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Online image databases as multi-purpose resources: discovery of a new host ant of *Rickia wasmannii* Cavara (Ascomycota, Laboulbeniales) by screening AntWeb.org

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Abstract

Public awareness has been raised on the importance of natural history and academic collections for science and society in a time when reduced financial support and staff cuts are prevalent. In the field of biology, new species and new interspecies associations are constantly discovered by making use of museum collections, digitalised materials or citizen science programs. In our study, the *Myrmica* Latreille, 1804 image collection of AntWeb.org was screened for fungal ectoparasites. A total of 397 imaged specimens from 133 species were visually investigated. A single specimen of *M. hellenica* Finzi, 1926, collected in Greece by U. Sahlberg, showed a conspicuous fungal infection. The parasite was identified using microscopic methods as *Rickia wasmannii* Cavara, an ectoparasitic fungal species specialised to *Myrmica* ants. This finding represents a new country record and a new *Myrmica* species for the host spectrum of *R. wasmannii*. According to our results, online entomological databases can be screened relatively easily for ectoparasitic fungal infections from new hosts and new regions. However, depending on quality of the insect voucher photos, additional investigation of the material could be needed to confirm the identity of the parasite.

Keywords

Biological collections, database, *Myrmica hellenica*, distribution, Greece

Introduction

Natural history collections have served as the fundament of taxonomic and biogeographical research over centuries. These biological collections could be important to reveal new geographical distributions of several species (Ponder et al. 2001, Suarez and Tsutsui 2004, Solow and Roberts 2006, Pyke and Ehrlich 2010). Examining biological samples in these collections may also reveal previously unrecorded associations between different species (Frey et al. 1992), e.g. in the case of parasites that remain observable and identifiable after the host is conserved and deposited (Haelewaters et al. 2015a). Among the Fungi, this approach in recording parasites' distributions and host associations have been applied in the case of plant-infecting fungi (e.g. Denchev and Denchev 2016) and insect ectoparasites. The latter category includes works describing new species (e.g. Thaxter 1924, Haelewaters and Rossi 2015, 2017, Santamaria et al. 2016, Wang et al. 2016) or presenting new distributional and host association records (e.g. Weir and Hammond 1997, Báthori et al. 2014, 2015, Haelewaters et al. 2014, 2015a, 2017). Records of parasitic fungi have also originated from photo-sharing websites such as Flickr and iNaturalist (e.g. fungi parasitizing *Harmonia axyridis* see Haelewaters et al. 2017) and the image collections of further citizen science projects may similarly be utilized (e.g. Lost Ladybug Project in the USA, the UK Ladybird Survey and iSpot in South Africa, see also Roy et al. 2016). Regarding the family of ants (Formicidae), our group has recently found that the thorough screening of museum specimens can lead to new distributional and host-association records of the otherwise rarely observed ectoparasites in the order Laboulbeniales, providing the first Central European records of the species *Laboulbenia camponoti* Batra (Báthori et al. 2014) and that of *Rickia lenoirii* Santam. (Báthori et al. 2015).

Natural history collections experienced a decline in funding in the last decade with growing concern over the vast potential of biological information waiting to be uncovered from these resources (Pyke and Ehrlich 2010). On the other hand, digitization of conserved material and the possibility to remotely access an ever-growing number of high resolution photographs from collections around the globe has opened up previously unimaginable possibilities (e.g. Beaman and Cellinese 2012) in recent years. Although most researchers are in agreement that new species, taxa and their distributions should not be described merely on digital photos under most circumstances (Amorim et al. 2016, Krell 2016), digital collections can serve as a powerful resource leading to their discoveries as they can point to the material for later direct examination scattered over collections, or direct to the sampling localities.

In the present study, we extend our previous work by screening preserved ant specimens for fungal ectoparasites utilizing the AntWeb website, currently the world's largest online database of images, specimen records, and natural history information on ants (AntWeb 2017). Our aim was to test whether the relatively easily recognizable fungus *Rickia wasmannii* Cavara, an ectoparasite of *Myrmica* Latreille, 1804 ants, can be observed on AntWeb's collection of digitized material of this species-rich and widespread ant genus, thereby providing new distributional and host records for the fungus.

Material and methods

To survey *R. wasmannii* infections, all the specimens in the genus *Myrmica* (workers, males, and queens) digitized in the online database of AntWeb.org were examined. Based on their current statistics (ver. 6.58), 594,399 specimen records and 199,352 total specimen images can be found in this online database from all over the world. In the case of the genus *Myrmica*, 263 species are present in the database, but only 133 species have imaged specimens. In total 397 specimens of these *Myrmica* spp. (44 queens, 30 males, 323 workers) are represented in the database with 1823 photographs (1409 if images of specimen labels are excluded), originating from Europe, Africa, Asia and North America (see Supplementary file 1). Eleven specimens were not identified to species level and one specimen (FOCOL0709) was misplaced.

We examined all the available images of the genus *via* opening each in a web browser. Identifying imaged specimens as being infected was based on the comparison with specimens in our collection of dried infected *Myrmica* ants. The thalli of *R. wasmannii* are relatively easy to identify on the basis of their morphology (De Kesel et al. 2016). Their appearance is conspicuously whitish, hyaline in the case of dried host material, therefore one can quickly differentiate it from hairs. The microscopic examination of slide mounted material and the comparison with the detailed morphological description and illustrations by De Kesel et al. (2016) and Báthori et al. (2017) is sufficient for species identification of the fungal parasite.

As described previously (e.g. Haelewaters et al. 2015a), the strong attachment of Laboulbeniales thalli to the host (Tragust et al. 2016) may last for decades even in the case of dried host specimens. To test whether the mode of host collection and initial conservation affects the appearance of the fungal infection, we killed infected hosts previously collected in Hungary (Pfliegler et al. 2016) by freezing or with ethanol or chloroform then mounted them on pins. No substantial loss of thalli was observed during the procedure and the appearance and density of thalli was unchanged after months of subsequent storage. The fungal infection remained easily observable on photographs taken of these dried specimens (Fig. 1). Thus, we concluded that the image collection of AntWeb can reliably be used to screen for *R. wasmannii* infections, including older specimens.

From the infected host material identified during the survey, a single thallus (Fig. 2) was removed with an entomological pin dipped into gylcerol. The thallus was slide-mounted into PVA medium and deposited at Conservatoire and Jardin botaniques de Genève (CJBG, deposition number: G00562301). Photographs of the mounted thallus were made using an Olympus BD40 microscope equipped with an Olympus 100× Phase Contrast lens and an Olympus DP-70 digital microscope camera, with the DP Controller (Olympus) software. Subsequently, image background was removed in Photoshop CS6 (Adobe). Morphological species identification was conducted using available descriptions (De Kesel et al. 2016) and comparison with the slide collection of the Dept. of Evolutionary Zoology and Human Biology, University of Debrecen.

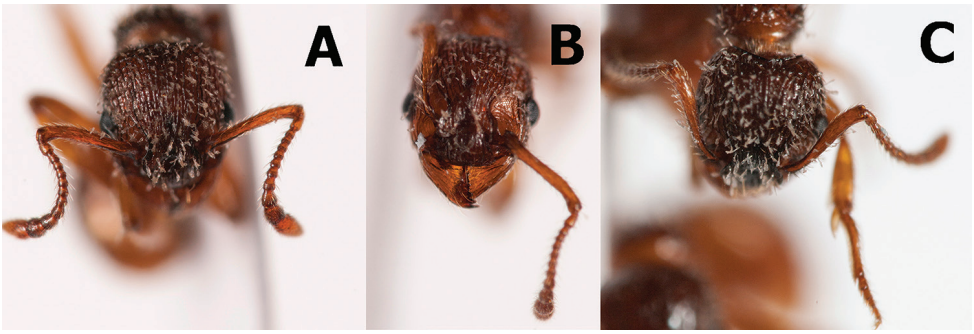

Figure 1. Appearance of the fungal infection on mounted specimens: *Rickia*-infected *Myrmica scabrinodis* hosts killed by freezing (**A**) or with ethanol (**B**) or chloroform (**C**), photographed one month after mounting and dry storage.

Figure 2. Photograph of a slide-mounted *Rickia wasmannii* thallus (deposition number: G00562301) from *Myrmica hellenica* host, recorded on the AntWeb (specimen: CASENT0907653).

Results and discussion

Most of the 397 examined *Myrmica* spp. specimens were represented by multiple photographs showing the ants from different angles in the AntWeb database. Due to the presence of important taxonomic characters, the head was imaged for all specimens. This is advantageous property of the database for our work, as the number of thalli is usually the highest on the head (Haelewaters et al. 2015b, De Kesel et al. 2016). This, together with the image quality and resolution in the database enabled us to identify any infected specimen.

We have identified a conspicuously infected *M. hellenica* Finzi, 1926 worker (Photo by Zach Lieberman, https://www.antweb.org/bigPicture.do?name=casent0907653&shot=h&number=1; Image: AntWeb 2002–2017. Licensing: Creative Commons Attribution License; downloaded on 09.05.2017) (Fig. 3) among the 397 available *Myrmica* specimens (0.25% of the investigated samples). The other 396 *Myrmica* specimens (99.75%) did not exhibit signs of *R. wasmannii* infection. Due to the occasional presence of dust particles (e.g. on photograph of specimen CASENT0900350) and the fact that host ants are occasionally parasitized by a very low number of thalli (Markó et al. 2016), which may be obscured in photographs, our method of screening the AntWeb database does not enable us to completely rule out infection among these specimens.

The single unequivocally infected specimen identified by us was collected in Greece, thus it represents a new country record and simultaneously a new host species for the fungus. The collection data of the specimen is as follows: Greece: Patras (approx. 38°14'47"N, 21°44'4"E), collected by U. Sahlberg. The fungal thalli covered the ant's head and body in high density. No other specimens with the same collection data were available in AntWeb. Further specimens of the host, *M. hellenica*, collected in Northern Greece and Iran were present in the database, but these were not photographed or did not show infection, respectively.

With our new record, the number of countries this fungus is recorded in is now increased from 17 to 18: Spain, France, Great Britain, Belgium, Netherland, Luxembourg, Germany, Switzerland, Austria, Italy, Czech Republic, Slovakia, Poland, Hungary, Romania, Slovenia, Bulgaria (Santamaria 2001, Espadaler and Santamaria 2012, Csata et al. 2013, De Kesel et al. 2016) and Greece (this paper). The known host species of the fungus is raised from nine: *M. gallienii* Bondroit, 1920; *M. rubra* (Linnaeus, 1758); *M. ruginodis* Nylander, 1846; *M. sabuleti* Meinert, 1861; *M. scabrinodis* Nylander, 1846; *M. slovaca* Sadil, 1952; *M. specioides* Bondroit, 1918; *M. spinosior* Santschi, 1931; and *M. vandeli* Bondroit, 1920 (De Kesel et al. 2016, Haelewaters et al. 2015a, 2015c) to ten by *M. hellenica* (this paper).

Our observations have proven that online image databases can be exploited to record parasitic infections, extend the distribution and host spectrum of parasitic species, thereby supplementing the direct examination of specimens in non-digitized collections. Making more high-quality micrographs and SEM images (see: Tragust et al. 2016) available for the public would facilitate similar efforts. Furthermore, raising

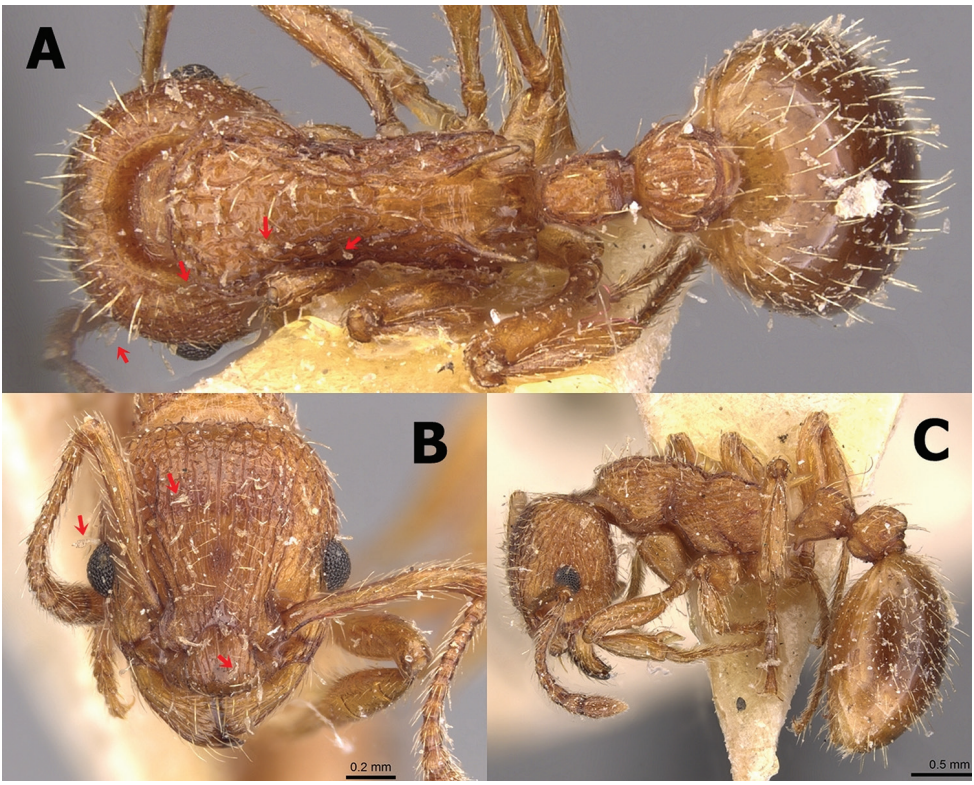

Figure 3. Dorsal (**A**), head (**B**) and profile (**C**) view of the *Rickia wasmannii* infected *Myrmica hellenica* worker, recorded on AntWeb (specimen: CASENT0907653), arrows indicate some clearly identifiable *R. wasmannii* thalli.

the number of photographs available for individual specimens would be beneficial to conduct fungal infection screenings (see: Fig. 3C where the infection is not clearly visible). Depending on the quality of the host photos, additional investigation of the material might be still needed to assess the identity of the fungal parasite due to their small size.

Conclusion

Our results highlight the possibility of using digitized collections to uncover host-parasite associations. The study of insect-Laboulbeniales associations have recently also benefited from the use of digital photo- and biological observation sharing websites (Flickr and iNaturalist; Haelewaters et al. 2016), further emphasising the new possibilities and non-trivial uses of resources in the digital era.

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Supplementary material 1

Online image databases as multi-purpose resources: *Rickia wasmannii* Cavara (Ascomycota, Laboulbeniales) on a new host ant from a new country by screening AntWeb.org

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Data type: occurence

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