

Comprehensive survey of Romanian myrmecoparasitic fungi: new species, biology and distribution

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Abstract. Several species of fungi exploit ants. Most of them are known from the tropics, where they may exhibit spectacular morphologies, and a handful of species are known to occur in Europe. Available data on their geographic range, host specificity and effect on the host is scarce and require acquisition. In addition, there is a significant geographic bias in the available data: mostly Western and Central European records are known. Herein, we provide an overview of the myrmecoparasitic fungal species from a hitherto barely explored country, Romania: *Aegeritella superficialis* Balazy et J. Wiśn. (1974), *Myrmecinosporidium durum* Hölldobler, 1933, *Pandora myrmecophaga* (Turian et Wuest) S. Keller (2005) and *Rickia wasmannii* Cava (1899) are presented. *P. myrmecophaga* is reported for the first time in Romania, and several new locations for *R. wasmannii* are given, and also a new host species for the fungus. General aspects of the biology of the four species, their distribution in Romania and their host range are presented.

Key words: parasites, fungi, ants, *Aegeritella*, *Myrmecinosporidium*, *Pandora*, *Rickia*, Romania.

Introduction

Interactions between fungi and insects are many-fold: from transient to obligate associations, some are known to kill the insect host, but in several cases they may benefit the insect or the fungus, or both (Vega et al. 2009, Blackwell 2010). Social insects, particularly ants, are no exception in this regard. There is a wide range of fungi associated with ants, the most spectacular being that of the leaf cutter ants. Parasitic fungi, e.g. species of the genus *Cordyceps* (Fr.) Link, due to their extreme morphology and bizarre host manipulation techniques, have also received much attention (Schmid-Hempel 1998, Blackwell 2010, Evans et al. 2011). Ant colonies are a most rewarding target for parasites, as they constitute a highly dense and genetically similar group of hosts (Schmid-Hempel 1998). Consequently, ants employ a number of hygienic behaviors to reduce virulence and transmission of entomopathogenic agents within the colony, e.g. increased grooming and nest cleaning, production of antibiotics, pathogen avoidance, removal of infected individuals, and sometimes even the relocation of the entire colony (Schmid-Hempel 1998, Roy et al. 2006). The intricate mechanisms of the evolutionary arms race in

ant-fungus interactions and its implications on the social structure of ants make this topic an appealing subject for research. However, in spite of an increasing number of studies on the behavioral ecology and evolutionary ecology of insect-associated fungi, and particularly ant-fungus interactions (see Roy et al. 2006), there is still a considerable lack of basic information as regards geographic distribution and host range of myrmecoparasitic fungi. Although a considerable part of information scarcity can also be attributed to problems in fungal systematics (see e.g. Blackwell 2010 for recent developments), the lack of specific attention paid to such parasitic fungi contributes to the prevailing gaps in our knowledge (Espadaler & Santamaria 2012).

Recently, Espadaler & Santamaria (2012) offered a comprehensive survey of myrmecoparasitic fungi present in Europe. The study delineated regions with no current data for these species, which are mostly Eastern European countries such as Romania (see e.g. Csósz et al. 2012). In Romania only three myrmecoparasitic species were known until now: *Aegeritella superficialis* Balazy et J. Wiśn. (1974) (Paşcovici 1983), *Myrmecinosporidium durum* Hölldobler, 1933 (Csósz et al. 2012) and *Rickia wasmannii* Cava (1899) (Tartally et al. 2007). Here

we report the occurrence of one more fungus species in Romania: *Pandora myrmecophaga* (Turian et Wuest) S. Keller (2005).

Our contribution brings important findings with regards to the distribution and host utilization of ant parasitic fungi.

Materials and methods

An extensive bibliographic search was carried out for any published information on myrmecoparasitic fungi species in Romania. Extensive field work was applied in the case of the fungi *Pandora myrmecophaga* and *Rickia wasmannii*.

A systematic search for *Pandora myrmecophaga* was conducted in a large polydomous system of *Formica exsecta* Nylander, 1846 near Voşlobeni in Harghita County (N 46.61649, E 25.611893) on 28.IX.2010. Altogether 500 nests were surveyed for infected individuals out of a total of 3347 nests (see Markó et al. 2012). Next year a similar field survey was carried out between 05–09.VII.2011, and five additional smaller *F. exsecta* polydomous systems were also included from the same area. Altogether 1066 nests were surveyed belonging to six polydomous systems. Four systems were entirely censused (12, 16, 22, 23 nests), while two large systems only partially censused (360 out of 400 nests, 633 out of 3347 nests). All dead *F. exsecta* individuals found attached to a grass blade in the vicinity of a nest were collected together with the grass blade, and were placed into small vials separately with a wet cotton ball underneath (see Boer 2008). The corpses were regularly checked for the infestation, which usually appeared after 6–10 hours. All nests with infected individuals were verified for infestation twice during the study period with a difference of 3–5 days.

Systematic searches were applied for *Rickia wasmannii*. On the base of Tartally et al. (2007) two infected populations were known previously, which were located at <30 km distance from each other. During the field survey similar habitats (wet grasslands of Northern exposure) were inspected in the wider region around the already known locations in spring 2011.

The taxonomical scheme and terminology of the fungi species follows Index Fungorum (<http://www.indexfungorum.org/>).

Results

Altogether four species of myrmecoparasitic fungi are known to occur in Romania.

Aegeritella superficialis Balazy et J. Wiśn. (1974)

Aegeritella superficialis is one of the five known *Aegeritella* species (Pezizomycotina, Ascomycota). All of them grow on the cuticle of workers of *Formica* species (Espadaler & Wiśniewski 1987, Espadaler & Monteserín 2003, Espadaler & Santamaria 2012).

They are believed to be epizotic fungi, since the contact of the fungus with the insect cuticle is superficial. *Aegeritella superficialis* forms small (up to 400 µm diameter) dark protuberances; bulbils looking like dirt on the ant body. Sometimes the bulbils are yellowish, and flat which makes them difficult to notice, especially if the host ants are reddish (Espadaler & Monteserín 2003).

Some studies (e.g. Chérix 1982, Wiśniewski & Buschinger 1982) suggest a reduced activity level and life span of the *Aegeritella* hosts, but the effects of the fungi on the ants have not been decisively elucidated yet (Espadaler & Wiśniewski 1987, Espadaler & Monteserín 2003). Infected ants do not seem to be affected by the presence of the fungus, which could also indicate that the metapleural secretions are effective and keep the fungi in an arrested state (Espadaler & Monteserín 2003). It is assumed that the frequent auto- and allogrooming on the head are of vital importance, which could contribute to a lower infestation level (Espadaler & Wiśniewski 1987, Espadaler & Monteserín 2003). Within-colony prevalence of the fungi varies from a few percent to as high as 60% (Wiśniewski 1977, Wiśniewski & Sokołowski 1983).

The known host range of *Aegeritella superficialis* includes various *Formica* species (see Espadaler & Santamaria 2012 for a review), but the fungus was reported only from a few European countries until now: the Czech Republic, Germany, Italy, Poland, Spain and Switzerland (Espadaler & Santamaria 2012). Based on available data it can be considered to be the most widely distributed myrmecoparasitic fungi in Romania. In a single article Paşcovici (1983) reported it on *Formica polyctena* Foerster, 1850, *F. pratensis* Retzius, 1783 and *F. rufa* Linnaeus, 1761 from nine localities (Fig. 1): Avrig (Sibiu County), Baraolt (Covasna County), Cehu Silvaniei (Sălaj County), Curtea de Argeş (Argeş County), Marginea (Suceava County), Mediaş (Sibiu County), Pojorita (Suceava County), Reghin and Sighişoara (Mureş County). New data are not available.

Myrmicinosporidium durum Hölldobler, 1933

The true systematic position of this fungus is still not clear (Sanchez-Peña et al. 1993, Espadaler & Santamaria 2012), but it is a member of the subphylum Entomophthoromycotina (the former order Entomophthorales). Infected ants are easy to recognize based on their conspicuous appearance: their body contain numerous dark, lentiform cap-

sules (ca. 30-50 µm in diameter), and these thick-walled spores of the fungi can easily be seen through the cuticle under a microscope (Sanchez-Peña et al. 1993, Buschinger et al. 2004, Pereira 2004, Gonçalves et al. 2012). In lightly colored ants the high number of the capsules turn the hosts' gaster darker, which makes them easy to pick from the nest (Buschinger et al. 2004, Pereira 2004). In the last stage of the infestation spores may be present massively not just in the gaster of the host, but also in the petiole, postpetiole, the thorax and even in the head (Sanchez-Peña et al. 1993, Pereira 2004, Gonçalves et al. 2012).

The exact nature of the fungus' effects on its host remains uncertain. Neither its life cycle nor the ways of infection are known (Sanchez-Peña et al. 1993). The infection is considered to become evident in fall, thus the infected ants hibernate and die in late spring (Buschinger et al. 2004, Pereira 2004). Within-colony prevalence of infestation is usually low but it can also reach quite extreme levels (Sanchez-Peña et al. 1993, Buschinger et al. 2004, Pereira 2004), while the behavior of infested workers and queens do not seem to be altered due to infestation, according to the observations of Buschinger et al. (2004). It is an uncommon but widespread ant parasite, which can reach a very extensive thallus development in the ant haemocoel without killing it (Sanchez-Peña et al. 1993).

Myrmicinosporidium durum is a generalist parasite: it infects an impressively wide range of ant species of three subfamilies, Myrmicinae, Formicinae and Dolichoderinae (see Espadaler & Santamaria 2012, Gonçalves et al. 2012 for detailed reviews). The fungus was reported from several localities all over Europe (Csősz et al. 2012, Espadaler & Roig 2012, Espadaler & Santamaria 2012, Gonçalves et al. 2012): Austria, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Portugal, Slovakia, Slovenia, Spain and Switzerland. It was also found in South- and North-America in the Galápagos Islands (Ecuador) (Espadaler 1997), and Texas (Sanchez-Peña et al. 1993), Alabama, Florida and Tennessee (Pereira 2004) in the USA. Most recently, this species has been reported from the Asian part of Turkey as well (Csősz et al. 2012).

In Romania it was recently discovered in two different species at three separate locations (Csősz et al. 2012) (Fig. 1): in a single *Solenopsis fugax* (Latreille, 1798) individual caught with a pitfall trap at Luna de Jos (Cluj County), central Romania, in three workers of *S. fugax* at Rimetea (Alba

County), central Romania, and in a single *Tetramorium* sp. E (sensu Schlick-Steiner et al. 2006) individual in the centre of the town Caransebeş (Caraş-Severin County), south-western Romania.

Pandora myrmecophaga (Turian et Wuest) S. Keller (2005)

Pandora myrmecophaga is also member of the subphylum Entomophthoromycotina. The fungus produces infective spores that attach to, germinate on, and penetrate the cuticle of its hosts, ultimately killing them. The fungal mycelium grows in the head, mesosoma, gaster, antennae, and legs of the ant. Production of conidia typically occurs at intersegmental parts of the gaster. The so-called 'summit disease' is present in many insect species: infected individuals look for an elevated position, usually vegetation parts (e.g. leaves, grass blades), from where then the wind can effectively spread the spores (Marikovskiy 1962, Roy et al. 2006, Boer 2008). In the case of ants, typically, infected workers climb on plants around their colonies, where they attach themselves by their mandibles and legs to distal parts of the plant (e.g. grass blades) (Fig. 2). Within hours the rhizoids of *P. myrmecophaga* grow out of the intersegmental parts of the basisternum and laterocervical plates and attach the ant to the leaf even more strongly. Then in one or two days, fur-like fungus appears at the intersegmental parts of the mesosoma and gaster, mainly from the dorsal parts, and somewhat later also near the bases of the mandibles and the antennal insertions (Marikovskiy 1962, Roy et al. 2006, Boer 2008) (Fig. 3). Seemingly warm and wet weather conditions favor the growth of the fungi (Marikovskiy 1962). Intriguingly, there are striking similarities in the host's behavioral modifications caused by *Dicrocoelium* spp. lancet fluke worms and *P. myrmecophaga*, suggesting that similar mechanisms may be involved.

The prevalence of the fungi within colonies is usually low: usually a few infected individuals can be found around a nest (Sosnowska et al. 2004, Boer 2008). On the other hand, the proportion of infected nests within a population can be high: Boer (2008) found that 22% of *Formica rufa* nests contained infected individuals in autumn.

The known host range of the fungus includes ant species of the genus *Formica*: *Formica exsecta*, *F. cf. lemani* Bondroit, 1917, *F. polyclena*, *F. Pratensis* and *F. rufa* (Turian & Wuest 1977, Balazy 1993, Sosnowska et al. 2004, Boer 2008).

The fungus is quite widely distributed in

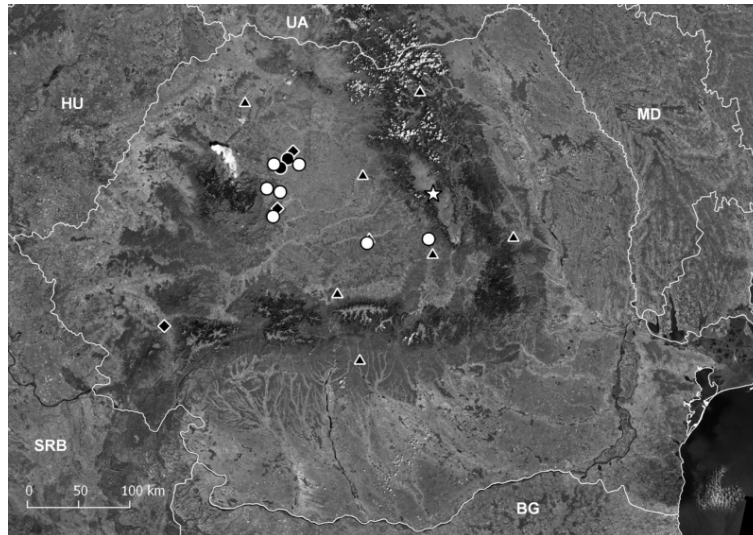


Figure 1. Currently known distribution of *Aegeritella superficialis* (black triangle), *Myrmicinosporidium durum* (black diamond), *Pandora myrmecophaga* (white star) and *Rickia wasmannii* in Romania (black dot – old data; white dot – new data) in Romania. One location of *M. durum* is overlapping with that of *R. wasmannii* at Luna de Jos.



Figure 2. *Pandora*-infected *Formica exsecta* worker before the outbreak of the fungi (photo by B. Markó).



Figure 3. *Pandora*-infected *Formica exsecta* worker after the outbreak of the fungi (photo by B. Markó).

Europe. It is known from the territory of the former Czechoslovakia (Balazy 1993), Germany (Balazy 1993), the Netherlands (Boer 2008), Poland (Balazy 1993, Sosnowska et al. 2004), Russia (Marikovsky 1962), Sweden (Balazy 1993), Switzerland (Turian & Wuest 1977, Balazy 1993), and the territory of the former Yugoslavia (Balazy 1993).

Balazy (1993) also reports a fungus with identical rhizoids, similar conidiophore and conidia found near Hancock, USA.

In Romania it is reported for the first time. It was found on *Formica exsecta* near the După Luncă protected marshland area at Voşlobeni in Harghita County (N 46.61649, E 25.611893, 28.IX.2010 and

05-09.VII.2011, leg. B. Markó et al.) (Fig. 1). In 2010, 500 nests belonging to one polydomous system were systematically searched and only three infected individuals at three nests were found (0.6% prevalence). In 2011, 1066 nests belonging to six polydomous systems were surveyed. Infected individuals were found only at the two biggest systems with 360 and 633 nests censused, the latter system being the same as the one investigated in 2010. Altogether, 17 (4+13) infected nests were found with 18 (4+14) infected individuals. The re-checking yielded positive results only in the case of a single nest, where an additional infected individual was detected after five days. The biggest system had a slightly higher prevalence (2.05 vs. 1.11%). Taking into account all polydomous systems the prevalence of infestation was 1.6% at a population level in the period of investigation in 2011.

Rickia wasmannii Cávára (1899)

Rickia wasmannii is a member of the widely distributed entomoparasitic order of fungi Laboulbeniales (Ascomycetes), which are ectoparasites of arthropods, mostly insects (Santamaria 2001, Herreraiz & Espadaler 2007). The host range of *Rickia* species specifically encompasses distantly related arthropods such as mites (Acari), millipedes (Diplopoda), mole crickets (Orthoptera: Gryllotalpidae), ants and various beetles (Coleoptera) (Weir & Blackwell 2005). *Rickia wasmannii* obligatorily exploits ants of the genus *Myrmica* Latreille (Espadaler & Santamaria 2012). The thallus consists of a multiseriate receptacle which is only one layer of cells thick. The fungal thalli penetrate the outer layer of the cuticle, and appear on the surface of the hosts as clubbed setae-like structures under the stereoscope. This makes the infected host easy to recognize for the myrmecologist, as highly infected ants appear unusually hairy.

Little is known about the effect of the Laboulbeniales fungi on their hosts, and while the fungi are parasitic and penetrate to the hemocoel of the host, they are usually regarded as neutral (see Tartally et al. 2007, García et al. 2010, Lapeva-Gjonova & Santamaria 2011, Bezděčka & Bezděčková 2011, Espadaler & Santamaria 2012). Clearly pathogenic relationships have been documented but it is not possible to generalize about host-parasite relationships for thousands of species in this order and the diverse array of arthropods which are utilized. Premature death or reduced fecundity has been observed in parasitized ladybird beetles, cock-

roaches, and staphylinid beetles (Kamburov et al. 1967, Gemeno et al. 2004, Bro Larsen 1952, Strandberg & Tucker 1974) but other studies using flies and cockroaches have shown no evidence of pathogenicity (Whisler 1968, Richards & Smith 1956).

The known host species of *Rickia wasmannii* are various *Myrmica* species, the most frequent being *M. scabrinodis* Nylander, 1846 (see Espadaler & Santamaria 2012 for a detailed review). The fungus was reported from many European countries (Espadaler & Santamaria 2012): Austria, Bulgaria, the Czech Republic, France, Germany, Hungary, Italy, Luxembourg, Slovakia, Slovenia, Spain, Switzerland and the United Kingdom.

In Romania, it was first reported by Tartally et al. (2007) on *Myrmica scabrinodis* from two localities (Fig. 1): Fănațele Clujului (Cluj-Napoca) and Luna de Jos, all in Cluj County, Transylvania, central Romania. Since then, seven more locations were found (Fig. 1): between Colțești and Izvoarele (N 46.40883, E 23.56011, 14.IV.2012, leg. B. Markó et al.) in Alba County, near Chinteni (N 46.87235, E 23.56003, 21.V.2011, leg. B. Markó et al.), near Săvădisla (N 46.65653, E 23.47477, 04.VI.2011, leg. B. Markó et al.), between Tureni and Micești (N 46.62551, E 23.64392, 19.V.2012, leg. B. Markó et al.) and near Vișea (N 46.87245, E 23.88208, 07.VI.2011, leg. B. Markó & Zs. Czokes) in Cluj County, near Sighișoara (N 46.18147, E 24.76574, 23.VII.2012, leg. B. Markó & K. Erős) in Mureș county and in the Vârghiș Gorge (N 46.2163, E 25.5507, 04.VII.2004, leg. S. Csósz) near Vârghiș in Covasna County, all in Transylvania, central Romania. In the majority of all known populations, *M. scabrinodis* was the only or the primary host, with the exception of the population between Micești and Tureni, where it was found only on *M. rubra* (Linnaeus, 1758). At Vișea it was found both on *M. scabrinodis* and *M. slovacica* Sadil, 1952, near Colțești on *M. gallienii* Bondroit, 1920 and *M. rubra* as well, while near Sighișoara on *M. ruginodis* Nylander, 1846. *M. ruginodis* is a new host species for *R. wasmannii* so far (Espadaler and Santamaria 2012).

Discussion

Ant-fungus relationships are intriguing study subjects due to the exquisite behavioral and ecological adaptations displayed by both sides of the symbiosis (Schmid-Hempel 1998, Roy et al. 2006).

Nevertheless, there is a considerable lack of knowledge not just regarding the details of host-parasite relationships or fungal morphology and development, but even basic information such as host range and geographic distribution (see Espadaler & Santamaria 2012). In the framework of this study we present distribution and host range data on four myrmecoparasitic fungal species in Romania. The scarcity of distribution data (e.g. in the case of *Pandora myrmecophaga*) or an unusual bias towards a specific region (e.g. in the case of *Rickia wasmannii*) indicates a lack of basic field studies, which should specifically target potential host species, as e.g. *Myrmica scabrinodis* in the case of *R. wasmannii*.

There are further species which could be present in Romania with considerable probability. *Laboulbenia camponoti* S.W.T. Batra, 1863 was recently identified in Bulgaria on *Camponotus aethiops* (Latreille, 1798), *C. universitatis* FOREL, 1890 and *C. pilicornis* (Roger, 1859) (Lapeva-Gjonova & Santamaria 2011), and one of the locations was very close to the Romanian Black Sea Coast. Consequently, field surveys targeting specifically *Camponotus* species in Southern Romania may yield the discovery of this seemingly rare parasitic fungus (see Espadaler & Santamaria 2012). The discovery of other Laboulbeniales is also expected. Herraiz & Espadaler (2007) recently found *Laboulbenia formicarum* Thaxter, 1902 on *Lasius neglectus* Van Loon, Boomsma & Andrásfalvy, 1990, an invasive ant species of European big cities. Since *L. neglectus* is also known from Romania it would be worth checking the known populations for the occurrence of this fungus as well.

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