

Fungi Associated with *Messor* Ants on the Balkan Peninsula: First Biogeographical Data

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Abstract: Ant nests' relatively stable and long-lasting microhabitats present ideal living conditions for many uni- and multicellular organisms, whose relationships range from mutualistic to parasitic. *Messor* harvester ants inhabit arid and semi-arid open areas where their colonies consist of large numbers of individuals. Due to the high number of other organisms associated with harvester ants, their nests can be defined as islands for unique biota. Despite significant progress in research on ant-associated fungi in Europe, little is still known about the recently described ectoparasitic fungus *Rickia lenoirii* Santamaria, 2015 (Laboulbeniales), found on two species of ants of the genus *Messor*. Here we report for the first time the occurrence of the ectoparasitic ant-associated fungus *R. lenoirii* from three countries (Albania, Bulgaria, and continental Greece) and multiple localities in the Balkans. The fungus was detected on four ant host species—*Messor structor* (Latreille, 1798), *M. wasmanni* Krausse, 1910, *M. hellenius* Agosti & Collingwood, 1987, and *M. mcarthuri* Steiner *et al.*, 2018 with the latter two representing new host records. Furthermore, spores of the widespread endoparasitic fungus of ants, *Myrmicinosporidium durum* Hölldobler, 1933 (Blastocladiomycota), were reported for the first time in *Messor structor* (Bulgaria). Images of the ant-associated *Rickia lenoirii* taken with a scanning electron microscope, a comparison with *R. wasmannii*, and a distribution map are also presented.

Keywords: ants; parasitic fungi; distribution; *Rickia lenoirii*; *Myrmicinosporidium durum*



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1. Introduction

Ants are well known as hosts of many parasitic organisms, including several endo- and ectoparasitic fungi [1,2]. To date, thirteen ant-parasitic fungal species have been reported in Europe [3,4]. Some of them are widespread, while others are known from only a few localities [5,6]. Four out of the thirteen myrmecoparasitic fungi are known to occur in the Balkans [7–9]. Three of them are ectoparasitic—*Aegeritella tuberculata* Balazy & J. Wisn., 1982 (Basidiomycota, Trichosporonales), *Rickia wasmannii* Cavara, 1899 and *Laboulbenia camponoti* S.W.T. Batra, 1963 (Ascomycota: Laboulbeniales), while the widespread fungus, *Myrmicinosporidium durum* Hölldobler, 1933 (Blastocladiomycota), is endoparasitic. The latter parasitizes a wide range of ant taxa [10,11]; the others are associated with specific genera. For example, the ectoparasitic *Aegeritella tuberculata* has been found on *Lasius* species [12–15] and less frequently on *Formica* [16–18], *R. wasmannii* and *L. camponoti* on *Myrmica* and *Camponotus*, respectively [3,19].

Rickia lenoirii Santam., 2015 was described from Aegina Island (Greece) and southern France (near Marseille), both findings being on *Messor* ants (*M. wasmanni* Krausse, 1910 and *M. structor* (Latreille, 1798)). It is the sixth species of the order Laboulbeniales known to parasitize ants, along with *Dimorphomyces formicicicola* (Speg.) I.I.Tav., *Laboulbenia camponoti*, *L. ecitonis* G.Blum, *L. formicarum* Thaxt., and *Rickia wasmannii* worldwide [6]. The description of *R. lenoirii* drew the attention of researchers, and the first distribution data in

the Carpathian Basin (six localities in Hungary and one in Romania) were reported [20]. Since the first description and distribution records in the Carpathian Basin, no further data on the species have been available, unlike the other ant-associated species of the genus, *R. wasmannii*, for which a number of studies on distribution, hosts utilization, and its impact on ants have been carried out in the last decade.

The Balkan Peninsula has long been understudied in this regard, and we have only recently begun to understand the diversity of myrmecoparasitic fungi and their host preference in this region. Therefore, we started a survey to explore this unique ant-associated biota better. In this study, we searched for the presence of parasitic fungal species (mainly *R. lenoirii* and *M. durum*) on *Messor* ants in Albania, Bulgaria, and continental Greece.

2. Materials and Methods

The fungal-infested ants were collected during ant inventory surveys in Bulgaria, Albania, and Northern Greece. Samples were collected by hand-searching. The ant specimens were stored in ethanol, and detection of the fungi was done by microscopic examination. In preparation for the scanning electron microscopy (SEM), the ant specimens were dehydrated in a graded series of ethyl alcohol, then air-dried and glued on thin aluminum boards. They were gold-coated in a vacuum unit, and images were taken by SEM-TM4000 series. A distribution map of previously known and new localities of *Messor*-associated fungi was created with QGIS (v. 3.26.3).

Although the identification of the ant hosts follows the latest taxonomic revisions, three nest samples (one each from Albania, Greece, and Bulgaria) were left to a species group due to the small number of specimens examined (5 each) and the lack of material from Albania in the latest revision of the *M. structor* group [21]. Thus, the final species identification of these specimens will be postponed after further studies (also molecular). Nest samples with the infected ants were deposited in the Sofia University collection (BFUS) and in the private collection of SC and were labeled with unique collection numbers.

To determine *Rickia lenoirii*, thalli were removed with an insect needle and prepared on slides in Heinz-PVA. The specimens were determined based on the shape, size, and number of cells of the thallus, as well as the shape of the antheridia and perithecia, and compared with the original description and diagnostic characteristics [6] and those described in [20]. Similar to the samples from the Carpathian Basin in [20], the apex of the perithecia was, in most cases, less truncated than in the original description. Specimens of *R. lenoirii* are deposited in the personal collection of FB at Eötvös Loránd University.

3. Results

Fungal-infected workers of *Messor* ants in the study fields were detected in 15 nest samples—14 with *Rickia lenoirii* and one with spores of *Myrmicinosporidium durum* (Table 1). In addition to the new data on the little-known *R. lenoirii*, previously known ones are also summarized in the same table (Table 1) and a map (Figure 1). For the new data, information on both the host ant species and the number of infected specimens is included. Scanning electron microscope images of *R. lenoirii* on *Messor* ants (Figure 2) and of the other species of *Rickia* infecting ants, *R. wasmannii* (Figure 3), are presented. A *Messor structor* ant that is heavily parasitized with *M. durum* is shown in Figure 4.

Table 1. Literature and new data on *Messor*-associated fungi.

<i>Rickia lenoirii</i> Santam., 2015 (Figure 2)						
Country	Collection Data	Latitude	Longitude	Altitude a.s.l. (m)	Ant Host, Number of Infected Specimens	Source, Collection Number
Greece	Aegina Island	37.7505	23.4337	28	<i>Messor wasmanni</i> Krausse	Santamaria & Espadaler 2015
France	Château Gombert, near Marseille	43.3497	5.4497	141	<i>Messor structor</i> (Latr.)	Santamaria & Espadaler 2015
Hungary	Budapest	47.5495	19.0340	118	<i>Messor structor</i> (Latr.)	Báthori et al. 2015
Hungary	Farkasrét	47.4822	18.9997	239	<i>Messor structor</i> (Latr.)	Báthori et al. 2015
Hungary	Ferenc-hegy	47.5164	19.0000	153	<i>Messor structor</i> (Latr.)	Báthori et al. 2015
Hungary	Balatonfüred	46.9641	17.8136	200	<i>Messor structor</i> (Latr.)	Báthori et al. 2015
Hungary	Révfülp	46.8333	17.6334	130	<i>Messor structor</i> (Latr.)	Báthori et al. 2015
Hungary	Badacsony	46.7863	17.4845	106	<i>Messor structor</i> (Latr.)	Báthori et al. 2015
Romania	Herkulesfürdő	44.8668	22.3993	398	<i>Messor structor</i> (Latr.)	Báthori et al. 2015
Albania	Dibër, Radomirë, 02.07.2021, leg. A. Lapeva-Gjonova	41.8127	20.4821	1275	<i>Messor</i> sp. (<i>structor</i> group), 1 worker	this study, BFUS-ALG-000507
Albania	Dibër, Zagrad, 02.07.2021, leg. A. Lapeva-Gjonova	41.7021	20.5001	1191	<i>Messor structor</i> (Latr.), 6 workers	this study, BFUS-ALG-000508
Greece	Falakro Mt., 27.06.2020, leg. A. Lapeva-Gjonova	41.2385	24.0557	942	<i>Messor</i> sp. (<i>structor</i> group), 5 workers	this study, BFUS-ALG-000506
Bulgaria	Ograzhden Mt., Churilovo, 10.09.2021, leg. A. Lapeva-Gjonova	41.4608	23.0067	671	<i>Messor hellenius</i> Agosti & Collingwood, 8 workers	this study, BFUS-ALG-000423
Bulgaria	Thracian plain, Belozem, 17.08.2022, leg. A. Lapeva-Gjonova	42.1900	25.0291	143	<i>Messor mcarthuri</i> Steiner et al., 5 workers	this study, BFUS-ALG-000615
Bulgaria	Besaparski hills, Trivoditsi, 15.04.2017, leg. A. Lapeva-Gjonova	42.1336	24.4530	191	<i>Messor structor</i> (Latr.), 2 workers	this study, BFUS-ALG-000499

Table 1. Cont.

<i>Rickia lenoirii</i> Santam., 2015 (Figure 2)						
Country	Collection Data	Latitude	Longitude	Altitude a.s.l. (m)	Ant Host, Number of Infected Specimens	Source, Collection Number
Bulgaria	Maleshevska Mt., Gorna Breznitsa, 27.03.2012, leg. A. Lapeva-Gjonova	41.7507	23.1093	484	<i>Messor structor</i> (Latr.), 2 workers	this study, BFUS-ALG-000500
Bulgaria	Vratsa karst Reserve, along Darvodeltsi river, 17.04.2016, leg. A. Lapeva-Gjonova	43.1783	23.5775	532	<i>Messor structor</i> (Latr.), 5 workers	this study, BFUS-ALG-000502
Bulgaria	Zemen gorge, Shegava river mouth, 08.05.2021, leg. I Georgiev	42.3931	22.7103	511	<i>Messor structor</i> (Latr.), 2 workers	this study, BFUS-ALG-000504
Bulgaria	Eastern Stara planina, Aytos, 19.06.2017, leg. A. Lapeva-Gjonova	42.7200	27.2380	263	<i>Messor wasmanni</i> Krausse, 1 worker	this study, BFUS-ALG-000306
Bulgaria	Sakar Mt., Radovets, 30.04.2011, leg. A. Lapeva-Gjonova	41.9277	26.4602	280	<i>Messor wasmanni</i> Krausse, 3 workers	this study, BFUS-ALG-000501
Bulgaria	Eastern Rhodopes Mt., Madzharovo, 04.06.2015, leg. A. Lapeva-Gjonova	41.6489	25.8703	134	<i>Messor wasmanni</i> Krausse, 3 workers	this study, BFUS-ALG-000503
Bulgaria	Dervent Heights, Golyam Dervent, 06.06.2021, leg. A. Lapeva-Gjonova	42.0034	26.7422	466	<i>Messor wasmanni</i> Krausse, 2 workers	this study, BFUS-ALG-000505
Bulgaria	South Black sea coast, Sinemorets, 11.11.1997, leg. A. Lapeva-Gjonova	42.0605	27.9679	22	<i>Messor wasmanni</i> Krausse, 2 workers	this study, BFUS-ALG-000616

Table 1. Cont.

<i>Myrmicinosporidium durum</i> Hölldobler, 1933 (Figure 4)						
Country	Collection Data	Latitude	Longitude	Altitude	Ant Host, Number of Infected Specimens	Source, Collection Number
Portugal	Baixo Alentejo	-	-	-	<i>Messor barbarus</i> (L.)	Gonçalves et al., 2012
Bulgaria	Vrachanska Mt., Lakatnik, 25.07.2014, leg. D. Gradinarov	43.0894	23.4000	460	<i>Messor structor</i> (Latr.), 1 worker	this study, BFUS-ALG-000509

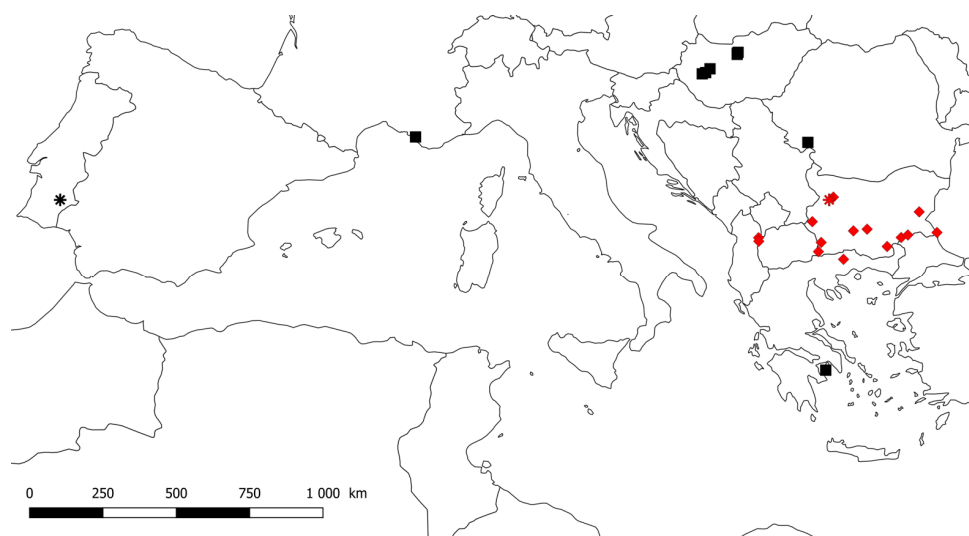


Figure 1. A map of previously known (in black) and new localities (in red) of *Messor*-associated fungi, *Rickia lenoirii* (squares) and *Myrmicinosporidium durum* (asterisks).



(a)



(b)

Figure 2. A worker of *Messor structor* infected by the fungus *Rickia lenoirii* (a) and its mature thallus (b). The arrow in Figure 2a indicates the attachment location of the fungus from Figure 2b.

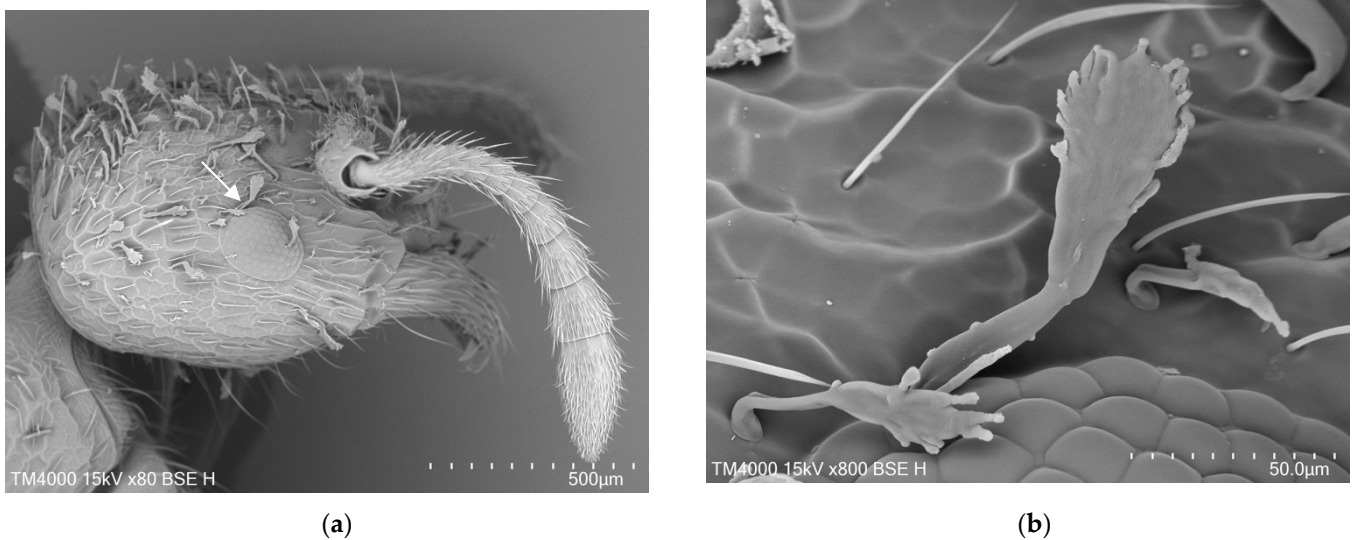


Figure 3. *Rickia wasmannii* on the *Myrmica* ant head (a) and its thalli (b). The arrow in Figure 3a indicates the attachment location of the fungi from Figure 3b.



Figure 4. A *Messor structor* ant with spores of *Myrmicinosporidium durum*. Scale: 1 mm.

4. Discussion

As a result of our study, two fungal species, *Rickia lenoirii* and *Myrmicinosporidium durum* were found to infect *Messor* ants. While the former is specific only to this ant genus, the latter fungus is a generalist with over 35 known host species among ants [8,10]. In this case, the discovery of *M. durum* in *Messor* ants is not unexpected, but the difficulty in detecting the infection due to the dark and thick cuticle of *Messor* ants may be the reason why this is the first time since the Portuguese report and why *Messor structor* has only now become known as a host (Figure 4).

Due to the relatively recent description of *R. lenoirii*, more data on the distribution, host species, and impact on them are still needed. The small thallus size of *R. lenoirii* (Figure 2) is the main reason for its late discovery. As can be seen from some of the data from our collections, infected ant individuals were present in collections long before the species was described.

The data on *R. lenoirii* in our study contribute novel biogeographic and ecological aspects. We report the first localities from the Balkans to the previously known ones of the fungus from southern France, Aegina Island, and the Carpathian Basin. We enlist 14 sites—2 in Albania, 1 in the continental part of Greece (Falakro Mt.), and 11 in Bulgaria (Table 1, Figure 1). The localities cover a wide altitudinal range from the Black Sea coast at 22 m a.s.l. (Bulgaria, Sinemorets) to nearly 1300 m a.s.l. (Albania, Korab Mt.). While *Rickia wasmannii* (Figure 3) is widespread, following the distribution of host species of the genus *Myrmica* in the Palaearctic, *R. lenoirii* is restricted to more southern parts of Europe, where ants of the genus *Messor* also occur. It should be noted that although *R. wasmannii* is widespread in Europe, its host spectrum is relatively narrow, parasitizing mainly *Myrmica* species that typically occur in moderately cool or moist microhabitats in a given region (e.g., *M. gallienii* Bondroit, 1920, *M. scabrinodis*, *M. rubra* Nylander, 1846, *M. vandeli* Bondroit, 1920, *M. ruginodis* Nylander, 1846) [22,23]. This is partly supported by a previous study showing a higher likelihood of *R. wasmannii* presence in habitats with low mean annual temperature and humidity in the Carpathian Basin, suggesting that climatic elements may significantly shape the distribution of this species [24]. Previously, [6,20] discussed that the distribution data on *R. lenoirii* so far have been found mainly near large bodies of water and that higher humidity might promote the presence of the fungus. However, most of the new localities from Bulgaria and Albania are far from large water bodies.

In our study, *R. lenoirii* was discovered on four ant hosts, as *Messor hellenius* and *M. mcarthuri* were added to the already known ones, *M. structor* and *M. wasmanni*. This confirms the close affiliation of this fungus to the ant genus but also indicates the potential for host range expansion after further exploration. Many species of *Messor* occur in the Balkans, and all of them prefer xerothermic biotopes. Despite unresolved taxonomic problems in some species groups, at least eight harvester ant species occur in the southeastern parts of the Balkans (Bulgaria, continental Greece, and the European part of Turkey), where more studies have been conducted [21,25–27]. For now, four of the known *Messor* species in the region have been found to be parasitized by *R. lenoirii*. Only *M. structor* reaches higher altitudes in the mountains, while the others (*M. wasmanni*, *M. hellenius*, and *M. mcarthuri*) are primarily lowland species. The other four *Messor* species, for which no records of infections are known so far, have habitat preferences in the Balkans like the latter. Furthermore, they are often sympatric. It would therefore be worthwhile to examine what habitat and climate factors might influence the occurrence of *R. lenoirii*, which infects *Messor* hosts with fundamentally different climate and habitat preferences.

In conclusion, the overlooked *Rickia lenoirii* seems to be more common than the small amount of data so far suggests, but its distribution is restricted in accordance with the range of *Messor* ant species, which are the most diverse in the Mediterranean region.

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