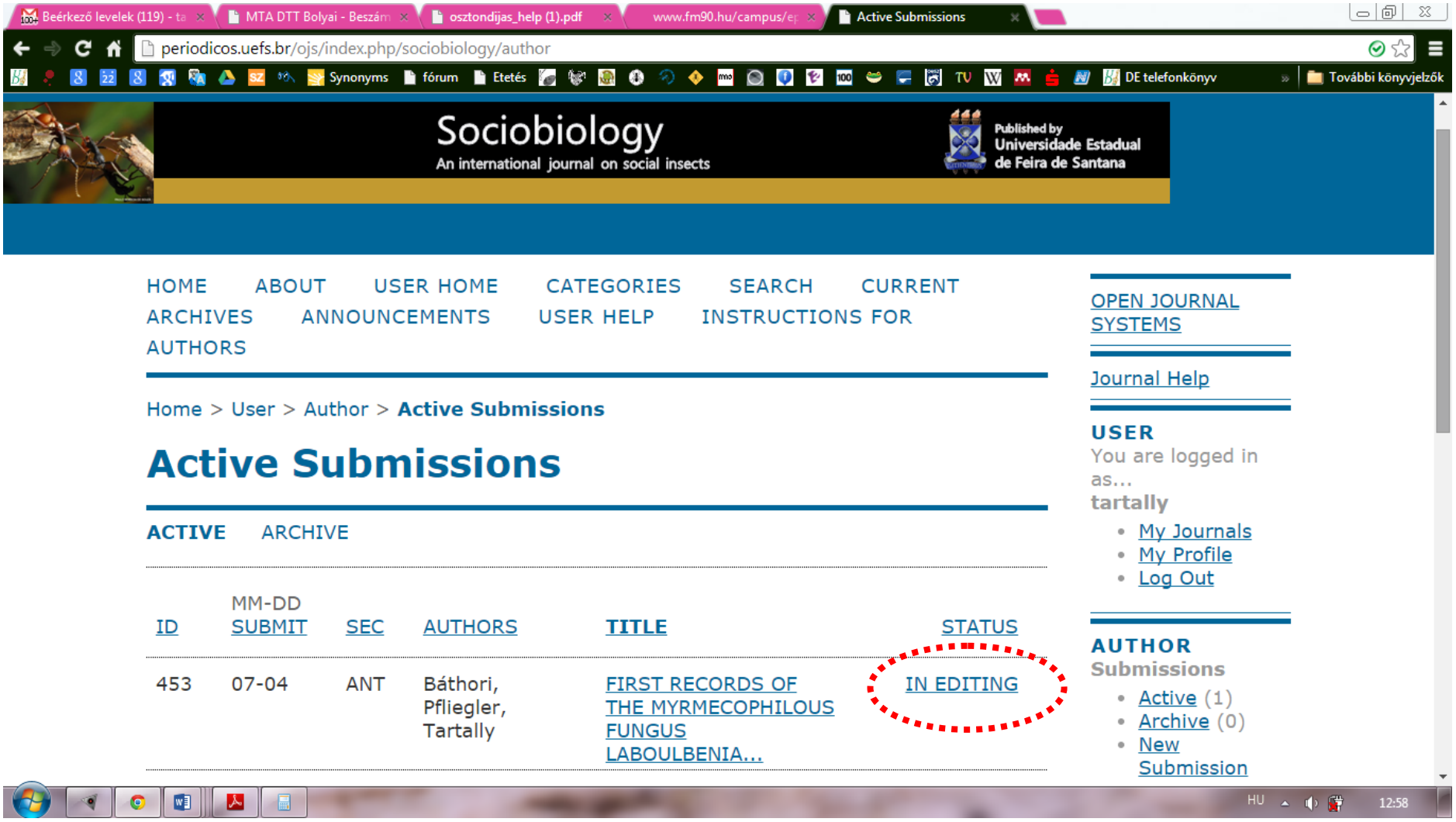

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Title: First records of the myrmecophilous fungus *Laboulbenia camponoti* Batra (Ascomycetes: Laboulbeniales) from the Carpathian Basin

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

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

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

1. Introduction:

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

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

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

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

2. Materials and Methods

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

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

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

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

3. Results and Discussion

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

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

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

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

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

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

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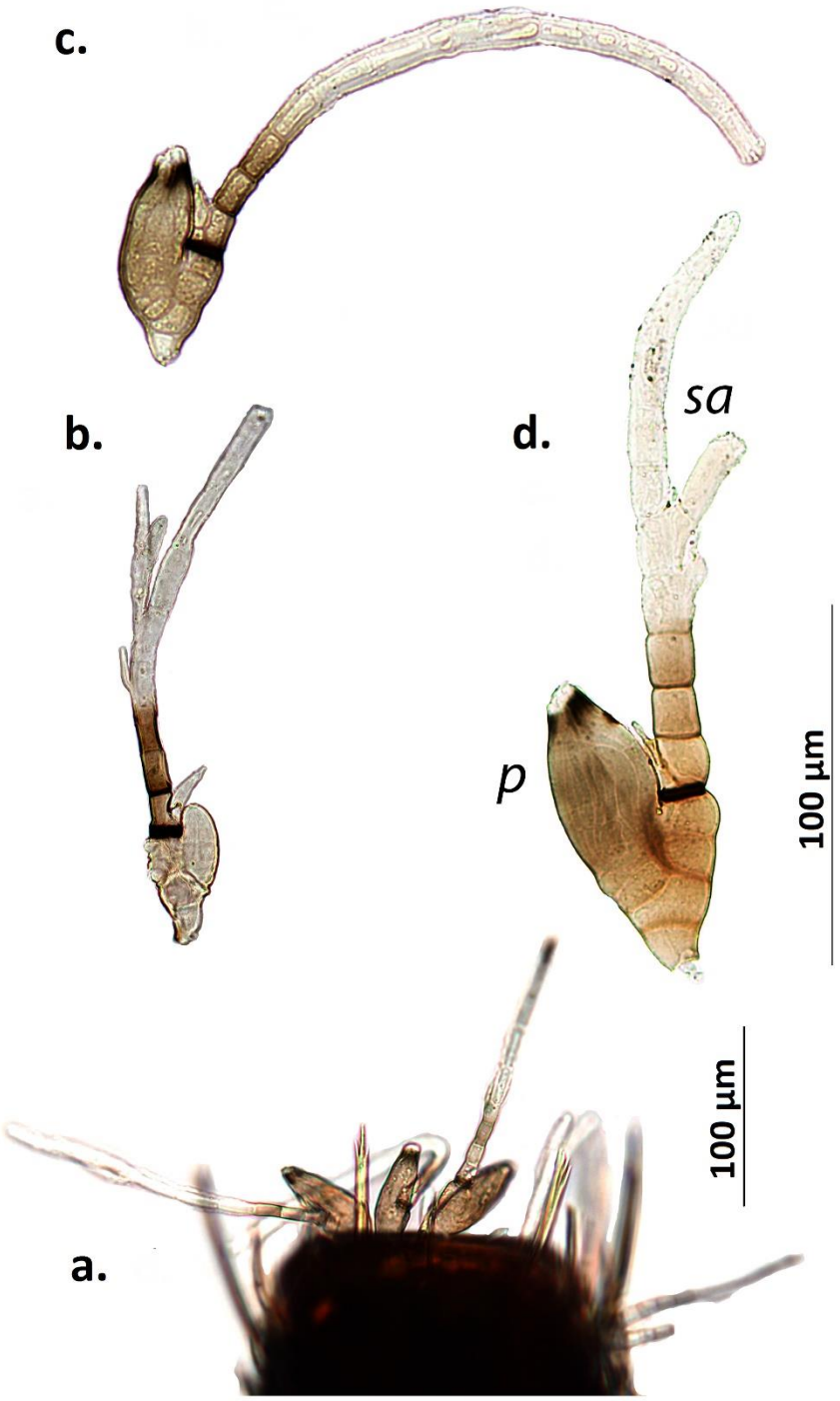

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188 Fig. 1. *Laboulbenia camponoti*. a. Group of thalli on antenna (Vienna). b. Young immature
189 thallus (Vienna). c. Immature thallus with developing perithecium (Vienna). d. Mature thallus
190 (Baziaş). Legend: p - perithecium; sa – sterile appendages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

Fig. 2. A *Laboulbenia camponoti* individual on the scapus of a *Camponotus aethiops* worker (Vienna), the figure illustrates how meticulous it is to find this small fungus on a large *Camponotus* individual, especially when dust on the host prevents easy recognition