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Rickia lenoirii, a new ectoparasitic species, with comments on world Laboulbeniales associated with ants

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ABSTRACT

A new species of the genus Rickia is described on ants of the genus Messor based on collections from Greece and France. Rickia lenoirii is distinguished from Rickia wasmannii by the smaller size of the thallus and by the fewer number of cells in each of the three series, as well by other characteristics. The newly described species belongs to the perlata morphological group and because of its diminutive thallus may be compared with similar small species, mostly on mites; therefore it could be included among acarophilous forms according to an old Thaxter definition. The database of ant-Laboulbenial interactions consists of 43 species, 10 genera, and three subfamilies of ants documented as hosts for the six Laboulbeniales known from ants worldwide. Some ant Laboulbenial species show a low host phylogenetic specificity while other species are much restricted in their host range. The highly biased known distribution of ant Laboulbeniales is probably an artifact and the database is far from being complete.

1. Introduction

Laboulbeniales are among the most important Orders of the Ascomycota, including around 2000 species of obligate ectoparasites from living arthropods, which are attached to the cuticle of their hosts. Most of these fungi (more than 1500 species) are known to parasitize Coleopteran Insects, followed at some distance by Diptera (about 200 species) (Weir and Hammond 1997). Only six species of Laboulbeniales parasitize Hymenoptera, and all of these attach to ants (Formicidae).

The genus Rickia Cavara is one of the largest known genera within the Laboulbeniales with 152 species, including the new one described here. Tavares (1985) reported 122 species and one variety. In the latest monographic work dealing with the genus Rickia, where 17 species from Sulawesi are reported, 5 of them new, Weir (1998) refers to approximately 145 species. According to the MycoBank databases (http://www.mycobank.org), the genus included 151 species, excluding
varieties and duplicities. This genus is mainly tropical, with 84% of the species living in such environments (Weir and Hammond 1997). Over 17 species were reported in Europe (Majewski 1994; De Kesel et al. 2013).

The aim of this paper is to increase the knowledge of Laboulbeniales living on ants with the description of a new species and to review the taxonomical and geographical distribution of these parasitic fungi which live on these social insects. Also, we intend to call the attention of myrmecologists and mycologists to fill the lack of data in huge areas of the world. The six species of Laboulbeniales parasitizing ants belong to three different genera: Dimorphomyces Thaxt., Laboulbenia Mont. and C.P. Robin in C.P. Robin, and Rickia (Table 1).

### 2. Materials and methods

Ant hosts were collected with an entomological aspirator during a routine myrmecological survey of the ants from southern region of Greece. The insects were preserved in 96\% ethanol. Upon inspection during the process of ant identification a sample was detected as infected with fungi. They turned to be a species of Laboulbeniales.

Permanent slides were prepared following previously described methods (Benjamin 1971) and are kept in the BCB Mycotheca of Universitat Autònoma de Barcelona (BCB-slides). Ant specimens with attached fungi are kept in the BCB-SS leg. A. Lenoir (Holotype, BCB-SS 36/18/C26) and C.P. Robin in C.P. Robin, and Rickia (Table 1).

### 3. Results

**Rickia lenoirii** Santam., sp. nov.  
Fig. 1.  
MycoBank no.: MB 807937.

Diagnosis: small species, less than 70 µm in length; each series of cells consisting of 2–3 (median), 2–3 (anterior), and 4 cells (posterior); flask-shaped antheridia above appendiculate cells; secondary-like sterile appendages formed after degeneration of antheridia; perithecium with a truncate tip.

Type: Greece, Aegina Island, 37°45’N 23°26’E, on *Messor wasmannii* Krausse (Hymenoptera, Formicidae), 8 March 2009, leg. A. Lenoir (Holotype, BCB-SS-E0512a; isotype, BCB-SS-E0512b).

Etymology: lenoirii, named after Prof. Alain Lenoir, myrmecologist who collected the first specimens of infected ants.

Thallus hyaline except for the blackened foot, trichogyne tan scar, and brown septa separating the appendiculate cells from appendages. Total length from foot to tip of perithecium 45–67 µm. Receptacle triseriate. Basal cell broadened distally, slightly longer than broad, more or less strongly curved towards the black foot; giving way distally to the two lower cells of the marginal series. Median series located above the lowermost pair of cells of the marginal series; consisting of 2, rarely 3, superposed cells, rounded in outline although very variable in shape and size, but the lower being the bigger; the distal cell near reaching the middle height of perithecium. Anterior series consisting of 2 (rarely 3) superposed, somewhat variable cells; the lower usually bigger and more or less outwardly inflated, but rectangular and flattened as well; the uppermost cell (and the second if exists) flattened and trapezoidal, giving rise to 1–2 triangular appendiculate cells which form the base for a constricted and brown septa and a single antheridia. Posterior series composed of 4 superposed cells; the lowermost cell flattened and rectangular, diagonal to vertically orientated in relation to thallial axis; second and third cells similar in shape and size, trapezoidal; the fourth or distal cell of the series is smaller, rounded, broader than long. Cells 1–2 of the posterior series each separating a triangular appendiculate cell and one antheridia, following the same pattern described above for anterior series. Third cell variable, lacking the appendiculate cell as well as supporting 1–2 appendiculate cells with their relative single antheridia. Fourth cell separating distally an appendiculate cell which forms the support for primary appendage, above a brown and constricted primary septum. Primary appendage short, rounded, globose, amorphous, appearing deteriorated and dirty even in young thalli or sporangia. Antheridia flask-shaped, straight to more or less falcate, 11–20 × 3–4 µm, bearing a long efferent neck; deteriorating with age and then reduced to amorphous secondary appendage-like structures. Perithecium 30–36 × 12–16 µm, stout, broadly fusiform, its maximum breadth near the third basal part; with a truncated tip; showing a persistent trichogyne brown scar at its upper, outer margin.

Hosts and distribution: on *Messor* spp. from Greece and France.

Additional specimens examined: France, Châteaux Combert, near Marseille, 43°20’58.98 N 5°26’58.84 E, on *Messor structor* (Latreille) (Hymenoptera, Formicidae), 17 July 2011, leg. O. Blight (BCB-SS-E0540ac).
Little variation has been observed between thalli found on *Messor structor* and *M. wasmanni*. Those thalli found on the former are slightly smaller, 45–60 µm in length whereas 55–67 µm in the later. Antheridia having lengths from 11 to 14 µm in *M. structor* to 16–20 µm in those of *M. wasmanni*. Nevertheless, perithecia do not differ significantly.

According to Tavares (1985) there are four basic structural types in the genus *Rickia*. The *perlata* type, which is short and broad, is widespread and includes *R. lenoirii*. Receptacle of the genus is flattened and typically triseriate, with two marginal series of cells supporting antheridia and or appendages, the anterior which represents the side where perithecium is located, and the posterior that supports the primary appendage; the median or axial series may be reduced and usually does not bear neither appendages nor antheridia (Thaxter 1926).

*Rickia lenoirii* may be compared with several species although they are very different from one to many characteristics. The other species of the genus living on ants, *R. wasmannii* Cavara, has no evidence of a morphological relationship with *R. lenoirii* and should be quickly separated from this by (1) cell I, very elongated and as long as the remainder thallus in *R. wasmannii*; (2) different number of cells in each series, 4–6 in the anterior, 5–9 in the posterior and 6–7 in the median, whereas in *R. lenoirii* we found respectively, 2–3, 4, and 2–3 cells; (3) lastly, *R. wasmannii* is much bigger in size 187–245 µm compared to the smallest 45–67 µm in *R. lenoirii*.

*Rickia lenoirii* is a very small species that should be compared with the smallest species in the genus as well as with species with similar ecology. With very few exceptions (e.g. *R. europsis* Thaxt.) these small species parasite mites and even Thaxter (1926, p. 471) wrote about them as the “group” of acarophilous forms: *R. anomala* Thaxt. on a “minute hairy mite belonging to a genus near Iphiopsis” from the West Indies (Thaxter 1926, p. 453) with a length of 48–56 µm; very different from our species by several characters such as its curved thallus having 9 cells in the posterior series; *R. arimensis* Thaxt.

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**Fig. 1** — *Rickia lenoirii* (A: BCB-SS-E0512b; B: BCB-SS-E0540b; C, D: BCB-SS-E540c; E: BCB-SS-E0512a). A, B, D: Mature thalli showing some details indicated by arrows; appendiculate cell in A, trichogyne scar in B, and primary septum in D. C: Detail of an immature thallus with undamaged trichogyne (arrow). E: Immature thallus where one antheridium is indicated with an arrow. Bar: A 20 µm, is the same for all figures.
which has a length of up to 68 μm, parasitises “a very large mite, parasitic on Passalidae” from Trinidad (Thaxter 1926, p. 455) differs by a larger number of cells in median and posterior series as well as by very different secondary appendages; R. celenopsis Thaxt. with a total length of 50 μm, which was found on a mite of the genus Celaenopsis from Trinidad (Thaxter 1926, p. 459) shows striking protuberant disposition of the lowermost cells in anterior and posterior series; R. depauperata Thaxt. which may be the smallest species in the genus, with only 35–40 μm of total length, also lives on Celaenopsis from Haiti (Thaxter 1926, p. 464) and has a perithecium with an erect projection; R. discreta Thaxt. is a small species of only 55–65 μm discovered “on various parts of a small undetermined gamasid mite” from Trinidad (Thaxter 1926, p. 466) well distinguished by its large, “discrete” appendages; R. elliptica Thaxt. which was found on the legs of mites of the genus Discopoma from Trinidad (Thaxter 1926, p. 468) has the series with more cells and an outgrowth on the perithecial tip; and many others up to the 59 species of Rickia described on mites.

Another species that must be morphologically compared with R. lenoirii is R. evesti Thaxt., described on Coleoptera Erotylidae belonging to the genus Eusteus from Philippines and Sumatra (Thaxter 1926, p. 471) which measures 50–90 μm and in its smaller forms (Thaxter 1926, Fig. 134) surprisingly resembles very much R. lenoirii except that the peritheicum of the later shows a conspicuously truncate perithecial tip; nevertheless the more typical forms of R. evesti illustrated in Fig. 133 by Thaxter show obvious differences between both species.

Deserving a special attention, are the three species from Poland described by Majewski (1994) on mites, which live with ants in nests. As will be mentioned below in the discussion, mites may play a role in dispersion of Laboulbeniales because some of them live in more or less close contact with arthropods as phoretics or, in a more particular way here related to the subject of this work, as myrmecophilous. These three species are: R. pachylaelapis T. Majewski on Pachylaelaps holothyroides (Leonardi) (Acarina, Mesostigmata, Pachylaelipsidae) from nests of Lasius flavus (Fabricius); R. stellata T. Majewski on Celaenopsis and Pleuronotocelaeano species (Acarina, Mesostigmata, Celaenopsidiae) from nests of Lasius sp.; and R. georgii T. Majewski on Hypoaspis cuneifer Michael (Acarina, Mesostigmata, Dermanyssidae) from nests of L. flavus. Characters distinguishing these species from R. lenoirii are: Rickia pachylaelapis is bigger, more rounded in shape, has long secondary appendages and a perithecium with a truncate apex but showing small acute lips; R. stellata shows unusual strikingly acute appendages on the thallus, without the typical narrow brown septa of most species in the genus; R. georgii is much bigger with many more cells in series, for example the 8–10 cells in median series, and a posterior series ending in an elongate distinct stalk of the primary appendage.

4. Discussion


This is the second Rickia species recorded for the Myrmicinae ant subfamily. The other Rickia species documented on ants — R. wasmannii — is found on seven species of Myrmica (Espadaler and Santamaria 2012; all in Europe), a genus well known for its moisture-loving habits (Radchenko and Elmes 2010). The extremely small size of Rickia lenoirii may have been the principal setback to its discovery. However, there are also ecological considerations that should be taken into account: Messor are granivorous ants, especially abundant in relatively dry habitats, even desert environments (Whitford 1996). Such contexts are clearly not the best moisture conditions for the general presence and development of fungi, although the general high air humidity expected in small islands, such as Aegina (−90 km²), or at Chateaux Gombert – 8 km in bee line from the sea — would allow for an ecological escape window for Rickia lenoirii. Over 110 names of Messor species rank and 40 names of subspecies rank are presently known (Bolton 2012), 15 of them in Greece (Legakis 2011). It is therefore highly likely that other Messor species will be detected as hosts for Rickia species.

How could R. lenoirii come into close contact with the ants? Mites are among the most diversified myrmecophiles (Hollodinber and Wilson 1990; Kronauer and Pierce 2011) and a large proportion of Rickia species (59 out of 151) have Acari as hosts (Tavares 1985, updated). The degree of physical contact between ants and myrmecophilous mites varies, from the free-living forms within nest galleries and scarce contact with ants to a tight, intimate relationship of long-time physical contact in phoretic mites and ants (Donisthorpe 1927; Kistner 1982). Consequently, it is tempting to suggest a direct evolutionary horizontal transfer of fungi between myrmecophilous Acari and their ant hosts. This hypothesis — mites as fungal vectors — has already been put forward for Laboulbeniales from beetles by Tavares (1985). A precise example is shown by Seeman and Nahrung (2000), after finding a minimum of two Laboulbeniales species growing on mites and on their associated beetle hosts. An indirect form of transmission, through spores in soil, is not to be dismissed (Lindroth 1948 in Madelin 1966; De Kesel 1995), although the short-lived spores (De Kesel 1995) impose limitations to this way of infection.

Acari diversity in ants, or in their nests, has been shown to be positively correlated with those ant species that have i) greater resource availability within nests; ii) populous colonies; and iii) a large body size (Campbell et al. 2013). Messor species comply with these three characteristics and an excellent example of such mite diversity are the results shown for alates by Uppstrom and Klompen (2011), who recorded a minimum of seven phoretic mite species — from several families — and five species of mites inhabiting the chaff-piles of Messor pergandei (Mayr). Other mite species are known from several Mediterranean Messor (Bernard 1967). A dedicated search for infested acari in the nests of M. wasmannii on Aegina Island would be justified.

Laboulbeniales seem to have effectively broken the battery of chemicals produced in the metapleural gland of ants. Messor structor was shown to be able to prevent mould development on seeds by removing mould from the seed integument (Celli and Maccagnani 1994). In addition, substances inhibiting fungal spore germination were detected in the Messor barbarus metapleural glands (Schildknecht and Koob 1970, 1971). Messor wasmannii is therefore also likely to have a good complement of behavioral and physiological mechanisms (Ugelvig and Cremer 2007) to clean them from ectoparasitic fungi. We are unaware of published evidence that convincingly suggests that...
Laboulbenia or Rickia may have any detrimental effects on their ant hosts, although this aspect is currently being explored (Markó et al. 2011; Tragust 2011). A different, non-alternative hypothesis should also be taken into account, namely, that Laboulbeniales in ants are close to, or have reached, a more or less avirulent stage, being akin to neutral symbionts. This was exactly the expectation set out by Boomsma et al. (2005) or Hughes et al. (2008) concerning the evolution of virulence in specialized ectoparasites, such as Laboulbeniales.

4.2. Taxonomical and geographical distribution of Laboulbeniales on ants

Forty-three species, 10 genera, and three subfamilies of ants are documented as hosts for the six Laboulbeniales known from ants worldwide (Table 1; Espadaler and Santamaria 2012). Although the data base of ant-Laboulbenial interactions is limited, a couple of tentative patterns seems to emerge. Insect-fungus interaction is highly asymmetric: any ant species hosts more than a single Laboulbenial, although three Laboulbeniales have seven to 21 ants as hosts. In addition Laboulbenia formicarum Thaxt. shows a low host phylogenetic specificity — sensu Foulin et al. 2011 — since it is found on six genera that belong to three different tribes (Bolton 2003). In contrast, Laboulbenia camponoti S.W.T. Batra on Camponotus, and the two Rickia species — each with its own ant host genus — show a high host phylogenetic specificity. The dichotomy “host-specialist” versus “host-generalist” seems to apply here (Komatsu et al. 2009; Espadaler and Olmo-Vidal 2011).

Only ants from four biogeographical regions are involved with Laboulbeniales (Fig. 2), with only two points being known from the tropics. Rickia on ants are limited to Continental Europe, while their hosts (Myrmica, Messor) are known from the Neotropical, Nearctic, Palearctic, Ethiopian and Oriental regions (Bolton 1995). The highly biased distribution of ant Laboulbeniales that is currently known probably points to a remarkable mismatch between the geographical location of taxonomists and biological diversity (Gaston and May 1992). In all likelihood, the database is far from complete for both Laboulbeniales on ants and for ants infested with Laboulbeniales. Dedicated attention from myrmecologists is needed to correct this shortcoming.

Fig. 2 — Global distribution of ant-Laboulbeniales interactions (up to January 2014) (see Table 1). In the Northern hemisphere they are documented from 28° N (India) to 51° N (United Kingdom). In the Southern hemisphere they are known from 0.58° S to 34.8° S. Higher magnification of European area (inset).

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REFERENCES


