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Rosalia longicorn *Rosalia alpina* (LINNAEUS, 1758) (Coleoptera: Cerambycidae) as a host of the entomopathogenic fungus *Cordyceps bassiana* LI, LI, HUANG & FAN, 2001 (Ascomycota: Hypocreales)

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ABSTRACT. The paper describes, for the first time, the occurrence of the entomopathogenic fungus *Cordyceps bassiana* (anamorph: *Beauveria bassiana*) on the imago of the endangered beetle Rosalia longicorn *Rosalia alpina* from the Low Beskid Mountains (the Carpathians, SE Poland). Furthermore, an isolate of the saprotrophic fungus *Hypoxylon fragiforme* was obtained as a result of laboratory tests on *R. alpina* specimens. Relationships between the identified fungi and *R. alpina* are discussed.

KEY WORDS: *Rosalia alpina*, *Cordyceps bassiana*, *Beauveria bassiana*, *Hypoxylon fragiforme*, endangered species, entomopathogenic fungi, saprotroph, Poland.

INTRODUCTION

The Rosalia longicorn *Rosalia alpina* (LINNAEUS, 1758) is an endangered and strictly protected beetle species with an Euro-Caucasian distribution (STARZYK 1992, 2004, EHNSTRÖM 2007, SHAPOVALOV 2012, IUCN 2014). In central Europe, *R. alpina* originally inhabited beech forests but was recently found to have spread into lowland beech-free forest stands (ČÍŽEK et al. 2009). *R. alpina* is a polyphagous species, although in central Europe it is mainly associated with the European beech *Fagus sylvatica* LINNAEUS, 1753 (ŠVÁCHA & DANILEVSKY 1988, BURAKOWSKI et al. 1990, SAMA 2002). The current

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distribution of the species in Europe is highly disjunct and there are only a few isolated areas of its occurrence in central Europe (BINNER & BUSSLER 2006). In Poland, *R. alpina* has withdrawn from most of its former sites and at present occurs only in certain parts of the Carpathians (MICHALCEWICZ & CIACH 2015). The main threats to *R. alpina* are related mostly to various aspects of silviculture, which affect the presence and preservation of habitats as well as the survival of the species at different developmental stages. Natural mortality is due to different groups of predators, such as birds, lizards and ants (WITKOWSKI 2007, ADAMSKI et al. 2013).

Cordyceps bassiana LI, LI, HUANG & FAN, 2001 is a fungus that grows naturally in the soil all over the world, parasitizes various species of arthropods and causes white muscardine disease. *Beauveria bassiana* BALSAMO-CRIVELLI, 1836 is an anamorph (asexual form) of the entomopathogenic fungus *Cordyceps bassiana*, from the phylum Ascomycota. When spores of the fungus are in contact with the host body, they germinate and penetrate the insect body through the epidermis, killing it within a few days. A white mycelium grows in the dead specimen and produces new spores. Conidia are unicellular, haploid and hydrophobic. *C. bassiana* growing on nutrient agar develops in the form of a white, powdery mycelium. *C. bassiana* parasitizes a wide range of arthropods: for this reason it is used in biological pest control (BARBARIN et al. 2012). However, the isolated strains differ in their range of hosts. Some of them, for example, are highly virulent to a small group of caterpillars, while others have a wide range of hosts (CASTRILLO et al. 2010).

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MATERIAL AND METHODS

On 24 August 2015, one dead imago of *R. alpina* with a white mycelium visible on its body (Fig. 1) was found in the Magura National Park near the village of Myscowa (Low Beskid Mts., SE Poland, UTM: EV38) (leg. et coll. R. KUBIŃSKI). The imago was found among the needle litter, on the edge of a forest dominated by European silver fir *Abies alba* MILLER, 1759, growing on a slope between a public road and the River Wisłoka. The white mycelium was visible at different locations on the insect's body.

Fig. 1. Mycelium of *Cordyceps bassiana* visible on the underside of the *Rosalia alpina* imago body. Photo by R. KUBIŃSKI.

The mycelium collected from this imago of *R. alpina* was placed in a number of Petri dishes containing PDA medium and incubated at room temperature for 7 days in the dark. Each colony emerging from the samples was subcultured to fresh PDA medium. The isolates were identified on the basis of their morphology and the DNA sequence comparison for two gene regions (ITS). DNA was extracted using the Genomic Mini AX Plant (A&A Biotechnology, Gdynia, Poland) using the manufacturer's protocol. The ITS rDNA region (ITS1-5.8S-ITS2) was amplified using the primers ITS 1F (GARDES & BRUNS 1993) and ITS 4 (WHITE et al. 1990). The sequences were compared with data from GenBank using the BLAST similarity search.

RESULTS AND DISCUSSION

As a result of this laboratory study, two species of fungi – *Cordyceps bassiana* and *Hypoxylon fragiforme* (PERSOON) J. KICKX, 1835 – were isolated (Table 1). On the nutrient agar, *C. bassiana* developed in the form of a white, powdery mycelium, while *H. fragiforme* grew in the form of a light brown cottony mycelium (Fig. 2).

Table 1. *Cordyceps bassiana* and *Hypoxylon fragiforme* isolated from the imago of *Rosalia alpina* (L.) (the Low Beskid Mountains, SE Poland); identification based on a comparison of ITS sequences in the GenBank database. Ls – length of the squences, Qc – query cover, E-v – E-values, Id – Identity.

Fungus species	Ls	Qc [%]	E-v	ITS rDNA acc. no.	Id [%]
Cordyceps bassiana	581	92	0.0	AB237657.1	99
Hypoxylon fragiforme	695	85	0.0	EF166624.1	100

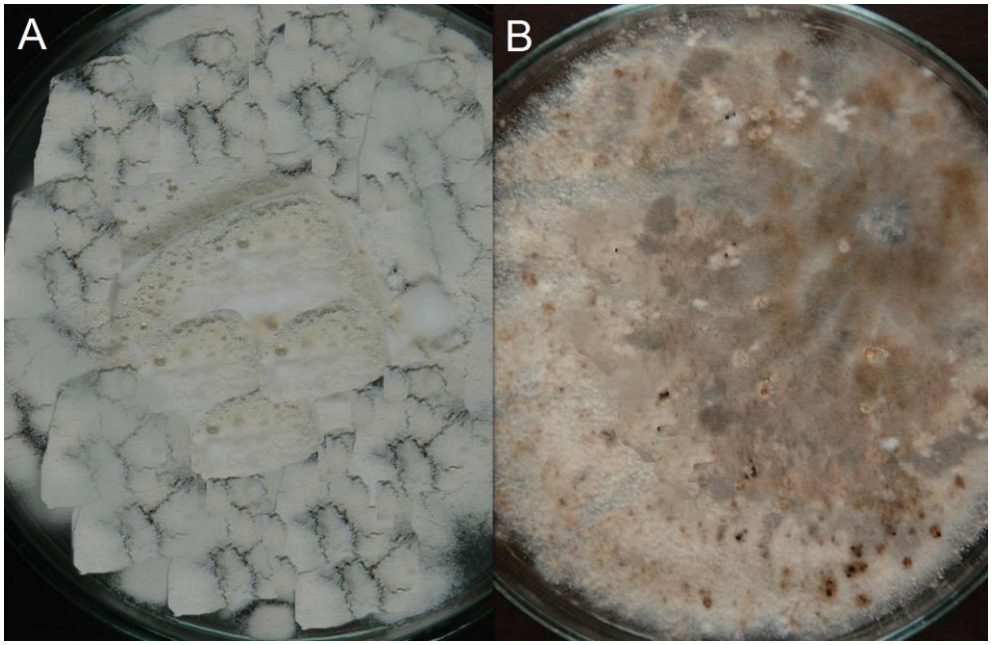

Fig. 2. Cultures of *Cordyceps bassiana* (A) and *Hypoxylon fragiforme* (B) on PDA nutrient medium. Photo by C. BARTNIK.

There are no reports in the world literature of any case of an imago of *R. alpina* being infected by *C. bassiana*. On the other hand, *C. bassiana* is known to be a pathogen of other beetle species from the family Cerambycidae and of other insects (e.g. BAŁAZY 1962, GŁOWACKA & ŚWIEŻYŃSKA 1993, EKEN et al. 2006). A number of European studies conducted in 2000 showed, for example, that the gypsy moth *Lymantria dispar* (LINNAEUS, 1758), a dangerous pest of deciduous trees, is very resistant to entomopathogenic fungi. Only one strain of *C. bassiana* was found to be capable of effective infection, but solely when administered in the form of dry spores; spraying spores in a suspension was

ineffective. Furthermore, although the fungus developed slowly (from 11 to 20 days depending on the stage of larval development), the final mortality rate was ca 80%. It appears that the immunity of *L. dispar* caterpillars is caused by their natural allies – bacteria living on their body integument. It also appears that the lack of a natural lipid cover on the exoskeleton of European spruce bark beetles makes them relatively vulnerable to *C. bassiana* (WRZOSEK & SIEROTA 2012).

The presence of *C. bassiana* on the imago of *R. alpina* is an interesting finding 1) in view of the fact that this fungus is highly pathogenic towards insects and has therefore been used in biological pest control (BARBARIN et al. 2012, CASTRILLO et al. 2010), and 2) since knowledge of the factors responsible for the limited occurrence of this endangered beetle species remains insufficient.

The second fungus, i.e. *H. fragiforme* (Ascomycota: Xylariales), one of the primary saprophytes of *Fagus sylvatica* (GUMIŃSKA & WOJEWODA 1985), was obtained as a result of examining the *R. alpina* imago in question. This finding should probably be attributed to the common occurrence of the beetle species on *F. sylvatica*. Because of the trophic relationships of this fungus and the beetle, one can assume that *R. alpina* may be a vector carrying *H. fragiforme*. At this point, however, it is difficult to determine the modes of transmission of this fungus by *R. alpina*, especially since various transmission strategies have been found to occur in saproxylic beetles. Basically, there are two means by which such beetles transmit fungal spores: externally by mycangia, and internally through the digestive tract or reproductive organs. Different types of mycangia on the body of beetles or their mandibles can be observed in representatives of bark beetles (Curculionidae: Scolytinae). Transmission of spores through the digestive tract occurs, e.g. in the large pine weevil *Hylobius abietis* (LINNAEUS, 1758), which spreads annosum root rot *Heterobasidion annosum* (FRIES) BREFELD, 1888. This type of fungal spore transmission also occurs, for example, in representatives of the following families: longhorn beetles, sap beetles (Nitidulidae) and round fungus beetles (Leiodidae) (CROWSON 1986, GUTOWSKI 2006). In this context, further detailed research would be necessary into the possible function of *R. alpina* as a vector contributing to the spread of saprophytic fungi and into the possible role of these fungi in the death of *R. alpina*.

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