PRELIMINARY DATA REGARDING BEETLE PARASITE SPECIES COLLECTED FROM DIFFERENT ECOSYSTEMS MET IN DOLJ COUNTY IN 2014-2015

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Abstract. The research on the diversity of parasite and parasitoid beetles from Dolj County exposed in this paper were made between 2009 and 2013. The beetle biological material (193 specimens, 3 of which displaying various parasite forms) was collected from terrestrial ecosystems (Urzicuţa and Breasta). The hosts, from the systematic viewpoint, belong to the order Coleoptera and 2 families: Scarabaeidae and Melolonthidae. The species on which parasites were found are *Orictes nasicornis* (Linnaeus 1758) and *Melasoma populi* (Linnaeus 1758). The identified parasite and predator species from the systematic viewpoint, are *Zicrona caerulea* (Linnaeus,1758) (Hemiptera: Pentatomidae: *Zicrona*) and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Sordariomycetes: Hypocreales: Clavicipitaceae: *Metarhizium*). In this paper we expose the results of research conducted in two species of parasites, the other will be set out in a forth coming paper.

Keywords: parasites, predator, beetles, *Zicrona caerulea, Metarhizium anisopliae.*

Rezumat. Date preliminare privind specii de paraziţi la coleoptere din diferite ecosisteme din judeţul Dolj colectate în perioada 2014-2015. Cercetările privind diversitatea paraziţilor şi parazitoizilor la coleoptere din judeţul Dolj expuse în lucrarea de faţă au fost realizate între anii 2014 – 2015. Materialul biologic de coleoptere (193 exemplare din care 2 exemplare au parazit, respectiv prădător) a fost colectat din ecosisteme terestre (Urzicuţa, Breasta). Gazdele, din punct de vedere sistematic, aparţin ordinului Coleoptera încadrandu-se în 2 familii: Scarabaeidae şi Melolonthidae. Speciile pe care s-au găsit paraziţi, respectiv prădător, sunt: *Orictes nasicornis* (Linnaeus 1758) şi *Melasoma populi* (Linnaeus 1758). Parazitul si prădătorul identificati în urma cercetărilor de specialitate, din punct de vedere sistematic sunt *Zicrona caerulea* (Linnaeus, 1758) (Hemiptera: Pentatomidae: *Zicrona*) şi *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Sordariomycetes: Hypocreales: Clavicipitaceae: *Metarhizium*). În lucrarea de faţă sunt expuse rezultatele cercetărilor efectuate la două specii de coleoptere, celelalte rezultate ale cercetărilor efectuate pe teren şi în laborator, urmând a fi expuse într-o lucrare viitoare.

Cuvinte cheie: paraziţi, prădător, coleoptere, *Zicrona caerulea, Metarhizium anisopliae.*

INTRODUCTION

The purpose of this paper is to present some contributions to the knowledge of the diversity of parasites and predator, analyzing beetle species present in different types of ecosystems in Dolj County. This paper renders the results of the researches conducted between the 2011 and 2013.

All the material found on land has been identified, analysed and then assessed the level of infestation.

The beetle biological material (193 specimens, were various parasitic forms) was collected from the terrestrial ecosystems Urzicuţa and Breasta. The hosts, from the systematic viewpoint, belong to the order Coleoptera and 2 families: Scarabaeidae and Melolonthidae and the identified parasites and predator, from the systematic view point, belong to the two orders: Pentatomidae (genre *Zicrona*) and Clavicipitaceae (genre *Metarhizium*).

MATERIALS AND METHODS

The material used in this paper consists in identifying, analyzing and researching a total of 193 specimens found in the field, on which, there were identified two species of parasites and predators.

The taxonomy and nomenclature of the identified species, is made according to the database Fauna Europea. The species of beetles are presented in systematic order according to the year they were collected and there are mentioned the species of parasite and parasitoids identified for each of them. The material was collected from Urzicuţa and Breasta settlements in 2014-2015. Collections were made at different times each year from April to June and continued. Collection date is mentioned for each species. Moreover, for every locality there are rendered the geographic coordinates, flora and fauna information.

Collection methods were different according to the analysed species.

Collection methods for *Oryctes nasicornis* and *Melasoma populi*: the insect was sampled from the ground with a pair of tweezers and put ajar containing filter paper soaked with alcohol. I took photos and transported the material to the Faculty of Biology, biology laboratory, where the specialists took samples from the surface of the insect body. To analyze the fungi, it was used the solid media culture method (the method of exhaustion and flooding method).

Collection method for *Metarhizium anisopliae*

The method by exhaustion:

- the product is discharged by means of platinum loop in a first sector on suitable solid growth medium;
- poured into petri dish;
- sterilize loop;
- disseminating product previously seeded as parallel grooves;

- sterilize the loop, continue the procedure until the entire surface is covered environment, finishing with a zigzag dissemination;

- is the most used to obtain isolated colonies (that will grow in the last sector).

Collection method for *Zicrona caerulea*

With forceps, after we took photographs and filmed, I got along with the predator of larvae to *C. populi*, and I deposited in the entomological jar. It was then given to Mr. V. Derjanski for determination.

RESULTS AND DISCUTIONS

Host: *Oryctes nasicornis* Linnaeus 1758 (rhinoceros beetle)
Parasites: *Metarhizium anisopliae*
Collection site: Urzicuţa (Fig. 1)
Collection date: March 6, 2015
According to IUCN Status: Threaten species, low risk.

Coordinates: 44°01′00″N 23°33′00″E

Urzicuţa village is located west of the Desnăţui valley, within Oltenia plain, with a plain relief represented by the Danube terraces with relative altitudes between 25 and 40 m; it is crossed by the Desnăţui river in the east and Baboia creek in the south. At the base of the terraces there emerged some springs that formed a chain of pools. In the southern part of the village, there is a lake whose waters were mineralized gradually gaining therapeutic properties in the treatment of rheumatic diseases, known as "Ionele Baths". The climate is temperate continental with a minimum between 5 and -20°C and maximum values between 28 and 38°C. The average amount of precipitation is about 520 mm / year.

Figure 1. Urzicuta and Breasta locality in Dolj county on the map (surse google maps).

Oryctes nasicornis

The species is found in the forest, forest steppe and steppe-like territories.

Adults are active during the period from April to August and survive only one year. The species was reported flying around light sources (PANIN 1957).

Measures of protection and preservation. Protecting old trees of deciduous forests; prohibiting the collection of the species by amateur collectors.

Species included in the Annexes of the Bern Convention as a threatened and rare species.

The insect was sampled from soil with a pair of tweezers and put in a entomologic jar. I took photos and transported the material to the Faculty of Biology, biology laboratory, where the specialists took samples from the surface of the insect body. After laboratory analysis was found muscardini green fungus *M. anisopliae* (Figs. 2, 3).

Figure 2. *Metarhizium anisopliae* to *Orictes nasicornis* female (original).

Metarhizium anisopliae (Metchnikoff) Sorokin

Muscardini green fungus was isolated from *Anisoplia austrian* beetle by Metchnikoff in 1879. He suggested that it can be used as antimicrobial agent against insect pests. Cultures of *M. anisopliae* produce destruxins A, B, C, D, and E and desmethyldestruxin B, toxic to insects. The production of destruxins quickly kills the larvae. *M. anisopliae* produced also toxic proteolytic enzymes (MARK et al., 1989).

Infections of arthropods by *Metarhizium* species are easily recognized a few days after death, when the fungus grows out of the arthropod integument and forms reproductive structures. Initially, one only sees fungal hyphae that appear white, but, as conidia form and mature they often take on a characteristic olive green color. However, depending on the species and strain of *Metarhizium*, spores can range in color from white to yellow to brown and green (TANADA & KAYA, 1993) in TODD UGINE 2014.

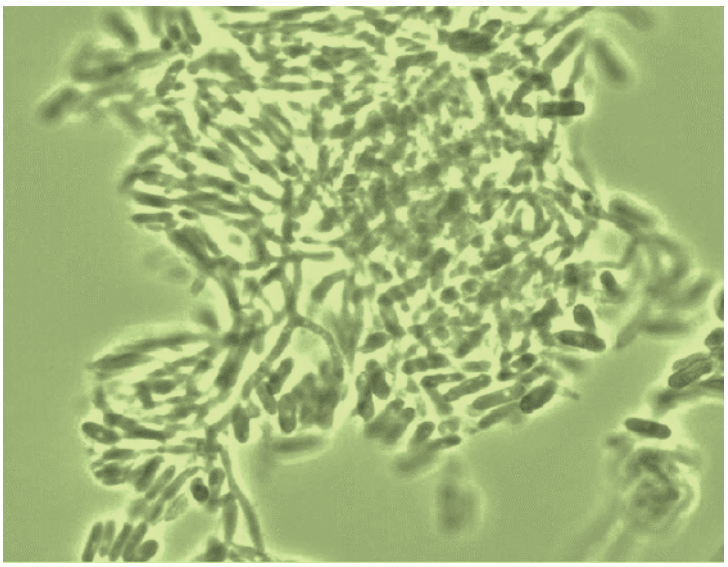

Figure 3. Hyphae at *Metarhizium anisopliae* (original).

On the larvae, *M. anisopliae* also form an opaque white, which turns green at the formation of conidia. Conidiophores are branched, a chain of conidia is formed on each of the conidiophores. The mass of the spore chains becomes dense and in cohorts with other spores produces prismatic masses of columns from the spore chains.

The infection takes place generally through the skin. However, the exact stage of infection depends on the stage of insect, environmental conditions and opportunity. The cuticle is penetrated by enzymes secreted by the penetrating tip of the hyphae. A penetrating hypha gives birth to the hyphal body before the host death. A hyphal body distributed throughout the body cavity gives rise to secondary hyphae.

In humid, warm environments, hypha occurs some days after the death of the insect, usually by weakening the tegument. This fungus also produces more toxic components that can kill the host.

Geographic distribution and hosts – *Metarhizium anisolpliae* is reported to be able to infect more than 100 different species of insects belonging to a wide variety of insect orders.

Host: *Melasoma populi* (Stephens, 1834)
Predator: *Zicrona caerulea* (Linnaeus, 1758)
Collection site: Breasta commune, village Obedin (Fig. 1)
Collection date: July 22, 2014
In this area we have not done research. Therefore the situation is first detected (Figs. 4a, 4b).

The territory of Breasta (coordinates: 44°10′N 23°42′E) settlement is situated in the southern part of the Getic Plateau, within Bălăciţa hilly plain, located west of the Jiu. The relief is represented by wide and long fields separated by the tributaries of the Jiu, NW-SE and W-E directed.The NE part of the territory is crossed by the Rasnic stream, a tributary of the Jiu and in the central part, by Breasta creek, a tributary of the Rasnic. Their beds are generally broad and high, providing favourable conditions for the development of the surrounding settlements. Along the wider sectors of the valleys, there develop floodplains and lower terraces formed from alluvial gravels and sand deposits. Generally, the relief of a hilly plain is one of lacustrine accumulation composed of gravels, sands and thick layers of clays.

Zicrona caerulea can reach an adult size of about 5–8 millimetres (0.20–0.31 in). The body is uniformly metallic blue-green (latin name *caerulea*, is blue). In the immature stage, the abdomen is red with black markings. These bugs are useful predators of leaf beetles in the genus *Altica* (BUG GUIDE), of larvae of various beetles and caterpillars of moths, but it also feeds on plants. Eggs are laid in spring. New adults of this univoltine species can be found from July onwards. This bug overwinters as an adult.

Figure 4a . Early instar nymph (the 4th stage of development) of *Zicrona caerulea* preying a larva of *M. populi* (original).

Figure 4b. Early instar nymph (the 4th stage of development) of *Zicrona caerulea* preying a larva of *M. populi* (original).

Melasoma (Crysomela) populi (Stephens, 1834) (Fig. 5)

This chrysomelid as well as the majority of about 50 Central-European dendrophagous species lives on species of the family Salicaceae and also often as a larva on some of them. Similarly as in many other insect pests its activity is mainly affected by moderate winters and dry and warm springs. Frequent climatic anomalies at the end of the last century and at the beginning of this century markedly affected the water balance of trees and their resistance to insect defoliators. Increased food quality (particularly the higher proportion of sugars) and favourable living conditions manifested themselves in the general increase of the population density of Chrysomelidae (including *C. populi*). The species was identified in localities Bucovăț Forest- 4 specs., July 5, 2005;; Ciuperceni - 1 spec. May 22, 2004; Craiova (the Botanical Garden) - 1 spec., June 20, 2005; Negoi - 7 specs., May 3, 2005; 3 specs., May 2, 2008; Secui - 1 spec., May 26, 2007 (MAICAN & CHIMIȘLIU, 2013) and in Varvoru de Jos (ILIE, 2007).

Figure 5. Larva and adult to *Melasoma (Crysomela) populi* (original).

Records from the patrimony of the museum: Baia de Fier (BOBÎRNAC et al., 1999); Coțofenii din Față, Craiova, Desa (ILIE & CHIMIȘLIU, 1999); Craiova (the Botanical Garden), Zvorsca (CHIMIȘLIU & MOGOȘEANU, 2009) in MAICAN & CHIMISLIU, 2013, but there were no reported parasites, parasitoids or predators.

Natural enemies of *C. populi* are discussed recently, e.g. by TEODORESCU (1980) in Romania and ZEKI &TOROS (1990) in Turkey. Findings on Central-European parasitoids of Chrysomelidae (including *C. populi*) from the family Tachinidae were summarized by TSCHORSNIG & HERTING (1994). For example, *Hexamermis albicans* (v. Sieb.) (Mermithidae) (POINAR 1988) and *Linobia coccinellae* (Sc.) TARASI et al., 2001) rank among parasitoids of *C. populi. Schizonotus sieboldi* (Ratz.) (Pteromalidae) as a widely distributed and important parasitoid of pupae is recently mentioned, e.g. by PETTERSEN (1976) from Norway, DŽANOKMEN (1978) from the European part of the former USSR and LOTFALIZADEH & AHMADI (1998) from Iran. DELLEDONNE et al. (2001) found that transgenic *P. alba* was resistant to larvae of *C. populi.*

They explain the resistance by the enzymatic activity of papain in tissues of this genetically modified poplar inhibiting the digestive proteinases of larvae. The inhibitor could be used in clone programmes for the selection of new poplar genotypes resistant to main insect pests (in URBAN 2006).

CONCLUSIONS

The degree some phyllophagous species of Chrysomelidae feed on leaves leading to the defoliation of trees was used for the study of their occurrence, bionomics and harmfulness. In recent years, attention was also paid to *C. populi*. Generally, with respect to appearance abundance of the pest and its wide area of distribution and considering size and varied colouring, *C. populi* is rather well known. It is dealt with by numerous special entomological and entomological/forest protection papers.

M. anisopliae is well known for its ability to control pest insects. It has been developed into commercial products for use in several countries. A few examples include: Bio-Green and Bio-Cane granules for control of soil grubs of pasture and sugar cane in Australia, Green Muscle for control of locusts in Africa, Ago Biocontrol for control of various pests of ornamental crops in South America, and BioPath for control of cockroaches in the United States. In general, different strains of *M. anisopliae* are species specific, meaning that *M. anisopliae* found to infect one insect species will not necessarily infect other insect species. While this limits its use as a general pest control, it makes the fungus safer by limiting its effects on non-target organisms.

ACKNOWLEDGMENT

Thanks to Mrs. PhD. Ana Popa and Mr. Hab. PhD. Valeriu Derjansky for the material determination.

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Received: March 26, 2015
Accepted: September 4, 2015