

DOI: 10.17387/BULLENTSOCMALTA.2019.07

# Ant-associated fungi in Bulgaria

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**ABSTRACT.** The current study gives an overview of the ant-associated fungi in Bulgaria. After the recent first records for the country of *Myrmicinosporidium durum* Hölldobler, 1933, *Rickia wasmannii* Cavara, 1899, and *Laboulbenia camponoti* S.W.T. Batra, 1963, new data on their host range and distribution have been obtained. Additionally the ectoparasitic fungus *Aegeritella tuberculata* Bałazy & Wiśniewski, 1982, which has not been previously recorded from the Balkan Peninsula was found on the host ant *Lasius brunneus* (Latreille, 1798) in the Bulgarian part of Strandzha Mountain, in a locality near the border of Turkey.

**KEY WORDS.** Balkan Peninsula, insect parasitic fungi, *Aegeritella*, *Laboulbenia*, *Rickia*, *Myrmicinosporidium*.

## INTRODUCTION

In Europe, ant-associated fungi, both ecto- and endoparasitic, have been the subject of scientific interest for the past century, with most of the extensive studies on their taxonomy, distribution and host-parasite relations carried out in the last decade (BÁTHORI *et al.*, 2015a, 2017; CSATA *et al.*, 2014, 2017; DE KESEL *et al.*, 2016; ESPADALER & SANTAMARIA, 2012; FERENC *et al.*, 2014; GÓMEZ *et al.*, 2016; HAELEWATERS *et al.*, 2015; PERAL *et al.*, 2017; PFLIEGLER *et al.*, 2016; SANTAMARIA & ESPADALER, 2015; WRZOSEK *et al.*, 2016). Although ants have developed strong anti-fungal defenses, they have also become specific hosts of several parasitic fungi, most often affecting individual specimens in the colony (ESPADALER & SANTAMARIA, 2012; HÖLLDOBLER & WILSON, 1990). In the last review that was published on the ant specific fungi from the Holarctic region, ESPADALER & SANTAMARIA (2012) list ten ecto- and one endoparasitic fungal species accompanied with information on their taxonomy, distribution, host utilization and corresponding references. Ectoparasitic fungi are represented by five species of *Aegeritella*, one *Hormiscium* species and four *Laboulbeniales* species, while *Myrmicinosporidium durum* Hölldobler, 1933 is the only typical endoparasitic species. The recently described *Rickia lenoirii* Santamaria, 2015, an ectoparasite and a *Laboulbeniales* species on *Messor* ants, found in Greece, France, Hungary, and Romania (BÁTHORI *et al.*, 2015b; SANTAMARIA & ESPADALER, 2015) should now also be included in the list. Insect pathogenic entomophthoralean fungi are rarely noticed on ants, such as *Pandora formicae* (Humber & Bałazy) Humber, an unusual example of a fungus adapted to exclusively infect wood ants from the genus *Formica* (MALAGOCKA *et al.*, 2017).

Recently, three fungal species, *Myrmicinosporidium durum* Hölldobler, 1933, *Rickia wasmannii* Cavara, 1899 and *Laboulbenia camponoti* S. W. T. Batra, 1963 were found to infest ants in Bulgaria (CSÓSZ *et al.*, 2012; LAPEVA-GJONOVA & SANTAMARIA, 2011).

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Here we provide a first record of *Aegeritella tuberculata* Balazy & Wiśniewski, 1982 from the Balkan Peninsula, as well as new data on host range and localities of hitherto known ant-associated fungi found in Bulgaria.

## MATERIAL AND METHODS

The fungal infested ants were collected during ant inventory studies in Bulgaria. Samples were collected by hand-searching and by the means of pitfall traps. The ant specimens were stored in 70% ethanol and detection of the fungi was made by microscopic examination. Laboulbeniales thalli were removed from the ants with an insect pin and mounted in a glycerol medium on slides. The studies of *Myrmicinosporidium durum* were made under a microscope through the cuticle of the parasitised ants. Its spores were easily detected, especially on the lightly coloured ant species. The observations of *Aegeritella* were performed using both dissecting and light microscopes. Preparations were stained with methylene blue solution in lactophenol to facilitate fungi identification. For confirmation of systematic affinity, molecular analysis was also carried out. Patches of fungi were scraped with a scalpel directly from the cuticle of the ants preserved in ethanol. Genomic DNA was extracted using an Isolate II Plant DNA kit (Bioline Ltd., UK) following the manufacturer's instructions. The complete ITS region was amplified and sequenced using the primer pair ITS1f and ITS4 (WHITE *et al.*, 1990) as described by RÓZALSKA *et al.* (2013).

Ant host specimens and fungi mounted on slides were deposited in the zoological collection of Sofia University. Material of *Aegeritella tuberculata* was also stored in the mycological collection of the University of Warsaw.

## RESULTS

### *Aegeritella tuberculata* Balazy & Wiśniewski, 1982

**Material examined:** Strandzha Mountain, Kondolovo vill., Byalata prast loc., 42°5'47.70"N 27°39'55.00"E, 275 m, pitfall traps, vii-viii.2010, leg. R. Kostova, host ant: *Lasius brunneus* (Latreille, 1798).

**Ecology.** Pitfall traps were set in *Fagus orientalis* Lipsky forest with undergrowth of the relict *Vaccinium arctostaphylos* L. The following ant species were recorded in the same area: *Aphaenogaster* cf. *subterranea*, *Camponotus fallax* (Nylander, 1856), *C. lateralis* (Olivier, 1792), *C. vagus* (Scopoli, 1763), *Colobopsis truncata* (Spinola, 1808), *Crematogaster schmidtii* (Mayr, 1853), *Dolichoderus quadripunctatus* (Linnaeus, 1771), *Formica cunicularia* Latreille, 1798, *Lasius fuliginosus* (Latreille, 1798), *L. platythorax* Seifert, 1991, *L. psammophilus* Seifert, 1992, *Myrmica scabrinodis* Nylander, 1846, *Prenolepis nitens* (Mayr, 1853) and *Temnothorax crasecundus* Seifert & Cs6sz, 2015.

*Aegeritella tuberculata* is a poorly understood fungal ectoparasite on ants. A detailed phylogenetic study confirmed the position of *A. tuberculata* within the fungal group Basidiomycota, order Trichosporonales (WRZOSEK *et al.*, 2016). All five known species of the genus form compact dark patches on the ant surface (ESPADALER & SANTAMARIA 2012; WRZOSEK *et al.*, 2016). No significant harm appears to be caused to the ant host, although restrictions in movement and feeding as well as a shortening of their lifespan are effects associated with the infestation (ESPADALER & OROMI, 1997; WRZOSEK *et al.*, 2016).

Previously, *A. tuberculata* has been reported from the Czech Republic, Poland and Spain in Europe (ESPADALER & SANTAMARIA, 2012; ESPADALER & SUÑER, 1989). This new record from Bulgaria is the most southeastern record of the fungus.

In Europe, the fungus has been found mainly on ants of the *Lasius* genus: *L. brunneus* (Latreille, 1798), *L. distinguendus* (Emery, 1916), *L. flavus* (Fabricius, 1782), *L. grandis* Forel, 1909, *L. niger* (Linnaeus, 1758), *L. nitidigaster* Seifert, 1996, and *L. umbratus* (Nylander, 1846). More rarely, it has also been found on *Formica* ant host species: *F. fusca* Linnaeus, 1758, *F. lemni* Bondroit, 1917, *F. pressilabris* Nylander, 1846, *F. rufa* Linnaeus, 1761, and *F. rufibarbis* Fabricius, 1793 (WRZOSEK *et al.*, 2016). Previous to this study, *Lasius brunneus* (Fig. 1), the ant host of *A. tuberculata*, was reported only from Poland (WRZOSEK *et al.*, 2016).



**Figure 1:** Worker of *Lasius brunneus* infested on the head, thorax, legs and gaster with bulbils (left) and a colony of *Aegeritella tuberculata* (right).

*Aegeritella tuberculata* bulbils reach up to 100 to 150  $\mu\text{m}$  in diameter; they are flat and easily detached from the cuticle. The oval cells, usually 5 to 8  $\mu\text{m}$  in diameter, always form one single layer lying directly on top of the cuticle. The sporogenous cells were not observed.

### *Myrmicinosporidium durum* Hölldobler, 1933

**Material examined:** Eastern Rhodopes, Byal izvor vill. (Ardino district), 41°32'55.64"N 25°3'55.91"E, 570 m, 27.iv.2003, ant host *Plagiolepis pygmaea* (Latreille, 1798), leg. A. Lapeva-Gjonova; Central Balkan Mountains, Karnare vill., 42°43'10.24"N 24°37'53.40"E, 645 m, 20.vi.2014, ant host: *Strongylognathus* cf. *karawajewi*, leg. A. Lapeva-Gjonova; Eastern Rhodopes, Svirachi vill. (Ivaylovgrad district), 41°28'37.57"N 26°6'18.37"E, 303 m, 02.vii.2014, ant host *Pheidole* cf. *pallidula*, leg. A. Lapeva-Gjonova.

*Myrmicinosporidium durum* was first recorded from Bulgaria in 2012 (Csösz *et al.*, 2012). Its spores were found in the following ant hosts: *Solenopsis fugax* (Latreille, 1798), *Tetramorium* sp. D and *Tetramorium* sp. E (*sensu* SCHLICK-STEINER *et al.*, 2006). After the recent revision of *T. caespitum* species complex (WAGNER *et al.*, 2017), the last two hosts are most likely referring to *T. staerckei* Kratochvil, 1944 and *T. immigrans* Santschi, 1927, respectively.

A new ant host of *M. durum* from *Strongylognathus testaceus*-species group was identified (Fig. 2). An infested *Strongylognathus* worker appears to be distinct from typical *S. karawajewi* Pisarski, 1966 and its status needs further studies. However, until now, only one *Strongylognathus* species, *S. caeciliae* Forel, 1897, in Spain, was recorded as a host of *M. durum* (GARCÍA & ESPADALER, 2010). Although *Plagiolepis pygmaea* and *Pheidole pallidula* (Nylander) are well known as hosts of *M. durum*, they have not been previously recorded as hosts in Bulgaria. Additionally, these are the first records of the fungus for Eastern Rhodopes and the Central Balkan Mountains.



**Figure 2:** Worker of *Strongylognathus* cf. *karawajewi* infested with spores of *Myrmecinosporidium durum*.

#### ***Rickia wasmannii* Cavara, 1899**

**Material examined:** Strandzha Mountain, Malko Tarnovo, 41°59'18.77"N 27°31'30.00"E, 318 m, 18.iv.2010, ant host: *Myrmica scabrinodis* Nylander, 1846, leg. A. Lapeva-Gjonova.

Until now, *Myrmica scabrinodis* is the only known ant host of *R. wasmannii* in Bulgaria (LAPEVA-GJONOVA & SANTAMARIA, 2011). The fungus was found in Strandzha Mountain (southeastern Bulgaria) in very wet sites.

#### ***Laboulbenia camponoti* S. W. T. Batra, 1963**

**Material examined:** Western Balkan Mountains, Vratsa karst Reserve, 43°11'39.01"N 23°29'47.01"E, 960 m, 16.iv.2016, ant host: *Camponotus aethiops* (Latreille, 1798), leg. A. Lapeva-Gjonova; Eastern Rhodopes, Gorni Glavanak vill., 41°40'34.067"N 25°49'7.167"E, 308m, 01.v.2016, ant host: *Camponotus aethiops* (Latreille, 1798), leg. A. Lapeva-Gjonova.

The fungus was reported on the carpenter ant hosts: *Camponotus aethiops*, *C. universitatis* Forel, 1890 and *C. sp.* (given as *C. pilicornis* (Roger, 1859)) from the southern Ograzhden, Eastern Rhodopes and Strandzha Mountains, as well as from the Northern Black sea coast in Bulgaria (LAPEVA-GJONOVA & SANTAMARIA, 2011). The Western Balkan Mountains is a new distribution area of the fungus in Bulgaria. New data confirm *C. aethiops* (Latreille, 1798) as one of the most frequent host of *L. camponoti*.

### **CONCLUSION**

Considering host utilization by ant parasitic fungi and their distribution range, at least four more species, *Aegeritella superficialis* Bałazy & Wiśniewski, 1974, *Rickia lenoirii* Santamaria, 2015, *Laboulbenia formicarum* Thaxter, 1902 and the entomopathogenic fungus *Pandora formicae* (Humber & Bałazy) Humber, are expected to occur in Bulgaria.

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