ZooNotes 57: 1-4 (2014)
www.zoonotes.bio.uni-plovdiv.bg
ISSN 1313-9916

Chaetonyx robustus (Scarabaeidae: Orphninae) – new natural host of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae)

DENIS GRADINAROV

Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tzankov Blvd., 1164 Sofia, Bulgaria, e-mail: dgradinarov@abv.bg

Abstract. *Heterorhabditis bacteriophora* Poinar, 1976 was isolated from single larva of *Chaetonyx robustus* Schaum, 1862 (Scarabaeidae: Orphninae) from soil of a riverside habitat near town of Zemen, SW Bulgaria. Insects from the subfamily Orphninae are reported for the first time as natural hosts of heterorhabditids. In laboratory conditions larvae of *C. robustus* exhibited a low susceptibility to nematodes, which probably results from adaptation to avoid nematode invasion, arising from continuous coexistence in natural habitats.

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

Introduction

Entomopathogenic nematodes (EPNs) (Rabditida: Steinernematidae, Heterorhabditidae) are lethal parasites of insects used for biological control (Burnell & Stock 2000, Georgis *et al.* 2006). Infective juveniles of the genus *Heterorhabditis* Poinar, 1976 release into the host haemolymph entomopathogenic bacteria from the genus *Photorhabdus*, which causes rapid host death and gives specific reddish color of the dead insects. Despite the low host specialization exhibited in laboratory conditions, the natural host range of EPNs is insufficiently investigated (Peters 1996, Lewis & Clarke 2012). For Bulgaria, only *Drasterius bimaculastus* (Rossi, 1790) (Coleoptera: Elateridae) has been reported as a natural host of heterorhabditid nematodes (Gradinarov 2003). In the present work a case of naturally infected with *Heterorhabditis bacteriophora* Poinar, 1976 larva of *Chaetonyx robustus* Schaum, 1862 (Scarabaeidae: Orphninae) and data on the host susceptibility to nematode infection are reported.

Material and Methods

The soil excavations were conducted on 15 June 2013 in a riverside habitat with alluvial soil near town of Zemen (SW Bulgaria, 42° 27.8'N, 22° 42.85'E). In the same locality EPN species *Steinernema feltiae* (Filipjev, 1934) has been previously established (Gradinarov 2012). The soil from a sample with dimensions 30x30x30 cm was examined for the presence of infected insect larvae. The infective juveniles (IJs) from the infected host were reared on larvae of *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae) for obtaining a

Accepted: 09 June 2014
Published: 16 June 2014
Университетско издателство "Паисий Хилендарски"
Plovdiv University Press "Paisii Hilendarski"

laboratory culture (ZGS-isolate). The nematode identification was performed on the morphology of IJs and male individuals (according to Nguyen 2007), reared on larvae of *G. mellonella*. The prepared microscope slides are deposited in the collection of the Department of Zoology and Anthropology, Sofia University (slides HR-Ch-1-18).

In the laboratory experiment larvae of *Chaetonyx robustus* (weight 8-10 mg), collected in the same locality on 01 September 2013, were used. Before the experiment the larvae were kept individually at 20°C for three days in quarantine. Infectivity tests were carried out in 2 ml Eppendorf tubes with perforated lids, filled with 1.5 cm^3 quartz sand (0.6 mm) (after Fairbairn *et al.* 2000). Approximately 500 IJs from ZGS-isolate were added on the sand surface with 100 µl of water, and a single *Chaetonyx* larva was release in each tube. Controls received tap water only. In additional treatment *G. mellonella* larvae, treated according the same experiment scheme, were used. Experiment was conducted in 30 replicates for treatments and the control. Tubes were incubated at room temperature (19-21°C) and mortality of the test insects was recorded on the fourth and sixth day.

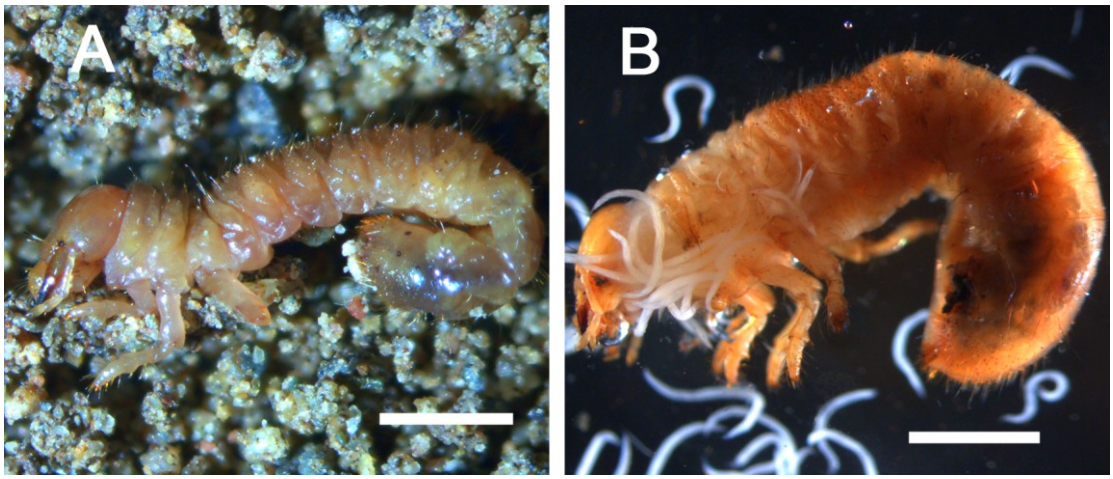

Fig. 1. Larvae of *Chaetonyx robustus* infected with *Heterorhabditis bacteriophora*. A: Naturally infected larva; B: Larva, infected at the laboratory experiment. Scale bars: = 1 mm.

Results and Discussion

In soil excavations 72 larvae of *Chaetonyx robustus* were detected. One larva (1.39%) was infected with EPNs (Fig. 1A). By morphology of IJs and male individuals (Fig. 2) nematodes were identified as *Heterorhabditis bacteriophora* Poinar, 1976. The measurements of IJs (n=30) are as follows: body length (L): 608.1 µm ± 21 (552 – 648); greatest body width (W): 23.5 µm ± 1 (22 – 25); distance to nerve ring: 85.2 µm ± 2.5 (78 – 89); distance to excretory pore (EP): 108.8 µm ± 2,7 (103 – 114); pharynx length (ES): 128.6 µm ± 3.9 (119 – 134); tail length (T): 96.5 µm ± 4 (85 – 103); ratio *a* (L/W): 25.9 ± 1(23.9 – 28.3); ratio *b* (L/ES): 4.7 ± 0.2 (4.4 – 5.3) and ratio *c* (L/T): 6.3 ± 0.2 (6.0 – 6.7).

In the laboratory experiment four larvae of *C. robustus* (13% mortality) were infected with nematodes on the fourth day. No further mortality was recorded on the sixth day. The nematode development was confirmed by dissection of one infected larva (Fig. 1B). In the control no host mortality occurred. All treated larvae of *Galleria mellonella* were dead and infested on the fourth day after infection.

Insects from Scarabaeidae are among the most commonly found natural hosts of *H. bacteriophora* (Peters 1996), and the present report is in accordance with this specialization. Previous studies have shown the ability of *Steinernema feltiae* to reproduce in larvae of *C. robustus* (Gradinarov & Petrova 2012). Both EPN species are common in soils at Zemen Gorge region (Gradinarov 2012, Gradinarov *et al.* 2012), and apparently utilize the same

insect species. In the present research, as well as in experiments with *S. feltiae*, larvae of *C. robustus* are less susceptible to nematode infection than *Galleria* larvae. This may resulted from some morphological, physiological or behavioral adaptations of the host. Especially, in the case of *H. bacteriophora* these adaptations are displayed both at natural and experimental conditions. In other studies in Bulgaria, concerning the relations between soil insects and EPNs in habitats with low anthropogenic impact, the host infection was sporadic or not observed at all (Gradinarov 2012, Mutafchiev & Gradinarov 2003). It is most probably that the continuous coexistence of EPNs and their hosts in natural habitats decreases the antagonistic relationships between their populations.

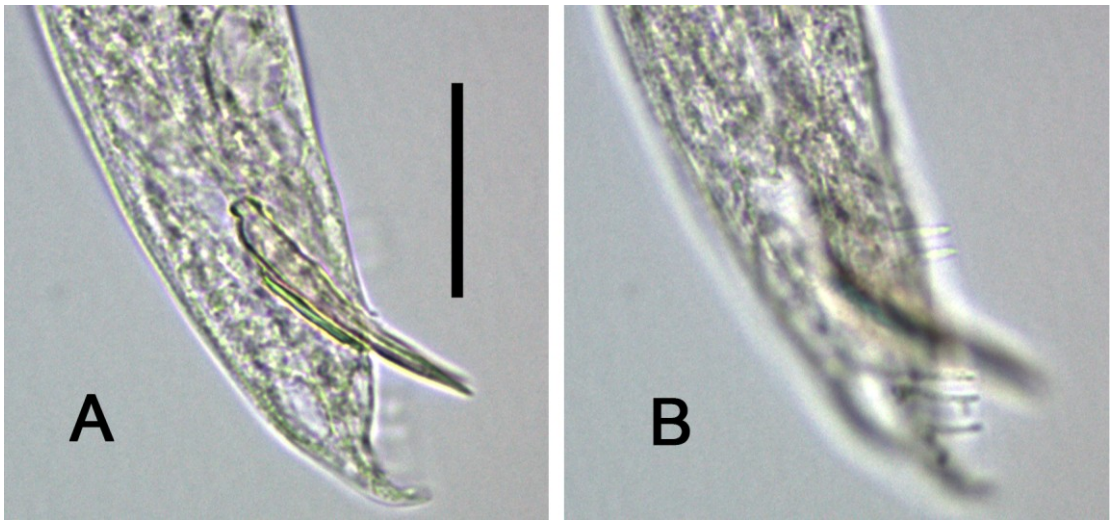

Fig. 2. Male of *Heterorhabditis bacteriophora* ZGS-isolate, obtained from *Galleria mellonella* larvae. A: Spicules and gubernaculum; B: Bursal papillae. Scale bar = 30 μm.

References

Burnell, A. M. & Stock, S. P. (2000). *Heterorhabditis*, *Steinernema* and their bacterial symbionts-lethal pathogens of insects. *Nematology*, 2 (1): 31-42.

Fairbairn, J. P., Fenton, A., Norman, R. A. & Hudson, P. J. (2000) Re-assessing the infection strategies of the entomopathogenic nematode *Steinernema feltiae* (Rhabditidae; Steinernematidae). *Parasitology*, 121 (2): 211-216.

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. (2003) New Natural Hosts of Entomopathogenic Nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) from Bulgaria. *Acta Zoologica Bulgarica*, 55 (3): 59-64.

Gradinarov, D. (2012) Asilidae (Diptera: Brachycera) – natural hosts of *Steinernema feltiae* (Rhabditida: Steinernematidae) at the Zemen Gorge region. *ZooNotes*, 34: 1-4.

Gradinarov, D. & Petrova, Y. (2012) Development of *Steinernema feltiae* (Rhabditida: Steinernematidae) in larvae of *Chaetonyx robustus* (Coleoptera: Orphnidae). *ZooNotes*, 36: 1-4.

Gradinarov, D., Petrova, E., Mutafchiev, Y. & Karadjova, O. (2012) Distribution of entomopathogenic nematodes of the genus *Heterorhabditis* (Rhabditida: Heterorhabditidae) in Bulgaria. *Nematologia Mediterranea,* 40: 173-180.

Lewis, E. E. & Clarke, D. J. (2012) Nematode parasites and entomopathogens. *In:* Vega, F. E. & Kaya, H. K. (Eds.), *Insect Pathology, 2nd ed.*, Elsevier, Amsterdam, The Netherlands, pp. 395-424.

Mutafchiev, Y. & Gradinarov, D. (2003) Entomopathogenic Nematodes of the Families Steinernematidae and Heterorhabditidae (Rhabditida) in the Vicinity of the Zheleznitsa Village, Vitosha Mt., Bulgaria. *Acta Zoologica Bulgarica*, 55 (2): 67-74.

Nguyen, K. B. (2007) Methodology, morphology and identification. *In:* Nguyen, K. B. & Hunt, D. D. J. (Eds.), *Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts*, Brill Publishing, Leiden, The Netherlands, pp. 59-119.

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.