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Ant-fungus Interactions: *Laboulbenia camponoti* Batra in Italy and a New Host for *L. formicarum* Thaxter (Fungi: Ascomycota, Laboulbeniales)

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Abstract

One *Laboulbenia* species is added to the checklist of Italian fungi. *Laboulbenia camponoti* was detected on the ant *Camponotus aethiops.* Additionally, *L. formicarum* was found on a new host (*Lasius niger*) in France. An updated map of world distribution for the two *Laboulbenia* is presented. Based on present knowledge, *L. camponoti* shows a much higher structural and phylogenetic host specificity than *L. formicarum*.

Introduction

Laboulbeniales, with over 2000 species, are obligate ectoparasites living attached to the cuticle of arthropod hosts (Tavares, 1985). Most are known to parasitize Coleoptera, although Diptera, Dermaptera, Dyctioptera, Acari and Diplopoda are also known to harbour specific Laboulbeniales (Weir & Hammond, 1997). Only six species of Laboulbeniales parasitize Hymenoptera, and all attach to ants (Formicidae). Ant Laboulbeniales are globally distributed, being known from the Palaearctic, Nearctic, Neotropical and Indo-Malayan regions, with the Afrotropical and Australasian regions still lacking any register (Santamaria & Espadaler, 2015). Here we deal with two species from this last, and small, group of Laboulbeniales.

Material and Methods

A total of 35 localities (8 in France and 27 in Italy) were haphazardly sampled during a road trip in October 2015. Most of the sampled locations habitats were urban or ruderal. Ants were directly preserved in alcohol and examined under a dissecting microscope. All ant samples were scrutinised with a Leica SMZ16 with magnifications ranging from 40x to 115x. Special attention was given to femora and tibiae as those parts use to be the surfaces were fungi are most visible. Due to the non-systematic sampling method, our results have to be taken more as descriptive of the scarcity of fungi rather than a quantitative measure of infestation prevalence in a given area.

Permanent slides were prepared following 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 private collection of K. Gómez (KGAC). Although the database is meagre, we explore host specificity of the two ant-parasite *Laboulbenia*, which accumulate 61 world records. Following Poulin et al. (2011), we use Simpson and Shannon indices as metrics for host structural diversity. Indices were obtained using EstimateS (Colwell, 2013).

Distribution maps were created with CartoDB (www.cartodb.com)

Results

A total of 145 *Camponotus* workers of six species and 322 *Lasius* (233 workers, 65 queens, 24 males) of five species were searched for parasites, and only three workers (one *Camponotus* and two *Lasius*; 0.6% prevalence in workers) were positive for *Laboulbenia* species. Two out of 33 *Camponotus* or *Lasius* samples were infested (6% prevalence in samples). Table 1 summarizes the localities where *Lasius* or *Camponotus* species were collected.

***Laboulbenia camponoti* S.W.T. Batra, 1963** (Fig 1a, 1b)

On *Camponotus (Tanaemyrmex) aethiops* (Latreille). One out of eight collected workers was infested with the fungus. Italy: Toscana: Montopoli 43º 40.4'N 10º 44.98'E; 50m Leg: Gómez, K. 03/10/2015. Garden with *Acer* sp., *Pinus* sp. and *Olea europaea*. Foraging on *Pinus* trees. [KG03133].

The fact that the worker was captured among other non infested workers seems to reinforce the notion that

Table 1. Mediterranean localities with *Camponotus* or *Lasius* species. 1: *Camponotus aethiops* (Latreille); 2: *Camponotus cruentatus* (Latreille); 3: *Camponotus lateralis* (Olivier); 4: *Camponotus piceus* (Leach); 5: *Camponotus sylvaticus* (Olivier); 6: *Camponotus vagus* (Scopoli); 7: *Lasius lasioides* (Emery); 8: *Lasius myops* Forel; 9*: Lasius niger* (Linné); 10: *Lasius paralienus* Seifert. X: Species present. **L**: species infested with *Laboulbenia*.

Ant species	1	2	3	4	5	6	7	8	9	10	11
FRANCE											
Bouches-du-Rhône: Bus station (Arles) 43º 40,92'N 4º 37,88'E 215m								X			
Bouches-du-Rhône: Cathedral Square (Arles) 43º 40,92'N 4º 37,88'E 215m										**L**	
Languedoc-Roussillon: Gruissan Pond (Aude) 43º 7,02'N 3º 4,74'E 10m		X			X						
Var: Chemin des Costettes 43º 23,91'N 6º 20,64'E 200m	X	X									
ITALY											
Lazio: Camping (Lughezza) 41º 55,92'N 12º 42'E 60m				X							
Liguria: Balzi-Rossi caves (2) (Grimaldi) 43º 47,15'N 7º 31,86'E 5m			X								
Liguria: Camping il Giglio (Monterosso al Mare) 44º 9,3'N 9º 39,6'E 250m			X			X	X				
Liguria: Marinella di Sarzana (Carrara) 44º 2,59'N 10º 1,46'E 5m								X			X
Liguria: Ruta 9 a Monterosso (1) (Monterosso al Mare) 44º 9,25'N 9º 39,47'E 200m				X							
Liguria: Ruta 9 a Monterosso (2) (Monterosso al Mare) 44º 9,34'N 9º 39,63'E 310m			X								
Liguria: Torre di Santamaria Square (San Bartolomeo al Mare) 43º 55,21'N 8º 6,19'E 10m							X				
Toscana: Meadow close to parking lot (Vinci) 43º 40,4'N 10º 45,12'E 60m									X		X
Toscana: Montopoli 43º 40,4'N 10º 44,98'E 50m	**L**			X							X
Toscana: Montopoli 43º 40,45'N 10º 44,74'E 40m											X
Toscana: Parking lot (Montepoli) 43º 40,4'N 10º 45,12'E 60m	X		X	X							
Toscana: PKG Decathlon (Cascina) 43º 40,53'N 10º 28,29'E 10m											X
Toscana: Via Scandicci 221 (Florence) 43º 45,75'N 11º 12,53'E 50m						X					
Umbria: Castle garden (Orvieto) 42º 43,36'N 12º 7,22'E 275m	X								X		
Umbria: Catedral Square (Orvieto) 42º 42,99'N 12º 6,8'E 320m						X	X		X		X

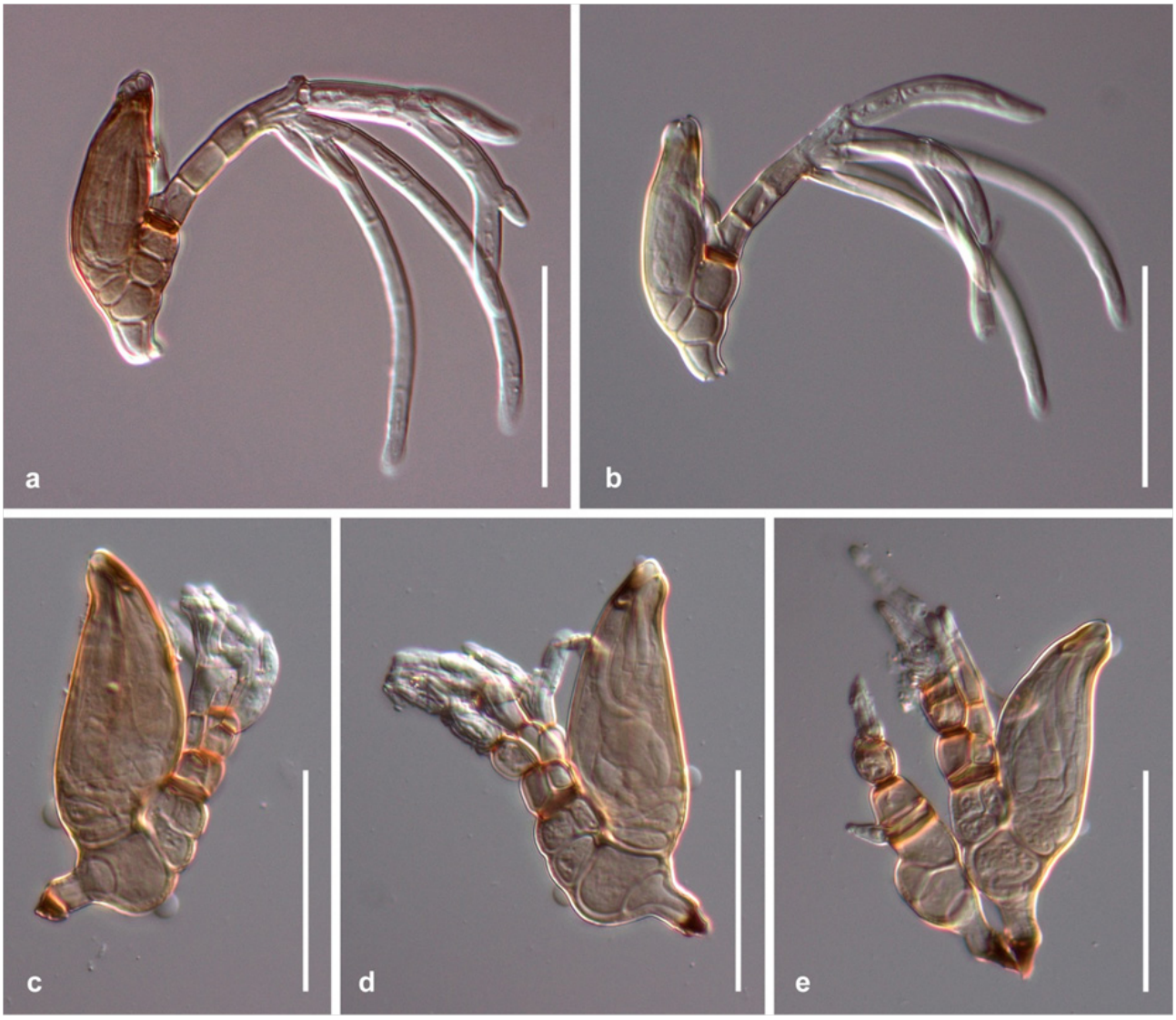

Fig 1. a, b: *Laboulbenia camponoti* Batra. a, mature specimen, b, slightly immature specimen. Darkened foot was broken in slide mounting; c, d, e: *Laboubenia formicarum* Thaxter. c, d, mature female specimens. In e, paired thalli, male at left and female at right. Scales: 50 µm.

Laboulbenia fungi does not seem to be a strong handicap for infested ants while foraging.

L. camponoti was previously known from 19 world records, from six countries (Fig 2): India *loc. typ.* (Batra, 1963), Austria (Báthori et al. 2014), Bulgaria (Lapeva-Gjonova & Santamaria, 2011), Romania (Báthory et al., 2014), Spain (Balazuc et al., 1982; Espadaler & Blasco, 1991), Turkey (Espadaler & Lodos, 1983).

***Laboulbenia formicarum* Thaxter, 1902** (Fig 1c, 1d, 1e)

On *Lasius (s.str.) niger* (Linnaeus). 2 out of 2 collected workers were infested, both drowned in a fountain. This species is a new natural host for the fungus, although it had already been proved to be susceptible using experimental infestations in the laboratory (Tragust et al. 2015). France: Bouches-du-Rhône. Cathedral Square (Arles) 43º 40.92'N 4º 37.88'E 215m. Leg: Gómez, K. 28/09/2015. Urban environment. [KG03116]. Curiously enough, this site matches the pattern of coastal localities where this fungus has been collected −4 out of 5 localities− in Europe.

L. formicarum was previously known from 41 world records, from five countries (Fig 3): U.S.A. *loc. typ.* (Thaxter, 1902), Canada (Judd & Benjamin, 1958), France (Espadaler et al., 2011), Portugal (Espadaler & Santamaria, 2003), Spain (Herraiz & Espadaler, 2007).

Other ant genera collected (# of species) in France (*Cataglyphis* (2), *Crematogaster* (1), *Hypoponera* (1), *Messor* (2), *Plagiolepis* (1), *Pheidole* (1), *Tapinoma* (1), *Tetramorium* (1)) or Italy (*Aphaenogaster* (2), *Crematogaster* (1), *Formica* (1), *Hypoponera* (1), *Linepithema* (1), *Messor* (1), *Pheidole* (1), *Plagiolepis* (2), *Solenopsis* (1), *Tapinoma* (1), *Temnothorax*(1), *Tetramorium* (1)) were not infested.

Fig 2. Known distribution for *Laboulbenia camponoti* Batra, up to February 2016 (blue dots, known distribution; orange dot, new record).

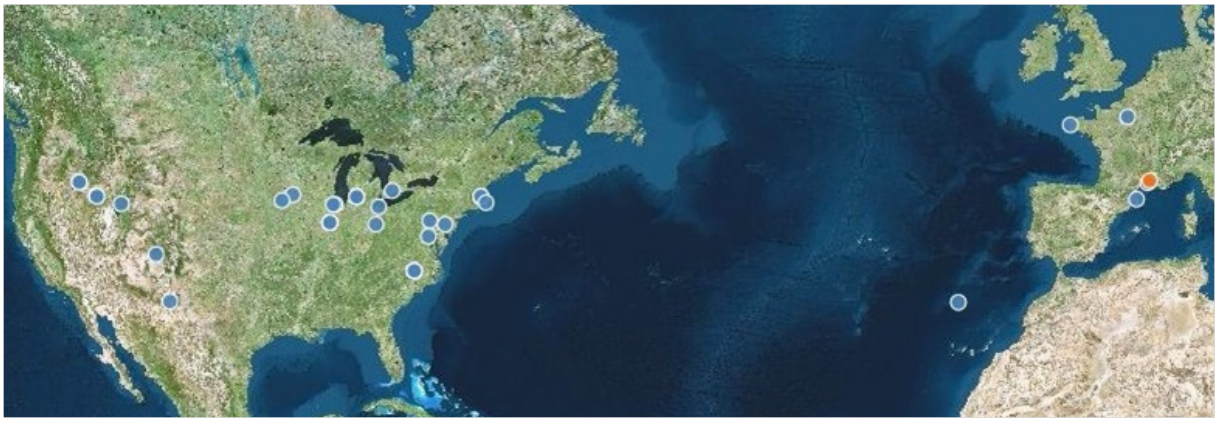

Fig 3. Known distribution for *Laboulbenia formicarum* Thaxter, up to February 2016 (blue dots, known distribution; orange dot, new record).

Discussion

One hundred years have elapsed since the first instance of an ant-infesting Laboulbenial was collected in Italy. *Rickia wasmannii* Cavara was detected by Spegazzini (1914) on workers of *Myrmica scabrinodis* Nylander from Conegliano (Veneto). We are unaware of any other register of ant-Laboulbenial interaction in Italy.

The two *Laboulbenia* species here discussed seem to be limited to the Northern hemisphere. The genus *Camponotus* has a world distribution. Instead, the diverse ant genera known as hosts for *L. formicarum* (see below) have a Northern hemisphere distribution (http://www.antwiki.org/wiki/Category :Genus_Distribution_Map accessed March 2016). The absence of data for ant Laboulbeniales from Central and Eastern Eurasia calls for a dedicated search.

It is perhaps noteworthy the contrasting host range of both species. For *Laboulbenia camponoti*, 17 out of 19 known hosts belong in six species of *Camponotus* (*Tanaemyrmex*). Subgenus host identity for the two citations from India remains unknown. Instead, for *Laboulbenia formicarum*, known host belong in 25 ant species from five genera (several subgenera): (*Lasius* (s.str.), *Lasius* (*Acanthomyops*), *Formica* (*Neoformica*), *Formica* (*Raptiformica*), *Formica* (*Serviformica*), *Myrmecocystus, Prenolepis, Polyergus*) (Espadaler & Santamaria, 2012), in two tribes (Ward et al. 2016).

Host specificity encompasses several components: 1) structural (=basic) specificity or the number, and proportion, of host use; 2) phylogenetic specificity, the range of phylogenetic spectrum of hosts; 3) geographic specificity, the consistency of host use across the parasite geographical distribution. Depending on the quantity and quality of available knowledge, those different components may be singly explored, or in their interactions (Poulin et al. 2011). A simple analysis of the structural and phylogenetic components of host specificity indicates a lower specificity in *L. formicarum* (Table 2). The low host phylogenetic specificity in *L. formicarum* – sensu Poulin et al. 2011 – is the exception, rather than the rule, with host-Laboulbeniales relationships (Tavares, 1985; Weir & Hammond, 1997). Geographic specificity is likely low in *L. camponoti* as shows its detection on three *Camponotus* (*C. aethiops, C. pilicornis, C. sylvaticus*) at a single organic citrus grove in La Selva del Camp (Tarragona, Spain ; 41º13’07”N, 01º08’35”E) (unpub. obs.).

Table 2. Ant host structural and phylogenetic diversity of two *Laboulbenia* species.

	Shannon diversity	exponential Shannon diversity (Hill's N1)	Simpson diversity (Hill's N2) (inverse form)	Tribes	Genera	Subgenera	Species	# global records
L. camponoti	1.66	5.26	3.77	1	1	1	7-8	20
L. formicarum	3	20.16	16.00	2	5	>5	25	41

Although a mere six *Camponotus* species are known from the state of Delhi, the genus has 83 species in India (Bharti et al. 2016). We take the conservative approach to consider the two registers of *L. camponoti* from India to belong in two different host species.

A similar example of extremely low phylogenetic specificity is exhibited by the ant parasitic fungus *Myrmicinosporidium* Hölldobler. Known from the Palaearctic, the Nearctic and a single location in the Southern hemisphere, its spores have been detected in a wide host range of 38 species, 17 genera, and three ant subfamilies (Gonçalves et al., 2012; Lapeva-Gjonova, 2014; Giehr et al. 2015). Furthermore, geographic specificity seems to be also very low in *Myrmicinosporidium* since it was documented on seven ant hosts, belonging to seven genera, from five tribes, and three subfamilies at a single olive grove from Póvoa de São Miguel (Portugal) (Gonçalves et al., 2012).

With this note we add one more Laboulbenial to the list of the Italian mycoflora and prove right experimental, cross-infection laboratory results by Tragust et al. (2015) in the wild for *Laboulbenia formicarum* infecting *Lasius niger* in France. The ant genera *Camponotus* (subg. *Tanaemyrmex*) with eight species in Italy, and *Lasius* (s.str.) with nine species (Baroni Urbani, 1971; Poldi et al., 1995) offer ample host opportunities for the fungi. The genus *Messor*, with eight species in Italy (op. cit.), provides also possible hosts for the recently described *Rickia lenoirii* Santam. from Greece (Santamaria & Espadaler, 2015). Thus, it can be only expected an enlargement of the database for those interesting ant-fungus interactions when a dedicated search for is undertaken, either in the field as in this paper, or in Museum collections (Suarez & Tsutsui, 2004; Báthori et al., 2014, 2015). Ant genera *Myrmica, Camponotus* (*Tanaemyrmex*)*, Lasius* (s.str.) and *Messor* should be specifically focused.

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