PRELIMINARY DATA REGARDING BEETLE PARASITE SPECIES COLLECTED FROM DIFFERENT ECOSYSTEMS MET IN DOLJ COUNTY IN 2009-2013

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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 (296 specimens, 6 of which displaying various parasite forms) was collected from aquatic (Obedin, Craioviţa Nouă Pool) and terrestrial (Bucovăţ, Secui, Park Romanescu) ecosystems. The hosts, from the systematic viewpoint, belong to the order Coleoptera and 4 families: Cerambycidae, Scarabaeidae, Dynastiidae, Melolonthidae. The species on which parasites were found are *Cerambyxcerdo* Linnaeus in 1758; *Oryctesnasicornis* Linnaeus in 1758; *Pentodon idiota idiota* Herbst; *Copris lunaris* (Linnaeus 1758); *Melolontha melolontha* (Linnaeus 1758). The parasites and parasitoids identified from the systematic viewpoint, are varied and grouped as follows: saprophytic fungi (Ascomycetes: Hypocreales: Clavicipitaceae) of the genus *Beauveria* (*Beauveria bassiana*) and genus *Metarhizium* (*Metarhizium anisopliae*); arachnids - *Uropodoidea* sp. (Mesostigmata: Uropodoidea) and nematodes - *Gordius*sp. (Gordioidea: Gordiidae: *Gordius).* The dominant species was the fungus *Beauveria bassiana* identified in *Cerambyx cerdo*, new for Romania in case of this species. In this paper we expose the results of research conducted in two species of parasites (*Beauveria bassiana* identified in *Cerambyx cerdo* and *Uropoda* sp. to *Copris lunaris*); the other will be set out in a forth coming paper.

Keywords: parasites, parasitoids, beetles, ecosystems.

Rezumat. Date preliminare privind specii de paraziţi la coleoptere din diferite ecosisteme din judeţul Dolj colectate în perioada 2009-2013. Cercetările privind diversitatea paraziţilor şi parazitoizilor la coleoptere din judeţul Dolj expuse in lucrarea de faţă au fost realizate între anii 2009 – 2013. Materialul biologic de coleoptere (296 exemplare din care 6 exemplare au diverse forme parazite) a fost colectat din ecosisteme acvatice (Obedin, Balta Craioviţa Nouă) şi terestre (Bucovăţ, Secui, Parcul Nicolae Romanescu). Gazdele, din punct de vedere sistematic, aparţin ordinului Coleoptera încadrandu-se în 4 familii: Cerambycidae, Scarabaeidae, Dynastidae, Melolonthidae. Speciile pe care s-au găsit paraziţi sunt: *Cerambyx cerdo* Linnaeus, 1758; *Oryctes nasicornis* Linnaeus, 1758; *Pentodon idiota idiota* Herbst; *Copris lunaris* (Linnaeus 1758); *Melolontha melolontha* (Linnaeus 1758). Paraziţii şi parazitoizii identificaţi în urma cercetărilor de specialitate, din punct de vedere sistematic, sunt variaţi şi sunt încadraţi astfel: ciuperci saprofite (Ascomicete: Hypocreales: Clavicipitaceae) din genul *Beauveria* (*Beauveria bassiana)* şi genul *Metarhizium (Metarhizium anisopliae)*; arahnide - *Uropodoidea* sp. (Mesostigmata: Uropodoidea) şi nematode - *Gordius* sp. (Gordioidea: Gordiidae: *Gordius*). Specia dominantă este ciuperca *Beauveria bassiana* identificată la *Cerambyx cerdo*, nouă pentru Romania la această specie. Această specie de ciupercă este destul de răspandită însă nu este menţionată frecvent în lucrări de specialitate. În lucrarea de faţă vom expune reultatele cercetărilor efectuate la două specii de paraziţi (*Beauveria bassiana* identificată la *Cerambyx cerdo* şi *Uropoda* sp. la *Copris lunaris*), celelalte urmand a fi expuse într-o lucrare viitoare.

Cuvinte cheie: paraziţi, parazitoizi, coleoptere, ecosisteme.

INTRODUCTION

The purpose of this paper is to present some contributions to the knowledge of the diversity of parasites and parasitoids, analyzing beetle species present in different types of ecosystems in Dolj County.

In recent decades, as a response to environmental issues such as climate changes, land use change or habitat fragmentation intensified the concerns regarding species and spatial variability, especially in the light of the delimitation of areas for conservation.In this context, insects undergo the complex action of ecological factors (climatic factors, soil factorsand biotic) affecting biological cycles of insects, spread emergence of mass propagation or decrease the number of the specimens of certain species, the emergence of new pests, etc.As a result, the number of beetle specimens found in the studied ecosystems was low.

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

The beetle biological material (296 specimens, were various parasitic forms) was collected from aquatic (Obedin Pool, Craioviţa Nouă Pool) and terrestrial (Bucovăţ, Secui, Park Romanescu) ecosystems. The hosts, from the systematic view point, belong to the order Coleoptera and 4 families: Cerambycidae, Scarabaeidae, Dynastiidae, Melolonthidae.

The parasites and parasitoids identified from the systematic view point, are varied and grouped as follows: saprophytic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*); arachnids - *Uropodoidea* sp. and nematodes – *Gordius* sp. The dominant species was the fungus *Beauveria bassiana* identified in *Cerambyx cerdo*, new for Romania in case of this species.

MATERIALS AND METHODS

The material used in this paper consists in identifying, analyzing and researching a total of 296 specimens found in the field, on which, there were identified five species of parasites/parasitoids.

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 from the aquatic ecosystem was collected from Obedin Pool, Craiovița Noua Pool while the one from the terrestrial ecosystem from Bucovăț, Secui, Romanescu Park, between 2011 and 2013. Collections were made at different times each year from April to September. 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 host species.

1. Collection methods for *Cerambyx cerdo* and *Copris lunaris*:

The insect was sampled from the ground with a pair of tweezers and put in a jar 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) and for mites, after taking photos, they were placed in a solution of paraffin and sent to the expert for determination.

2. Collection and research methods for mites

The nests examined in this study were built in sandy soil, in an area where cattle used to rest and, thus, the vegetation was damaged. The top soil was removed using a hoe to uncover the opening of the tunnel, which was of about 2 cm in diameter. In case of *Copris lunaris*, th most of the tunnel passes in a short gallery of about 5 cm deep, leading to the nest chamber. Each of the two examined nests contained one female that was stored in separate glass jars at room temperature until they were transported and examined carefully under the microscope. Using tweezers, mites were collected from *C. lunaris* females, more precisely from the feet and the ventral side of the abdomen.For identification, the mites were prepared in paraffin.

To determine the collected material, there were used the works of PANIN (1957) and MEDVEDEV (1960), the determination of the species of beetles being done by Mrs. Cornelia Chimişliu, PhD, in the entomology laboratory of the Department of Natural Sciences Museum of Oltenia Craiova.The determination of the ascomycetes species was made by Professor Ana Popa, PhD., Faculty of Medicine, microbiology laboratory. Some of the photos were made with DMC-FZ62 Panasonic FullHD digital camera by Lila Gima and another category were made by Marilena Popescu - chemist in the laboratory of Restoration - Oltenia Museum Craiova by means of the stereomicroscope OLYMPUS 3D.

The taxonomy and nomenclature of the identified species is made according to Fauna Europea.

RESULTS AND DISSCUTION

The analysed material–is represented by 296 specimens of which 5 specimens–had parasites. The material was collected between 2011 and 2013 in the following locations: Bucovăț Forest, Secui, Obedin, Craiova - Romanescu Park, Craiova - Craiovița Nouă–Pool. There are rendered the collection sites, the species of collected beetles and the identified parasites (Table 1).

Table 1. Material collected and their parasites.

No.	Host	Collection sites	Date of collection	Parasites
1	*Copris lunaris* (LINNAEUS 1758)	Craiova – Craiovița Nouă Pool	May 09, 2011	*Uropoda* sp. (*Copridis parasites)*
2	*Cerambyx cerdo* LINNAEUS, 1758	Bucovăț	June 24, 2012	*Beauveria bassiana*

Host: *Copris lunaris* (Linnaeus 1758)
Parasite: *Uropoda* sp.
Collection site: Craiova – Craiovița Nouă Pool
Date of collection: May 9, 2011

Figure 1. Craiova – Craiovița Nouă Pool (surse Google maps).

Figure 2. Collection site: Craiova – Craioviţa Nouă Pool and Bucovăţ locality.

Copris lunaris (Linnaeus 1758)

They are coprophagous, feeding on various mammalian excreta, mainly large herbivores.

Of Coprinae only *Copris lunaris* (Linnaeus, 1758) is spread throughout Central Europe. His behaviour in nesting was described by several authors, including LENGERKEN (1952), TEICHERT (1960) ROMMEL (1967) and KLEMPERER (1982a, b) in BAHRAMI et al., 2011.

The female excavates a room in cow dung and together with the male, fill it with fresh manure (Figure 1). These two parent beetles bring with them a community of phoretic mites. Manure is shaped into a paste and left to ferment for a while. The female shapes 4-8 brood pear-shaped balls and deposits one egg in a small hole on top of each ball.

The female, sometimes with the male, stays underground in the incubation chamber until descendants appear, and protects the juveniles from larvae or pupae contained in the balls. Finally, the female leaves the nest underground chamber with the juveniles. When a pair of *C. lunaris* builds a reproductive room, manure is clean and moist, and an initial period of fermentation has to pass. Beetles then divide the mass of manure and shape four or five pear-shaped balls, and the female lays egg in each. When brood balls are fully formed and the larva is developing, dung gradually gets dry on the outside and is consumed inside by the larva; when the larva turns into pupa the nest is relatively dry. In short, before the hatch of the adult specimen, the ball walls are so dry and hard that the migration of mites out of the balls becomes impossible.

The penetration of the mites in the brood balls must start earlier, shortly after laying eggs and can only continue until the walls get hard. The *C. lunaris* brood ball has a small area where the wall is thin and porous, apparently to allow ventilation (KLEMPERER 1982a, b in BAHRAMI et al., 2011). It is not known if mites can pass through the porous area, but the presence of different species of mites inside versus outside the ball, suggests that they have a great ability to adapt. When pupae have completed their development, juvenile beetles and the female leave the brood chamber.

A lot of researchers believe deuteronymph transport mites as drill. Drill (from the greek phoresis = to be worn to be transported) is a method of spreading in the animal world by which individuals of a species less mobile are transported long distances by representatives of other species, without the two bodies to have relationships parasitism. This method is adopted by various animals who are looking for new food sources, or by parasites when looking for a new host. Drilling may be temporary or permanent and widespread dust mite.

Figure 1. Female of *Copris lunaris* (original).

Uropodoidea (tortoise mites) associated with *Copris lunaris* dung beetle (Coleoptera: Scarabaeidae)

(Arthropoda: Chelicerata: Arachnida: Acari: Parasitiformes: Mesostigmata: Monogynaspida: Uropodina)

Superfamily: Uropodoidea

It comprises over 30 families. Their systematics is uncertain.

Mites are an order of arachnids from the arthropod class. They are small organisms, sometimes microscopic; the head, thorax and abdomen are generally undifferentiated, and the mouthparts (an elongated tube) are adapted to chew, suck, sting. They live on plants, soil, water, dead or living animals. A large number of species are agricultural pests, others are parasites of man and animals. Many mites, such as ticks, are transmitters of serious diseases as relapsing fever, encephalitis in humans, piroplasmosis in animals, etc. (KONTSCHÁN, 2007). Many mites can be vegetable or animal parasites or intermediate hosts (e.g. for tapeworms). Predatory mites are sometimes used in the biological control of insect pests (e.g. *Phytoseiulus persimilis* against red spider).

The superfamily Uropodoidea or tortoise mites is represented by over 2,000 species described worldwide, many of them in irregular habitats such as, nets, wood scraps and dung.

Phoresy (ability of mites to attach to the body of an insect) is therefore a prerequisite for their distribution between these habitats. Their eating habits are little known, but usually they are considered to be omnivorous, feeding on hyphae of fungi, slow moving prey, etc. The deutonymphs of certain species feed on nematodes and fungi, as well as on the eggs and larvae of their hosts (BAHRAMI et al., 2011).

The most commonly encountered species of mites at *Copris lunaris* are *Pelethiphis altocumulus, Macrocheles copridis, Parasitus copridis, Uropoda copridis, Copriphis pterophilus* and *Hispanic onchodellus*.

Many papers have been published on mesostigmatid mites that live in association with *Copris* species, but few of the authors have paid special attention to the behavioural relationship between mites and their hosts. The mites have developed different strategies for dispersal, as shown by their preferential attachment either to adults or their offspring (BAHRAMI et al., 2011).

Of these, only *Copridis parasitus* is more abundant at adults compared to brood balls.

Phoresy (ability of the deutonymphs to attach to the host) of this type requires certain life behaviours and adaptations to ensure that the mite dispersal phase is completed (Figures 2, 3, 4).

These adaptations have become particularly complex when associated with mite dung beetles subfamily Coprinae, demonstrating a high level of parental brood care. The care to Coprinae juveniles by adults, especially females, offers mites the rare opportunity to have access to both generations: parents and offsprings (BAHRAMI et al., 2011).

All specimens of this species were in the phoretic stage, deuteronymph. Interesting to note is the only known female model *Uropoda copridis* initially found at a larva into a ball of *Copris lunaris* and described by MAŠÁN & HALLIDAY, 2009. This suggests a different release strategy, and it seems that the deutonymph *U. copridis* is not attached to the emerging individual until it leaves the brood ball.

The deutonymph *U. copridis* attaches to the host using an anal pedicel, as found in many species of *Uropodina* (ATHIAS-BINCHE, 1984).

Parasitus copridis showed a strong preference for the parent beetles instead of brood balls.

This species of mitesis susceptible to feed-on nematodes, tiny insect eggs, mites and others.

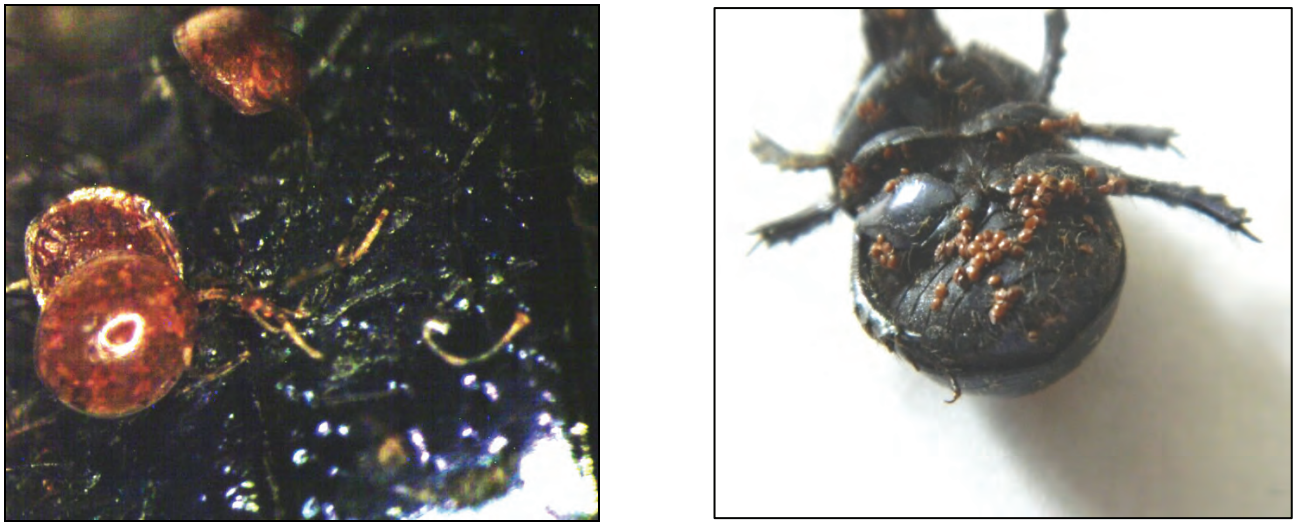

Figure 3. Deuteronymph fixed with the uropod from ventral abdomen (original).

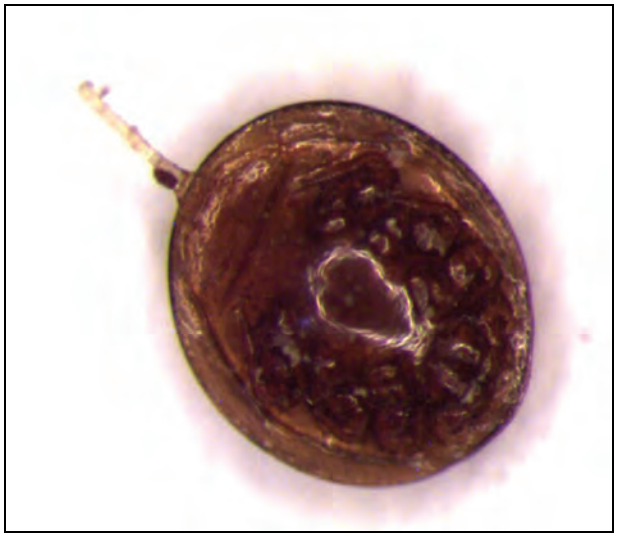

Figure 2. Deuteronymph *Uropoda* sp. (original).

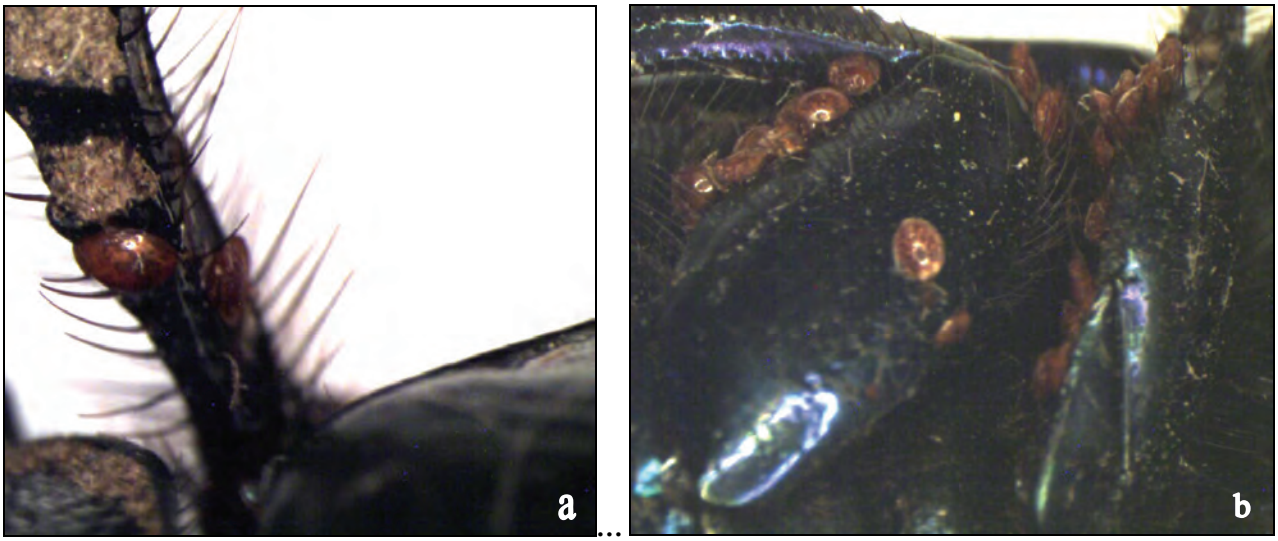

Figure 4. Deuteronymph fixed to the talus (a) and to the legs, in general (b) (original).

Host: *Cerambyx cerdo* Linnaeus
Parasites: *Beauveria bassiana* (Bals.-Crivat.) Vuill.
Collection site: Bucovăţ Forest
Collection date: June 24, 2012

Bucovăţ Forest is located in the central - north-eastern part of the Dolj County.

Bucovăţ Forestis located at the exit of Bucovăţ settlement and is situated in the western part of the Romanian Plain, in the sector known as Oltenia plain. The forest is situated in the north-eastern extremity of Băileşti Plain at an altitude of 83 m. The forest where we made the collection is made up of Turkey oak (*Quercus cerris*) and Hungarian oak (*Quercus frainetto*), tall temperate tree species with strong, gnarled branches and a large and rich crown. (CĂLINESCU, 1969*).*

Collection was performed on June 24, 2014, I the afternoon, at 5.46 p.m. The temperature was 28°C, clear sky, atmospheric pressure: 1017 hPa, humidity: 64%, precipitation: 0 mm, Dew Point: 16°C.

Cerambyx cerdo Linnaeus, 1758 (or great capricorn beetle) is one of the largest European beetles.
(Coleoptera: Cerambycidae: Cerambycinae: *Cerambyx*)
According to IUCN Status: endangered species.

Adults appear from May to June and are usually active at night. They feed on tree sap seepage through cracks in the bark (BALACHOWSKY, 1962-1963). Their habitat is represented by old forests with deciduous species, preferring especially those of oaks; sometimes it can be found in parks.The flight takes place from May to August (PANIN & SĂVULESCU, 1961).

The species is included in the Annexes of the Bern Convention as a threatened and rare species. It is also included in the Red Book of Moldova and Lithuania, Ukraine, Belarus and the IUCN Red List.

The fungus *Beauveria bassiana* was identified on the collected specimen. After being collected from the body with tweezers was placed in a glass Petri box and *B. bassiana* growing out on selective agar medium.

Beauveria bassiana (Bals.-Crivat.) Vuill.
(Sordariomycetes: Hypocreales: Clavicipitaceae: *Beauveria*)

The first microorganism recognized as a pathogen fungus was *Beauveria bassiana* (Bass, 1835). The genus *Beauveria* attacks all stages of all groups of insects. They were also occasionally found in wild rodent lungs and nasal cavity of horses, humans and giant turtles. *B. bassiana* develops world wide, has one of the longest lists of hosts among imperfect fungi and it appears in the soil as a ubiquitous saprophyte.

B. bassiana generally infects through the skin. *B. bassiana* eliminated mycotoxins such as beauvericin in culture media. Toxic compounds rapidly weaken the insects after the invasion through hemolymph. The larvae, pupae and adults derived from insects infected with *Beauveria* have white external mycelium and become conidia within one or two days after their death (Figures 5a, b, c; 6).

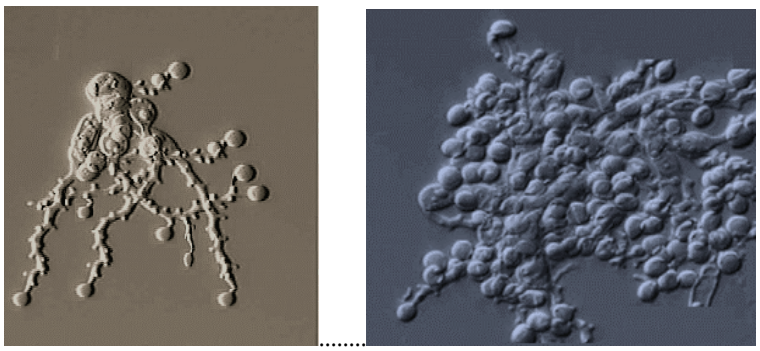

Figure 6. Conidiogenous cells and conidia of *Beauveria bassiana* (original).

Figure 7. *B. bassiana* growing out on selective agar medium (original).

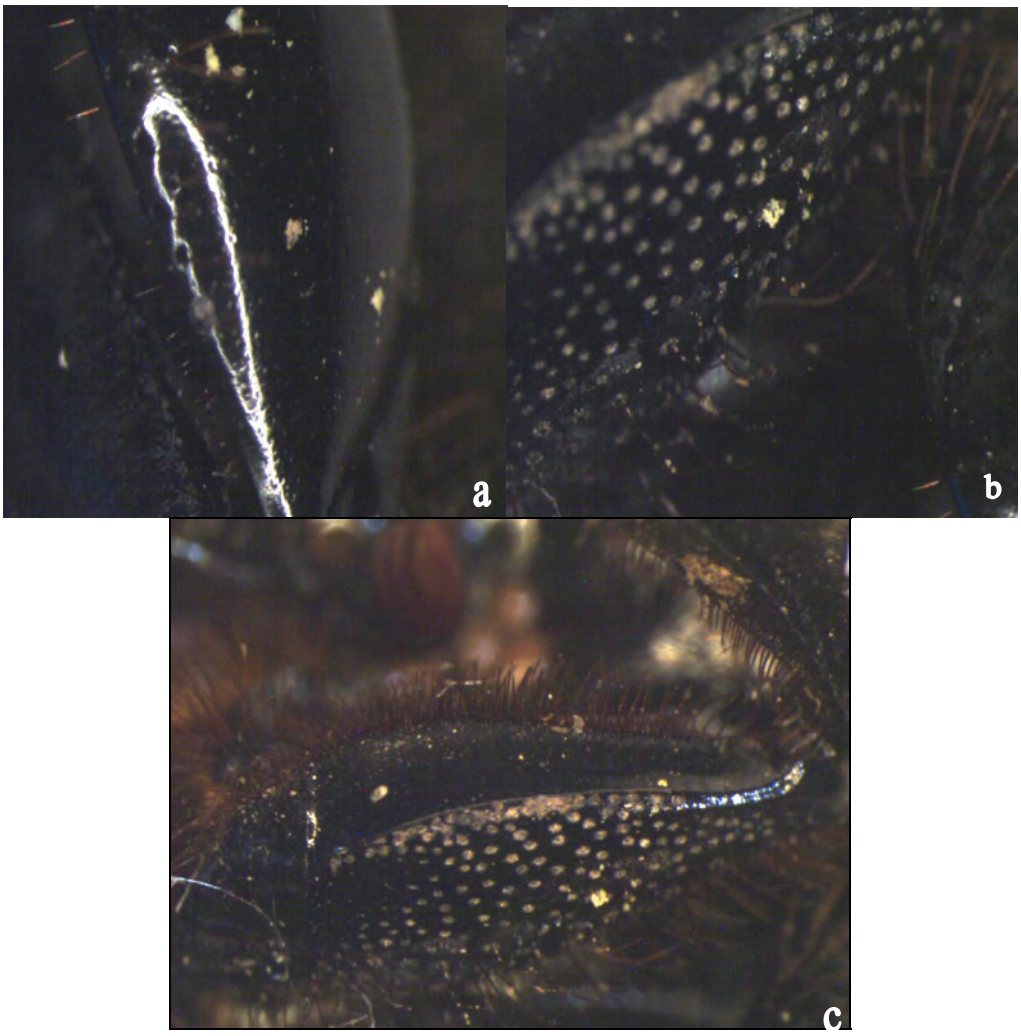

Figure 5 a, b, c. *Beauveria bassiana* to *Cerambyx cerdo* (original).

Geographic distribution and hosts

Beauveria bassiana occurs world wide. The hosts are mainly Lepidoptera, Coleoptera and Homoptera. Some of the most important economic insects that are susceptible to this fungus are *Ostrinia nubilalis*, *Laspeyresia pomonella*, *Popillia japonica*, *Colorado potato* beetle, *Leptinotarsa decemlineata.* Host colonization occurs when the fungus can overcome the immune defense-mechanisms and invade the insect hemolymph.

When insects die, the fungus produces an antibiotic, oosporin, which will enable it to overcome the competition of bacteria from the insect intestinal tract.Saprophytic phase is characterized by body mummification transformed into sclerotia. The hyphae from across the skin prefer certain segments and then they are covered by a white cottony-like mycelium, which will initiate the formation of conidia (SUSAN MAHR, 2010).

Beauveria is a cosmopolitan anamorphic genus, a facultative pathogen fungus of necrophagous arthropods. In addition, *Beauveria* eliminated a diverse array of biologically active secondary metabolites that include pigments, non-peptides and polyketides, peptidic antibiotics, synthesized nonribisomal peptides and other secreted metabolites involved in the pathogenesis of insects and a virulence with industrial, pharmaceutical and agricultural potential (VEY et al., 2001) in SUSAN MAHR, 2010.

CONCLUSIONS

The work joins the efforts of professionals who contribute to the knowledge of the entomofauna diversity. Of the identified species of parasites, we published the results for two species we have studied more closely; further results of the research on other parasites and their hosts will be published in anup coming paper.

Many different species of mites have developed different strategies for dispersal, as shown by their preferential attachment to either the parental or progeny generations of beetles.

Beauveria bassiana fungus identified in *Cerambyx cerdo* is new for Romania in this species. Moreover, the scientific research conducted in *Copris* nests, too, are the first reported in Romania.

The advantage of using *B. bassiana* against other microbial control agents consists in the broad-spectrum activity and its virulence which allowed them to be used successfully in agricultural areas of the former USSR and Eastern Europe. *B. bassiana* microfungus control agent is very interesting because it can infect the host through ingestion or through simple contact, unlike other microbiological control agents (SUSAN MAHR, 2010).

B. bassiana can be mass produced at low cost and can be applied by conventional methods. It is not harmful to vertebrates. In addition, they have a long environmental persistence of conidia, which can allow naturally certain diseases such as enzootic or epizootic diseases (EFSA, 2012).

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