Fundam. appl. Nematol., 1992, 15 (5), 427-430

Steinernema feltiae (Steinernematidae : Rhabditida) parasitizing adult fungus gnats (Mycetophilidae : Diptera) in California

George O. POINAR, Jr.

Department of Entomological Sciences, University of California, Berkeley, CA 94720, USA.

Accepted for publication 12 November 1991.

Summary — Natural populations of *Steinernema feltiae* (Filipjev) are here reported for the first time from North America. The present population, designated the fungus gnat strain, parasitizes the adult stages of *Mycetophila fungorum* (Mycetophilidae : Diptera) feeding in the gilled mushroom, *Agrocybe praecox* (Bolbitiaceae : Agaricales) in Northern California. This is the first instance of a steinernematid that habitually parasitizes the adult stages of a holometabolous insect.

Résumé — Steinernema feltiae *(Steinernematidae : Rhabditida) parasite des adultes du moucheron des champignons (Mycetophilidae : Diptera) en Californie* — Des populations naturelles de *Steinernema feltiae* (Filipjev) ont été récoltées pour la première fois en Amérique du nord. La présente population, désignée comme la souche du moucheron des champignons, parasite les stades adultes de *Mycetophila fungorum* (Mycetophilidae : Diptera) se nourrissant sur l'agaric *Agrocybe praecox* (Bolbitiaceae : Agaricales) en Californie du nord. C'est le premier exemple d'un Steinernematide parasitant naturellement les stades adultes d'un insecte holométabole.

Key-words : Nematodes, *Steinernema,* insect parasites, California.

Up until the present, there have been no reports of *Steinernema feltiae (Filipjev)* [= *S. bibionis* (Bovien)] in North America although this species has been reported from Europe, Australia and New Zealand (Poinar, 1990). During an investigation of insects occurring in mushrooms in Northern California, the author discovered a strain of *S. feltiae* parasitizing adult mycetophilid flies. This strain is characterized, compared with the SN strain from France and the hybrids of crossings between the two strains are examined. This is the first time that a steinernematid nematode has been found that habitually parasitizes the adult of a holometabolous insect.

Materials and methods

The mushrooms containing the parasitized fungus gnats were collected from a park in Albany (Alameda Co.), California during February and March, 1990. The fungus gnats were identified as *Mycetophila fungorum* (De Geer) (Mycetophilidae : Diptera). They were reared from larvae feeding in the mushroom, *Agrocybe praecox* (Fr.) Fayod (Bolbitiaceae : Agaricales). The fungus gnat strain infected larvae of *Galleria mellonella* in the laboratory and its development and life cycle appeared similar to other steinernematids.

Crosses between the fungus gnat strain of *S. feltiae* with the SN strain of *S. feltiae* from France were conducted using the hanging blood drop method. This consists of placing surface sterilized (with a 1 % solution Hyamine 10X) infective juveniles in separate hanging drops of wax moth *(Galleria mellonella)* blood. After reaching the pre-adult stage, males and females of the same and different strains were placed together in separate blood drops and observed over a period of 10 days.

For morphological examinations and measurements, infective juveniles and adults that emerged from parasitized wax moth larvae were heat killed (60 °C fixed in 3 % TAF and processed to glycerin. Microscopic examinations were conducted with a Nikon Optiphot microscope equipped with Differential Interference Contrast.

Results

The present strain of *S. feltiae* was only collected from adult mycetophilids that emerged from pupae in soil under decaying specimens of *A. praecox*. No other insect stages were found infected although this does not imply that mycetophilid larvae and pupae are immune to the infection.

Infected adult flies survived for several days after emergence before succumbing to the infection. During this period, developing nematodes (from three to twenty) could be found in the hemocoel. Thus, the nematodes matured to the adult stage and mated while

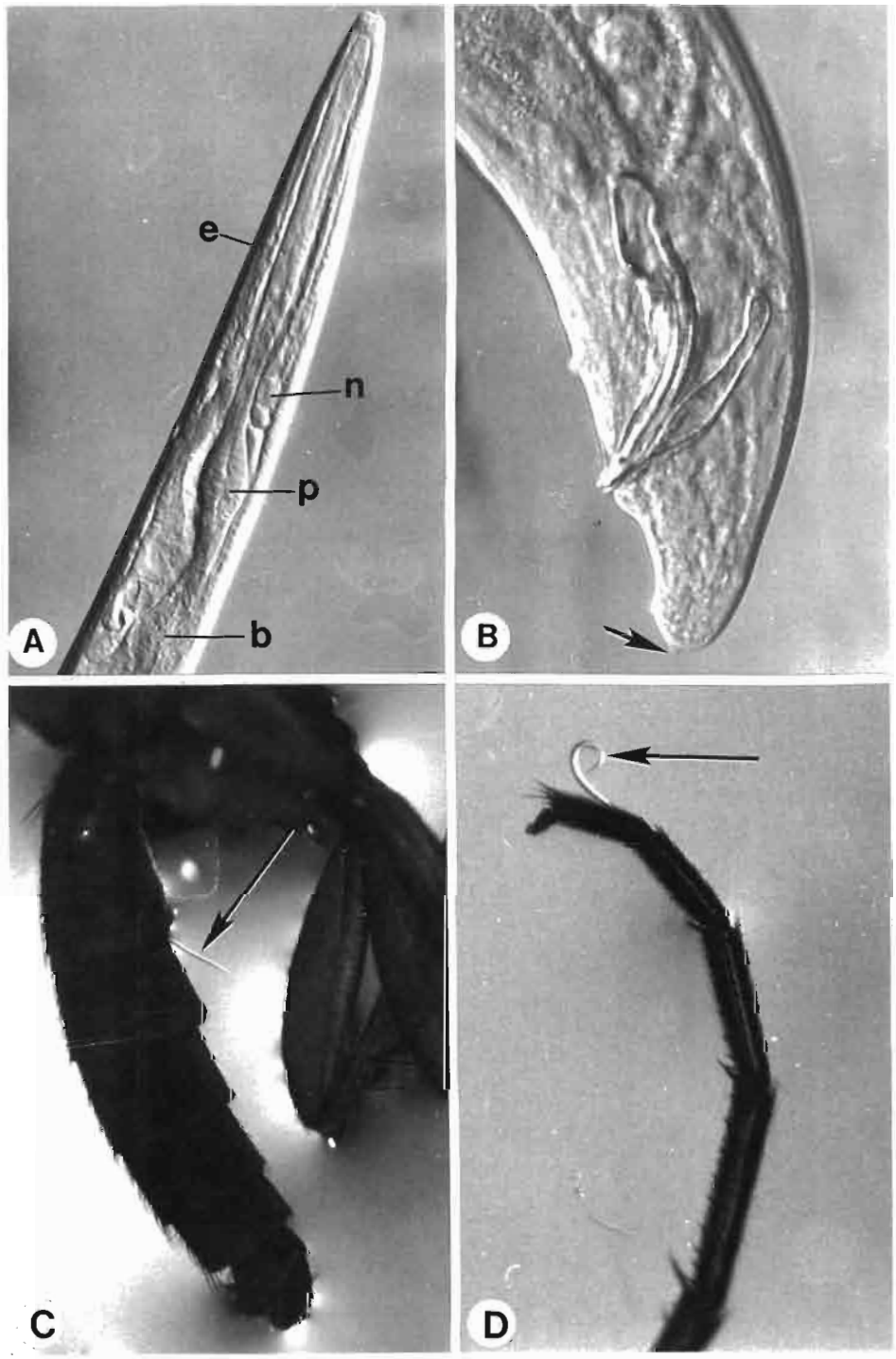

Fig. 1. Fungus gnat strain of *Steinernema feltiae* — A : Lateral view of anterior portion of infective juvenile (e = excretory pore; n = nerve ring; p = basal bulb of pharynx; b = bacterial pouch); B : Lateral view of first generation male. Note characteristic shaped spicules and short, broad tail projection (arrow); C : Infective stage juvenile (arrow) emerging from an abdominal intersegmental membrane of *Mycetophila fungorum;* D : Infective stage juvenile (arrow) emerging from a tarsal segment of *Mycetophila fungorum.*

the fly was still alive. During this time, cells of *Xenorhabdus* could be found in the hemolymph of infected flies.

The parasitized hosts died only after the first generation nematode eggs had hatched. Within one or two days after death, the infective stage juveniles of *S. feltiae* began emerging through the intersegmental membranes of the dead adult flies. Many of the latter had not decomposed and the nematodes often struggled for some time (up to one hour) before finally leaving the host cadaver. At this time, groups of emerging infectives could be seen in rapid motion in attempts to free themselves from the adult fly. Preferred exit areas were intersegmental membranes of the abdomen (Fig. 1C) but even tarsal segments were an exit route (Fig. 1D). Many of the infective juveniles remained attached to the host by their head (with tail moving) during emergence, suggesting that they left the host tail first.

Although quantitative differences occur between the infective juveniles of the fungus gnat strain and the SN strain of *S. feltiae* (see Table 1), they are not significant. The fungus gnat strain does have the shortest known infective juveniles of all previously described strains although the average value (784 µm) is within the range of the known species variation (736-950 µm; Poinar, 1990). Infectives of the fungus gnat strain also have somewhat smaller values in all other measurements except body width and tail length, thus they can be typified as being shorter but stouter than the typical *S. feltiae* infective juveniles (Table 1). These differences are reflected in the ratios which vary slightly from those of the typical strains. It is interesting to note that the quantitative values obtained for the infective stages of the F_1 cross were greater than those of either of the parent strains, yet they still fall within the range for the species. Whether this represents some type of intraspecific hybrid vigor is not known.

An interesting variation in the first generation males of the fungus gnat strain is the length of the cuticular tail spine (Table 2). In the first generation males, this spine is short and broad at its base, measuring only 2.0 µm (0-3.2 µm) in length, which is shorter than is normally found in this species (4-13 µm; Poinar, 1986). However, second generation adult males have a longer cuticular tail spine that is 5.9 µm (4.8-8.0 µm) in length and falls within the normal range of this species. The SN strain had a normal value for this character in both generations and the cross possessed intermediate values for the first generation and the second generation tail spine values were identical to those of the SN strain (Table 2).

Discussion

This is the first report of *Steinernema feltiae* from North America. In Denmark, this nematode parasitizes larvae of bibionid flies (Bibionidae : Diptera), while in

Table 1. Comparison of infective juvenile quantitative characters of the fungus gnat and SN strains and the F_1 cross of *Steinernema feltiae* (all measurements in µm).

Character	Fungus gnat strain (η = 15)	SN strain (from Poinar. 1986)	F_1 cross (Fungus gnat ♀ x SN ♂) (η = 15)
L	784 (617-857)	817 (736-896)	820 (769-926)
Max. body diam.	29 (22-35)	26 (25-29)	27 (24-29)
Ant. to excret. pore	58 (50-62)	61 (56-66)	65 (62-69)
Ant. to nerve ring	92 (72-101)	98 (88-112)	106 (98-112)
Ant. to pharynx base	122 (107-138)	136 (128-147)	145 (136-152)
Tail length	80 (67-83)	79 (70-88)	83 (80-86)
Ratio a[1]	27 (24-32)	31 (29-33)	32 (31-35)
Ratio b[2]	6.5 (5.4-7.3)	6.0 (5.3-6.4)	6.0 (5.5-6.4)
Ratio c[3]	9.9 (9.2-10.7)	10.4 (9.2-12.6)	10.5 (10.0-10.8)
Ratio d[4]	0.47 (0.41-0.51)	0.45 (0.42-0.51)	0.45 (0.43-0.47)
Ratio e[5]	0.72 (0.65-0.76)	0.78 (0.69-0.86)	0.78 (0.74-0.84)

1. L. divided by maximum body diameter.
2. L divided by distance from anterior end to pharynx base.
3. L divided by tail length.
4. Distance from anterior end to excretory pore divided by distance from anterior end to pharynx base.
5. Distance from anterior end to excretory pore divided by tail length.

Table 2. Length of the cuticular tail spine in first and second generation males of the SN strain, fungus gnat strain and F_1 cross of *Steinernema feltiae* (all measurements in µm).

Strains	*First generation*	*Second generation*
SN	5.4 (3.2-8.0)	8.6 (8.0-9.6)
Fungus gnat	2.0 (0-3.2)	5.9 (4.8-8.0)
F_1 cross (Fungus gnat ♀ × SN ♂)	4.3 (0-8.0)	8.6 (8.0-9.6)

New Zealand, it is commonly found in tussock grass where it attacks lepidopterous larvae.

The fungus gnat strain is the first steinernematid that habitually parasitizes an adult stage of a holometabolous insect. Several adaptations have occurred which makes this possible. The first is the unavailability of the larval fungus gnats. To reach them, the infective stages would have to crawl up out of the soil and enter the mushroom. Since this can not occur, the nematodes only have contact with the mature prepupae, pupae and emerging adult flies which occur in the soil. It is probable that the infective stages collect around the pupae and then enter the adult fly through the anus as soon as it emerges. Entrance into the host's hemocoel would occur shortly after. There are cases of *Steinernema glaseri* being transported by adult Japanese beetles (*Popillia japonica* Newm.), eventually leading to the insects death, but larval parasitism is much more frequent and the former situation was considered important only for nematode dispersal and not development (Girth *et al.*, 1940).

Most hosts of steinernematids are killed by the nematode's symbiotic bacteria within 24 hours after the infective juveniles have penetrated their hemocoel. It is extremely rare for an insect to live 2-3 days after being invaded with a bacterial-carrying infective juvenile. The reason for the prolonged survival of infected fungus gnats in the present case is unknown. The bacteria may multiply more slowly in the fungus gnat's hemolymph which would favor nematode development since if the host was killed too quickly, the cadaver might break down and decompose before the nematodes could mature. The infective juveniles of the fungus gnat strain clearly harbor cells of *Xenorhabdus* since the latter could be isolated from infective stage nematodes in the blood drop crossing experiments. The possibility of this being an attenuated strain of *Xenorhabdus* also exists.

In representing a new strain of *S. feltiae* from North America, the fungus gnat strain provides added variation to the species, not only in quantitative values, but also in host interactions. Host selection in steinernematid nematodes is an unresolved question. However, the present study lends credence to the idea that different strains of *Steinernema* species have adapted to specific host groups in different geographical areas under different environmental conditions (Poinar, 1990). Thus, *S. feltiae* apparently has a bibionid fly strain in Denmark, a lepidoptera strain in New Zealand and a fungus gnat strain in Western North America. None of these strains, however, are restricted to members of their respective host order under laboratory conditions.

Acknowledgments

The author thanks Harry D. Thiers, Department of Biology, San Francisco State University for identifying the mushroom and Paul H. Arnaud, Jr., Department of Entomology, California Academy of Sciences, San Francisco for determination of the fungus gnat.

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