Parasitylenchus nearcticus sp. n. (Tylenchida : Allantonematidae) parasitizing Drosophila (Diptera : Drosophilidae) in North America

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Summary - Parasitylenchus nearcticus sp. n. (Tylenchida : Allantonematidae) is described as a parasite of Drosophila recens (Diptera : Drosophilidae) collected from the Adirondack Mountains in New York State, USA. The new species is compared to the single previously known species of Parasitylenchus from Great Britain and a key to the allantonematid parasites of Drosophilidae is presented. The females of the two parasitic generations show dimorphism in regards to the form of the stylet and pharyngeal glands. P. nearcticus n.sp. sterilizes females of D. recens, thus having an effect on the population ecology of this host.


Key-words : Allantonematidae, Drosophila recens, insect parasitism, nematodes, Parasitylenchus nearcticus sp. n.

Allantonematids were reported from drosophilid flies by Gershenzon (1939) (see Poinar [1975] for citations of nematodes in general from drosophilids), however Welch (1959) was the first to formally describe members of this nematode family from lesser fruit flies. The earlier reports and Welch's descriptions of Howardula aoronomyphium Welch and Parasitylenchus diplogenus Welch suggested that this host-parasitic relationship was confined to the Palearctic.

However, Montague and Jaenike (1985) reported the presence of H. aoronomyphium in mycetophagous Drosophila in North America. A report of a similar association in Japan (Kimura & Toda, 1989) and the presence of an allantonematid infected drosophilid in Dominican amber (Poinar, 1984) demonstrated that this parasitic association was not only global but that it had evolved millions of years ago.

In 1994, the junior author (I.D.) discovered a species of Parasitylenchus in Drosophila recens from the Adirondack Mountains in New York State, USA. Members of the genus Parasitylenchus are unusual in their development because there are two amphilnictic parasitic generations in their life cycle, both of which are passed in the body of the host. Only a single amphilnictic generation occurs in Howardula and the majority of the other allantonematid species.

A detailed examination of the New York material showed that it differed from the British P. diplogenus and a description follows. This study presented an opportunity to study all stages of the life cycle for the first time with a drosophilid infection by a Parasitylenchus and demonstrated that dimorphism does indeed occur between the first and second generation females. A key to the Allantonematidae parasitizing Drosophilidae is presented.

Materials and methods

Adult Drosophila were captured from June through August in 1994 and 1995 by sweep netting over decaying fungi in mixed forests at 500 to 600 m elevations around Big Moose Lake and Moss Lake in Herkimer County, NY, USA. Wild-caught individuals of D. recens, D. falleni and D. neotesacea were dissected in Drosophila ringer's solution (Montague & Jaenike, 1985), and scored for infection by Howardula sp. and Parasitylenchus sp. A strain of Parasitylenchus established from these flies has been maintained continuously in the laboratory for 18 months, using the following protocol. New emerged parasitized flies were placed in culture vials containing Instant Drosophila Medium (Carolina Biological Supply) plus a piece of commercial mushroom (Agaricus bisporus). They were transferred every 4 days to fresh food until they were 3 weeks old. At this age, two parasitized male flies were placed in a culture vial and two female flies from an uninfected culture were
added to the same culture vial after 2 days. All cultures were maintained at 19°C.

Living parasitized adult flies were dissected at different times to obtain nematodes at various stages of development of the first and second generations. The dissections were made in Ringer's solution and the nematodes were heat killed, fixed in TAF and processed to glycerin. All measurements were made on slide mounted fixed material, however certain morphological details, i.e., stylet structure and pharyngeal gland morphology, were best made by observing living individuals.

**Parasitylenchus nearcticus** sp.n. (Figs 1, 2)

**Measurements**

*Free-living, first generation, vermiform female* (Fig. 1C) (n = 10): L = 239 (228-254) μm; greatest width = 10 (8-11) μm; head to excretory pore = 57 (45-65) μm; head to base of pharynx = 54 (48-70) μm; head to nerve ring = 41 (37-58) μm; head to tip of gonad = 133 (118-144) μm; tail length = 40 (35-45) μm; vulva to tail tip = 69 (61-74) μm; V = 71 (68-73); stylet length = 15 (13-16) μm; a = 25 (21-29); c = 6 (5-7).

*Parasitic, second generation, vermiform female* (Fig. 2B): This stage is similar, quantitatively, to the free-living female. The two important characters which differ are the structure of the stylet which is smaller (11 [9-12] μm) and thinner and the penetration glands which have atrophied.

*Parasitic, first generation, swollen female* (Fig. 2A) (n = 10): L = 488 (435-582) μm; greatest width = 78 (64-90) μm; excretory pore faint = 25 (23-27) μm; tail length = 19 (16-27) μm; vulva to tip of tail = 40 (24-54) μm; V = 91 (88-96)%; stylet length = 16 (13-18) μm; length eggs = 56 (50-61) μm; width = 28 (23-32) μm; a = 6 (5-9); c = 27 (17-40). Eggs normally show one division before oviposition. Oviparous.

*Parasitic, second generation, swollen female* (Fig. 2B) (n = 10): Length = 368 (307-467) μm; greatest width = 58 (50-80) μm; excretory pore and anus not visible; vulva to tail tip = 32 (24-42) μm; V = 91 (88-94)%; stylet length = 8 (6-11) μm; one to three eggs occur in uteri; length eggs = 60 (52-66) μm; width = 25 (20-29) μm; a = 7 (5-9); eggs normally undergo one division before oviposition. Oviparous.

*Males* (Fig. 1A) (n = 10): The males from both generations were similar in all aspects. Length = 230 (197-257) μm; greatest width = 10 (8-11) μm; head to excretory pore = 51 (43-56) μm; tail length = 38 (32-45) μm; tail width = 8 (7-10) μm; stylet faint, length = 7 (6-8) μm; head to tip of testis = 82 (72-93) μm; width of testis = 79 (64-96) μm; length of spicules (both same size) = 9 (8-10) μm; bursa and gubernaculum absent.

**Description**

Body of the free-living, first generation, vermiform female slender. Entire body often surrounded by a loose cuticle, even in mated specimens. Head truncate, lip region not off-set. Stylet curved dorsally. Dorsal gland opening located just posterior to the stylet base. Nerve ring distinct and in some specimens, hemizonid present posterior to the nerve ring. Excretory pore about one stylet length behind the nerve ring. Near the nerve ring the pharynx swelling slightly and forming a small, but distinct bulb. Three pharyngeal (penetration glands) extending to and sometimes overlapping the ovary. Uterus and ovary short. Sperm elongate rather than oval. Tail tip often curved dorsally.

The corresponding second generation females do not enter a host and exhibit the absence of pharyngeal glands and a shorter and thinner stylet.

Males identical in both generations. Thin and short stylet similar to that of the second parasitic generation females. Tip of the testis nearly reaching the nerve ring and vas deferens packed with developing sperm. Spicules small, separate and diverging at the cloacal opening: their proximal half broadening with a central constriction, and distal half thin and narrow, making the entire spicu-
Parasitylenchus nearcticus sp. n.

Fig. 2. Parasitylenchus nearcticus sp. n. A: First generation swollen parasitic female removed from the hemocoel of Drosophila reccents; B: Second generation swollen parasitic female removed from the hemocoel of Drosophila reccents (Scale bars: A = 41 μm; B = 45 μm).

Free-living female of this latter species was not described in detail so comparisons between the two species have to be made with males and swollen parasitic females. However the presence of a longer tail and stylet in P. nearcticus sp. n. males make this species distinct from P. diplogenus. The longer stylet and division of the eggs before oviposition in P. nearcticus sp. n. first generation swollen females separates this species from P. diplogenus.

The following key uses diagnostic characters to separate P. nearcticus sp. n. from the two previously described allantonematid parasites of drosophilids.

1 - Swollen parasitic females over 1 mm in length, with juveniles in uteri (ovoviparity); vulva not visible; male length > 300 μm, with spicule length > 15 μm; a single amphimictic generation in host .................. P. diplogenus

- Swollen parasitic females < 1 mm in length, with only eggs in uteri (oviparity); vulva distinct; male length < 300 μm, with spicule length < 15 μm; two amphimictic generations in host .................................. 2

2 - Male lacking a stylet; male tail < 30 μm; first generation swollen; female with stylet length < 12 μm; eggs deposited without undergoing embryonization; egg length < 45 μm .................. P. nearcticus

- Male with a thin stylet; male tail > 30 μm; first generation swollen female with stylet length > 12 μm; eggs with at least one division before oviposition; egg length > 45 μm.................. P. nearcticus

BIOLICAL OBSERVATIONS

Among the flies captured in 1994-1995 in the Adirondacks, the percentage of flies parasitized by P. nearcticus sp. n. were as follows: D. recens (5%, n = 116); D. falleni (0%, n = 328); and D. neotestacea (0%, n = 276). In these same flies, 6% of D. recens, 11% of D. falleni and 20% of D. neotestacea were infected by Howardula sp. Interestingly two females of D. recens were simultaneously infected with both Parasitylenchus and Howardula. The field data indicate that P. nearcticus sp. n. is restricted to a single host, D. recens, in the Adirondacks. To determine if the latter two species were resistant to infection by P. nearcticus sp. n. several wild-caught adults of D. recens, D. falleni and D. neotestacea were placed in a single culture vial and allowed to breed. Among the emergent F1 generation, 50% of D. recens were infected but none of the other two species contained Parasitylenchus; thus it appears that P. nearcticus sp. n. is physiologically restricted to D. recens in the Adirondacks.

In both wild-caught and laboratory-reared D. recens, all females parasitized by P. nearcticus sp. n. were sterile (no mature eggs were detected).

Discussion

Characteristic of the genus Parasitylenchus are two amphimictic generations inside the host. The first generation is initiated by a free-living fertilized female that penetrates into the body cavity of a larval host, in this
case a drosophilid larva. The free-living females of *P. nearcticus* are equipped with a sturdy stylet and three penetration glands which make entry possible. After entering the host’s hemocoel, the nematode enlarges into an egg-producing, swollen first-generation parasitic female. Eggs deposited by this female develop into juveniles which pass into the adult stage and initiate the second amphimictic generation in the host. The second generation female again becomes swollen and produces eggs which develop into juveniles which mature to males and third and fourth stage females inside the host. These stages then leave the host and mature in the environment. Mating occurs and the fertilized female is ready to initiate a first generation parasitic cycle. It is noteworthy that the second generation females do not have penetration glands nor as well developed stylets as the first generation. Since this stage does not penetrate a host, these structures are no longer needed. This is the first time that this type of dimorphism has been noted in the genus *Parasitylenchus*. Both first and second generation males are similar in morphology and behaviour. Their stylets are thin and really have no function although the second generation males that mature in the host could use their stylets to assist them in leaving the host.

Welch (1959) noted two molts in both sexes of the free-living generation of *P. diplogenus*. The third occurred in the host and the fourth after the nematodes had left the host, leading Welch to speculate that a molt may occur in the egg. A molt was observed in the egg of *P. nearcticus* and many newly formed females were still surrounded by three cast cuticles, thus accounting for all of the molts.

Siddiqi (1986) characterized the genus *Parasitylenchus* as having basal stylet knobs, similar stylets of the first and second generation and the opening of the excretory pore near the base of the stylet. In the case of *P. nearcticus*, there are no stylet knobs (nor did Welch describe any for *P. diplogenus*), the female stylets of the two generations are different and the opening of the excretory pore is clearly posterior to the nerve ring. Thus, either some modification will have to be made regarding the generic description of *Parasitylenchus* or the drosophilid parasites should be placed in their own separate genus.

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**References**


