

## Development of *Steinernema feltiae* (Rhabditida: Steinernematidae) in larvae of *Chaetonyx robustus* (Coleoptera: Orphnidae)

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**Abstract.** *Steinernema feltiae* (Filipjev, 1934) infects and reproduces in larvae of *Chaetonyx robustus* Schaum, 1862 (Coleoptera: Orphnidae), isolated from soil of the same habitat. Insects of the family Orphnidae are reported for the first time as hosts of entomopathogenic nematodes. Lack of establishment of natural infected larvae may be due to the lower susceptibility of *C. robustus* to nematode invasion and random factors related to the spatial distribution of infective juveniles in soil.

**Key words:** entomopathogenic nematodes, non-target hosts, parasite ecology.

### Introduction

Infective juveniles (IJs) of entomopathogenic nematodes (EPNs) of the family Steinernematidae (Rhabditida) release pathogenic bacteria of the genus *Xenorhabdus* Thomas and Poinar, 1979 into the host haemolymph. Insects die from septicemia and nematodes feed with the decomposed tissues. Depending on the host size, one or more amphimictic generations develop and the new IJs leave the cadaver (Woodring & Kaya 1988). Laboratory experiments for susceptibility are mainly implemented with pests in order to use the nematodes as biological control agents (Georgis *et al.* 2006). Little attention is paid to non-target species, some of which can be natural hosts of nematodes, and could be affected by the use of EPNs as bioagents (Harvey *et al.* 2012). The aim of this work is to verify the capability of *Steinernema feltiae* (Filipjev, 1934) to infect and reproduce on larvae of *Chaetonyx robustus* Schaum, 1862 (Coleoptera: Orphnidae), isolated from soil of the same habitat as nematodes.

### Materials and methods

Culture of *Steinernema feltiae*, isolated from soil of riverside forest in Zemen Gorge (SW Bulgaria, N 42°27'48'', E 22°42'51'', 02.10.2011) and maintained on larvae of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) was used for the laboratory

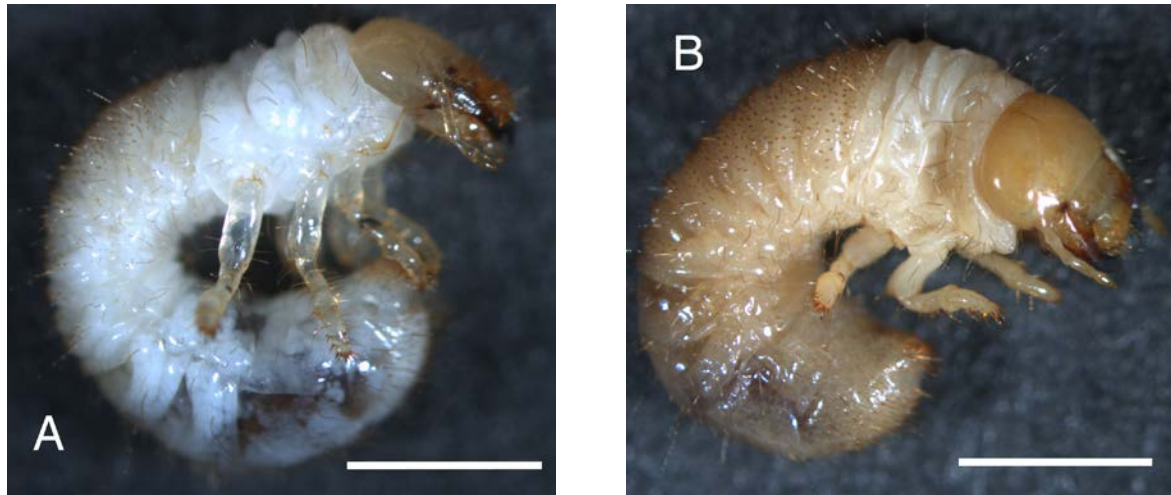
experiments. At the same location 70 live larvae of *Chaetonyx robustus* were collected on 30.04.2012. Imago and larvae of this species has been established there in previous studies (Fig. 1). In the laboratory experiments 20 of the 70 larvae collected (weight 9-15 mg, average 12, 2 mg) were exposed to IJs of *S. feltiae* with a dose of 150 IJs per larva. The experiments was carried out in Petri dishes on filter paper (after Woodring & Kaya 1988) at  $20 \pm 2^\circ\text{C}$ . Larvae of *G. mellonella* (n=20), infected in the same conditions and dose, were used as a control. New IJs, emerged from the *C. robustus* larvae and obtained after dissection of cadavers in 0, 9% NaCl were counted. The IJs, obtained from *C. robustus* were used to infect 10 new larvae of *G. mellonella* under the same experimental conditions.



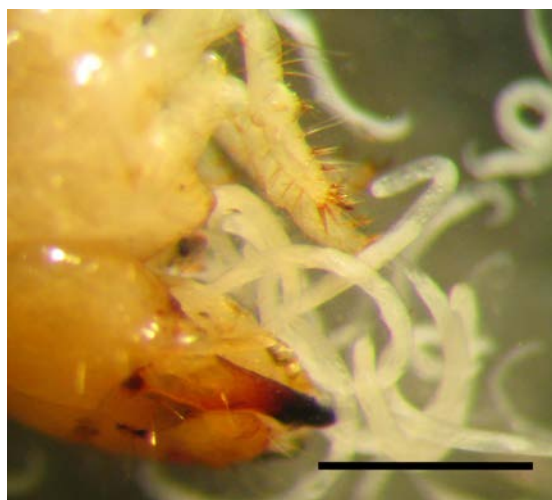
**Fig. 1.** Imago and larvae of *Chaetonyx robustus*, collected in soil of the investigated habitat (22.04.2011, D. Gradinarov leg.). Scale bar: 10 mm.

### Results and discussion

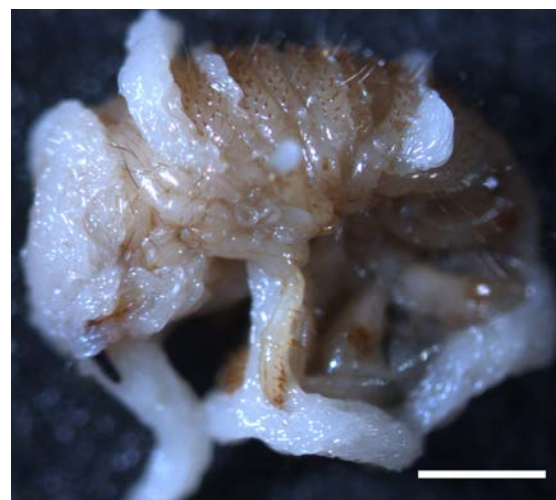
Dead larvae for both tested insect species were registered on the second day after the start of the experiment. At the third day 100% mortality was recorded for *Galleria mellonella* in the control and 60% for *Chaetonyx robustus* (12 larvae died, with typical symptoms of bacterial infection) (Fig. 2). For two of the infected larvae of *C. robustus* was observed atypical migration of mature individuals from the first parasitic generation with no further development of nematodes (Fig. 3). Migration of new IJs from the rest *Chaetonyx* larvae, as well as from *Galleria* larvae, started on the seventh day (Fig. 4). On the eleventh day seven larvae of *C. robustus* with apparently completed parasite development have been washed and dissected for collecting the nematodes. The number of the IJs, obtained by both methods is presented in Table 1. In all inspected host larvae the parasite development was completed to new IJs. In two cases, a sparse mature second generation of nematodes was observed. The IJs, obtained from *C. robustus*, were capable to infect larvae of *G. mellonella*, causing 100% mortality, with subsequent development of nematodes.



**Fig. 2.** Liva (A) and infected larva (B) of *Chaetonyx robustus*. Scale bar: 2 mm.



**Fig. 3.** First parasitic generation emerging from host. Scale bar: 1 mm.



**Fig. 4.** Juveniles migration from a host. Scale bar: 1 mm.

**Table 1.** Nematodes, collected from *Chaetonyx* larvae.

Nematodes	Individual <i>Chaetonyx</i> larvae							Mean ± STD
	1	2	3	4	5	6	7	
IJs (washing)	5593	3961	3621	2354	649	1376	957	2644 ± 1821
IJs (dissecting)	229	1143	391	1262	498	147	1012	669 ± 459
TOTAL	5822	5104	4012	3616	1147	1523	1969	3313 ± 1816
Adults (2 <sup>nd</sup> gen.)	-	-	-	-	-	+	+	

Experiment performed showed that *S. feltiae* is capable to infect and reproduce in larvae of *C. robustus*. Insects of the family Orphnidae are reported for the first time as hosts of EPNs. At the dose used development completed generally with one parasitic generation. The mortality of *C. robustus*, resulting from nematode invasion at a dose of 150 IJs per host,

is not high and not in all infected larvae parasites complete their development. The quantity of new IJs is significant, compared to the host size, and they are capable to invade new hosts.

*Chaetonyx robustis* often forms aggregations in riverside soils of forest habitats in Zemen Gorge (Gradinarov, Petrova, unpublished data). In these habitats *S. feltiae* is also a common species (Gradinarov 2012). Correlation between insect aggregations and the presence of EPNs in the soil is shown by Mráček & Bečvař (2000). Considering the ability of nematodes to fully complete their development in larvae of *C. robustis* and its abundance at the investigated habitat, it is probably one of the hosts of *S. feltiae* there. At the same locality *S. feltiae* was found in naturally infected larva of Asilidae (Diptera) (Gradinarov 2012). *S. feltiae* is among EPNs species with numerous known natural hosts (Peters 1996) and it possibly utilises more than one insect species at the investigated site. The lack of finding naturally infected *Chaetonyx* larvae may be due to the moderate species susceptibility to the nematode invasion, shown in this experiment, as well as random factors related to the spatial distribution of IJs in the soil and the absence of nematode aggregation with required for successful invasion density (Spiridonov *et al.* 2007) in our study.

### References

- Georgis, R., Koppenhöfer, A. M., Lacey, L. A., Bélair, G., Duncan, L. W., Grewal, P. S., Samish, M., Tan, L., Torr, P. & van Tol, R. W. H. M. (2006) Success and failures in the use of parasitic nematodes for pest control. *Biological Control*, 38: 103-123.
- Gradinarov, D. (2012) Asilidae (Diptera: Brachycera) – natural hosts of *Steinernema feltiae* (Rhabditida: Steinernematidae) at the Zemen Gorge region. *ZooNotes*, 34: 1-4.
- Harvey, C. D., Alameen, K. M. & Griffin, C. T. (2012) The impact of entomopathogenic nematodes on a non-target, service-providing longhorn beetle is limited by targeted application when controlling forestry pest *Hylobius abietis*. *Biological Control*, 62: 173–182.
- Mráček, Z. & Bečvař, S. (2000) Insect aggregation and entomopathogenic nematode occurrence. *Nematology*, 2: 297-301.
- Peters, A. (1996) The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect population. *Biocontrol science and technology*, 6: 389-402.
- Spiridonov, S. E., Moens, M. & Wilson, M. J. (2007) Fine scale spatial distributions of two entomopathogenic nematodes in a grassland soil. *Applied Soil Ecology*, 37: 192-201.
- Woodring, L. J. & Kaya, K. H. (1988) *Steinernematid* and *Heterorhabditid* nematodes: hand book of biology and techniques. *Southern Cooperative Series Bulletin. A publication of the nematode subcommittee of the Southern Research Project S135- Entomopathogens for use in Pest Management Systems*. Arkansas Agricultural Experimental Station, Fayetteville, 331: 1-30.