

NEW DATA ABOUT PREVALENCE OF *ENDORETICULATUS*  
*POECILIMONAE* (MICROSPORIDIA: ENCEPHALITOOZONIDAE)  
IN THE GRASSHOPPER *POECILIMON THORACICUS*  
(ORTHOPTERA: TETTIGONIIDAE)

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**Abstract:** A microsporidium belonging to the genus *Endoreticulatus* was found in a Bulgarian population of the bellied bright bush cricket *Poecilimon thoracicus* (Tettigoniidae), a widely distributed grasshopper species in Europe. The hosts were collected in 2014, 2015 and 2016 in Gabrovnitsa site. The pathogen produced intense infections in the mid gut and gastric caeca of the host. The lowest prevalence of *E. poecilimoni* was recorded in mid June of 2016 (40.0%). The highest infection rate was observed in the beginning of July 2016 (94.4%). No statistical significant differences in the prevalence of *E. poecilimoni* in males (55.9%) and females (56.4%) of *P. thoracicus* were detected.

## INTRODUCTION

Microsporidia are eukaryotic, unicellular pathogens related to the Fungi. Together with the Aphelida and Cryptomycota they form a sister group (Opisthosporida) of the true Fungi (Karpov et al. 2014). Microsporidia infect invertebrate and vertebrate animals from all major taxa. The most commonly recorded hosts are insects. So far it has been found that at least 80 microsporidian genera infect ninety insect species (Solter et al. 2012). Microsporidia often play an important role in the regulation of insect populations. They cause rather chronic infection which leads to low or moderate mortality. Microsporidian infection frequently decreases host reproduction and feeding, and epizootics in the host

population can reduce population size. In general microsporidia are probably more suited for use as classical biological control agents that are introduced to augment epizootics or into naïve host populations to initiate establishment.

Twenty two microsporidian species belonging to ten genera have been reported or described from grasshoppers in the families Acrididae, Tettigoniidae, Gryllidae, Pyrgomorphidae, Romaleidae and Tristiridae (Henry, 1967, 1971; Sprague, 1977, Issi and Krylova, 1987; Toguebaye et al., 1988; Wang et al., 1991, Streett and Woods, 1993; Lange et al. 1995, 1996, 2009; Sokolova et al. 2006). These include eight *Nosema* species, three *Liebermannia* species, two *Paranosema* species, and single species in the genera *Perezia*, *Tubulinosema*, *Vairimorpha*, *Heterovesicula*, *Johenrea*, *Encephalitozoon* and one *Endoreticulatus* species. Three species have not been identified to genus level (Sokolova et al. 2006; Lange et al. 2009).

*Endoreticulatus poecilimoni* was described from *Poecilimon thoracicus* (Tettigoniidae) collected in one site in Northwestern Bulgaria. Field population of *P. thoracicus* persisted at high densities over a 3-year period. Prevalence of the microsporidium increased over the active feeding season yearly and averaged 22.2%, ranging from a low of 5.7% in early June to a high of 88.2% in late July 2011 (Pilarska et. al. 2015).

The aim of this study is to present new data about persistence of *Endoreticulatus poecilimoni* in one *Poecilimon thoracicus* population, and also data about its prevalence depending on the host gender.

## MATERIALS AND METHODS

Nymphs and adults of *P. thoracicus* were collected from the village of Gabrovnitsa (43°05.331'N: 023°27.626'E; Northwestern Bulgaria in 2014, 2015 and 2016 (Table 1). The grasshoppers were transported to the laboratory and refrigerated to reduce activity and prevent transmission of the pathogen prior to dissection. The individuals collected in 2014 and 2015 were frozen and kept until 2016 for dissection and diagnosis. Internal organs of *P. thoracicus* were examined for presence of microsporidia under light microscopy (400x). Gut tissues of infected insects were smeared on slides, fixed with methanol and stained with Giemsa (Sigma Diagnostic Accustain) (Becnel 2012).

$\chi^2$  test was used for statistical calculation of differences in infection prevalence in male and female individuals of *Poecilimon thoracicus* (Essi 1987).

## RESULTS AND DISCUSSION

The microsporidium *Endoreticulatus poecilimonae* was found in a population of the bellied bright bush cricket *Poecilimon thoracicus* (Tettigoniidae) from Northwestern Bulgaria, near the village of Gabrovnitsa. The lowest prevalence of *E. poecilimoni* was recorded in mid-June of 2016 (40.0%) (Table 1). The highest infection rate was observed in the beginning of July 2016 (94.4%). The average infection rate in 2016 was 68.1% and the average prevalence for the three

collection years was 56.1%. Similarly to Pilarska et al. (2015) it was found that prevalence of the microsporidium increased within the feeding season of the host every year (Table 1.). However, density of the host was high in the collection site each year which is in accordance with the observation of Pilarska et al. (2015). This suggests that the pathogen does not impact seriously the host density.

The lowest prevalence of the microsporidium in male individuals was 53.8% recorded in late June and the highest was in beginning of July - 83.3%. The highest infection rate -100%, was detected in female individuals collected in the beginning of July (end of the feeding season). However, no statistical significant differences in the prevalence of *E. poecilimoni* in males and females in different collections of *P. thoracicus* was detected ( $P>0.05$ ).

Table 1. Prevalence of *E. poecilimoni* in male and female individuals of *Poecilimon thoracicus*

Collection date	Number investigated			Number infected/ prevalence (%)		
	males	females	Total	males	females	Total
<b>2014</b>						
28.06.	17	15	32	11/64.7	7/46.7	18/56.3
<b>2015</b>						
14.06.	50	50	100	20/40.0	20/40.0	40/40.0
<b>2016</b>						
12.06.	20	17	37	11/55.0	6/35.2	17/45.9
27.06.	13	14	27	7/53.8	8/57.1	15/55.5
3.07.	21	32	53	17/80.9	26/81.3	43/62.3
9.07.	6	12	18	5/83.3	12/100.0	17/94.4
<i>Subtotal</i>	<i>60</i>	<i>75</i>	<i>135</i>	<i>40/66.6</i>	<i>52/69.3</i>	<i>92/68.1</i>
<b>Total</b>	<b>127</b>	<b>140</b>	<b>267</b>	<b>71/55.9</b>	<b>79/56.4</b>	<b>150/56.1</b>

*E. poecilimoni* infects intensively the mid gut and gastric caeca of the host. Mature spores contained single nucleus with oval shape. They measured 2.58 x 1.34  $\mu\text{m}$  with 16 to approximately 32 spores situated in a parasitophorous vacuole formed by the host cell. We consider that *E. poecilimoni* is transmitted only orally while Goertz and Hoch (2008) reported that another *Endoreticulatus* - *E. schubergi* infecting the gut host was transmitted both orally and via a transovum route, most likely on the surface of eggs.

Our study reported here confirmed the persistence of *E. poecilimoni* in one *P. thoracicus* population and revealed that there is no differences in the prevalence of the pathogen in both host gender. Despite the intense gut infection it seems that this microsporidium does not affect the host density. However further long term studies are needed to characterize the impact of *E. poecilimoni* on *P. thoracicus* density.

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