Global patterns to molecular problems in lichenology – a summary of the most important results

Studies of biodiversity in various taxonomic groups

In our sudies we followed the practise of recent taxonomic works in lichenology combining features originating from microscopic morphological studies, analysis of lichen secondary metabolites (LSMs), information on habitat and geographical distribution. In some groups molecular genetic markers were also considered.

Furthermore 21 species of lichen-forming and lichenicolous fungi were found from Hungary (2 spp), Romania (2spp) and or Serbia (1 sp) or from smaller regions (17 spp) as new distribution records published in scattered literature sources (**Papp et al. 2020**, **Aszalósné et al. 2021**, **Farkas and Lőkös 2021**, **Farkas et al. 2022**).

29. Papp, B., Erzberger, P., **Lőkös, L.**, Szurdoki, E., Németh, Cs., Buczkó,K., Höhn, M., Aszalósné Balogh, R., Baráth, K., Matus, G., Pifkó, D., **Farkas, E.** 2020: Taxonomical and chorological notes 12 (126–136). *Studia bot. hung.* 51(1): 77–98.
31. Aszalósné Balogh, R., Buczkó, K., Erzberger, P., Freytag, Cs., Homm, Th., **Lőkös, L**., **Matus, G.**, Nagy, Z., Papp, B., **Farkas, E**. 2021: Taxonomical and chorological notes 15 (153–163). *Studia bot.hung.* 52(2): 165–184.
36. Farkas, E.; **Lőkös, L**. 2021: Distribution of *Absconditella lignicola* (Stictidaceae, lichenised Ascomycetes) in Hungary. *Studia bot. hung.* 52(2): 115–124.
59. Farkas, E., A. Balogh, R., Bauer, N., **Lőkös, L.**, **Matus, G.**, Molnár, Cs.,Papp, B., Pifkó, D., **Varga, N.** 2022: Taxonomical and chorological notes 16 (164–177): *Studia bot. hung.* 53(1): 249–266.

Lichen-forming fungi

Focus on lichen secondary metabolites

Using various microscopic methods (DIC, fluorescence microscope) and the high performance thin layer chromatography (HPTLC) several thousand specimens (herbarium and freshly collected) were identified or revised and deposited in lichen collections BP, BTM, EGR, VBI (see indexherbariorum.org for abbreviations). The number of earlier detected 55 LSMs analysed by HPTLC in similar studies in Hungary increased to c. 100 during the years of current project (2017–2023).

1) Soredia, pseudocyphellae, rhizines, features of lower surface are the main morphological characters in the specimens of the genus *Cetrelia* W.L. Culb. & C.F. Culb.analysed against the presence of cortical pigment, atranorin and medullary α-alectoronic acid, anziaic acid, α-collatolic acid, β-alectoronic acid, β-collatolic acid, imbricaric acid, 4-O-demethylimbricaric acid, olivetoric acid, perlatolic acid, physodic acid and 4-O-methylphysodic acid (twelve metabolites). The originally known two taxa proved to be rare (*C. cetrarioides* (Delise) W.L. Culb. & C.F. Culb.) or less frequent than regarded earlier (*C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb.). The very rare *Cetrelia chicitae* and *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. (as the most frequent species of the genus) are new for the Hungarian lichen flora. (**Farkas et al. 2021**)

   34. Farkas, E., **Biró, B.**, **Sinigla, M.**, **Varga, N**., **Lőkös, L**. 2021: Analysis of lichen secondary chemistry increased the number of Cetrelia species (Parmeliaceae, lichenised Ascomycota) in Hungary. *Cryptogamie, Mycologie* 42(1): 1-16. [Q1, IF 2.245]

2) Thalli of the *Cladonia chlorophaea* species group with horizontal primary thalline lobes and sorediate, cup-, funnel-, goblet- or wineglasss-shaped podetia contain twelve different LSMs (atranorin, congrayanic, cryptochlorophaeic, 4-O-methyl-cryptochlorophaeic, fumarprotocetraric, grayanic, 4-O-demethyl-grayanic, homosekikaic, merochlorophaeic, norrangiformic, quaesitic, rangiformic and thamnolic acids) in various combinations. Six taxa are currently distinguished at species level based mainly on these substances: *C. asahinae* J.W.Thomson, *C. cryptochlorophaea* Asahina, *C. grayi* G.Merr. ex Sandst., *C. merochlorophaea* Asahina *C. novochlorophaea* (Sipman) Brodo & Ahti and *C. chlorophaea* (Flörke ex Sommerf.) Spreng. Three species – *C. asahinae*, *C. grayi* and *C. novochlorophaea* – represent new distribution records to Hungary.

A detailed morphometric study – considering the size of podetia, cup, stalk, soredia and squamules – was also carried out. Although *Cladonia asahinae* and *C. cryptochlorophaea* were usually smaller than the robust *C. chlorophaea* or *C. merochlorophaea*, the chemical characteristics supplied more stable results than morphological metrics (**Farkas et al. 2023**).

80. Farkas, E., **Lőkös, L.**, **Veres, K.** 2023: Analysis of lichen secondary metabolites and morphometrics in the Cladonia chlorophaea species group (Cladoniaceae, lichenized Ascomycota) inHungary. *Cryptogamie Mycologie* 44(5): 61-82. [Q2, IF 1.4]

3) *Lepraria* species of Hungary were investigated and their morphological and chemical divesity were presented on two posters (**Farkas et al. 2017**, **2018**), including geographical distribution maps and identification key to species. *Lepraria caesioalba* (B. de Lesd.) J. R. Laundon, *L. crassissima* (Hue) Lettau, *L. diffusa* (J. R. Laundon) Kukwa, *L. eburnea* J. R. Laundon, *L. elobata* Tønsberg, *L. incana* (L.) Ach., *L. jackii* Tønsberg, *L. lobificans* Nyl., *L. membranacea* (Dickson) Vainio, *L. neglecta (*Nyl.) Lettau, *L. rigidula* (B. de Lesd.) Tønsberg, *L. vouauxii* (Hue) R. C. Harris, These species were compared also to the following leprarioid taxa: *Botryolepraria lesdainii, Lecanora compallens, Lecanora rouxii, Leprocaulon microscopicum, Leproplaca spp, Phlyctis spp, Psilolechia lucida*. *L. lobificans* was found to be the most frequent in Hungary.

5. Farkas, E., **Lőkös, L**., **Sinigla, M.**, **Varga, N.** 2017: The genus Lepraria (lichen-forming fungi) in Hungary: Lepraria zuzmófajok Magyarországon. *Acta Biologica Plantarum Agriensis* 5(1): 50. [4th Conference on Cryptogams, Eger, 2017.november 30 – december 1.]
9. Farkas, E., **Lőkös, L.**, **Sinigla, M**., **Varga, N.** 2018: Lepraria zuzmófajok elterjedése Magyarországon. Distriburion of lichens in Hungary. In: Molnár, V.A.,Sonkoly, J., Takács, A. (eds) XII. AFVK. Progr. és összefogl: 12th IntConf Adv in res fl and veget Carp-Pann reg. Programme and Abstracts. p. 65.

4) Field studies, morphological and chemical investigations of two brown *Xanthoparmelia* species (*X. pokonyi* and *X. ryssolea*, both protected by law in Hungary; **Farkas et al. 2017**) confirmed our concept about the priority of morphological characters over differences in LSM production. We analysed a large amount of herbarium material and freshly collected specimens, but our findings were not in agreement with the relevant literature, a former widely accepted monographic treatment (Esslinger 1977) emphasizing the role of secondary metabolites (occurrence of gyrophoric acid with stenosporic acid (often with additional terpenoid / zeorin) in *X. ryssolea* and with divaricatic acid in *X. pokornyi*). Stenosporic acid was found most frequently in both morphologically different species similarly to findings of Amo de Paz et al. (2012). *X. ryssolea* differs by its vaganoid growth form and the lack of dorsiventrality and a very similar pigment production on both sides of the thallus, spot reaction N+. The few rhizines are usually found on both surfaces and on some large thalli they alternate between the two surfaces, while *X. pokornyi* is clearly dorsiventral, closely attached to the substrate often among mosses carrying rhizines on the lower surface only, spot reaction N– (cf. Esslinger 1977, Senkardesler 2010). However, Amo de Paz et al. (2012) indicates N+ for both species. Probably an analysis of so far less frequently studied genetic markers might lead to a better separation of *X. pokornyi* and *X. ryssolea.*

2. Farkas, E., **Lőkös, L.**, **Sinigla, M.** 2017: An update on protected lichen species in Hungary. In: Martin Kukwa (ed.) XX Symposium of Baltic Mycologists and Lichenologists: Book of abstracts. Gdansk: Fundacia Rozwoju Universitetu Gdanskiego, p. 9.

5) Generic key to lichen-forming fungi in Hungary (**Farkas submitted**) has been submitted to ABPA. A key to treat 268 genera of lichen-forming fungi including 945 species from Hungary is compiled (cf. Farkas et al. in prep.). The key sorts lichens according to traditionally distinguised morphological groups based on vegetative and reproductive structures, fruticose, foliose and crustose thalli with apothecia and perithecia. Elongated and stalked reproductive structures are also distinguished, as well as the type of photobiont (cyanobacterium, green alga) is considered. Due to recent phylogenetical-taxonomic studies several taxa with recent nomenclature grouped under larger, morphologically related genera following the practice of published national identification keys from Germany (Wirth et al. 2013) and Great Britain (Smith et al. 2009).

76. Farkas, E. (2023): Generic key to lichen-forming fungi in Hungary. *Acta Biologica Plantarum Agriensis* (submitted)
79. Farkas, E., **Lőkös, L.**, **Varga, N.**: Checklist of the Hungarian lichen-forming and lichenicolous fungi. (Magyarországi zuzmók és zuzmólakó mikrogombák fajlistája). Manuscript in preparation.

Focus on molecular genetic analysis

6) *Cladonia magyarica* was the main object that we were studying in the most details by molecular genetic methods. Fungal ITS (primary barcoding region), RPB2, IGS and TEFα sequences were generated from samples of *Cladonia magyarica* from main distribution areas (including the type locality) of the Carpathian Basin. We amplified the ITS region (commonly used as barcode) of greenalgae (*Asterochloris* sp.) with specific primers from the same samples as the lichenized fungi. Based on our studies we concluded that our samples contain more than 2 species of algae. Morphologically related species (*C. chlorophaea*, *C. fimbriata*, *C. foliacea*, *C. pyxidata*) were also sampled from nearby locations for comparison. A manuscript is under preparation based on c. 180 sequences generated on the basis of extended field work in a wider area of the type locality (**Varga et al. 2023**, Varga et al. in prep).

90. Varga, N., Kanyungulu, C.N., **Farkas, E.**: Deeper insight into the phylogenetics of *Cladonia magyarica* in Hungary. manuscript in preparation for Lichenologist [Q2; IF 1.4]
91. Varga, N., **Lőkös, L.**, **Farkas, E.** 2023: Contributions to the lichen-forming and lichenicolous fungi of the Aggtelek National Park (NE Hungary). *Studia Botanica Hungarica* 54(2): 155-174.

7) The study of the yellow-green *Xanthoparmelia* species was continued (57-58. **Bauer et al. 2022a**, **2022b**). Recenly two further species (X. mougeotii and X. verrucigera) were recognised from Hungary in this group. The manuscript (treating morphological chemical and molecular genetic markers) on usnic acid containing taxa is in preparation (Varga et al. in prep.). The study of brown species of the genus with different secondary chemistry (divaricatic acid, gyrophoric acid, loxodic acid, stenosporic acid and others) is planned to be published separately.

57. Bauer, N., Hüvös-Récsi, A., **Lőkös, L.**, **Farkas, E.** 2022a: A new steppeelement in the Vienna Basin, the first record of *Xanthoparmelia pulvinaris* (Parmeliaceae) for Austria. *Herzogia* 35(1): 22-31.[IF 0.613]
58. Bauer, N. Hüvös-Récsi, A., **Lőkös, L**., Matus, G., **Sinigla, M.**, **Farkas, E.** 2022b: Distribution of *Xanthoparmelia pulvinaris* (Parmeliaceae) in Hungary. *Studia Botanica Hungarica* 53(2): 113-135.
Varga, N., **Molnár, K.**, **Lőkös, L.**, Kanyungulu, C.N., **Farkas, E.** The usnic acid containing species in the genus *Xanthoparmelia* (lichenised Ascomycota) in Hungary. Manuscript in preparation

8) Four *Vezdaea* species (*Vezdaea aestivalis*, *V. leprosa*, *V. retigera* and *V. rheocarpa*) found in Hungary were studied and described morphologically, an identification key, illustrations and distribution maps were added (Sinigla et al. in prep.). Some of the ITS sequences were not successful, we were only able to barcode *V. aestivalis*. The plan to use additional markers was interrupted. Three of the four species represent new distribution record for Hungary.

Sinigla, M., **Lőkös, L.**, **Varga, N.**, **Farkas, E.** The genus *Vezdaea* (lichenised Ascomycota) in Hungary. Manuscript in preparation

We had contributions to phylogenetic studies of higher taxa (Lecanoraceae, Physciaceae, Teloschistaceae and Trapeliaceae – Kondratyuk et al. 2019, 2021, 2022). We took part in species identification and further analysis of sqeuences originated from other projects.

9) From the combined phylogenetic analysis of multi-locus sequence data of the Lecanoraceae including two nuclear protein-coding markers (RPB2 and RPB1), the internal transcribed spacer nrITS) and a fragment of the mitochondrial small subunit (12S mtSSU) sequences, found that the originally monotypic genus *Verseghya* is positioned within the *Verseghya-Lecidella-Pyrrhospora* clade of the Lecanoraceae and includes one more taxon *Verseghya thysanophora* widely distributed in Northern Hemisphere (**Kondratyuk et al. 2019**).

20. Kondratyuk, S.Y., **Lőkös, L**., Jang, S.-H., Hur, J.-S., **Farkas, E.** 2019: Phylogeny and taxonomy of *Polyozosia, Sedelnikovaea* and *Verseghya* of the *Lecanoraceae* (Lecanorales, lichen-forming Ascomycota). *Acta Bot. Hung.* 61(1–2): 137–184. [Q2]

10) A combined phylogeny analysis based on a combined matrix of nrITS and 12S mtSSU sequences lead to the description of seven genera of the family Physciaceae new to science with strong support, i.e.: *Helmutiopsis*, *Huriopsis*, *Johnsheardia*, *Klauskalbia*, *Kudratovia*, *Kurokawia* and *Poeltonia* (**Kondratyuk et al. 2021**).

42. Kondratyuk, S. Y., **Lőkös, L.** , Kärnefelt, I., Thell, A., Jeong, M.-H.;Oh, S.-O., Kondratiuk, A.S , **Farkas, E.**, Hur, J.-S. 2021: *Contributions* to molecular phylogeny of lichen-forming fungi 2. review ofcurrent monophyletic branches of the family *Physciaceae*. *Acta Botanica Hungarica* 63(3-4): 351-390. [Q2]

11) The new genus *Kudratoviella* has been described for the former *Caloplaca zeorina* group with high level of bootstrap support in the phylogenetic tree of the Teloschistaceae, based on combined dataset of nrITS, 28S nrLSU DNA and 12S SSU mtDNA sequences (**Kondratyuk et al. 2022**).

64. Kondratyuk, S.Y., Persson, P.-E., Hansson, M., **Lőkös, L.**, Kondratiuk, A.S., Fayyaz, I., Kouser, R., Afshan, N.S., Niazi, A.R., Zulfiqar, R. et al. 2022: Contributions to molecular phylogeny of lichens 4. New names in the *Teloschistaceae*. *Acta Botanica Hungarica* 64(3-4): 313-336. [Q2]

Lichenicolous fungi

12) An annotated checklist of the lichenicolous fungi of Hungary has been published (**Varga et al. 2021**) with 104 lichenicolous species in 64 genera, including 53 new species for the country. Old records of 5 species were confirmed by new collections. Key characteristics of some of the most interesting species were illustrated by microscopic views and 2 distribution maps. Although lichenicolous fungi have been less well studied in Hungary in the past, the relative diversity of lichenicolous fungi in the country (0.112), as indicated by Zhurbenko's (2007) lichenicolous index, was found to be slightly higher than the mean value calculated for the world (0.103) (Table 1). [This value expresses the diversity of lichenicolous fungi related to its hosts' diversity within an area, see also **Shivarov et al. 2021**]. In a further publication 13 species of lichenicolous fungi were listed with new distribution records from the Aggtelek National Park (**Varga et al. 2023**).

Table 1. Lichenicolous index values worldwide and in selected European countries, USA and Canada, and the numbers of species the calculation based on.

Country or region	**Lichenicolous fungi**	**Lichens**	**LI[1] (lichenicolous index)**	**References** *found in the original publication*
Bavaria (Germany)	399	1624	0.246	[86]
Great Britain	384	1677	0.229	[18,87]
Belgium, Luxembourg & N France	201	930	0.216	[88]
Germany	392	1946	0.201	[89]
Italy	492	2565	0.192	[90–91]
France, 2020	592	3185	0.186	[92]
Fennoscandia	430	2387	0.180	[38]
France, 2017	546	3082	0.177	[93]
France, 2014	513	3528	0.145	[94]
USA and Canada	631	4880	0.129	[95]
Ukraine	246	1910	0.129	[96]
Hungary	104	926	0.112	present paper
World	2000	19387	**0.103**	[97–98]
Albania	38	398	0.095	[21–22,26–27]
Russia	276	3388	0.081	[99–100]
Greece	64	1353	0.047	[101]
Bulgaria	45	1120	0.040	[20]
Romania	40	1194	0.034	[102]
Serbia	15	668	0.022	[22,23–25]

[1] LI = species number of lichenicolous fungi/species number of lichens [Zhurbenko 2007].

47. Shivarov, V.V., **Varga, N.**, **Lőkös, L**., Brackel, W.v., Ganeva, A., Natcheva, R., **Farkas, E.** 2021: Contributions to the Bulgarian lichenicolous mycota – an annotated checklist and new records. *Herzogia* 34: 142-153. [Q4, IF 0.613]

50. Varga, N., **Lőkös, L.**, **Farkas, E.** 2021: Annotated checklist of the lichenicolous fungi of Hungary. *Diversity* 13: 557. [Q1, IF 3.029]

91. Varga, N., **Lőkös, L.**, **Farkas, E.** 2023: Contributions to the lichen-forming and lichenicolous fungi of the Aggtelek National Park (NE Hungary). *Studia Botanica Hungarica* 54(2): 155-174.

The study of indicator nature of lichens at various scales

Further studies revealed the relation of lichen populations to a series of environmental conditions and confirmed the bioindicator nature of lichens at various scales by combined methods (of ecophysiology, coenology, multivariate statistical analysis, etc.).

At national scale

1) Indicator groups of species based on characteristic distribution patterns were established at national scale compared to global / European tendencies (**Farkas et al. 2022**). Distribution data originating from earlier herbarium collections and recent biodiversity records form the basis of distribution analyses in lichen species with different ecological requirements, where the records allowed comparisons or showed clear trends. However, about 40 % of the 926 species currently known from Hungary were categorised as “distribution types to be established”.As the occurrences of lichens are strongly correlated to background environmental conditions (e.g., air pollution, global warming), confirmed byWirth’s ecological indicator values, the analysis of distribution types has a great value for bioindication and the establishment of current and future climatic and pollution situations.

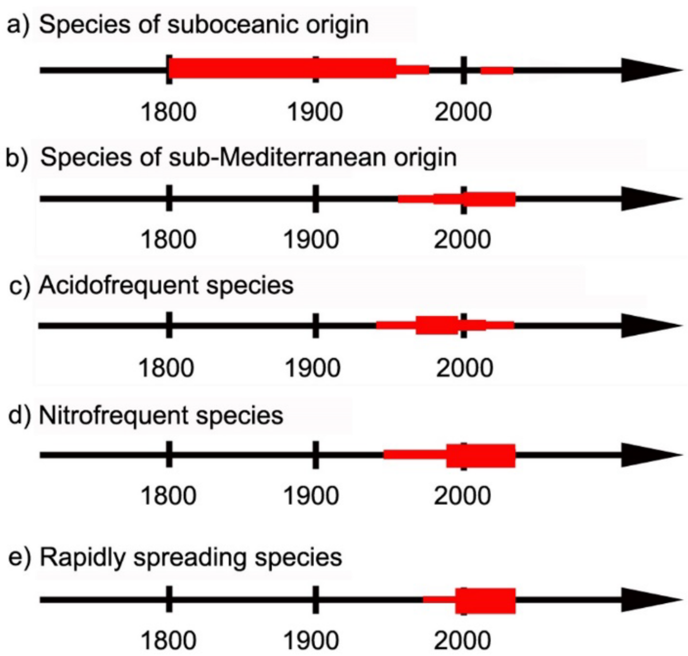

Figure 1. The five changing distribution types of lichen species in time (years) according to possible explanations represented by the thickness of the red line.

Five distribution types were introduced (Fig. 1) – presented by characteristic examples – according to lichen distribution maps prepared in different periods of time (representing changing environmental conditions): (1) species of decreasing occurrences by time (e.g., *Lobaria pul-monaria*, *Menegazzia terebrata*, suboceanic, acidic pollution sensitive species), (2) species with no or few former records but with increasing occurrences in recent decades (e.g., *Flavoparmelia soredians*, *Hyperphyscia adglutinata*, *Solenopsora candicans*, sub-Mediterranean species), (3) species with increasing and then (from c. 2000) decreasing occurrences (e.g., *Scoliciosporum chlorococcum*, *Straminella conizaeoides*, acidofrequent species), (4) species with widely increasing occurrences in recent decades (e.g., *Physcia aipolioides*, *Piccolia ochrophora*, *Xanthoria parietina*, nitrofrequent species), and (5) species with rapidly increasing occurrences (e.g., *Absconditella lignicola*, *Coenogonium pineti*, *Evernia divaricata*, rapidly spreading species). The proposed distribution types of lichen species may be applied to wider regions (the European or the global level).

62. Farkas, E., **Varga, N.**, **Veres, K.**, **Matus, G.**, **Sinigla, M.**, **Lőkös, L.** 2022: Distribution types of lichens in Hungary that indicate changing environmental conditions. *Journal of Fungi* 8, 600. [Q1, IF 5.724]

At regional scale

2) Habitat preferences of three protected *Cladonia* species (*C. arbuscula*, *C. mitis* and *C. rangiferina*) growing on underlying rocks of redsandstone, basalt, Pannonian sandstone and gravel were investigated in the Bakony Mts. Sporadic populations of these species mostly exist at the top of hills and mountains in open acidofrequent oak forests. *C. rangiferina* was found to grow beneath higher canopy cover than either *C. arbuscula* or *C. mitis*. It was established that *Cladonia rangiferina* is a good indicator species of natural habitats in Hungary due to its restricted distribution and low ecological tolerance. These results may lead to the adoption of effective conservation methods (e.g. game exclusion, artificial dispersal) in the future. (**Sinigla et al. 2021**)

   48. Sinigla, M., Szurdoki, E., **Lőkös, L.**, Bartha, D., Galambos, I., Bidló,A., **Farkas, E.** 2021: Distribution and habitat preference ofprotected reindeer lichen species (Cladonia arbuscula, C.mitis and C. rangiferina) in the Balaton Uplands (Hungary). *Lichenologist* 53(3): 271-282 [Q2, IF 1.651]

3) We found that site had a significant effect on species richness that might reflect the different types and severity of previous disturbance events. Most of the frequent species were negatively affected by higher moss cover. Some lichen species were more abundant or found only on south-facing (more arid), while others preferred or occurred only on north-east (more humid) dune sides. (**Veres et al. 2021**)

   52. Veres, K., Csintalan, Zs., Kovács, B., **Farkas, E.** 2021: Factors at multiple scales influence the composition of terricolous lichen communities in temperate semi-arid sandy grasslands. *Lichenologist* 53: 467–479 [Q2, IF 1.651]

4) Cryptogams, compose a large part of biomass and contribute to the biodiversity of sandy grasslands (**Aszalós-Balogh et al. 2023a**). The lichen and bryophyte dynamics were studied at two Eastern Hungarian dry sandy grassland sites. The sites of *Festuco vaginatae–Corynephoretum* community have been monitored for three aims: (1) quantifying the diversity and biomass of the cryptogamic communities; (2) exploring the cryptogamic response to management changes; and (3) studying the effect of fencing on the cryptogamic assemblages. Fencing has led to increased biomass of cryptogams within a few years. Lichens in general benefited comparatively more from exclosure than bryophytes. The increase in lichen biomass (especially that of *Cladonia rangiformis*) is clearly due to the over 10-year absence of grazing. The only lichen favored by moderate grazing is the legally protected *C. magyarica*. Short spells of low-intensity grazing can promote the species richness of cryptogams in the community.

   72. Aszalósné Balogh, R., **Farkas, E.**, Tüdősné Budai, J., **Lőkös, L**., **Matus, G**. 2023a: Cryptogamic biomass in pannonic acidic sand steppes subject to changing land-use. *Plants* 12: 2972. [Q1, IF 4.5] https://doi.org/10.3390/plants12162972

5) Cryptogams of ten urban flatroofs, contrasting in their age and size, were studied between 2016 and 2018 (**Aszalós-Balogh et al. 2023b**). Siliceous and calcareous substrata occurred at each site. Microclimate at two sites of contrasting shading was recorded from September 2016 to January 2017. Biomass of two differently aged, exposed flatroofs was sampled in October 2018. A total of 61 taxa (25 bryophytes, 36 lichens), mostly widespread synanthropic species, have been detected with an explicit difference of species composition between shaded and exposed sites. Floristically interesting species included acidophilous bryophytes (*Hedwigia ciliata*, *Racomitrium canescens*) and lichens (*Xanthoparmelia conspersa*, *Stereocaulon tomentosum*) of montane character. The most widespread lichen is *Cladonia rei* which accounted for a significant part of the biomass at selected sites. Species-area curves for bryophytes at exposed sites have become saturated at 100–

150 m^2. In contrast, saturation of lichen diversity has not been reached even at the largest sites. Flatroofs can harbour relatively diverse microhabitats and species-rich synanthropic vegetation. It is urgent to study these sites before renovation eliminates them. Diversification of urban surroundings is possible in the future via application of various substrates in renovated and newly constructed roofs.

74. Aszalósné Balogh, R., **Matus, G.**,·**Lőkös, L.**, Adorján, B., Freytag, C., Mészáros, I.,·Oláh, V., Szűcs, P., Erzberger, P. **Farkas, E.** 2023b: Cryptogamic communities on flatroofs in the city of Debrecen (East Hungary) *Biologia Futura* 74: 183–197. [Q12, IF 2.1] https://doi.org/10.1007/s42977-023-00166-3

At microhabitat scale / seasonality

6) The effect of seasonal variation and different micro-environmental conditions (aspect) were explored on the metabolic activity of five terricolous lichen species. Higher photosynthetic activity, and a higher level of photoprotection, were detected in lichens from north-east-oriented microsites compared to south-west populations. We confirmed a species-specific response in lichen functions to the spatially and termporarily changing environment. Therefore the investigation of more than one species of different growth forms is suggested to draw a general conclusion (**Veres et al. 2020**).

30. Veres, K., **Farkas, E.**, Csintalan, Zs. 2020: The bright and shaded sideof duneland life: the photosynthetic response of lichens toseasonal changes is species-specific. *Mycological Progress* 19: 629–641. [Q1, IF 2.847]

UV protection mechanisms in two partner lichen symbiosis

In intact thalli

7) In lichens, the protection of the photosynthetic apparatus is essential for the survival of its thallus. The changes in the concentrations of three UV protectant metabolites of 3 Cladonia species, as well as the plastid pigment concentration, were determined. Both the UV protectant metabolite and plastid pigment concentrations showed seasonal fluctuations according to changing light and humidity conditions. The interspecific difference of the adaptation were more pronounced than that of the intraspecific one among seasons and microhabitat types. (**Veres et al. 2022a**)

67. Veres, K., Csintalan, Zs., Laufer, Zs., **Engel, R.**, **Szabó, K.**, **Farkas,E.** 2022a: Photoprotection and high-light acclimation in semi-aridgrassland lichens – a cooperation between algal and fungal partners. *Symbiosis* 86: 33–48. [Q1, IF 3.109]

In aceton treated thalli in a long–term field experiment

8) Results on the long term effect of removing the UV protectant LSMs lichen thalli was summarised (**Veres et al. 2022b**). Since the long term effect of diminishing UV-protectant lichen metabolites was unknown, a major part of LSMs was removed from C. foliacea thalli by acetone rinsing. The lichens were then maintained under field conditions for 3 years. Photosynthetic activity and light protection were checked by chlorophyll a fluorescence kinetics measurements every 6 months. The concentrations of fumarprotocetraric and usnic acids were monitored by chromatographic methods (HPLC, HPTLC). Our results proved that seasonality had a more pronounced effect than that of acetone treatment on the photoprotective function of lichens over a long term scale. Even after 3 years, the treated thalli contained half as much usnic acid as the control thalli, and the level of photoprotection remained unchanged in the algae. The amount of available humidity was a more critical limiting environmental factor than the amount of incoming irradiation affecting usnic acid production. The lichenicolous fungus *Didymocyrtis cladoniicola* became relatively more abundant in acetone treated than in control samples, indicating a slight change caused by the treatment.

68. Veres, K., **Sinigla, M.**, **Szabó, K.**, **Varga, N.**, **Farkas, E.** 2022b: The long-term effect of removing the UV-protectant usnic acid from the thalli of the lichen Cladonia foliacea. *Mycological Progress* 21:83; Q1, IF 2.538]

Further studies on lichen secondary metabolites – production, localization and application

While studying LSMs, our studies pointed out that there are several fields of limited knowledge on these metabolites. Therefore a few studies were initiated on these fields.

Quantitative aspects

Effect of geographic location of habitat – at global scale

The cortical pigment usnic acid is generally known to protect lichens from extreme radiation. According to earlier studies the parmelioid lichen *Flavoparmelia caperata* (L.) Hale contains (+)-usnic acid. Since the production of LSMs, is influenced by the environmental conditions, we supposed that especially the different temperature, radiation and humidity between European and African habitats resulted in difference in the LSM concentrations in lichen samples from various geographical areas (**Farkas et al. accepted**). Therefore we analysed samples collected in populations in Hungary (17), Serbia (3), Kenya (8) and Tanzania (2). By the application of a chiral chromatographic method described earlier, the presence of (+)-usnic acid was confirmed in thirteen of the above specimens. The usnic acid content was measured by HPLC-PDA. The content of usnic acid shows a substantial variation in both continents being slightly, but not significantly higher in Africa. This result can be explained by the supposedly similar microclimatic conditions of the habitats (within macroclimatically different sites) that are most probably consistent with the specific niche requirements of *F. caperata*.

77. Farkas, E., Kirika, P.M., **Szabó, K.**, Muhoro, A.M. (2023): Concentration data of (+)-usnic acid enantiomer from some European and African samples of Flavoparmelia caperata (Parmeliaceae, lichenised Ascomycota). *Cryptogamie Mycologie* (submitted and accepted manuscript) [Q2, IF 1.4].

Effect of habitat features and biological composition – at regional scale

LSMs have interesting biological activities, and considerable quantitative variations may be present intraspecifically. Such variations of medullary fumarprotocetraric acid (FA) and cortical usnic acid (UA) were observed in the lichen *Cladonia foliacea*, but the mechanism of variation is far from being understood (**Farkas et al. 2020**, **Farkas et al. submitted**). The current study aimed to characterise the quantitative variation of FA and UA and to investigate the association between LSM contents and ecological / biological variables. Fungal and algal trees were constructed using fungal (nrITS, RPB2) and algal (nrITS) loci, respectively. With a chiral chromatographic method, the contents of (-)-UA were determined in 29 *C. foliacea* specimens, ranging from 6.88 to 34.27 mg/g dry weight (d.w.). FA contents are lower and vary from 1.44 to 9.87 mg/g d.w. Even though the fungal tree showed two well resolved clades, no significant differences of UA or FA contents were found between the two fungal clades. However, significantly higher UA/FA content ratio as well as unique habitat was found in specimens associated with the alga *Asterochloris lobophora* than the ones associated the other *Asterochloris* algae. Taking all predictive variables together, our multivariate data analysis incidates that photobionts explain the most variance of LSM contents in the lichen *C. foliacea*. Our study suggests that future LSM biosynthetic studies take the photobiont into consideration when dealing with intraspecific quantitative variation of LSMs.

28. Farkas, E., **Biró, B.**, **Szabó, K.** , **Veres, K**. , Csintalan, Zs., **Engel, R.** 2020: The amount of lichen secondary metabolites in *Cladonia foliacea* (Cladoniaceae, lichenised Ascomycota). Acta Bot. Hung. 62(1–2): 33–48 [Q2]
81. Farkas, E., Xu, M., Muhoro, A.M., **Szabó, K.**, Heiðmarsson,S., Viktorsson, E.Ö., Ólafsdóttir, E.S. (2023):Algal partnership isassociated with quantitative variation of lichen specializedmetabolites: an example of Cladonia foliacea in Central andSouthern Europe. *Symbiosis* (submitted manuscript) [Q1, IF 2.5]

Localization of lichen secondary metabolites

It is hypothesised that the thickness of morphological layers is in correlation with concentration of LSMs, therefore measurements were carried out in the sections of *Cladonia foliacea* thalli compared with concentration measurements in the long term acetone rinsing experiment (control samples).

Farkas, E., **Veres, K**. The quantitative aspects of thalline layers and lichen secondary metabolites. Manuscript in preparation

Corticolous, terricolous and saxicolous *Lepraria* and *Cryptothecia* species were studied by fluorescence, some are quenching, others (e.g., *L. eburnea*, *L. incana*) show various coloured fluorescence at different vawelength (**Farkas et al. 2017**).

5. Farkas, E., **Lőkös, L**., **Sinigla, M.**, **Varga, N.** 2017: The genus Lepraria (lichen-forming fungi) in Hungary: Lepraria zuzmófajok Magyarországon. *Acta Biologica Plantarum Agriensis* 5(1): 50. [4th Conference on Cryptogams, Eger, 2017.november 30 – december 1.]

Photobiont *Rhizonema* sp on fungal hyphae of *Tamasia fijiensis* was illustrated by a micrograph under fluorescence in NIKON SMZ18 research stereo microscope (**Farkas et al. 2023**).

75. Farkas, E. 2023: Foliicolous lichens of the Fiji islands. *Acta Botanica Hungarica* 65(1-2): 87-111. [Q2]

The foliicolous *Calopadia erythrocephala* was studied by fluorescence in Nikon SMZ 18 (**Farkas et al. 2021**). The surface of the quenching naphtaquinone containing campylidium parts were shown in contrast with the fluorescent thallus.
A great number of other taxa (*Xanthoparmelia*, *Caloplaca*, *Cladonia* and *Pseudocyphellaria* species) with usnic acid and antraquinone pigments or other LSMs (e.g. stictic acid related compounds and terpenoids) were studied by Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS). A cooperation has been developed with Dr. László Márk (University of Pécs, Medical School) for further examinations by this sensitive method, MALDI-MS imaging, ideal for loclization studies. The work is promising but not ready for a journal publication, yet.

40. Farkas, E., **Varga, N.**, **Veres, K.**, **Lőkös, L.**, Márk, L. 2021: From spot reactions to high-end analytical methods in the study of lichen secondary metabolites. Program and abstract book IAL9: International Association for Lichenology 9thSymposium - online, p.108.[oral presentation]

Bioactive role of lichen secondary metabolites – at global scale

In a review paper we focused on lichens with potential insecticidal and antiprotozoal activity due to their LSMs (Muhoro and Farkas 2021). Insecticidal and antiprotozoal effects of crude extracts and 7 LSMs (mostly usnic acid) of 32 lichen species were determined.

45. Muhoro, A.M., **Farkas, E.É.** 2021: Insecticidal and antiprotozoalproperties of lichen secondary metabolites on insect vectors and their transmitted protozoal diseases to humans. *Diversity* 13: 342 [Q1, IF 3.029

During the years of the project (2017–2023) we prepared 37 reviewed journal publications mentioned above or listed in the publication list online (EPR) in a total IF value of 38.993 (without the values of the submitted and so far unpublished maunuscripts). The number of Scimago Q1 journal publicattions are 8, Q2 publications are 14.

Furthermore 47 abstracts were published and 3 manuscripts were submitted (one of them has already been accepted for publication). Further manuscripts are also in preparation.

New scientific degrees

During the research period our young scientist participants achieved PhD degrees or had a considerable progress in their PhD studies.

Katalin Veres (2022), Mónika Sinigla (2023) have received their PhD degrees. Rebeka Aszalósné-Balogh (supervised by our participant Gábor Matus) defended her PhD thesis in 2023.

A further participant (Nóra Varga) and PhD students (Arthur Muhoro, Coretor Kanyungulu) supervised by the PI are also preparing their PhD theses and planning their defences in 2024 or the following years.

References

Amo de Paz, G., Cubas, P., Crespo, A., Elix, J.A., Lumbsch, H.T. 2012: Transoceanic dispersal and subsequent diversification on separate continents shaped diversity of the Xanthoparmelia pulla group (Ascomycota). *PLoS ONE* 7(6): e39683. doi:10.1371/journal.pone.0039683

Esslinger, T.L. 1977: A chemosystematic revision of the brown Parmeliae. *Journal of the Hattori Botanical Laboratory* 42: 1-211.

Şenkardeşler, A. 2010: New lichen records from Turkey. *Mycotaxon* 111: 379-386.

Smith, C.W., Aptroot, A., Coppins, B.J., Fletcher, A., Gilbert, O.L., James, P.W., Wolseley, P.A. (Eds.) 2009: *The lichens of Great Britain and Ireland.* British Lichen Society, London, UK. pp. 1–1046.

Wirth, V., Hauck, M., Schultz, M. 2013: *Die Flechten Deutschlands*; Ulmer Verlag: Stuttgart, Germany; pp. 1–1244.

Zhurbenko, M.P. 2007: Lichenicolous fungi of Russia: History and first synthesis of exploration. *Micol. Phitopatol.* 41: 481–486. (In Russian).

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