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Asilidae (Diptera: Brachycera) – natural hosts of *Steinernema feltiae* (Rhabditida: Steinernematidae) at the Zemen Gorge region

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Abstract. Invaded with entomopathogenic nematode *S. feltiae* (Filipjev, 1934) larva from family Asilidae was found in an alluvial soil of riverside forest in the region of Zemen Gorge, SW Bulgaria. It is the first record of Asilidae as natural hosts of *S. feltiae*. It seems that insects from the family Asilidae are generally among the suitable hosts of the genus *Steinernema* Travassos, 1927. Members of Asilidae are important predators in the soil, and their susceptibility to nematode invasion must be considered when assessing the impact of entomopathogenic nematodes on beneficial soil insects.

Key words: entomopathogenic nematodes, faunistics, host range, beneficial soil fauna.

Introduction

Entomopathogenic nematodes (EPNs) from the family Steinernematidae (Rhabditida) are an object of diverse studies in many countries, mainly in relation with their potential as biological control agents of insect pests (Hominick 2002). At the same time, as a result of the extensive use of the "*Galleria*-bait" method in faunistical and ecological researches (Bedding & Akhurst 1975) data about natural hosts of EPNs and, therefore, their role in the natural habitats are scarce (Peters 1996). Several cases of finding naturally infected with EPNs insects have been recorded in Bulgaria (Gradinarov *et al.* 2000, Gradinarov 2003). In the present work a new natural host of *Steinernema feltiae* (Filipjev, 1934) from the Zemen Gorge region is reported.

Materials and methods

Nematodes were isolated from soils of riverside habitats in Zemen Gorge (SW Bulgaria) using larvae of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) as a bait insects (Bedding & Akhurst 1975). A total of 296 soil samples were processed during the period of 1998-2011. The infective juveniles were further cultured on *G. mellonella* to provide mature individuals for species identification. The identification of nematodes from the genus *Steinernema* Travassos, 1927 was carried out mainly on the basis of morphometrical characteristics of the male individuals from the first parasitic generation and the infective juveniles (according to Adams & Nguyen 2002) after mounting them on glycerol-paraffin microscopic slides. In order to check for presence of naturally infected hosts, soil excavations were performed at positive for EPNs sites.

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Results and discussion

The species *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae) was repeatedly isolated from soils in the region of Zemen Gorge during the period of investigation (Table 1). It was found in 23 samples, collected from 13 localities. *S. feltiae* is a common species in soils of lowlands and low mountain habitats in Bulgaria and was previously reported from the Zemen Gorge, but without accurate locations (Shishiniova *et al.* 2000). The present study indicated that the species occurs regularly in the alluvial soils of meadows, grasslands and riverside forests, which are typical for the gorge (Table 1).

Table 1. Localities of *S. feltiae* in Zemen Gorge.

Collection date	Site	Habitat
23.05.1998	Near Zemen, N42°28'16'', E22°43'58'', 580m	Riverside grassland
17.09.1998	Vicinity of Razhdavitsa Vill., N42°23'47'', E22°42'08'', 500m	Riverside forest
17.09.1998	Vicinity of Razhdavitsa Vill. N42°23'47'', E22°42'28'', 500m	Riverside forest
17.09.1998	Near Shegava Riv. N42°23'34'', E22°42'35'', 500m	Riverside meadow
20.09.1998	Near the railway tunnel 2, N42°26'49'', E22°42'44'', 570m	Riverside forest
15.03.2000	Shegava Riv. Canion, N42°23'39'', E22°43'05'', 545m	Orchard
14.05.2000	Zemenska Planina Mts. N42°28'27'', E22°43'22'', 650m	Oak-hornbeam forest
29.10.2000	Near the railway tunnel 2, N42°26'49'', E22°42'43'', 580m	Riverside meadow
27.03.2001	Near Shegava Riv., N42°23'34'', E22°42'35'', 500m	Riverside forest
14.04., 25.05.2001	Near Zemen, N42°28'16'', E22°43'58'', 580m	Riverside grassland
16.03.2002	Near Zemen, N42°28'07'', E22°43'41'', 580m	Riverside forest
05.09.2009	Near the railway tunnel 1, N42°28'04'', E22°43'12'', 580m	Riverside forest
24.09., 02.10.2011	Near the railway tunnel 1, N42°27'47'', E22°42'51'', 575m	Riverside forest

The presence of naturally infected insects in one of the positive for *S. feltiae* sites (September and October 2011, N42°27'47'', E22°42'51'') was monitored monthly during May-September of 2012. The investigated habitat was a riverside forest on a deep alluvial soil next to an orchard, with black alder (*Alnus glutinosa* L.), black locust (*Robinia pseudoacacia* L.), dogwood (*Cornus sanguinea* L.), and *Aegopodium podagraria* L. (Apiaceae) as predominant grass plant species (Fig. 1). A total of 19 larvae of the family Asilidae (Diptera: Brachycera) were found in soil of the site on 01.07.2012. Of these, one larva was dead, with typical for steinernematids symptoms of infection - soft, uniformly yellow colored body. After dissection three days later five ♀♀ and one ♂ nematodes from the first parasitic generation of the species *S. feltiae* (Fig. 2, 4) were isolated.

Fig.1. Fragment of the investigated habitat with *Aegopodium podagraria.*

Fig.2. Nematodes emerging from the asilid host larva. Scale bar: 1 mm.

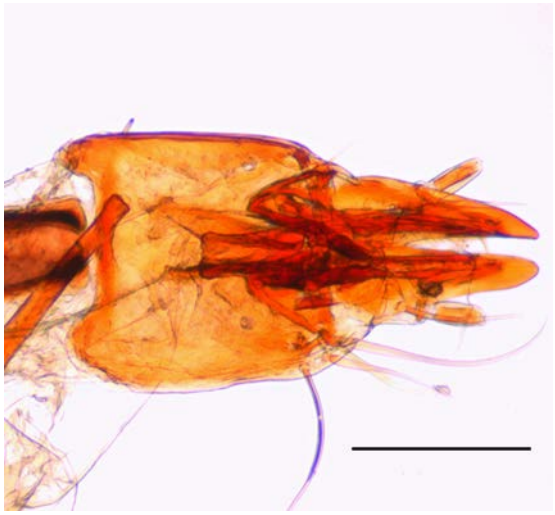

Fig. 3. Head capsule of the host larva. Scale bar: 0.3 mm.

Fig. 4. Caudal view of the male *S. feltiae* isolated from the host. Scale bar: 25 μm.

Because of the difficulties of identification of preimaginal stages, the host remains unidentified to a species level. The presence of mandibles and the morphology of the maxillary complex show that it belongs to Asilinae (Fig. 3) (Giljarov 1964). At the same site live larvae of other Asilidae, Therevidae (Diptera), live larvae and imago of Orphnidae, Elateridae, Curculionidae, Tenebrionidae (Coleoptera) and larvae of some other insect families were also found.

It is the first record of Asilidae as natural hosts of *S. feltiae*. Even though *S. feltiae* is frequently found in soils of different habitat types at low altitudes, this is the first report of natural host for this species in Bulgaria. Despite the relatively low host specialization, revealed by experimental studies, it appears that in the natural habitats EPN show preferences to a few insect orders (Peters 1996). This fact reflects the frequency of finding natural hosts from different insect orders for concrete nematode species, as well as their suitability as biocontrol agents (Gergis *et al.* 2006). Thus, *S. feltiae* has repeatedly been

found in different species from order Diptera (Poinar & Lindhardt 1971, Poinar 1992) and has been used successfully in the biological control of its representatives (Gergis *et al.* 2006). According to Poinar (1992), some populations of the species can be adapted to hosts from Diptera. Our observation may confirm this opinion. On the other hand, other species of the genus *Steinernema* (*S. bicornutum* Tallosi, Peters & Ehlers, 1995 and probably *S. kraussei* (Steiner, 1923)) were found to infect larvae of Asilidae in other studies in Bulgaria (Gradinarov 2003), which shows that the insects of the family are generally suitable hosts of the genus. Larvae of Asilidae are among the major predators in the soils of many habitats, and their susceptibility to nematode invasion has to be considered when assessing the impact of EPNs on beneficial soil insect fauna.

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