bifurcatus Poinar & Steenberg, 2012	Coleoptera: Coccinellidae	Palearctic (Denmark)
P. coccinellinae Iperti &Waerebeke, 1968	Coleoptera: Coccinellidae	Palearctic (France)
P. curvidentus (Fuchs, 1914) Micoletzky, 1922	Coleoptera: Curculionidae: Scolytinae	Palearctic (Germany)
P. dispar (Fuchs, 1915) Micoletsky, 1922	Coleoptera: Curculionidae: Scolytinae	Palearctic (central Europe)
P. diplogenus Welch, 1959	Diptera: Drosophilidae	Palearctic (United Kingdom)
P. klimenkorum Kortentchenko, 1987	Coleoptera: Curculionidae: Scolytinae	Palearctic (Russia)
P. macrobursatus Blinova & Gurando, 1977	Coleoptera: Curculionidae: Scolytinae	Palearctic (Russia)
P. myiophagus Poinar & Runyon, **n. sp.**	Diptera: Dolichopodidae	Nearctic (USA)
P. nearcticus Poinar, Jaenike & Dombeck, 1997	Diptera: Drosophilidae	Nearctic (USA)
P. orthotomici Ho, Kaya & Shea, 1989	Coleoptera: Curculionidae: Scolytinae	Palearctic (South Korea)
P. pseusobifurcatus Camino, Rodríguez Gil, Reboredo & González, 2019	Coleoptera: Coccinellidae	Neotropical (Argentina)

Parasitylenchus myiophagus n. sp.

(Figs 1–12)

Description (Table 2). *First generation parasitic female* (n = 1): Ovoviviparous, yellow, body ventrally coiled with 3 bends, filled with reproductive and gut cells; body length, 5.4 mm; greatest body width, 205 μm; stylet thin, narrowing toward apex, lacking basal thickenings; stylet length, 26 μm; length head to nerve ring, 176 μm; length tip of intestine to tip of tail, 112 μm; excretory pore and anus not visible; vulva sub-terminal; tail rounded.

Second generation parasitic females (n = 10): Ovoviviparous, body yellowish-brown, slightly curved but without bends; body mostly filled with reproductive and gut cells; body length, 370–400 μm; body width, 36–42 μm.

Second generation parasitic males (n = 5): Body yellowish brown, slightly curved to straight (some individuals have a constriction near the tail tip); body length, 320–330 μm; body width; 34–40 μm; distance tail tip from cloaca, 160 μm; spicules paired, separate, length 12–16 μm; bursa faint, length 14 μm; gubernaculum absent.

TABLE 2. Morphological measurements of *Parasitylenchus myiophagus* **n. sp.**

Character	Value
First generation parasitic female (N=1)	
Length	5.4 mm
Width	205 μm
L/W ratio	26.3
Stylet length	26 μm
Length head to nerve ring	176 μm
Length tip of intestine to tip of tail	112 μm
a	26.3
V	0.42
Second generation parasitic females (N=10)	
Length	370–400 μm
Width	36–42 μm
L/W ratio	9.5–10.0
a	9.8
Second generation parasitic males (N=5)	
Length	320–330 μm
Width	34–40 μm
L/W ratio	8.2–9.0
Distance tail tip to cloaca	160 μm
Length spicules	12–16 μm
Length bursa	14 μm
a	8.5
c	8.6
c’	1.0
T	0.6
Developing juveniles (N=10)	
Length	46–190 μm
Width	10–16 μm
L/W ratio	4.6–11.8

Developing juveniles (n = 10): Length, 46–190 μm; width, 10–16 μm.

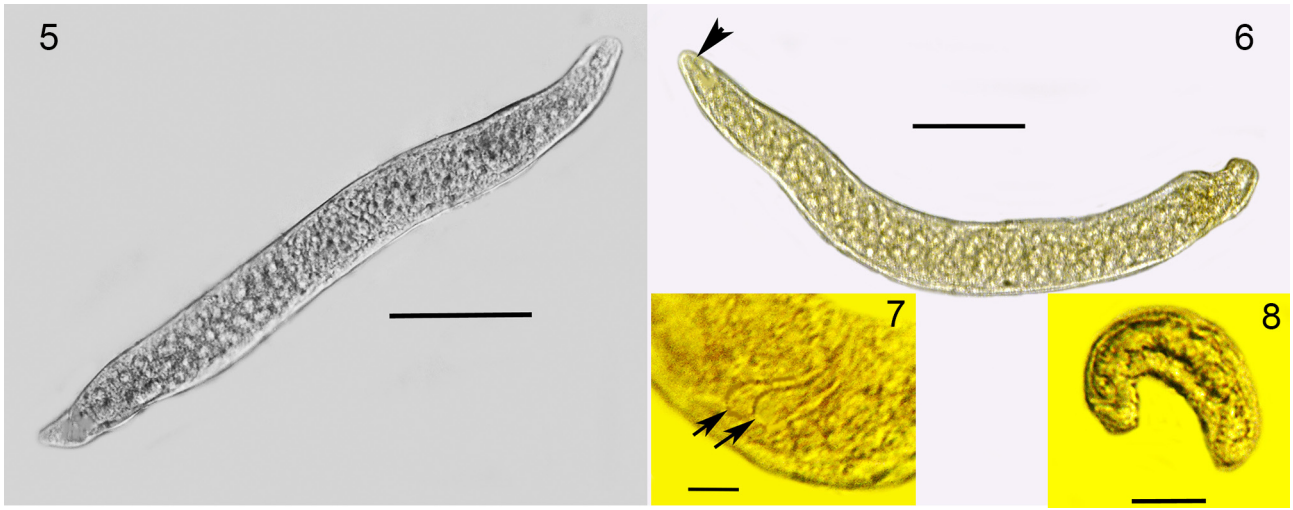
Comments. The first generation parasitic female (Figs 2,3,9,12), second generation parasitic females (Fig. 5), second generation parasitic males (Figs 6,7,10,11) and early developmental stages (Fig. 8) occur in the host’s body cavity. First generation parasitic female yellowish, body extended with 3 bends, ovoviviparous, containing faint stylet lacking knobs or basal thickenings; second generation parasitic females and males light yellow with indistinct stylets lacking knobs or basal thickenings; males with paired, separate spicules with cephalated heads; faint bursa; gubernaculum lacking. Development of the second generation stages occurs in clusters (Fig. 4), which include eggs, developing juveniles and adults.

Typological characters and diagnosis of *P. myiophagus*. Placement of *P. myiophagus* in the family Parasitylenchidae Siddiqi, 1986 and subfamily Parasitylenchinae Siddiqi,1986 is based on the presence of a primary heterosexual generation alternating with a secondary heterosexual generation in the host, parasitic females ventrally curved, slender simple, ventrally arcuate spicules, the presence of a bursa, the absence of a gubernaculum and a dipteran host (Siddiqi, 2000).

FIGURE 1. Male *Tachytrechus sanus* (Diptera: Dolichopodidae) parasitized by *Parasitylenchus myiophagus* **n. sp.** Arrow shows exposed portion of abdomen containing first and second generation adult nematodes. Scale bar: 1.4 mm.

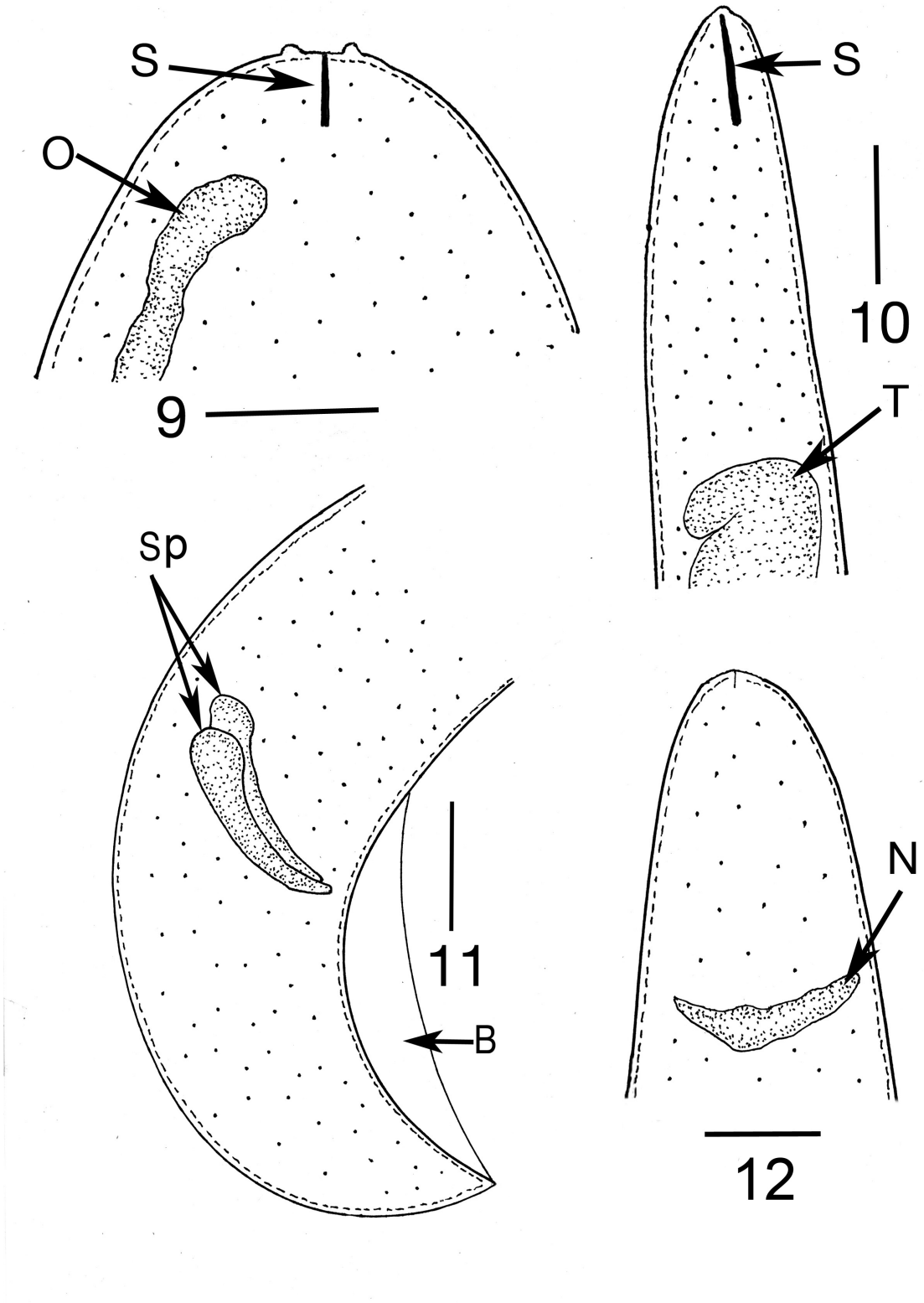
FIGURES 2–3. First generation parasitic female of *Parasitylenchus myiophagus* **n. sp. 2.** Female removed from the abdomen of *Tachytrechus sanus* (Diptera: Dolichopodidae), arrow shows nerve ring. **3.** Head, arrow shows tip of extended ovary with developing eggs, arrowhead shows stylet. Scale bars: 2 = 380 μm; 3 = 34 μm.

FIGURE 4. Clusters containing eggs, developing juveniles and second generation adults of *Parasitylenchus myiophagus* **n. sp.** Scale bar: 100 μm.



FIGURES 5–8. Second generation parasitic *Parasitylenchus myiophagus* **n. sp. 5.** Female. **6.** Male, arrowhead shows stylet. **7.** Spicules (arrows) of male. **8.** Newly hatched second generation juvenile. Scale bars: 5 = 70 μm; 6 = 50 μm; 7 = 7 μm; 8 = 12 μm.

Placement in the genus *Parasitylenchus* Micoletzky, 1922 is based on three types of adults in the host (primary heterosexual generation female, secondary heterosexual generation female, and male), with the secondary heterosexual forms mating in the host.



FIGURES 9–12. *Parasitylenchus myiophagus* **n. sp. 9.** Head of first generation parasitic female. **10.** Head of male. **11.** Male tail. **12.** Tip of first generation parasitic female showing location of nerve ring. Abbreviations: B= bursa; N = nerve ring; O= tip of ovary; S= stylet; Sp = spicules; T= tip of testis. Scale bars: 9 = 64 μm; 10 = 25 µm; 11 = 10 µm; 12 = 58µm.

Parasitylenchus myiophagus can be distinguished from other species in the genus *Parasitylenchus* by its dipteran host, a character shared only by *P. diplogenus* Welch, 1959 and *P. nearcticus* Poinar, Jaenike & Dombeck, 1997. *Parasitylenchus myiophagus* can be distinguished from the former species by its short, straight first and second generation oviparous females that are subequal in size and the longer stylet of the first generation parasitic females. From *P. diplogenus, P. myiophagus* differs in having relatively short, swollen second generation oviparous females that are subequal in size and by the absence of a stylet in the male.

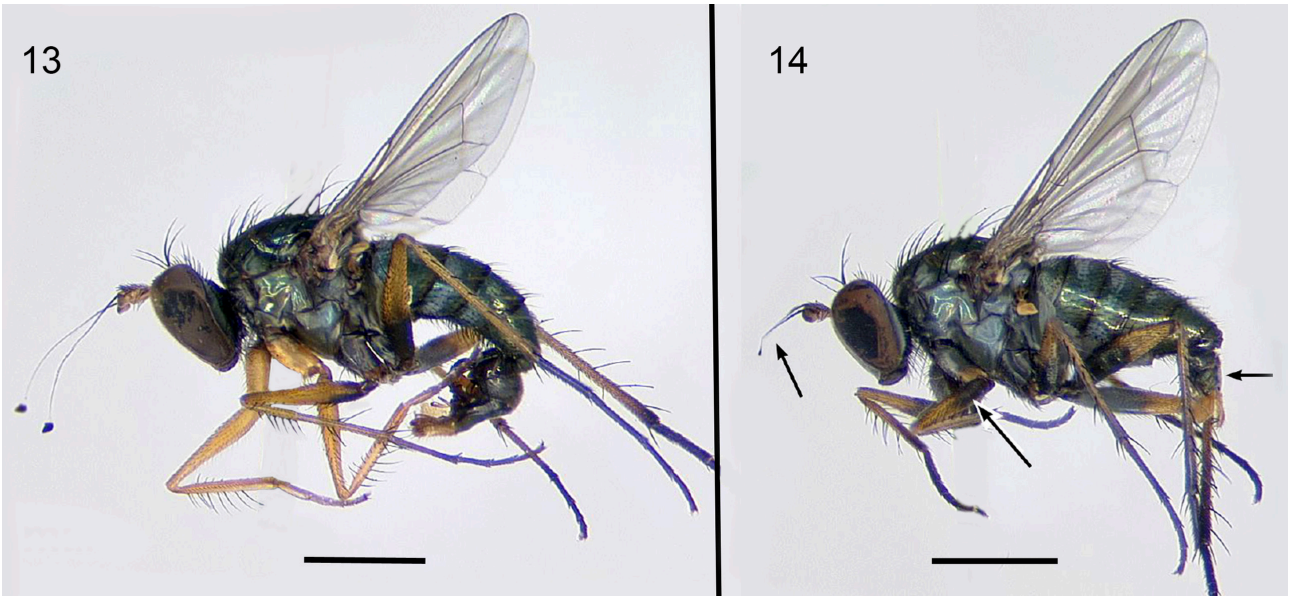
Etymology. The specific epithet is derived from the Greek "myia"= fly and the Greek "phagos"= to eat.

Type-locality. USA, Montana, Gallatin County, Bridger Mountains, Johnson Canyon, large hillside spring, 30 July 2010, N 45°57.73' W111°01.61', JB Runyon.

Type material. Holotype (T-560t) first generation parasitic female deposited in the USDA Nematode Laboratory, Beltsville, Maryland, USA. Paratypes (parasitic females and males) deposited in the Montana Entomology Collection, Montana State University, Bozeman, Montana, USA.

Host. Males of *Tachytrechus sanus* Osten Sacken, 1877 (Diptera: Dolichopodidae). Four demasculinized males were collected at the type locality. One was dissected for this study and the remaining three were pinned and deposited in the Entomology Collection at Montana State University, Bozeman.

Effect on host. Most tylenchid parasites do not kill their hosts outright, but many can cause a variety of behavior and developmental host changes. This is normally expressed as a reduction in body size but can involve sterility and even death. In *T. sanus*, *P. myiophagus* causes demasculinization (a reduction in development of male primary and secondary sexual characters) that is similar to effects caused in other male dolichopodids by mermithid nematode infection (Kahanpää, 2008). The most obvious sign of infected males is the much smaller and poorly rotated genitalia (cf. Figs 13,14). Other *P. myiophagus*-induced changes to male *T. sanus* include having a wider face that is light yellow to silver (face very narrow and golden in uninfected males), arista of antenna shorter with reduced apical lamella, and darker legs especially the fore coxa and fore femora (which are yellow in uninfected males) (Fig. 14). Demasculinization also occurs in other dolichopodids infected with nematodes, including several species of *Dolichopus* in the Palearctic (Kahanpää, 2008; Germann *et al*. 2010) and some *Dolichopus* and *Rhaphuim* species in the Nearctic (Runyon, in review), but these cases are so far known to be caused by mermithids.



FIGURES 13–14. Effect of tylenchid parasites on morphological structures of male *Tachytrechus sanus*. **13.** Non-infected male. **14.** Infected male. Note signs of demasculinization including shorter antenna with smaller apical lamella (left arrow), darker legs (middle arrow) and smaller, poorly rotated genitalia (right arrow). Scale bars: 13 = 1.5 mm; 14 = 1.6 mm.

Microbial parasites of **Parasitylenchus myiophagus n. sp.**

The second generation females of *Parasitylenchus myiophagus* **n. sp.** have uteri filled with eggs, however very few developing juveniles could be found. In a normal *Parasitylenchus* life cycle, juveniles emerging from the second

generation females would be common and eventually leave the host through the digestive or reproductive openings. After maturing and mating in the environment, the fertilized infective stage females would search out a new host, which would probably be a larva or pupa. The developing nematodes would then be carried into the adult stage.

The rarity of juvenile stages of second generation females may be due to a microsporidial infection that was prevalent throughout the population of both second generation parasitic males and females. Cysts (pansporoblasts) ranging up to 10 μm in diameter contained developing round spores measuring roughly between 2.7 and 3.6 μm in diameter (Figs 15,16). Microsporidians (Division Microsporidia), especially members of the genus *Dubosquia* Pérez but also representatives of the genera *Thelohania* Henneguy, *Nosema* Nägeli, *Pleistophora* Gurley and *Microsporidium* Balbiani are known to infect nematodes (Dollfus, 1946; Kudo, 1954; Sprague, 1977). The present infection in *Parasitylenchus myiophagus* sp. n, which may have initially been obtained from the fly host, could account for the absence of juveniles in the second generation parasitic females.

FIGURES 15–16. Microsporidian infections of secondary generation females of *Parasitylenchus myiophagus* **n. sp. 15.** Head area showing clusters of developing spores (arrowheads). **16.** Head area showing spores adjacent to swollen excretory pore area (arrowhead). Scale bars: 15 = 17 μm; 16 = 13 μm.

This infection could represent a case of environmental or habitat host selection, where a parasite infects new hosts based on availability (versus phylogenetic or physiological host selection, where the parasite develops in a new host phylogenetically related to its present host). It is known that some species of microsporidia have a wide host range. For instance, the mosquito parasite, *Nosema algerae*, is capable of infecting members of the orders Lepidoptera, Hemiptera, Odonata and Orthoptera, aside from its natural host (Undeen & Maddox, 1973).

Discussion

Dolichopodidae is an ancient group, with fossils in Lebanese amber extending back to the Early Cretaceous (Poinar & Milki, 2001). However, most fossil dolichopodids (representatives of some 23 genera) occur in Baltic amber (Poinar, 1992). An examination of Baltic amber dolichopodids revealed a male (*Gheynia* sp.) that was parasitized by a tylenchid nematode very similar to *P. myiophagus* **n. sp.** The fossil fly had its abdomen torn open, possibly from the actions of a predator. This action released an elongate first generation parasitic female, very similar in form to the parasitic female of *P. myiophagus* **n. sp.** (see Fig. 2). Also released were clusters of second generation females that are similar to those of the second generation females of *P. myiophagus* **n. sp.** (see Fig. 4). This fossil nematode was recently described and placed in *Palaeoparasitylenchus* Poinar (Poinar & Brown, 2021). It is apparent that dolichopodid-tylenchid associations have existed at least for some 40–55 million years (Wolfe *et al*. 2016).

This is the first description of an extant tylenchid nematode parasite of a dolichopodid fly. The only previously known nematode parasites of dolichopodids are apparent mermithid nematodes that were found in Finland (Kahanpää, 2008), Norway (Hedström, 1976), and the United States (Runyon, in review). Mermithids can easily be distinguished from insect-parasitic tylenchids since only juveniles of the former group occur in the host while the adults are free-living in the host's environment.

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George Poinar thanks Alex Brown for supplying the parasitized Baltic amber dolichopodid. Dan Bickel assisted with identification of the dolichopodid in Baltic amber. This research was supported [in part] by the USDA Forest Service, Rocky Mountain Research Station. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy.

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