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5
6 **Title:** First records of the myrmecophilous fungus *Laboulbenia camponoti* Batra
7 (Ascomycetes: Laboulbeniales) from the Carpathian Basin

8
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16
17 **Running title:** *Laboulbenia camponoti* is reported from the Carpathian Basin

18

19 **Abstract** – *Laboulbenia camponoti* Batra, 1963 (Ascomycetes: Laboulbeniales), has been
20 found on *Camponotus aethiops* (Latreille, 1798) (Hymenoptera: Formicidae) workers in the
21 Carpathian Basin: in Baziaș, Caraș-Severin (Romania), and Vienna (Austria). Vienna is the
22 northernmost known locality of this fungus (48°12' N). These new observations expand the
23 area of *L. camponoti* from regions with Mediterranean and subtropical climatic influences to
24 the common borders of the Continental and Pannonian regions. These results show that
25 *Camponotus* samples from other climatic regions should be examined more closely for this
26 fungal parasite.

27

28 **Keywords:** Austria, *Camponotus aethiops*, Central-Europe, mycology, Romania, social
29 parasite.

30

31 **1. Introduction:**

32 The order Laboulbeniales comprises more than 2000 species in about 140 genera (Santamaria,
33 2001; Weir & Blackwell, 2005; Kirk *et al.*, 2008). They are obligate ectoparasites of
34 arthropods, and approximately 80% of the described Laboulbeniales species parasitize
35 Coleoptera species (Santamaria, 2001; Henk *et al.*, 2003; Weir & Blackwell, 2005).

36

37 In the order Hymenoptera, only ants are known to be hosts of certain species of
38 Laboulbeniales (Espadaler & Santamaria, 2003). Thus far, four species of these fungi have
39 been reported to be associated with ants in Europe: *Rickia wasmannii* CAVARA, 1899, is found
40 in 14 countries on seven *Myrmica* species; *Laboulbenia formicarium* THAXT, 1908, in France,
41 Portugal and Spain on two *Lasius* species; *Laboulbenia camponoti* BATRA, 1963, in Bulgaria
42 and Spain on five *Camponotus* species; and *Rickia lenoirii* SANTAMARIA and ESPADALER,
43 2014, in Greece and France on two *Messor* species (Herraiz & Espadaler, 2007; Lapeva-
44 Gjonova & Santamaria, 2011; Espadaler & Santamaria, 2012; Santamaria & Espadaler, 2014).
45 The effect of these ant parasitic fungi on their hosts is rather understudied except for the work
46 of Csata *et al.* (2014). They found that under laboratorial conditions the lifespan of *Myrmica*
47 *scabrinodis* NYLANDER, 1846 individuals infected with *R. wasmannii* was significantly
48 reduced in comparison with the lifespan of uninfected ants. Moreover auto- and allogrooming
49 increased in infected nests. These facts support the parasitic character of ant-associated
50 Laboulbeniales fungi.

51

52 Only *R. wasmannii* has been reported among these four species in the Carpathian Basin
53 (Tartally *et al.*, 2007). As *Camponotus aethiops* (Latreille, 1798) is a relatively common
54 species in this region (Csősz & al., 2011; pers. observ.), which is one of the known hosts of *L.*

55 *camponoti* (Espadaler & Santamaria, 2012), we suspected the possibility to record *L.*
56 *camponoti* from the Carpathian Basin. Our aim was therefore to prove the presence of *L.*
57 *camponoti* within this region by checking museum specimens of *C. aethiops*. Though the
58 other known (Espadaler & Santamaria, 2012) host ants (*C. universitatis* Forel, 1890; *C.*
59 *pilicornis* (Roger, 1859); *C. sylvaticus* (Olivier, 1792)) are not known from this region (Csősz
60 & al., 2011), we aimed to search for individuals among museum specimens from the
61 Carpathian Basin. Finding *L. camponoti* for a new region may call the attention of
62 myrmecologists and mycologists to check *Camponotus* specimens more intensively for the
63 presence of this small and understudied fungus.

64

65

66 **2. Materials and Methods**

67

68 To reveal the presence of *L. camponoti*, all the *Camponotus aethiops* (Hymenoptera:
69 Formicidae) specimens (workers, males, and queens) in the Hymenoptera Collection of the
70 Hungarian Natural History Museum were examined under an Olympus SZX9
71 stereomicroscope at magnifications of 12.6x-114x. No *C. universitatis*, *C. pilicornis* or *C.*
72 *sylvaticus* specimens were found in this collection from the Carpathian Basin.

73

74 Pinned specimens of the host that were found to be infested were soaked in 70% ethanol for
75 5-12 hours and examined using transmitted light under a binocular microscope at 10x
76 magnification. Thalli were removed with an insect pin and cleared in lactic acid (12 hours)
77 before being mounted in a PVA-glycerol medium and photographed with an Olympus digital

78 camera through an Olympus BX-40 microscope equipped with 40x and 100x lenses.
79 Measurements were taken with the manufacturer's image acquisition software (DP
80 Controller).

81

82 Specimens are deposited in the Fungi Collection of the Hungarian Natural History Museum
83 on slides (inventory numbers: BP 105023, BP 105024).

84

85

86 **3. Results and Discussion**

87

88 More than 200 *C. aethiops* specimens were examined, originating from 34 parts of the
89 Carpathian Basin (sites in Hungary, Romania, Slovakia, Austria, and Serbia). Only three
90 specimens (less than 1.5% of the investigated samples) of *C. aethiops* workers were found to
91 be parasitized by *L. camponoti*: two workers from Vienna, Austria (48°12' N, 16°22' E, 180 m
92 a.s.l.), and one from Baziaș, Romania (44°48' N, 21°23' E, 85 m a.s.l.). The fungus grew from
93 the cuticle of different body parts of the workers, mainly on the head and the legs (Fig. 1-2).
94 No infested queens or males were found. However, the numbers of queens and males in the
95 museum collection were small.

96

97 The number of thalli observed on infected *Camponotus* specimens was relatively small. A
98 dozen (mostly immature) thalli were found in two groups on an antenna of one specimen from
99 Vienna, while the other worker from the same location had only two immature thalli with
100 developing perithecia (the spore-producing fruiting body of the fungus) on one leg. A single,

101 mature thallus with visible spores was found on the head of the Romanian specimen collected
102 at Baziaş (Fig. 1). Variation in the length and number of the sterile appendages was
103 observable, as also noted in the species' original description (Batra, 1963), where explanations
104 of life stages and morphology are also available.

105

106 The ectoparasitic fungus *L. camponoti* was found for the first time in Romania and Austria
107 (see: Espadaler and Santamaria, 2012 and references therein). The number of countries this
108 fungus is recorded in is now increased from four to six: it has previously been found only in
109 Spain, Bulgaria, Turkey (for a review: Espadaler and Santamaria, 2012 and references
110 therein) and India (Batra, 1963). In its prior known localities, the Mediterranean or
111 subtropical climatic influence is strongly expressed. This may have led myrmecologists and
112 mycologists to consider *L. camponoti* to be distributed solely in such climatic areas. However,
113 the two newly recorded localities are in the common borders of the Continental and
114 Pannonian regions (see: EEA, 2011), and the new locality at Vienna is the northernmost
115 (48°12' N) known latitude of *L. camponoti* in the world. These facts give a new picture of the
116 potential distribution of this fungus.

117

118 The inconspicuous nature of *L. camponoti* has undoubtedly contributed to the scarcity of its
119 distribution records. As illustrated by Fig. 2., the thalli are very hard to locate, especially on
120 older museum specimens with dust particles. Determination of the fungus must be validated
121 by light microscopy. Because European *Camponotus* species are usually large (see e.g.
122 Seifert, 2007), and therefore usually easily observed with the naked eye, myrmecologists
123 rarely examine them by microscopy. However, these results demonstrate that a thorough

124 examination of *Camponotus* specimens from other climatic regions may reveal the presence
125 of this little-known parasitic fungus.

126

127

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135

136

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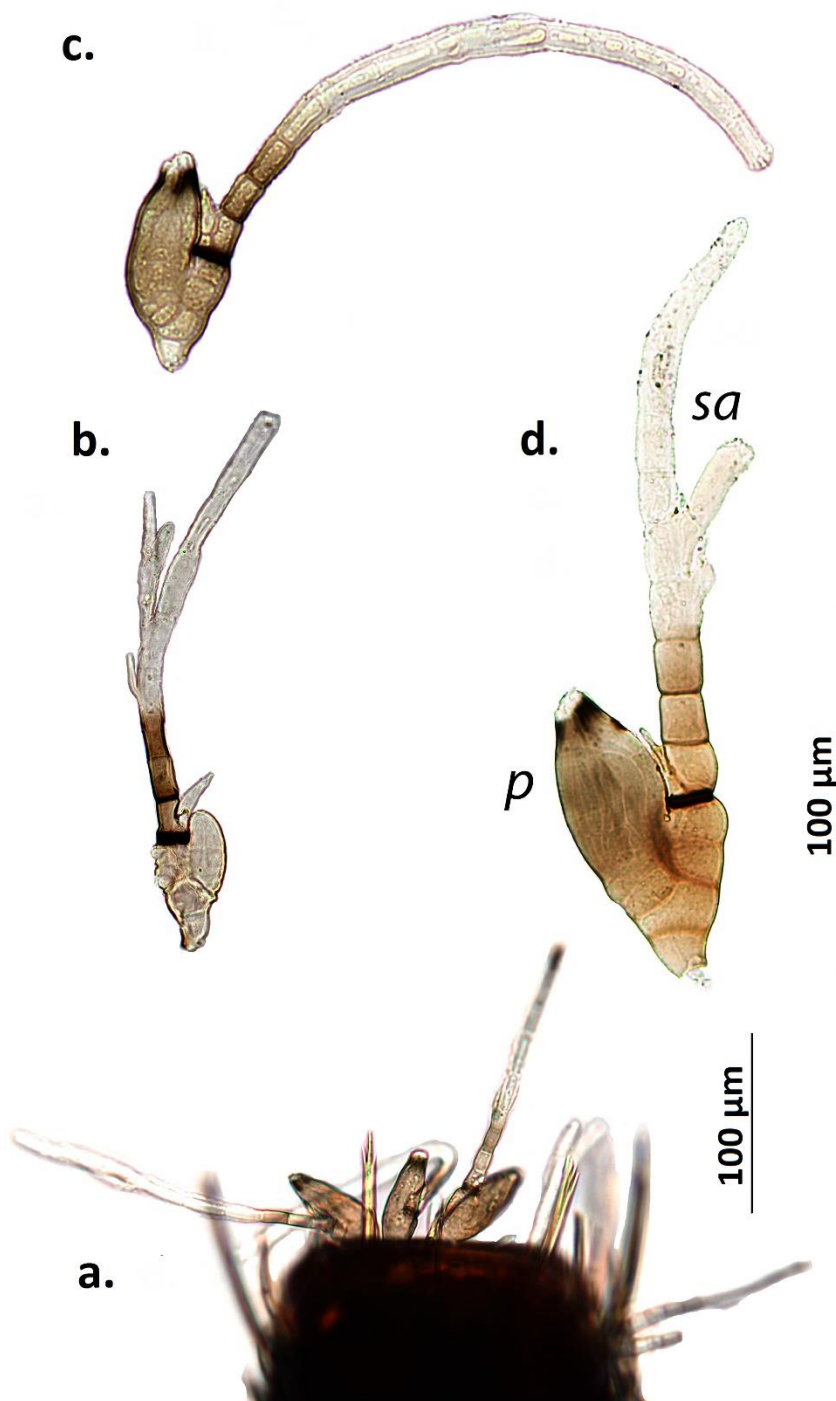
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186



187

188 Fig. 1. *Laboulbenia camponoti*. a. Group of thalli on antenna (Vienna). b. Young immature
 189 thallus (Vienna). c. Immature thallus with developing perithecium (Vienna). d. Mature thallus
 190 (Bazias). Legend: p - perithecium; sa – sterile appendages (their number shows individual
 191 differences).



192

193 Fig. 2. A *Laboulbenia camponoti* individual on the scapus of a *Camponotus aethiops* worker

194 (Vienna), the figure illustrates how meticulous it is to find this small fungus on a large

195 *Camponotus* individual, especially when dust on the host prevents easy recognition