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New insights on the phylogeny and biology of the fungal ant pathogen *Aegeritella*

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ABSTRACT

This paper evaluates the phylogenetic position of the ectoparasitic fungus *Aegeritella tuberculata* Bałazy & Wiśniewski, and broadly discusses its presence on ants in southern Poland. Field work was conducted in the Silesian Beskid Mountains in 2011–2013. The fungus was found on four species of ants: *Lasius niger* L., *Lasius brunneus* Latr., *Formica lemani* Bondr. and *Formica fusca* L. The first three species have not been noted previously in the literature as hosts of *Aegeritella* fungi. The infection rate ranged from 1% for *Formica lemani* to 21% for *L. brunneus*. Molecular analysis based on ITS and SSU rDNA sequences revealed close relationships between *Aegeritella* and *Trichosporon* isolates. We conclude that the genus *Aegeritella-inceratae sedis* until now, should be placed within the fungal group Basidiomycota, Tremellomycetes, Tremellomycetidae, Tremellales, *Trichosporon*aceae.

1. Introduction

As social insects ants are exposed to disease outbreak more dire than solitary living insects. Elevated risk of disease transmission is considered a severe cost of sociality. It is worth noting, that epidemics are quite rare. Ants developed various systems of protection – including immunological and behavioral (Konrad et al., 2012, 2015; Kappeler et al., 2015). The ants' diseases could be caused by bacteria, viruses, nematodes (Allen et al., 2011; Porter et al., 2013; Russell et al., 2012; Poinar et al., 2006) as well as microsporidia and other fungi (Chen et al., 2004; Oi et al., 2005; Csősz et al., 2012). The well known ants' parasites are *Ophiocordyceps myrmicarum* and *Ophiocordyceps unilateralis* with their anamorphs (Simmons et al., 2015; Mongkolsamrit et al., 2012), some members of Entomophthorales ex. *Pandora formiceae* (Małagocka et al., 2015). The generalist insect pathogens like *Beauveria bassiana* or *Metarhizium anisopliae*, and *Metarhizium brunneum* are also studied in contexts of ants' survival (Hughes et al., 2004; Konrad et al., 2015; Siebeneicher et al., 1992; Heinze and Walter, 2010). Recent research focuses on the influence of external parasites as *Rickia wasmanii* or *Laboulbenia formicarum* to the hosts' fitness (Báthori et al., 2015; Konrad et al., 2015). The risk of ants' infection by species of genus *Aegeritella* is still not clear. Found only on ants, this genus includes five species of fungi whose phylogenetic position and biology are poorly understood. All form cushion-like structures called sporodochia that are built of a compact pseudo parenchymal tissue, which contains short phialideae-like conidiogenous, spore-producing cells. Such formations occur as compact patches of brown cells on the surface of the cuticle of living ants, causing no visible damage (Bałazy and Wiśniewski, 1974). *Aegeritella* species are ectoparasitic fungi that live on the host's body surface without clearly causing harm, which distinguishes them from true pathogenic fungi. They are sometimes even considered commensal species. The presence of two members of a genus *Aegeritella* has been confirmed in Poland. *Aegeritella superficialis* Bałazy and Wiśniewski (1974) occurs on ants of the genus Formica, mainly *Formica rufa* and *Formica polyctena*. *Aegeritella tuberculata* Bałazy and Wiśniewski (1982) has been described from ants of the genus Lasius, but has also been observed on Formica (Bałazy and Wiśniewski, 1974, 1982). The latter fungus has been reported previously in Europe (Czech Republic, Poland, Spain) and North

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Table 1
Infection rate of *Aegeritella tuberculata* on several ant species based on direct insect examination (4625 specimens).

Ant species	Occurrence	Nest type	Number of samples	Number of ant specimens	Average number of specimens per sample	Standard deviation	Number of samples infected with *Aegeritella* sp.	% of samples infected with *Aegeritella* sp.	Number of ant specimens infected with *Aegeritella* sp.	% of ant specimens infected with *Aegeritella* sp.	% of infected ants within whole sample where at least one *Aegeritella* colony was observed
Formica fusca	Common, in open areas and in the forests	Under rocks, clumps of grass, and rotten stumps	29	174	6.00	2.89	1	3.44	6	3.45	50.00
Formica lemani	Mountains	Under rocks, clumps of grass, and rotten stumps	25	189	7.56	4.17	1	4.00	2	1.06	33.30
Formica rufa	Forests	On the ground, near trees, nests from needles	17	206	12.12	6.66	1	5.90	9	4.37	40.10
Lasius brunneus	Forests	Under the bark and wood of dead and live trees in forests	8	60	7.50	6.54	3	37.50	17	28.33	100.00
Lasius niger	Common, including in areas of heavy human influence	In the ground or under rocks that often form considerable mineral soil mounds	58	942	16.24	11.6	3	5.20	13	1.38	12.03
other species			281	3054	10.87	4.25	0				
Total			418	4625	11.06		9	2.15	46	0.99	31.29

America (Espadaler and Santamaria, 2012). Another new species (not yet described) that appeared to differ from known taxa was observed by X. Espadaler on ants from Aegina Island in Greece (Bałazy, pers. comm.).

The main objectives of this study were (1) to clarify the phylogeny of *A. tuberculata*, and (2) to assess its occurrence on ants in southern Poland.

2. Material and methods

2.1. Collection, culturing of ants and morphological characterization of fungus

In 2011–2013, intensive field surveys were conducted on ants that showed symptoms of infestation by ectoparasitic fungi in the Beskid Mountains (Beskid Śląski and Beskid Mały) of Southern Poland. A total of 4625 ant specimens from 418 samples were collected (Table 1). Insects were collected directly from nests, using glycol traps placed in the ground or sweep netting on herbaceous vegetation. Ants were preserved in 70% ethanol, and then examined under a stereoscopic microscope in order to identify host species and screen for mycelial growth. Insects were identified using available keys (Radchenko et al., 2004; Seifert, 2007). When fungal structures corresponding to *Aegeritella* mycelium were observed, we made microscopic preparations stained with methylene blue solution in lactophenol to facilitate species identification of fungi. The source of *A. tuberculata* for DNA studies and ecological observation was a *Lasius niger* colony affected by a fungus keeping indoor following the instructions of Czechowski and Pisarski (1992). The ant colony, consisting initially of the queen and several workers, was observed over a one-year period in 2013 indoor, in a pot. Microscopic examination of the queen and some workers revealed the presence of *A. tuberculata* on the ants' body surfaces. A culture was passed to formicarium in February 2014. It was placed in a test tube that consisted of several chambers partitioned with corks. Ants were fed with a honey–water solution and small dead insects. The queen began to lay eggs after a few days, and the colony then grew quickly. When the colony had reached 100–200 workers it had to be transferred to a larger container.

More than 100 workers, along with the queen, deposited eggs, larvae and pupae were transferred to a formicarium that was constructed of transparent plastic to allow observation under a stereoscopic microscope. A humidification system was created by filling a tube made of porous material, with one end submerged in a container of water. 50 workers were placed in another part of the formicarium built of cork in which hollow chambers had been created. Both colonies were kept shaded at room temperature. Fresh food was provided for the ants every few days, and water was supplemented as needed. Selected ants with *Aegeritella* on cuticle were preserved in 70% ethanol and designated for molecular study.

For scanning electron microscopy the infected ants that had been preserved in 70% ethanol were dehydrated in 10-min incubations in increasing concentrations of ethanol, an ethanol/acetone mixture, and pure acetone. They were subsequently critical point dried, coated with gold, and observed under a LEO 1430 VP microscope (Carl-Zeiss).

2.2. Fungal culture

Freshly dead ants bearing visible colonies of *A. tuberculata* were washed trice with sterile saline solution and left to dry. Legs with visible patches were cut off with sterile scalpel and placed on potato dextrose agar (PDA), yeast morphological agar (YMA) and malt yeast peptone agar (MYP) media plates, three repetitions each. The washed ant material was also suspended in sterile saline