Микроспоридиални и гъбни инфекции в пеперуди и правокрили от България

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Microsporidian and fungal infections in Lepidoptera and Orthoptera in Bulgaria

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Резюме: Четиристотин шестдесет и три гъсеници от 10 вида пеперуди, събрани от дървета и храсти през пролетта и лятото на 2017, 2018 и 2019 г. в 5 находища от Северозападна и Южна България са изследвани за наличието на микроспоридиални и гъбни патогени. Също така са изследвани 77 екземпляра на скакалеца *Poecilimon thoracicus* (Orthoptera, Tettigoniidae), събрани от различни храсти и многогодишни растения през пролетта и лятото на 2017 г. Проведените микроскопски анализи показват наличието на микроспоридиална инфекция, причинена от *Endoreticulatus poecilimonae* в скакалеца *Poecilimon thoracicus* и гъбна инфекция в гъсеници на *Erannis defoliaria,* причинена от *Entomophaga auliciae*. Изследванията покават, че средната екстензивност на заразяване с микроспоридията *E. poecilimonae* е 57,1%. При наблюдаваната епизоотия е установена 100% зараженост с *E. auliciae*. *E auliciae* е ефикасен гъбен патоген, който причинява силни епизоотии и може да бъде използван като класически или подсилващ биологичен агент.

Ключови думи: микроспоридии, ентомопатогенни гъби, скакалци, пеперуди

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Abstract: Fourteen hundred and sixty three larvae of 10 lepidopteran species collected from trees and bushes in the spring and summer of 2017, 2018 and 2019 from 5 localities in Northwest and South Bulgaria were investigated for presence of microsporidian and fungal pathogens. Also, 77 grasshopper individuals of *Poecilimon thoracicus* (Orthoptera, Tettiigoniidae) collected from various shrubs and perennial plants in the spring and summer of 2017 were examined. Conducted microscopic analyses showed the presence of microsporidian infection caused by *Endoreticulatis poecilimonae* in *P. thoracicus* and fungal infection in the larvae of mottled umber, *Erannis defoliaria* caused by *Entomophaga auliciae*. The studies showed that the average infection rate with the microsporidium *E. poecilimonae* was 57.1%. The prevalence of the fungus *Entomophaga auliciae* was 100% during the observed epizootic. *E. auliciae* is an efficient fungal pathogen which causes strong epizootics and can be used as classical or augmentation biological agent.

Keywords: microsporidia, entomopathogenic fungi, grasshoppers, moths

One of the most important pest species in Bulgaria are the lepidopterans of the families Erebidae, Tortricidae, Geometridae, Noctuidae, Lasiocampidae the invasive representatives of Crambidae and some grasshoppers of the order Orthoptera, family Tettigoniidae [Zaemdzhikova et al. 2019].

The need to reduce the negative impact of insecticides and preserve biodiversity in natural ecosystems requires the search for and use of host specific biological agents (fungi and microsporidia) and means of controlling these pests.

In order to explore the potential for the use of biological insect pest control agents, it is crucial to investigate their pathogens by examining their species composition and distribution.

Microsporidia are obligate, intracellular pathogens infecting all major taxa of animals. They form a sister group, Opisthosporida, of the true Fungi [Karpov et al. 2014]. More than 90 microsporidian species are reported from insect hosts [Solter et al. 2012]. These pathogens are regarded as candidates for classical biological control agents. So far, 14 microsporidian species have been reported from lepidopterans (Erebidae, Tortricidae and Noctuidae) [Pilarska et al. 2015; Vladova et al. 2017] and 3 microsporidia from grasshoppers (Tettigoniidae and Acrididae) [Golemansky et al. 1998; Pilarska et al. 2015].

Entomopathogenic fungi are regarded also as efficient biological agents which cause severe mortality and reduce the population density of the pest populations. In Bulgaria Draganova et al., [2004], Draganova, Lecheva [2001], Draganova et al. [2013] and Pilarska et al. [2001, 2018] have conducted investigations of the naturally occurring mycoses of butterflies and grasshoppers and reported 6 fungal species from Ascomycota (Hypocreales and Eurotiales) and 2 species from Entomophthoromycotina (Entomophthorales).

In this paper we present the results of studies of microsporidia and fungi of lepidopteran and orthopteran pest from natural populations.

Material and Methods

Fourteen hundred and sixty three larvae of lepidopterans belonging to the families Geometridae, Noctuidae, Lasiocampidae, Erebidae and Crambidae were collected from trees and bushes in the spring and summer of 2017, 2018 and 2019 from 5 localities in Northwest and South Bulgaria [Table 1]. Seventy seven grasshopper individuals of *Poecilimon thoracicus* (Orthoptera, Tettiigoniidae) were collected from various shrubs and perennial plants in the spring and summer of 2017.

The collected insects were transported to the laboratory. The identification of lepidopteran larvae was carried out according Patocka et al. [1999], Brutovsky et al. [2004] and of the grasshoppers by Dr. Dragan Tchobanov from the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

In order to prevent horizontal transmission of the pathogens prior dissections and reduce activity the collected insects were refrigerated.

Temporary microscopic preparations were prepared from the internal organs of the hosts (digestive system, reproductive system, Malpighian tubes, fat body, silk glands) and were examined for the presence of pathogens with a Zeiss Axiostar microscope at magnification 100 and 400x. When microsporidia were detected, tissues of infected individuals were smeared on slides, fixed with methanol and stained with Giemsa (Sigma Diagnostic Accustain) [Becnel 2012; Solter et al. 2012]. In the case of fungal infections the lepidopteran larvae were found dead in one of the studied locations.

Results and Discussion

The conducted microscopic analyses of the collected insects showed the presence of microsporidian infection caused by *Endoreticulatis poecilimonae* in specimens of *Poecilimon thoracicus* and fungal infection in the larvae of mottled umber, *Erannis defoliaria* caused by *Entomophaga auliciae* [Table 1]. No infections were found in the other 9 studied insect species.

E. poecilimonae was described in 2015 by Pilarska et al. [2015] from the same locality in Northwest Bulgaria. It infects the mid gut and gastric caeca of *P. thoracicus* nymphs and adults. The spores of the microsporidium are uninucleate measuring in average 2.58 x 1.34 µm [Fig. 1]. The species is transmitted only horizontally [Pilarska et al. 2015]. Later Vladova et al. [2017] found again the pathogen in this locality.

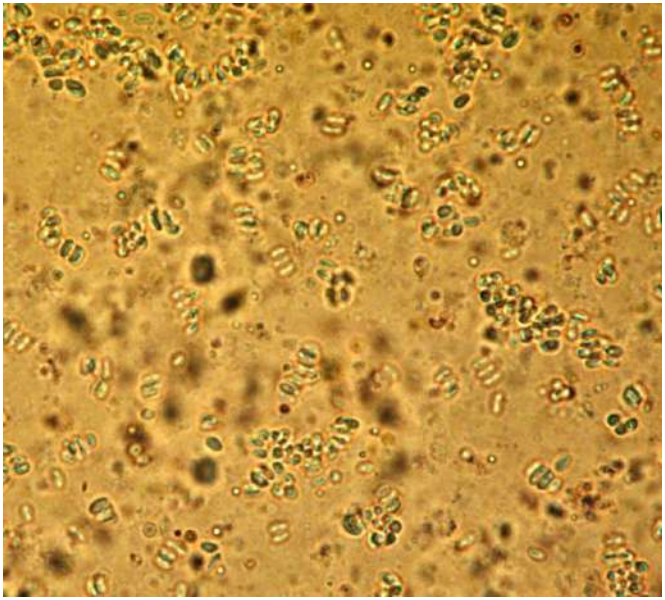

Fig. 1. Alive spores of *Endoreticulatus poecilomonae*, 400x

Our studies showed that the average infection rate with *E. poecilimonae* was 57.1%, which corresponds to the prevalence published by Vladova et al. [2017]. The authors investigated the occurrence of the microsporidium within 3 years [2014 – 2016] and showed that the average infection rate was 56.1%. Similarly to Pilarska et al. [2015] and Vladova et al. [2017] we found that prevalence of the microsporidium increased and was highest in the end of the feeding season of the host [Table 1].

However the density of *P. thoracicus* was not affected. Since the first record of the microsporidium in 2010 [Pilarska et al. 2015] it has been found almost every year ranging from 5.7% in the beginning of May, 2011 to 94.4% in the beginning of July, 2016 [Pilarska et al. 2015; Vladova et al. 2017]. This suggests that the pathogen persists in the population of *P. thoracicus* however does not impact seriously the host density.

The entomopathogenic fungus *Entomophaga auliciae* was detected in dead larvae of *Erannis defoliaria* collected in 2018 in village of Otchin Dol where it caused an epizootic [Table 1]. All larvae were hanging on the tree branches with head down showing symptoms typical for infections caused by the fungi of order Entomophtorales [Fig. 2]. Microscopical anlysis of the dead larvae showed the presence of hyphae, conidia and azygospores of *E. auliciae*.

Table 1. Investigated insect species and found pathogens

Insect hosts	Locality/Date	Number	Found pathogen/ Prevalence (%)
Lepidoptera			
Geometridae			
Erannis defoliaria	Kirkovo/5.05.2017 Slivnitsa/ 9.05.2018 Otchin Dol/14.05.2018 Levishte 22.05.2018 Panitchevo/22.05.18 Levishte/11.05.2018	15 8 56 10 5 10	– – *Entomophaga aulicae*/100 – –
Operophtera brumata	Kirkovo/5.05.2017	53	–
Agriopis leucophaearia	Kirkovo/5.06.2017	36	–
Agriopis aurantiaria	Kirkovo/6.06.2017	7	–
Noctuidae			
Orthosia cruda	Kirkovo/5.05.2017	17	–
Orthosia miniosa	Kirkovo/5.05.2017	17	–
Crambidae			
Cydalima perspectalis	Slivnitsa/1.08.2018 Slivnitsa/18.08.2019	50 48	– –
Lasiocampidae			
Erigaster lanestris	Levishte/5.06.2019	23	–
Erebidae			
Lymantria dispar	Kirkovo/5.05.2017 Levishte/5.06.2018 Levishte/11.05.2019	11 20 22	– – –
Tortricidae			
Tortix viridana	Kirkovo/5.05.2017	55	–
subtotal		**463**	
Orthoptera			
Tettiigonidae			

Poecilimon thoracicus	Levishte/30.06.2017	18	*Endoreticulatus poecilimonae*/44,4
	Levishte/1.07.2017	17	*E. poecilimonae*/58,8
	Levishte/9.07.2017	31	*E. poecilimonae*/61,3
	Levishte/15.07.2017	11	*E. poecilimonae*/63,6
subtotal		**77**	*E. poecilimonae*/ 57.1
Total		**540**	

Fig. 2. Dead larvae of *Erannis defoliaria* hanging from tree branches full with conidia and resting spores of *Entomophaga aulicae*

E. auliciae is distributed in the North hemisphere and has a wide range of lepidopteran hosts – Arctiidae, Geometriidae, Erebidae, Noctuidae, Pyralidae and Tortricidae [Balazy, 1993]. Epizootics caused by *E. auliciae* was observed for the first time in 2000 in larvae of the brown tail moth *Euproctis chrysorrhoea* in Bulgaria [Pilarska et al. 2001]. The authors established the pathogen in 16 out of 72 sites with brown tail moth infestation in Balkan, Sakar, Sredna Gora and Rodope Mountains. Later *E. auliciae* was found again in the same host in the region of town of Asenovgrad where it caused also a strong epizootic [Pilarska et al. 2018].

Conclusions

Despite the lack of established microsporidian infections in the investigated lepidopteran species it is necessary to continue parasitological research by increasing not only the number of the analyzed specimens, but also by covering a greater number of populations of these hosts from different sites.

E. auliciae is an efficient fungal pathogen which causes strong epizootics and can be used as classical or augmentation biological agent. The detection and identification of new pathogens in harmful insects is a step towards the widespread use of biological approaches that conserve natural ecosystems from contamination with chemicals such as pesticides.

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