FOLIA ENTOMOLOGICA HUNGARICA
ROVARTANI KÖZLEMÉNYEK
Volume 79 2018 pp. 195–201

First record of Laboulbeniales (Fungi: Ascomycota) infection on bat flies (Diptera: Nycteribiidae) from the Caucasus region

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Abstract – The ectoparasitic fungus, *Arthrorhynchus nycteribiae* (Peyritsch) Thaxter (Fungi: Laboulbeniales) is recorded for the first time in Georgia. It was found on the obligate, hematophagous bat fly, *Penicillidia conspicua* Speiser, 1901 (Diptera: Nycteribiidae), parasitizing the cave-dwelling bat species, *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Miniopteridae). This record represents the first observation of Laboulbeniales infection on bat flies from the Caucasus region. With one figure.

Key words – *Arthrorhynchus nycteribiae*, *Miniopterus*, *Penicillidia*

INTRODUCTION

Bats host a wide variety of highly specific ectoparasites, such as fleas, mites and bat flies (Marshall 1982). Bat flies (Diptera: Nycteribiidae and Streblidae) are obligate, hematophagous ectoparasites of bats. They belong to the superfamily Hippoboscoidea, along with louse flies, Hippoboscidae and tsetse flies, Glossinidae (Dittmar *et al.* 2006, Petersen *et al.* 2007). The family Nycteribiidae currently contains ~280 described species, showing higher diversity in Eastern Hemisphere, whereas family Streblidae have ~230 species, distributed mainly in the Western Hemisphere (Dick & Patterson 2006).

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In Europe, 16 species of nycteribiids and one species of streblids have been recorded (Szentiványi *et al.* 2016). The family Nycteribiidae represents four genera in Europe: *Basilia* Miranda-Ribeiro, 1903 (6 species), *Nycteribia* Latreille, 1796 (5 species), *Penicillidia* Kolenati, 1863 (3 species) and *Phthiridium* Hermann, 1804 (2 species). The family Streblidae represents one species, *Brachytarsina flavipennis* Macquart, 1851 (Szentiványi *et al.* 2016). Nycteribiids are wingless, and most species are eyeless due to their parasitic life-style. Bat flies show highly specialized reproductive strategy: one single larva develops within the female and as soon as it reaches the third larval stage it is deposited by the female on a substrate (e.g. cave wall). The deposited larva immediately pupates. After emergence the fly actively searches for its bat hosts. Female flies only leave their hosts during pupal deposition (Marshall 1982, Dick & Patterson 2006). This type of reproductive strategy known as obligate pseudo-placental unilarviparity (Meier *et al.* 1999), which is a common characteristic within the superfamily Hippoboscoidea.

Bat flies show different degree of specificity, being monoxenous (one host species), oligoxenous (multiple host species within one genus) or polyxenous (several host species in different genera or families). High degree of specificity is common in bat flies (Dick & Patterson 2006). Bat flies are known as vectors and potential vectors of several pathogens, such as *Polychromophilus* spp. and *Bartonella* spp. (Gardner & Molyneux 1988, Sándor *et al.* 2018).

Bat flies are also known to host different parasitic organisms, such as ectoparasitic fungi (Blackwell 1980, Marshall 1982, Haelewaters *et al.* 2017, Szentiványi *et al.* 2018). Laboulbeniales (Fungi: Ascomycota) are widely distributed and species rich microparasites of arthropods. Unlike other multicellular fungi, Laboulbeniales form thallus (plural: thalli) instead of hyphae or mycelia. These fungus species occur on a wide variety of host groups, such as Coleoptera, Diptera and Hymenoptera (Weir & Hammond 1997, Csata *et al.* 2013, Haelewaters *et al.* 2015, 2018*b*, Pfliegler *et al.* 2016). The most common species on nycteribiid bat flies are *Arthrorhynchus eucampsipodae* Thaxter and *Arthrorhynchus nycteribiae* (Peyritsch) Thaxter (Haelewaters *et al.* 2018*a*).

In Europe, *Arthrorhynchus* species mostly occur on *Nycteribia schmidlii* Schiner, 1853, *Penicillidia conspicua* Speiser, 1901 and *Penicillidia dufourii* (Westwood, 1835) (Haelewaters *et al.* 2017, Szentiványi *et al.* 2018). A comprehensive study showed that the main bat host of fungi infected flies is the cave-dwelling common bent-wing bat, *Miniopterus schreibersii* (Kuhl, 1817) (Haelewaters *et al.* 2017). Haelewaters *et al.* (2017) also presented a comprehensive literature search on fungi occurrence which showed that *Arthrorhynchus* spp. are not documented in Georgia, neither in neighbouring countries.

MATERIALS AND METHODS

Samples were collected from *Miniopterus schreibersii* during August 2014 in the Batumi Botanical garden, Batumi, Georgia (N41.694504°, E41.707491°) (Fig. 1). Bats were caught using mist nest near the concrete tunnel (20 m long), which they used as a day roost. All bat individuals were released after collection of parasites and taking parameters such as sex, age, body mass and forearm length. All manipulation with animals were done according to the ethic guidelines of the American Society of the Mammalogists (Sikes & Gannon 2011), and approved by permit no. 4752, 26 August 2014 issued by the Ministry of Environment and Natural Resources Protection of Georgia.

Parasites were collected by forceps from the bat host, and placed in 70% ethanol. Bat flies were identified based on Theodor`s (1967) key. Fungal thalli were counted, and species identification was based on Thaxter (1908) and Haelewaters *et al.* (2017). Voucher specimens of both bat flies and fungi are deposited at Museum of Zoology Lausanne, Switzerland.

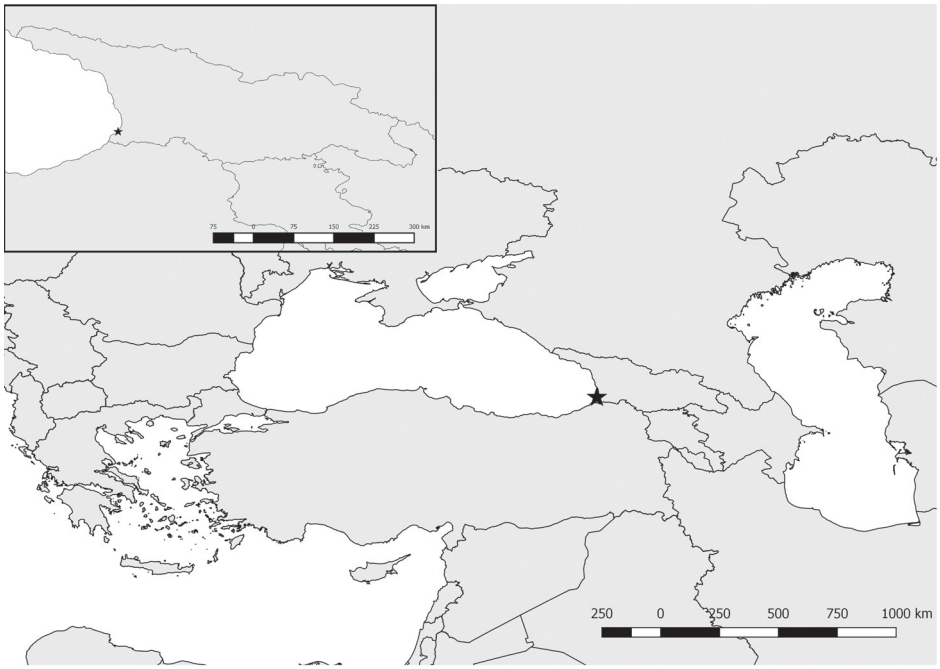

Fig. 1. Location of bat fly sampling is indicated on the map (black asterisk)

RESULTS

Bat flies were sampled from a single *Miniopterus schreibersii* (occasional sampling) in Georgia (Table 1). All sampled flies (n = 8) have been identified as *Penicillidia conspicua*. Two individuals (one female and one male) were found infected by *Arthrorhynchus nycteribiae* (prevalence = 25%).The female *Penicillidia conspicua* had 32 thalli, while the male individual was infected by 86 thalli. Both flies had infection by immature and fruiting fungal bodies. The position of the infection appeared on the ventral and dorsal parts of the abdomen as well as on the genitalia in both individuals. *Arthrorhynchus nycteribiae* infected *Penicillidia conspicua* were previously collected from various bat species, includ-

Table 1. Distribution and bat host records of *Arthrorhynchus nycteribiae* infecting *Penicillidia conspicua*

Country	Bat host	References
Australia	Not known	Blackwell (1980)
Bulgaria and/or Slovakia	*Miniopterus schreibersii*	Samšiňáková (1960)
"Central Europe"	*Rhinolophus euryale*	Kolenati (1857)
Croatia	*Miniopterus schreibersii*	Kolenati (1857), Szentiványi *et al.* (2018)
"Czecho-Slovakia"	*Miniopterus schreibersii*	Balazuc (1971)
France	*Miniopterus schreibersii/ Myotis myotis/ Rhinolophus euryale/ Rhinolophus ferrum-equinum/ Myotis capaccinii*	Balazuc (1971), Blackwell (1980)
Hungary	*Miniopterus schreibersii/ Rhinolophus euryale*	Moesz (1931), Balazuc (1971), Haelewaters *et al.* (2017), Szentiványi *et al.* (2018)
Georgia	*Miniopterus schreibersii*	This study
Portugal	*Miniopterus schreibersii*	Jensen *et al.* (2018), Szentiványi *et al.* (2018)
Romania	*Miniopterus schreibersii/ Myotis daubentonii / Rhinolophus ferrumequinum*	Blackwell (1980), Haelewaters *et al.* (2017)
Serbia	*Rhinolophus euryale*	Bechet & Bechet (1970)
Slovakia	*Miniopterus schreibersii*	Szentiványi *et al.* (2018)
Spain	*Miniopterus schreibersii*	Balcells (1954), Szentiványi *et al.* (2018)
Switzerland	*Miniopterus schreibersii*/not known	Bernasconi (1961), Balazuc (1971)

ing *Miniopterus schreibersii, Myotis capaccinii* Bonaparte, 1837*, Myotis daubentonii* (Kuhl, 1837)*, Myotis myotis* (Borkhausen, 1797), *Rhinolophus euryale* (Blasius, 1853) and *Rhinolophus ferrumequinum* (Lacépède, 1799) (Table 1).

DISCUSSION

Here we report the first occurrence of Laboulbeniales infection on bat flies from Georgia. The bat host, *Miniopterus schreibersii* is a widely distributed species in Southern Europe, North Africa and the Middle East to the Caucasus (Appleton *et al.* 2004). Two bat fly species, *Nycteribia schmidlii* and *Penicillidia conspicua* often parasitize *Miniopterus schreibersii* and both considered as highly specific on this bat host. Szentiványi *et al.* (2018) showed the presence of *Arthrorhynchus nycteribiae* on *Penicillidia conspicua* in Croatia, Hungary, Portugal, Slovakia and Spain, which suggests that *Arthrorhynchus* infection might be present within the whole distribution of its fly and bat host. The average prevalence of fungal infection on *Penicillidia conspicua* was 22.9% (n = 144) (Szentiványi *et al.* 2018). Similarly, Haelewaters *et al.* (2017) observed a 25% (n = 152) prevalence of *Penicillidia conspicua* in Central Europe (Hungary and Romania).

Arthrorhynchus nycteribiae infection also occurs on *Penicillidia dufourii*, but it is considered as an "accidental transfer" from *Penicillidia conspicua*, which seems to be the main host of this fungus species (Haelewaters *et al.* 2017). Haelewaters *et al.* (2017) found a 2% (n = 102) prevalence, while Szentiványi *et al.* (2018) observed a 7.7% (n = 52) prevalence of *Penicillidia dufourii* infected by *Arthrorhynchus nycteribiae*. In this study, we cannot conclude the infection patterns of the bat fly population due to the low sample number (due to occasional sampling). Additional parasite sampling of potential hosts in poorly researched countries will contribute to our understanding of the distribution patterns of Laboulbeniales species.

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Acknowledgements – We thank the administration of Batumi Botanical Garden for help and collaboration during the field work in Georgia, especially Nazi Leonidze for kind support with accommodation and organization of work in the area of the Garden. We thank the Ministry of Environment and Natural Resources Protection of Georgia for issuing the permit to conduct the field work. We thank to UNEP EUROBATS Projects initiative for grant support of the project "Transborder collaboration in bat migration research in Eastern Europe and the Black Sea region, 2014". Péter Estók was supported by the grant "Complex improvement of research capacities and services at Eszterházy Károly University" (EFOP-3.6.1-16-2016-00001).

REFERENCES

Appleton B. R., Mckenzie J. A. & Christidis L. 2004: Molecular systematics and biogeography of the bent-wing bat complex Miniopterus schreibersii (Kuhl, 1817) (Chiroptera: Vespertilionidae). – *Molecular Phylogenetics and Evolution* **31**: 431–439. https://doi.org/10.1016/j.ympev.2003.08.017

Balazuc J. 1971: Notes sur les Laboulbéniales. III. Rectifications, synonymies et mises au point (suite). – *Bulletin mensuel de la Société linnéenne de Lyon* **40**:211–216.

Balcells R. E. 1954: Quirópteros de cuevas catalanas: campaña de 1952–53. – *Speleon* **5**: 105–110.

Bechet M. & Bechet I. 1970: Un champignon rare, Arthrorhynchus nycteribiae (Peyr.) Thaxt., parasite des diptères pupipares (Insecta). – In: Orghidan T. (ed.): *Livre du centenaire Emile G. Racovitza 1868–1968.* – Académie De La République Socialiste De Roumanie, Bucharest, pp. 487–489.

Bernasconi R. 1961: Note sur la flore mycologique cavernicole de Suisse. – *Schweizerische Zeitschrift für Pilzkunde* **39**: 85–92.

Blackwell M. 1980: Incidence, host specificity, distribution, and morphological variation in Arthrorhynchus nycteribiae and A. eucampsipodae (Laboulbeniomycetes). – *Mycologia* **72**:143–158. https://doi.org/10.2307/3759427

Csata E., Czekes Z., Erős K., Német E., Hughes M., Csősz S. & Markó B. 2013: Comprehensive survey of Romanian myrmecoparasitic fungi: new species, biology and distribution. – *North-Western Journal of Zoology* **9**: 23–29.

Dick C. W. & Patterson B. D. 2006: Bat flies – obligate ectoparasites of bats. – In: Morand S., Krasnov B. R. & Poulin R. (eds): *Micromammals and macroparasites from evolutionary ecology to management*. – Springer Japan, Tokyo, pp. 179–194. https://doi.org/10.1007/978-4-431-36025-4_11

Dittmar K., Porter M. L., Murray S. & Whiting M. F. 2006: Molecular phylogenetic analysis of nycteribiid and streblid bat flies (Diptera: Brachycera, Calyptratae): implications for host associations and phylogeographic origins. – *Molecular Phylogenetics and Evolution* **38**: 155–170. https://doi.org/10.1016/j.ympev.2005.06.008

Gardner R. & Molyneux D. H. 1988: Polychromophilus murinus: A malarial parasite of bats: life-history and ultrastructural studies. – *Parasitology* **96**: 591–605. https://doi.org/10.1017/S0031182000080215

Haelewaters D., Boer P., Gort G. & Noordijk J. 2015: Studies of Laboulbeniales (Fungi, Ascomycota) on Myrmica ants (II): variation of infection by Rickia wasmannii over habitats and time. – *Animal Biology* **65**: 219–231. https://doi.org/10.1163/15707563-00002472

Haelewaters D., Hiller T. & Dick C. W. 2018*a*: Bats, bat flies, and fungi: a case of hyperparasitism. – *Trends in Parasitology* **34**: 784–799. https://doi.org/10.1016/j.pt.2018.06.006

Haelewaters D., Page R. A. & Pfister D. H. 2018*b*: Laboulbeniales hyperparasites (Fungi, Ascomycota) of bat flies: independent origins and host associations. – *Ecology and Evolution* **8**(16): 8396–8418. https://doi.org/10.1002/ece3.4359

Haelewaters D., Pfliegler W. P., Szentiványi T., Földvári M., Sándor A. D., Barti L., Camacho J. J., Gort G., Estók P., Hiller T., Dick C. W. & Pfister D. H. 2017: Parasites of parasites of bats: Laboulbeniales (Fungi: Ascomycota) on bat flies (Diptera: Nycteribiidae) in Central Europe. – *Parasites & Vectors* **10**(96): 1–14. https://doi.org/10.1186/s13071-017-2022-y

Jensen K., Rodrigues L., Pape T., Garm A., Santamaria S. & Reboleira A. S. 2018: Hyperparasitism In caves: bats, bat flies and ectoparasitic fungus. – *Arpha Conference Abstract* **1**: 1–2. https://doi.org/10.3897/aca.1.e29967

Kolenati F. A. 1857: Epizoa der Nycteribien. – *Wiener Entomologische Monatsschrift* **1**: 66–69.
Marshall A. G. 1982: Ecology of insects ectoparasitic on bats. – In: Kunz T. H. (ed.): *Ecology of bats*. Springer, Boston, pp. 369–401.
Meier R., Kotrba M. & Ferrar P. 1999: Ovoviviparity and viviparity in the Diptera. –*Biological Reviews* 74: 199–258.
Moesz G. 1931: Mykológiai Közlemények VIII. (Mykologische Mitteilungen VIII.) – *Botanikai Közlemények* **28**: 161–175.
Petersen F. T., Meier R., Kutty S. N. & Wiegmann B. M. 2007: The phylogeny and evolution of host choice in the Hippoboscoidea (Diptera) as reconstructed using four molecular markers. – *Molecular Phylogenetics and Evolution* **45**: 111–122. https://doi.org/10.1016/j.ympev.2007.04.023
Pfliegler W. P., Báthori F., Haelewaters D. & Tartally A. 2016: Studies of Laboulbeniales on Myrmica ants (III): myrmecophilous arthropods as alternative hosts of Rickia wasmannii. – *Parasite* **23**(50): 1–7. https://doi.org/10.1051/parasite/2016060
Samšiňáková A. 1960: Příspěvek poznání entomofytnich hub na muchulovitých (Nycteribiidae). (Beiträge zur Kenntnis der entomophagen Pilze auf den Nycteribiiden.) – *Zoologické Listy* **9**: 237–238.
Sándor A. D., Földvári M., Krawczyk A. I., Sprong H., Corduneanu A., Barti L., Görföl T., Estók P., Kováts D., Szekeres S., László Z., Hornok S. & Földvári G. 2018: Eco-epidemiology of novel Bartonella genotypes from parasitic flies of insectivorous bats. – *Microbial Ecology* **76**(4): 1076–1088. https://doi.org/10.1007/s00248-018-1195-z
Sikes R. S. & Gannon W. L. 2011: Guidelines of the American Society of Mammalogists for the use of wild mammals in research. – *Journal of Mammalogy* **92**(1): 235–253. https://doi.org/10.1644/10-MAMM-F-355.1
Szentiványi T., Estók P. & Földvàri M. 2016: Checklist of host associations of European bat flies (Diptera: Nycteribiidae, Streblidae). – *Zootaxa* **4205**(2): 101–126. https://doi.org/10.11646/zootaxa.4205.2.1
Szentiványi T., Haelewaters D., Pfliegler W. P., Clément L., Christe P. & Glaizot O. 2018: Laboulbeniales (Fungi: Ascomycota) infection of bat flies (Diptera: Nycteribiidae) from Miniopterus schreibersii across Europe. – *Parasites and Vectors* **11**(395): 1–7. https://doi.org/10.1186/s13071-018-2921-6
Thaxter R. 1908: Contribution toward a monograph of the Laboulbeniaceae. Part II. – *Memoirs of the American Academy of Arts and Sciences* **13**: 217–469.
Theodor O. 1967: *An illustrated catalogue of the Rothschild Collection of Nycteribiidae in the British Museum (Natural History), with keys and short descriptions for the identification of subfamilies, genera, species and subspecies.* – British Museum (Natural History), London, 506 pp.
Weir A. & Hammond P. M. 1997: Laboulbeniales on beetles: host utilization patterns and species richness of the parasites. – *Biodiversity And Conservation* **6**: 701–719. https://doi.org/10.1023/A:1018318320019