Hidden biodiversity in herbarium collections: experience of searching for lichenicolous fungi in lichen herbaria

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Abstract. Lichen herbaria contain a large number of parasitic fungi accidentally collected along with lichens. Various aspects of searching for lichenicolous fungi in lichen herbaria are discussed. The productivity of such searches, including the discovery of species new to science, may be higher than when these fungi are searched in nature. In one day's work, 20–25 specimens of lichenicolous fungi can be found in the herbarium, and 2–15 specimens can be found in field studies.
Keywords: lichen parasites, voucher collections.

Скрытое биоразнообразие в гербарных коллекциях: опыт поиска лихенофильных грибов в гербариях лишайников

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Резюме. Гербарии лишайников содержат большое количество случайно собранных вместе с лишайниками паразитических грибов. Обсуждаются различные аспекты поиска лихенофильных грибов в гербариях лишайников. Продуктивность таких поисков, включая обнаружение новых для науки видов, может быть выше, чем при поиске этих грибов в природе. За один день работы в гербарии можно найти 20–25 образцов лихенофильных грибов, а при полевых исследованиях — 2–15 образцов.
Ключевые слова: паразиты лишайников, референсные коллекции.

Voucher collections of organisms are not only a 'pillar' of modern nomenclature, but also the material for scientific research of various kinds (Agerer, 2002). In particular, plant and fungi specimens collected for herbaria are often inhabited by parasitic fungi, which together with their host accidentally find their way into herbaria and can later serve as a subject of independent research (Kohlmeyer, 1975; Döbbeler, 1997; Denchev, Denchev, 2016; Ristaino, 2020). The aim of this article is to reflect on our own experience of searching for lichenicolous fungi in herbarium lichen collections, including a comparison of the results of searching for these fungi in herbaria and in nature.

Lichenicolous fungi are non-lichenized fungi obligately inhabiting lichens. According to the latest checklist of lichenicolous fungi (Diederich *et al.*, 2018), about 2000 species of these fungi from 341 genera and 8 classes of the kingdom Fungi were

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known by 2018; about 95% of these species belong to the Ascomycota, and 5% belong to the Basidiomycota. The true species richness of this ecological-trophic group of fungi is estimated at 3000–5000 species (Diederich *et al.*, 2018). It may be even much higher, with Diederich *et al.* (2022) recently estimating the global diversity of lichenicolous heterobasidiomycetes alone at over 1000 species.

For some of the most fully studied regions of the Holarctic, the ratio of the number of lichenicolous fungi species to the number of lichen species [Lichenicolous Index according to Zhurbenko (2007)] is approximately 0.2, i.e., one lichenicolous fungi species per five lichen species (Zhurbenko, 2011).

The size of fruiting bodies of lichenicolous fungi usually does not exceed 0.5 mm. Their conspicuity has not been specifically studied yet, but according to our estimates, in the Arctic only about 15% of the lichenicolous fungi species are visible to the naked eye, about 45% of species are clearly visible only at 10× magnification, and the remaining 40% can be confidently distinguished at 20–40× magnification (Zhurbenko, 2010). Thus, only about half of lichenicolous fungi species can be purposefully collected in nature (using a 10× lens), while the detection of the entirety of the lichenicolous mycobiota requires viewing lichen samples under a stereomicroscope. It should also be noted that the discovering of lichenicolous fungi requires: 1) knowledge of what these fungi look like and on which parts of lichens to look for them as some species of lichenicolous fungi are confined to certain parts of lichens, such as apothecia, cephalodia, or the backsides of the lobes; 2) readjustment of attention to objects 1–2 orders of magnitude smaller than the lichens themselves. It is therefore not surprising that in lichenological studies lichenicolous fungi are often overlooked and enter the herbarium unintentionally, without any notes on the labels about their presence. It is noteworthy that the first known image of a lichenicolous fungus, *Biatoropsis usnearum* Räsänen, growing on *Usnea* sp., (Dillenius, 1742) was also unintentional as the author mistook the basidiomata of the parasite for lichen organs (Diederich, Christiansen, 1994).

Only fragmentary data on the frequency of occurrence of lichenicolous fungi in nature are known so far. For example, it has been shown that in some arctic and alpine plant communities of the Holarctic 5–10% of the visually surveyed lichen thalli are infected with lichenicolous fungi (Zhurbenko, 2010; Fleischhacker *et al.*, 2015). On visual inspection of herbarium specimens of lichens of the genus *Cladia* from the southern hemisphere, about 5% of specimens were found to be infected with lichenicolous fungi (Zhurbenko, Pino-Bodas, 2015). Thus, in these studies, visually distinguishable lichenicolous fungi were found on every 10–20-th lichen sample. This suggests in favour of the recommendation that large herbaria, in which most lichen species are represented by dozens of specimens, are the most promising for searching for parasites.

Extremely promising for finding lichenicolous fungi are lichen collections from geobotanical sample plots, collected for further determination by lichenologists. Especially in cases where everything is collected in a row and in great repetition. For example, 617 of the 2511 (25%) specimens of lichenicolous fungi from the Russian Arctic cited by Zhurbenko (2010) were found in lichen collections of geobotanists.

An obvious advantage of herbarium studies over field studies is the wide geographical coverage of large herbarium collections. For example, during 40 days of work in the TNS herbarium, I found lichenicolous fungi species new to 21 countries from four continents: Argentina, Australia, Bhutan, Canada, China, Colombia, Costa Rica, Dominican Republic, India, Japan, Malaysia, Nepal, North Korea, Norway, Papua New Guinea, Peru, Russia, South Korea, Thailand, Taiwan, and the USA (Zhurbenko, Ohmura 2018a, 2019). It would clearly be impossible to find this material in nature in such a short period of time.

It is assumed that up to 95% of lichenicolous fungi species are confined to a particular host genus (Lawrey, Diederich, 2003), which makes it meaningful to revisit these fungi on certain lichen taxa. As our experience shows, such studies are very convenient to carry out in large herbaria (Table 1).

Table 1

Examples of searching for lichenicolous fungi on certain lichen taxa in herbaria

Lichen taxa surveyed	Number of lichenicolous fungi species discovered (new to science)	Herbaria	References
Baeomycetaceae, Icmadophilaceae	11(5)	TNS	Zhurbenko, Ohmura, 2020
Cladia	4(3)	H	Zhurbenko, Pino-Bodas, 2015
Siphula s. l.	16(6)	mainly H, HO, TNS, and UPS	Motiejūnaitė *et al.*, 2019
Sphaerophoraceae	9(4)	mainly UPS	Zhurbenko, 2023b

It has been supposed that lichen herbaria are less likely to contain ‘unhealthy’ specimens (Alstrup, 1985). However, this is unlikely to significantly affect their representativeness compared to natural biota, as saprotrophs and strong pathogens are rare among lichenicolous fungi (Lawrey, Diederich, 2003; Zhurbenko, 2013b). The relatively higher representation of rare species in herbaria seems more objective, as trivial mass species are usually of less interest to florists and taxonomists. An obvious advantage of herbaria is the revision of critical lichen groups by outside taxonomists, which makes the identifications of lichenicolous fungi hosts more reliable.

A major disadvantage of studying herbarium collections can be the limitations caused by the age of the specimens. However, as shown by the description of the ascomycete *Llimoniella bryonthae* Zhurb. et Diederich, based on a specimen collected 157 years ago (Zhurbenko, 2021), even at this age all diagnostic anatomical-morphological features of the fruiting bodies of fungi can be clearly seen. Sequencing ‘historical’ fungal specimens can be more problematic, as DNA is known to become highly fragmented with age. However, there are encouraging examples here as well. One of the oldest lichenicolous fungi specimens that could be sequenced is *Tremella umbilicariae* Diederich et Millanes, 33 years old (Diederich *et al.*, 2014). For lichens of the genera *Cladonia*, *Nephroma*, *Peltigera*, and *Ramalina*, it was possible to obtain full-length sequences for more than 100-year-old samples (Kistenich *et al.*, 2019). The oldest fungal specimen from which ITS sequences could be obtained is probably the 210-year-old *Hygrophorus cossus* (Sowerby) Fr. (Agaricales) (Larsson, Jacobsson, 2004).

As my experience shows, the productivity of lichenicolous fungi detection in lichen herbaria can be higher than in nature. During one day of work in herbarium I found 20–25 specimens of these fungi, while in field surveys — 2–15 specimens (Table 2). This proved equally true for the discovery of lichenicolous fungi species unknown to science, where my personal record is the discovery in one day of work in herbarium H of three species new to science subsequently described in Motiejūnaitė *et al.* (2019).

Table 2

Productivity of searching for lichenicolous fungi in nature and in herbaria

Territories or herbaria	Number of lichenicolous fungi specimens found (species described as new to science)	Number of work-days	Average number of lichenicolous fungi specimens found per day	References / data sources
India	49	11	4	Zhurbenko, 2013a
Mongolia	550(5)	37	15	Zhurbenko *et al.*, 2019, 2020b; M. P. Zhurbenko, unpublished data
Russia, Bastak Reserve	38	4	10	Zhurbenko, 2014
Russia, Caucasus Reserve	88	28	3	Zhurbenko, Kobzeva, 2016
Russia, Pechora-Ilych Reserve	110	10	11	Zhurbenko, 2004
Russia, Teberda Reserve	199	18	11	Zhurbenko, Kobzeva, 2014
Svalbard	185(3)	16	12	Zhurbenko, Brackel, 2013
Vietnam	34(4)	18	2	Zhurbenko *et al.*, 2020a
Herbarium H	25(3)	1	25	Motiejūnaitė *et al.*, 2019
Herbarium TNS	816(9)	40	20	Motiejūnaitė *et al.* 2019; Zhurbenko, Ohmura, 2018a, b, 2019, 2020; Zhurbenko *et al.*, 2017, 2018
Herbarium UPS	43(4)	2	21	Zhurbenko, 2023a, b

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