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Analysis of lichen secondary chemistry doubled the number of *Cetrelia* W.L. Culb. & C.F. Culb. species (Parmeliaceae, lichenised Ascomycota) in Hungary

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ABSTRACT
The distribution patterns of lichen secondary metabolites are often taxon specific. They represent cryptic chemical diversity additional to morphological-anatomical biodiversity. *Cetrelia* W.L. Culb. & C.F. Culb. species (*c*. 200 specimens) were checked and revised by thin-layer chromatography. Soredia, pseudocyphellae, rhizines, features of lower surface are the main morphological characters 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. The European occurrence of *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb. – described from America – is confirmed on the basis of identical secondary metabolite composition by seven lichen substances. Four species were revealed and mapped in Hungary. *Cetrelia chicitae* and *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. are new for the Hungarian lichen flora. From the originally known two taxa *C. cetrarioides* (Delise) W.L. Culb. & C.F. Culb. proved to be rare, *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. is less frequent than regarded earlier. Currently *C. monachorum* is the most frequent *Cetrelia* species in Hungary. *Cetrelia chicitae* and *C. cetrarioides* may need protection measures. *Parmelia cetrarioides* f. *pseudofallax* (Gyeln.) Gyeln. is lectotypified here.

KEY WORDS
Chemotaxonomy, geographical distribution, high performance thin-layer chromatography (HPTLC), lichen-forming fungus, lichen secondary metabolites (LSMs), lectotypification.

RÉSUMÉ

L'analyse de la chimie secondaire du lichen double le nombre d'espèces de Cetrelia *W.L. Culb. & C.F. Culb. (Parmeliaceae, Ascomycota lichénisées) en Hongrie.*

Les modèles de distribution des métabolites secondaires des lichens sont souvent des taxons spécifiques. Cela représente une diversité chimique qui s'ajoute à la biodiversité morphologique et anatomique. Les espèces de *Cetrelia* W.L. Culb. & C.F. Culb. (environ 200) furent contrôlées et révisées avec une chromatographie sur couche mince. Soredia, pseudocyphellae, rhizinae et les caractéristiques de la surface inférieure sont les principaux caractères morphologiques analysés par rapport à la présence de pigment cortical, d'atranorine et d'acide médullaire (acide α-alectoronique, acide anziaque, acide α-collatolique, acide β-alectoronique, acide β-collatolique, acide imbiricarique, acide 4-O-déméthylimbiricarique, acide olivétorique, acide perlatorique, acide physodique, acide 4-O-méthylphysodique) qui peuvent être trouvés dans les médulles. La présence de l'espèce *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb. (décrite en Amérique) en Europe est prouvé sur la base de l'apparition de sept composants de lichens différents. Nous avons démontré et cartographié la présence de quatre espèces en Hongrie. Les espèces *C. chicitae* et *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. sont nouvelles pour la flore lichenique Hongroise. L'espèce connue auparavant *C. cetrarioides* (Duby) W.L. Culb. & C.F. Culb. devient rare, et l'espèce *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. est moins fréquente qu'elle ne l'était. De nos jours *C. monachorum* est l'espèce la plus fréquente de ce genre en Hongrie. Les espèces *C. chicitae* et *C. cetrarioides* doivent être protégées. *Parmelia cetrarioides* f. *pseudofallax* (Gyeln.) Gyeln. est lectotypifié ici.

MOTS CLÉS

Chimiotaxonomie, distribution géographique, chromatographie en couche mince haute performance (CCMHP), lichen formant des fongus, lichen secondaire metabolite (LSM), lectotypification.

INTRODUCTION

Lichens produce approximately 1050 secondary metabolites, most of which are bioactive and unique to lichen-forming fungi (Stocker-Wörgötter 2008, 2015; Molnár & Farkas 2010). These chemically diverse (aliphatic and aromatic) lichen substances are produced by the mycobiont (Elix 1996; Huneck 1999). They are accumulated in the cortical (e.g. atranorin) and medullary layers (e.g. imbricaric, perlatolic or olivetoric acid and others) as extracellular crystals on the outer surfaces of the hyphae. Lichen secondary chemistry analyses (e.g. Bjerke *et al.* 2005; Wang *et al.* 2009; Duong *et al.* 2017; Brakni *et al.* 2018) – and study of their relation to environmental conditions (cf. Armaleo *et al.* 2008; Hauck *et al.* 2009; Hauck 2011) – clarify the background of the chemical diversity of the studied taxa. The production of lichen secondary metabolites (LSMs) is genetically controlled (Culberson & Culberson 2001), and often correlated with morphology and geography at the species and genus levels at different scales (Egan 1986; Zhou *et al.* 2006; Matteucci *et al.* 2017). The distribution patterns of taxa characterised by LSMs have been widely used in lichen taxonomy and systematics since they represent cryptic chemical diversity additional to morphological-anatomical biodiversity (Crespo & Lumbsch 2010). Thanks to standardised chromatographic studies (TLC – Culberson & Kristinsson 1970; Culberson 1972, 1974; HPTLC – Arup *et al.* 1993) the knowledge of LSMs has developed worldwide largely since the 1970s. Application of HPTLC was introduced for analysing samples of Hungarian herbaria later in 1998 (Farkas *et al.* 1998) contributing to morphological investigations during identifications and revisions (Biró *et al.* 2015; Farkas & Biró 2015; Farkas *et al.* 2016).

The role of LSMs in taxonomic identification of lichens has already been studied by scientists of the 19th century (cf. Nylander 1866a, b; Hawksworth 1976; Carlin 1987). Since the occurrence and composition of secondary metabolites are often taxon specific, they have been widely used in lichen taxonomy and systematics in several groups of lichens (e.g. Culberson 1969, 1970, 1986a, b; Schmitt & Lumbsch 2004; Nelsen & Gargas 2008; Leavitt *et al.* 2011). Though the number of LSMs produced by lichens varies from 1-3 to 12, not the number, but the composition of LSMs characterises them better (Elix 1996). The presence/absence and composition of LSMs were considered at various levels of taxa from chemosyndromes, chemical races – via species – to families (Hawksworth 1976; Nourish & Oliver 1976; Randlane *et al.* 2009; Osyczka & Skubała 2011; Lendemer 2012).

Differences in secondary chemistry within the genus *Cetrelia* W.L. Culb. & C.F. Culb. (Parmeliaceae, lichenised Ascomycota) were also considered at various taxonomic levels of taxa from chemical varieties to species level. Though their treatment as species is becoming widely accepted by now, there are other opinions in publications until quite recent years. Thell & Kärnefelt (2011) treated all three Nordic *Cetrelia* species under one name, *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb., thus the chemically different species *C. cetrarioides* (Duby) W.L. Culb. & C.F. Culb. and *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. were listed as synonyms. Olivetoric acid ("*C. olivetorum* s. str."), perlatolic acid ("*C. cetrarioides*") and imbricaric acid ("*C. monachorum*") containing chemotype specimens are mentioned in the paragraph "Chemistry". Similar taxonomic status and nomenclature are followed by Roux *et al.* (2017) for chemotypes, as *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. chémo. *olivetorum*, *C. olivetorum* chémo. *cetrarioides*, *C. olivetorum* chémo. *monachorum*, and *C. olivetorum* chémo. *chicitae*.

In Britain (Smith *et al.* 2009) similarly only *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. *s.l.* is mentioned with four

chemotypes characterised by spot reactions. However, Wirth *et al.* (2013) characterise four species mentioning them together as *C. olivetorum s.l.* from Germany. Stenroos *et al.* (2011, 2016) differentiated two species, *C. cetrarioides* and *C. olivetorum* on morphological (pseudocyphellae, soredia) and chemical basis (spot reactions, LSMs) in Finland. Degtjarenko *et al.* (2018) reported – with illustrations of TLC plates – that three species (*C. cetrarioides*, *C. monachorum*, *C. olivetorum*) are known from Estonia after revising specimens kept under different names.

According to the latest knowledge, the genus *Cetrelia* includes 18 species worldwide (Randlane *et al.* 2013; Mishra & Upreti 2015). Some of them are morphologically close to *Parmotrema*, *Platismatia* and *Punctelia* species. *Cetrelia* species are characterised by foliose thallus with laminal pseudocyphellae on the grey upper cortex, and on the mostly black and brown lower cortex with few rhizines. Apothecia are often lacking, therefore vegetative propagules (soredia, isidia), pseudocyphellae, rhizines, features of lower surface are the main morphological characters available for differentiating taxa (Fig. 1A-C). However, the genus is chemically diverse containing cortical pigment, atranorin and medullary substances: α-alectoronic acid, anziaic acid, α-collatolic acid, β-alectoronic acid, β-collatolic acid, imbricaric acid, 4-O-demethylimbricaric acid, microphyllinic acid, olivetoric acid, 4-O-methylolivetoric acid, perlatolic acid, physodic acid and 4-O-methylphysodic acid. Following the combined chemical and morphological species concept of Culberson & Culberson (1968) several revisions have been published from various parts of Europe (Randlane & Saag 1991; Obermayer & Mayrhofer 2007; Kukwa & Motiejūnaitė 2012; Kukwa *et al*. 2012; Wirth *et al.* 2013; Bely *et al.* 2014). Molecular genetic studies treating cetrarioid taxa confirmed, to some extent, chemical species of *Cetrelia* (Thell *et al.* 2002; Nelsen *et al.* 2011). Randlane *et al.* (2013) compiled a list of cetrarioid lichens, including all chemical species of *Cetrelia*, with updated phylogenetic information, Mishra & Upreti (2015), treating *Cetrelia* from India, prepared a worldwide key for the 18 *Cetrelia* species.

Recently Mark *et al.* (2018) analysed a dataset of four nuclear markers (ITS, IGS, Mcm7, RPB1) from 62 specimens within Bayesian and maximum likelihood frameworks and justified that chemotypes, distinguished according to the major medullary substance, clearly correlated with clades recovered within *Cetrelia*. The traditional species were generally monophyletic, with the exception of *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb. and *C. pseudolivetorum* (Asahina) W.L. Culb. & C.F. Culb. However, delimiting *Cetrelia* species based only on reproductive morphology was not supported phylogenetically.

Since *C. chicitae* was described from the United States (West Virginia, Pocahontas Co., Gaudiner Knob., Hale, Lich. Amer. Exs. 56 [holotype-DUKE]) and no American specimens were studied by Mark *et al.* (2018) American and European herbarium specimens available in BP and VBI were included together in the present study.

It seems that *Cetrelia cetrarioides*, *C. chicitae*, *C. monachorum* and *C. olivetorum* occur in a considerable part of Europe, at least in the so far studied geographical regions in various distance and direction from Hungary. The above-mentioned European studies contain very few or no data from Hungary. Therefore, our main aim was to improve the knowledge on the European distribution of *Cetrelia* species with Hungarian records via taxonomic revision of the *c*. 150 available Hungarian specimens. Up to now a former taxonomic concept (Wirth 1980) was followed in Hungary with two taxa treated at the level of variety of *C. olivetorum* (Verseghy 1965, 1973, 1988, 1994; Kiszelyné-Vámosi 1983; Seaward *et al.* 1985; Farkas 1990; Lőkös & Farkas 1997; Lőkös *et al.* 1997; Lőkös 2009, 2010; Lőkös & Balogh 2016). We were also interested if all the four species occur also in Hungary or the surrounding high mountains (Alps and Carpathians) make a kind of barrier for the distribution of some of these species. Since only spot reactions (hypochlorite probe: C+/-) were applied during identification process earlier, which allowed only an uncertain identification of olivetoric acid, present in *C. olivetorum* var. *olivetorum* and lacking in *C. olivetorum* var. *cetrarioides* (Fig. 1D), HPTLC method was essential for the chemical revision in this study.

The commonest substrate of these species is bark, both of deciduous and coniferous trees, but they seldom occur also on various, mossy rocks. Our further aim was to establish if there is any difference in habitat or substrate preferences of the species in Hungary.

Obermayer & Mayrhofer (2007) gave a detailed description of morphological characters of the species based on Central European, mostly Austrian specimens. Concerning to morphological characters we were mostly focused on the range of soredium size, since, according to Obermayer & Mayrhofer (2007), soredia differ between species, i.e., *C. cetrarioides* has fine soredia, *C. chicitae* and *C. monachorum* have coarse soredia, whereas both types are reported for *C. olivetorum*.

Before the revision, *Cetrelia olivetorum* (including both of these varieties at that time) was regarded a vulnerable species (Lőkös & Tóth 1997), thus we also planned to make an improved evaluation of *Cetrelia* species in red list categories or to make suggestions for legal protection in Hungary.

MATERIAL AND METHODS

The research object

The genus *Cetrelia* is characterised in detail in, for example, Smith *et al.* (2009), Wirth *et al.* (2013), Kukwa *et al.* (2012) and Obermayer & Mayrhofer (2007). The Hungarian *Cetrelia* species were originally identified under various names: e.g. *Parmelia cetrarioides* Delise, *Parmelia cetrarioides* var. *typica* Du Rietz, *Parmelia cetrarioides* f. *pseudofallax* (Gyeln.) Gyeln., *Parmelia cetrarioides* f. *sorediosa* (DC.) Fr., *Parmelia olivaria* Th. Fr., *Parmelia perlata* (Huds.) Ach. or *Parmelia trichotera* Hue (cf. Hazslinszky 1868, 1884; Borbás 1879; Boros 1925, 1929, 1931; Szatala 1925, 1926; Timkó 1925; Gyelnik 1926, 1928, 1931, 1933, 1934, 1937; Fóriss 1938, 1940; Kiszely 1968; Gallé 1975). From the 1980s they were kept in herbaria based on nomenclature applied in Verseghy (1994) under "*C. cetrarioides*" (as *C. olivetorum* var. *cetrari-*

TABLE 1. — Rf values of lichen secondary metabolites (LSMs) related to norstictic acid (N) and atranorin (A) in solvent systems A, B and C (cf. Arup *et al.* 1993).

LSM	Rf_{LSM}/Rf_N, Rf_A	Rf_{LSM}/Rf_N, Rf_A	Rf_{LSM}/Rf_N, Rf_A
–	in solvent system A	in solvent system B	in solvent system C
atranorin	36	30	28
perlatolic acid	30	32	24
imbricaric acid	30	32	22
4-O-methylphysodic acid	–	19	21
α-collatolic acid	24	21	18
anziaic acid	–	–	16
4-O-demethylimbricaric acid	–	21	15
β-collatolic acid	–	–	13
olivetoric acid	27	24	11
physodic acid	18	18	10
α-alectoronic acid	21	16	7
β-alectoronic acid	–	–	3

oides) and "*C. olivetorum*" (as *C. olivetorum* var. *olivetorum*). Specimens of bcMRDS, BP, BRA, BTM, CL, DE, EGR, JPU, SAMU, SZE, and VBI, herbaria [abbreviations mainly according to Thiers (continuously updated)] were revised by HPTLC and microcrystal tests. Altogether 166 specimens were studied from Hungary, 98 specimens were kept in the lichen collection BP (Budapest), furthermore 68 specimens were investigated from the other herbaria. One specimen of GZU mentioned from Hungary in literature (Obermayer & Mayrhofer 2007) was also considered. A further nine specimens of *C. chicitae* (8 BP, 1 VBI) from United States and Europe were investigated.

Morphological investigations

Soredia, pseudocyphellae, rhizines, as well as features of lower surface were investigated as the main morphological characters by stereo microscope following Obermayer & Mayrhofer (2007).

The morphology and anatomy were studied by using a NIKON Eclipse/NiU (DIC, epifluorescence) compound microscope, Nikon SMZ18 stereo microscope as well as Olympus SZX9 and Olympus BX50 (DIC) microscopes. Micrographs were prepared by Olympus E450 camera (with Quick Photo Camera 2.3 software) and Nikon DS-Fi1c and Fi3 camera (with NIS-Elements BR ML software) with the above-mentioned microscopes. If it was possible, 5-10 specimens by species were checked for measurements of soredia. Usually 15 micrographs of their sorediate lobe margins were taken. Diameters of soredia (n = 20) were measured on each views. Mean and standard deviation values were also established.

Presence of Lichen secondary metabolites

The presence of cortical pigment, atranorin and medullary LSMs: 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 were analysed by HPTLC (Fig. 2) and TLC (Fig. 3).

The cortical pigment atranorin is usually present in all species, but in some specimens this substance is present in trace amount (e.g. *C. cetrarioides*, coll. G. Matus, 2013, DE; *C. monachorum*, coll. M. Sinigla, 2015, BTM; coll. L. Lőkös, 2015, BP; coll. G. Matus, 2016) or lacking (e.g. *C. cetrarioides*, coll. E. Farkas and L. Lőkös, 1983, VBI, *C. chicitae*, coll. A. Feichtinger, 1875, BP; coll. A. Vězda, 1990, VBI).

Chemicals

All chemicals were of analytical or higher grade. HPLC acetone (VWR) was applied to extract LSMs from intact lichen samples for chromatographic analysis. Glycerine (ACIDUM-2), toluene (CARLO ERBA), acetic acid (LACH-NER), dioxene (REANAL, Sigma Aldrich), cyclo-hexane (LACH-NER), methyl-tert-butyl ether (Fisher Scientific UK), formic acid (LACH-NER), anisaldehyde (Sigma Aldrich), methanol (REANAL) and sulphuric acid (CARLO ERBA) were obtained from Reanal for TLC and HPTLC investigations and microcrystal tests.

Microcrystal tests

Microcrystal tests were prepared in GE solvent (glycerine – acetic acid, 3:1 v/v) following the methods described in Orange *et al.* (2010: 41-44). Microscopical views were compared to those published by Obermayer & Mayrhofer (2007) and Huneck & Yoshimura (1996).

Application of microcrystal test was especially useful for confirmation of the presence of imbricaric acid (Fig. 4A) and perlatolic acid (Fig. 4B). We could identify also olivetoric acid (Fig. 4C) and atranorin (Fig. 4D) by this method.

HPTLC method

HPTLC analysis was carried out according to standard methods for analysing lichen samples described by Arup *et al.* (1993) and Molnár & Farkas (2011). CAMAG horizontal chamber of 10 × 10 cm, CAMAG TLC Plate Heater III, 10 × 10 cm HPTLC plates (Merck, Kieselgel 60 F254) were used. The usual solvent systems (A: toluene – dioxene – acetic acid, 45:15:2 v/v/v; B: cyclo-hexane – methyl-tert-butyl ether – formic acid, 6.5:5:1 v/v/v) and most often solvent system C (toluene – acetic acid, 20:3 v/v) were applied. Plates were investigated under UV 254 nm and UV 366 nm after development, then fatty acids and water-repellent substances were studied, while sprayed with water, finally it was followed by spraying with 10% sulphuric acid and spots were observed at daylight.

The presence of α-alectoronic acid, anziaic acid, α-collatolic acid, atranorin, β-alectoronic acid, β-collatolic acid, imbricaric acid, 4-O-demethylimbricaric acid, olivetoric acid, perlatolic acid, physodic acid and 4-O-methylphysodic acid was investigated. Rf values of some of these LSMs are not given or slightly differing in Table 1 of Arup *et al.* (1993), therefore our data are summarised in Table 1 below. The presence of above LSMs in *Cetrelia* species is summarised in Table 2.

TABLE 2. — Presence of LSMs in *Cetrelia* W.L. Culb. & C.F. Culb. species (cf. Obermayer & Mayrhofer 2007). **+** = present; **(+)** = present in minor amount.

LSM/Species	***C. cetrarioides*** **(Delise) W.L. Culb. & C.F. Culb.**	***C. chicitae*** **(W.L. Culb.) W.L. Culb. & C.F. Culb.**	***C. monachorum*** **(Zahlbr.) W.L. Culb. & C.F. Culb.**	***C. olivetorum*** **(Nyl.) W.L. Culb. & C.F. Culb.**
atranorin (atr)	+	+	+	+
perlatolic acid (perl)	+	–	(+)	–
imbricaric acid (imbr)	(+)	–	+	–
4-O-methylphysodic acid (4mp)	–	+	–	–
α-collatotolic acid (α-col)	–	+	–	–
anziaic acid (anz)	+	–	+	–
4-O-demethylimbricaric acid (4dmi)	–	–	+	–
β-collatolic acid (β-col)	–	+	–	–
olivetoric acid (ol)	–	–	–	+
physodic acid (phys)	–	+	–	–
α-alectoronic acid (α-alec)	–	+	–	–
β-alectoronic acid (β-alec)	–	(+)	–	–

TLC METHOD

TLC analysis was carried out according to standard methods for analysing lichen samples described by Orange *et al.* (2010). 20 × 20 cm thin-layer chromatographic plates (Merck, Kieselgel 60 F254) were used. Solvent system C (toluene – acetic acid, 170:30 v/v) was applied according Mietzsch *et al.* (1994) and Elix (2014). Plates were investigated under UV 254 nm and UV 366 nm after development, it was followed by spraying with 10% anisaldehyde/sulphuric acid and spots were observed at daylight. The presence of α-alectoronic acid, anziaic acid, α-collatolic acid, atranorin, β-alectoronic acid, β-collatolic acid, imbricaric acid, 4-O-demethylimbricaric acid, olivetoric acid, perlatolic acid, physodic acid and 4-O-methylphysodic acid was investigated. Rf values were compared to published data (Orange *et al.* 2010 and Elix 2014). The presence of above LSMs in *Cetrelia* species is summarised in Table 2. Special attention was given to the *C. chicitae* samples.

DISTRIBUTION MAPS

Distribution maps were constructed by the computer program for geographical information system, QGIS 3.8 'Zanzibar', released in 2019, applying an adaptation of the Central European grid system (Niklfeld 1971; Borhidi 1984). The symbols (dots, circles) illustrated represent units of *c*. 5 × 6 km areas. Maps presenting distribution before and after revision were compared.

ABBREVIATIONS

LSM lichen secondary metabolite;
Rf retardation factor – here applied according to Arup *et al.* (1993): travelling distance expressed in mm for the studied LSM (Rf_{LSM}) compared to controls norstictic acid (Rf_N) and atranorin (Rf_A).

RESULTS AND DISCUSSION

Our results, based on morphological characters and LSMs, show that four species occur in Hungary as in several other European countries: *Cetrelia cetrarioides* (Fig. 5A, 16 specimens), *C. chicitae* (Fig. 5B, two specimens), *C. monachorum* (Fig. 5C, 100 specimens) and *C. olivetorum* (Fig. 5D, 29 specimens). *Cetrelia chicitae* and *C. monachorum* were discovered as new species for the Hungarian lichen flora.

Cetrelia cetrarioides (Delise) W.L. Culb. & C.F. Culb. (Figs 1C; 2; 5A; 6; 7; 12)

It is characterised by the presence of atranorin, perlatolic acid (major), ± imbricaric acid (minor) and anziaic acid. The lack of 4-O-demethylimbricaric acid is obvious and well observable already under UV 254 nm after developing the HPTLC plate (Fig. 2). Its soredia are fine [25-35(-40) µm in diam. – Obermayer & Mayrhofer (2007)], 32.3 ± 3.4 µm in Hungarian samples. Not or slightly raised pseudocyphellae of various size (*c*. 30-300 µm) occur on upper cortex, smaller ones on lower cortex. Its specimens were found most frequently on oak (*Quercus* sp. – 38%), beech (*Fagus sylvatica* L. – 15%) and other tree species (46%) at lower elevations than in Austria (Obermayer & Mayrhofer 2007), mostly at 200-600 m a.s.l., though in Belarus it occurs at even lower elevation (Bely *et al.* 2014). This species proved to be less frequent than expected (Figs 6; 7), and was previously confused with *C. monachorum*. *Cetrelia cetrarioides* is considered here as a critically endangered (CR) species in Hungary.

C. chicitae (W.L. Culb.) W.L. Culb. & C.F. Culb. (Figs 1A; 2; 3; 5B; 8; 12)

It is easy to recognise by a here revised characteristic composition of LSMs detected by HPTLC and TLC: atranorin, 4-O-methylphysodic acid, α-collatolic acid, β-collatolic acid, physodic acid, α-alectoronic acid, β-alectoronic acid. Since this species was described from the United States (West Virginia, Pocahontas Co., Gaudiner Knob, Hale, Lich. Amer. Exs. 56 – holotype-DUKE) and has not been confirmed from Europe by Mark *et al.* (2018), special attention was paid to its identification. All specimens available in BP and VBI were studied by TLC and HPTLC (Fig. 2). In addition to the

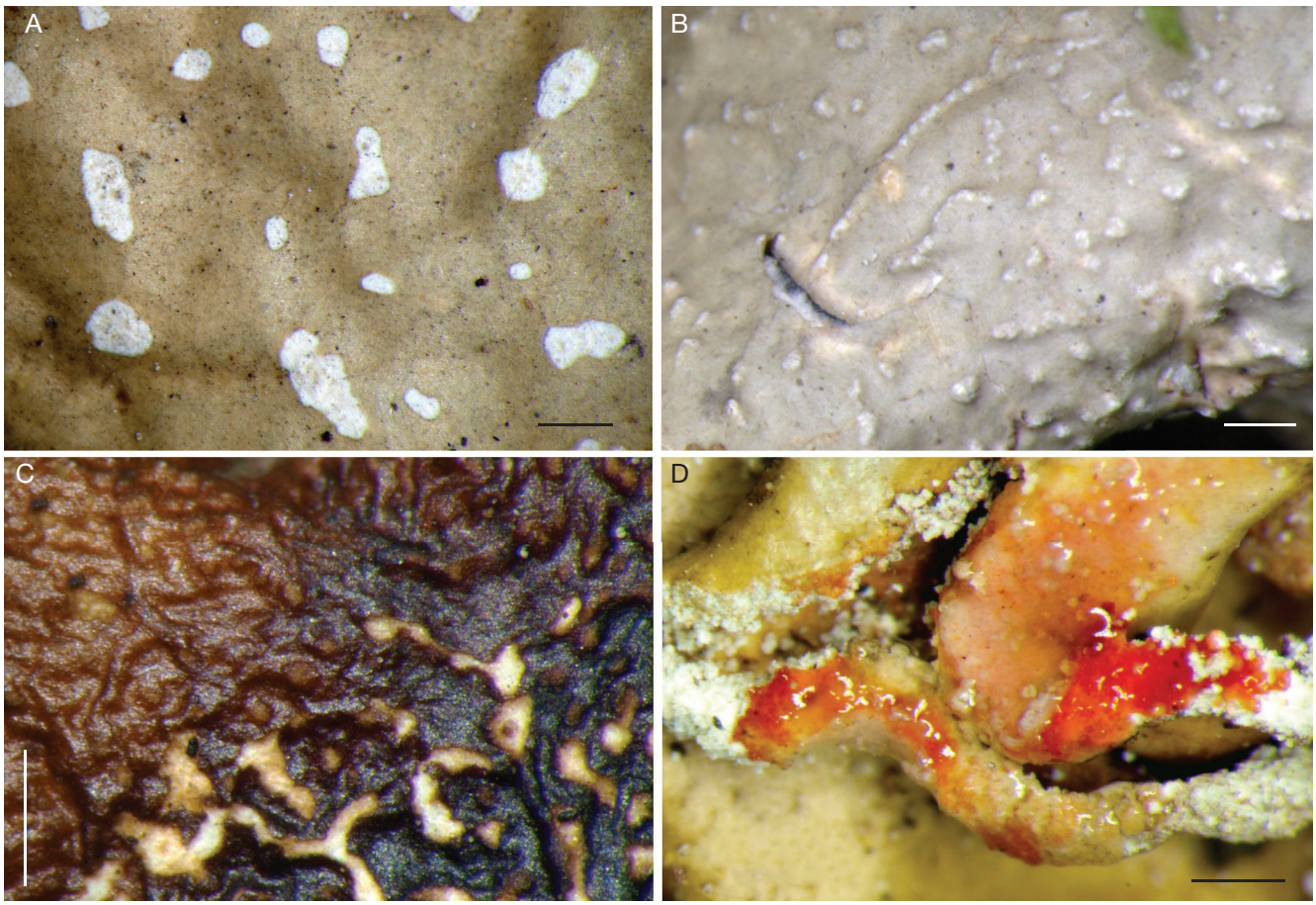

Fig. 1. — Differential morphological and chemical features in *Cetrelia* W.L. Culb. & C.F. Culb. species: **A**, large, not raised pseudocyphellae on upper cortex of *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb.; **B**, small, raised pseudocyphellae on upper cortex of *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb.; **C**, brown lower cortex of *C. cetrarioides* (Delise) W.L. Culb. & C.F. Culb. without rhizines; **D**, C+ reaction by NaOCl on marginal soralia of *Cetrelia olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. Scale bars: 500 µm.

previously studied compounds (cf. Culberson & Culberson 1968; Obermayer & Mayrhofer 2007), the identification of α-collatolic acid, β-collatolic acid, α-alectoronic acid and β-alectoronic acid (i.e., β-collatolic acid and β-alectoronic acid as newly identified susbstances of the above list) was possible by using an anisaldehyde/sulphuric acid spray on regular TLC plates (Fig. 3). Based on the seven substances above it was found that specimens from Hungary, Poland, Ukraine, Romania, Italy had the same pattern of substances as the three specimens from the United States, containing one from Pocahontas County (BP 91365), collected very near the type locality. Most of these specimens contained one or two additional unidentified minor substances, observable only under UV 366 prior to sulphuric acid treatment in position below atranorin and above 4-O-methylphysodic acid. The specimen collected near the type locality (BP 91365) contained both of these substances and a third one. This third unidentified component had the same travelling distance on the plate as that of 4-O-methylphysodic acid. One of the two Hungarian specimens (BP 49905) contained the upper unknown, the other (BP 71276) the lower one. The Polish sample (BP 21508) and one of the Ukrainian samples (BP 21504) contained both of these unknowns. Furthermore, the Italian (VBI 1742) and a part of a Ukrainian specimen (BP 22781) was lacking atranorin. The occurrence of above-mentioned unknown substances within specimens might be explained by the possibly non-monophyletic origin of *C. chicitae* suggested by Mark *et al.* (2018). It needs further studies, since the specimens from Virginia (United States) (BP 91365, BP 93416 and BP 75822) are not homogeneous either in the presence of these unknown substances.

Apart from the chemical composition, the somewhat twisted, wavy soralia are also characteristic features. Its soredia are coarse, 46.1 ± 7.3 µm in diam. (cf. (35-)40-55 µm in Obermayer & Mayrhofer 2007). Pseudocyphellae are large (150-500 µm), not raised on upper cortex, lacking from lower cortex.

Both Hungarian specimens of *C. chicitae* were found on beech (*Fagus sylvatica*) at 450-600 m a.s.l., one from the Bükk [BP 71276 sub *Parmelia cetrarioides* var. *typica*] and the other in the Zemplén Mts [BP 49905 sub *Parmelia cetrarioides*] (as *P. cetrarioides*) (Fig. 8).This is the rarest *Cetrelia* species in Hungary. As it has not been collected since 1961, *Cetrelia chicitae* is here proposed as critically endangered (CR(PE)) lichen species in Hungary.

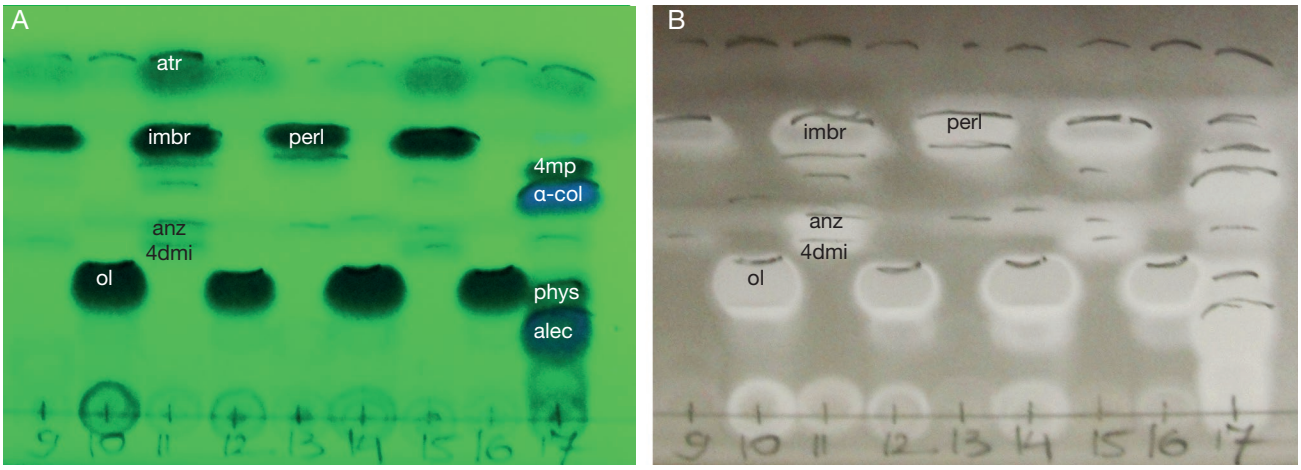

Fig. 2. — A detail of the chromatographic plate HPTLC nr 74/2014 developed in solvent system C presenting all species under UV 254 nm (**A**), and sprayed with water (**B**). Specimens in positions A10-A17 are A10: *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. (BP 21529), A11: *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. (BP 85318), A12: *C. olivetorum* (BP 84893), A13: *C. cetrarioides* (Delise) W.L. Culb. & C.F. Culb. (BP 21538), A14: *C. olivetorum* (BP 22787), A15: *C. monachorum* (BP 45013), A16: *C. olivetorum* (BP 21508), A17: *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb. (BP 93416). Abbreviations of LSMs are according to Table 2.

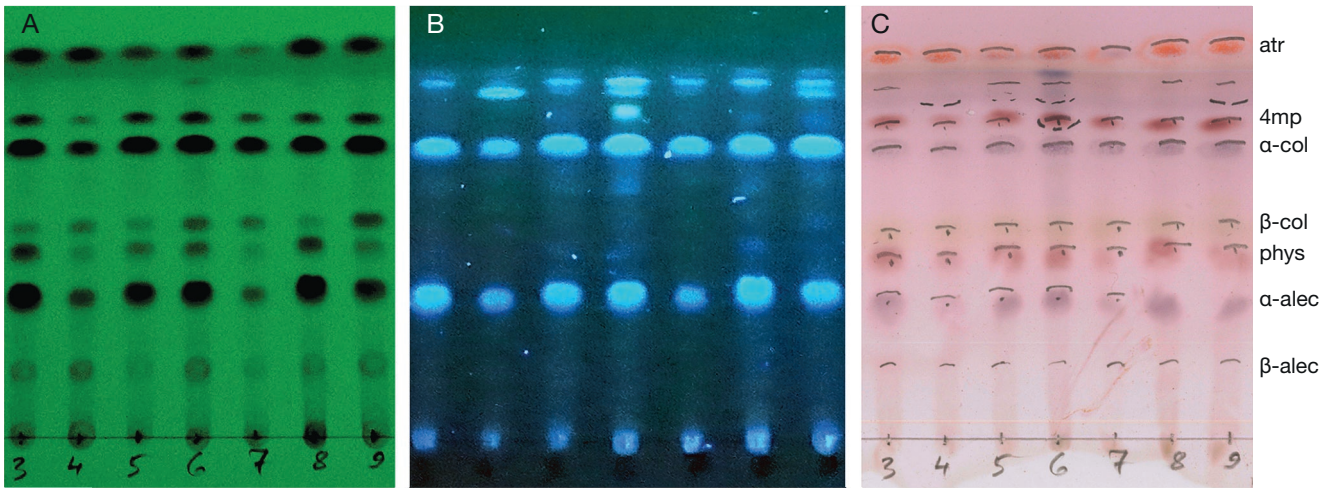

Fig. 3. — A detail of the chromatographic plate TLC nr 1903 developed in solvent system C presenting *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb. specimens under UV 254 nm (**A**), UV 366 nm (**B**) and sprayed with anisaldehyde/sulphuric acid (**C**). Specimens in positions 3-9 are: 3, from Page County, Virginia, United States (BP 75822); 4, from the Bükk Mts, Hungary (BP 71276); 5, from the Zemplén Mts, Hungary (BP 49905); 6, from Pocahontas County, West Virginia, United States (BP 91365); 7, from Ukraine (BP 44999); 8, from Romania (BP 85320); 9, from Poland (BP 21508). Abbreviations of LSMs are according to Table 2.

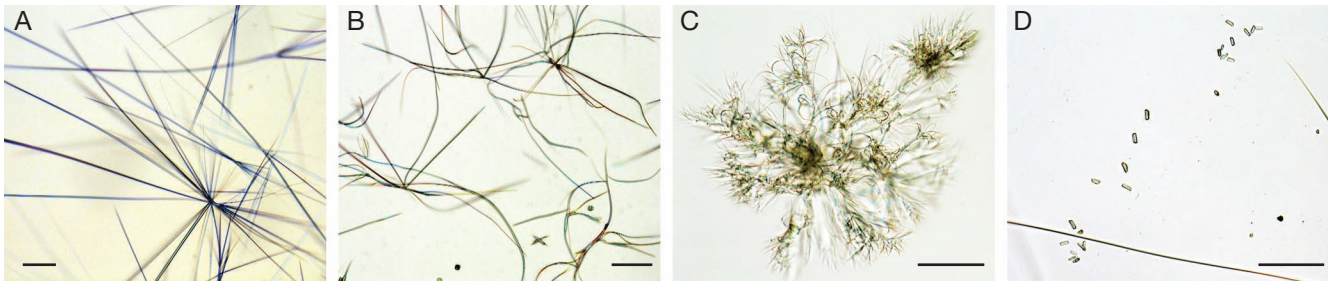

Fig. 4. — Microcrystal tests of **A**, imbricaric; **B**, perlatolic; **C**, olivetoric acids and **D**, atranorin in GE solvent (glycerine – acetic acid, 3:1 v/v). Scale bars: 50 µm.

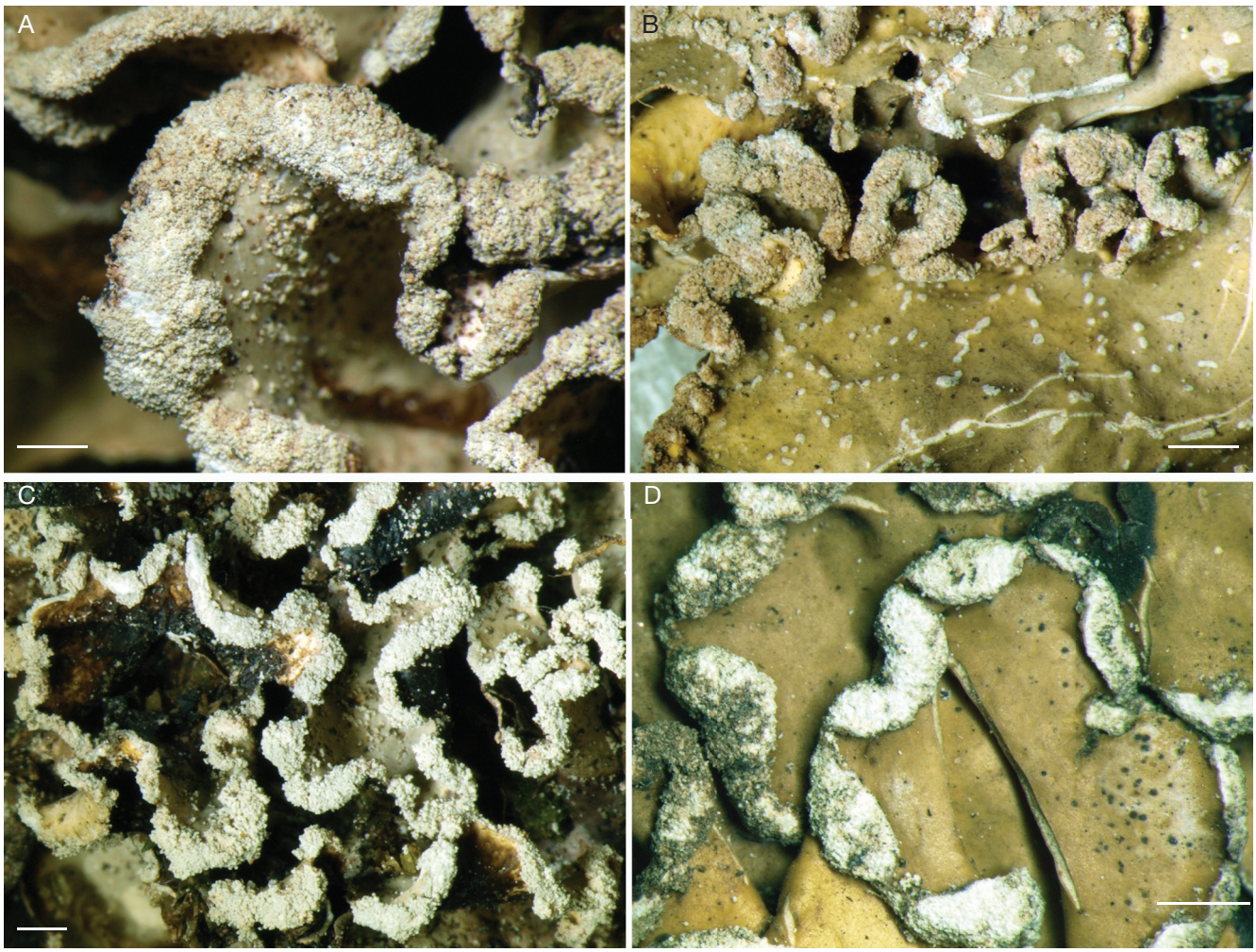

Fig. 5. — Thalline lobes with marginal soralia of **A**, *Cetrelia cetrarioides* (Delise) W.L. Culb. & C.F. Culb.; **B**, *C. chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb.; **C**, *C. monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb.; and **D**, *C. olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. Scale bars: 1 mm.

Cetrelia monachorum (Zahlbr.) W.L. Culb. & C.F. Culb.
(Figs 2; 5C; 9; 12)

It was not known before this revision as it was repeatedly misidentified before as either *C. cetrarioides* or *C. olivetorum*. It now turns out to be the most frequent *Cetrelia* species in Hungary (Fig. 9).

The presence of atranorin, imbricaric acid (major) ± perlatolic acid (minor), anziaic acid and 4-O-demethylimbricaric acid is characteristic. *Cetrelia sayanensis* Otnyukova, Stepanov & Elix (Otnyukova *et al.* 2009), a closely related species, has a slightly different chemical composition containing atranorin (minor), imbricaric acid (major), perlatolic acid (minor), divaricatic acid (minor)], anziaic acid (minor), 4-O-demethylimbricaric acid (minor), glomelliferic acid (trace) and loxodellic acid (trace). However, it has pustulate-capitate soralia with farinose soredia, while *C. monachorum* has only seldom laminal, capitate soralia and is further characterised by coarse soredia, 52.7 ± 5.6 µm in Hungarian samples [vs (35)40-55 µm diam. in Obermayer & Mayrhofer (2007)], small (50-150 µm), raised pseudocyphellae on upper cortex, but very rare or lacking on lower cortex.

Cetrelia monachorum is most frequently collected from rocks (70%), but also grows on *Quercus* (14%), *Fagus* (6%), *Carpinus* (2%), *Acer pseudoplatanus* L. (1%) and on unidentified bark (7%) between 100-1000 m a.s.l. reaching the highest possible elevation in Hungary (Mátra Mts). The species is proposed for the category near threatened (NT) in Hungary.

Cetrelia olivetorum (Nyl.) W.L. Culb. & C.F. Culb.
(Figs 2; 5D; 10-12)

It is the easiest of the four species to identify because olivetoric acid produces a characteristic, water repellent spot on the chromatographic plate, although a wider species concept previously (Wirth 1980; Verseghy 1988, 1994, Smith *et al.* 2009) also was applied for specimens not containing olivetoric acid (here revised mostly as *C. monachorum* by HPTLC). Cortical atranorin is also present. This species forms soredia varying

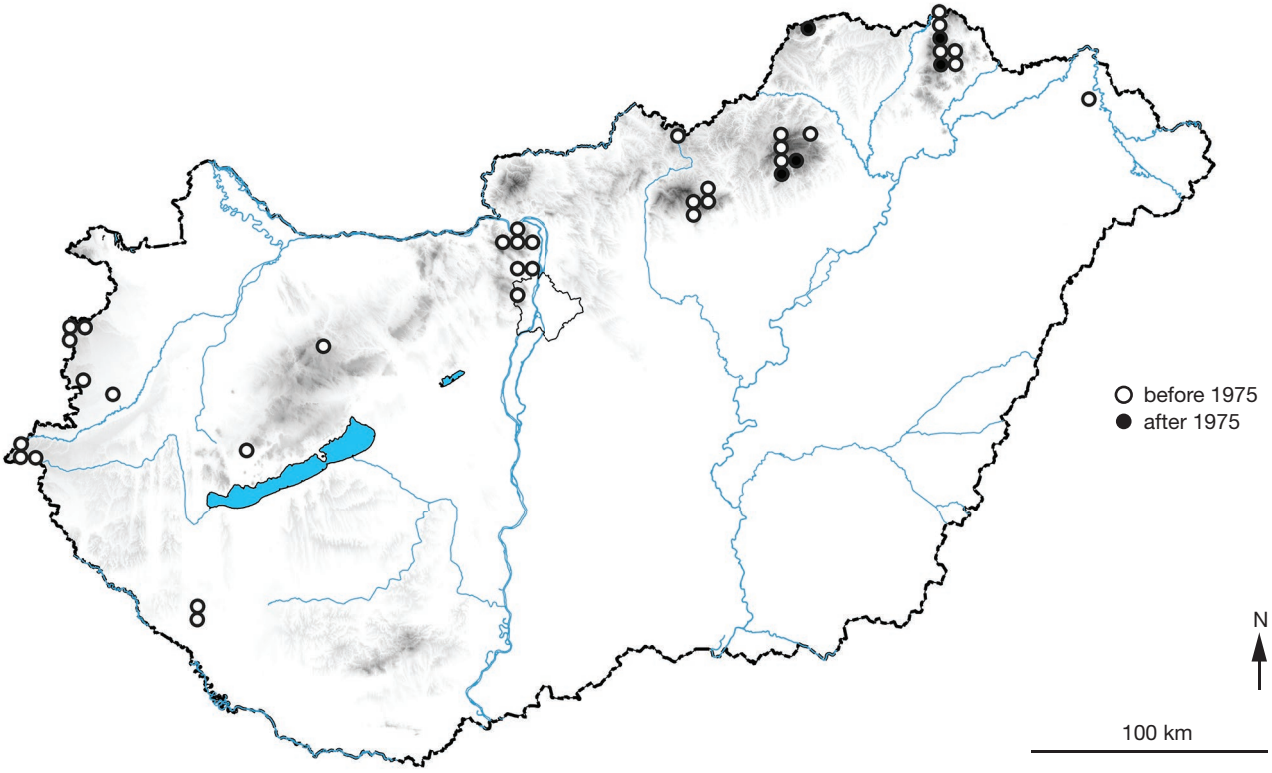

Fig. 6. — Distribution of *Cetrelia cetrarioides* (Delise) W.L. Culb. & C.F. Culb. in Hungary before revision.

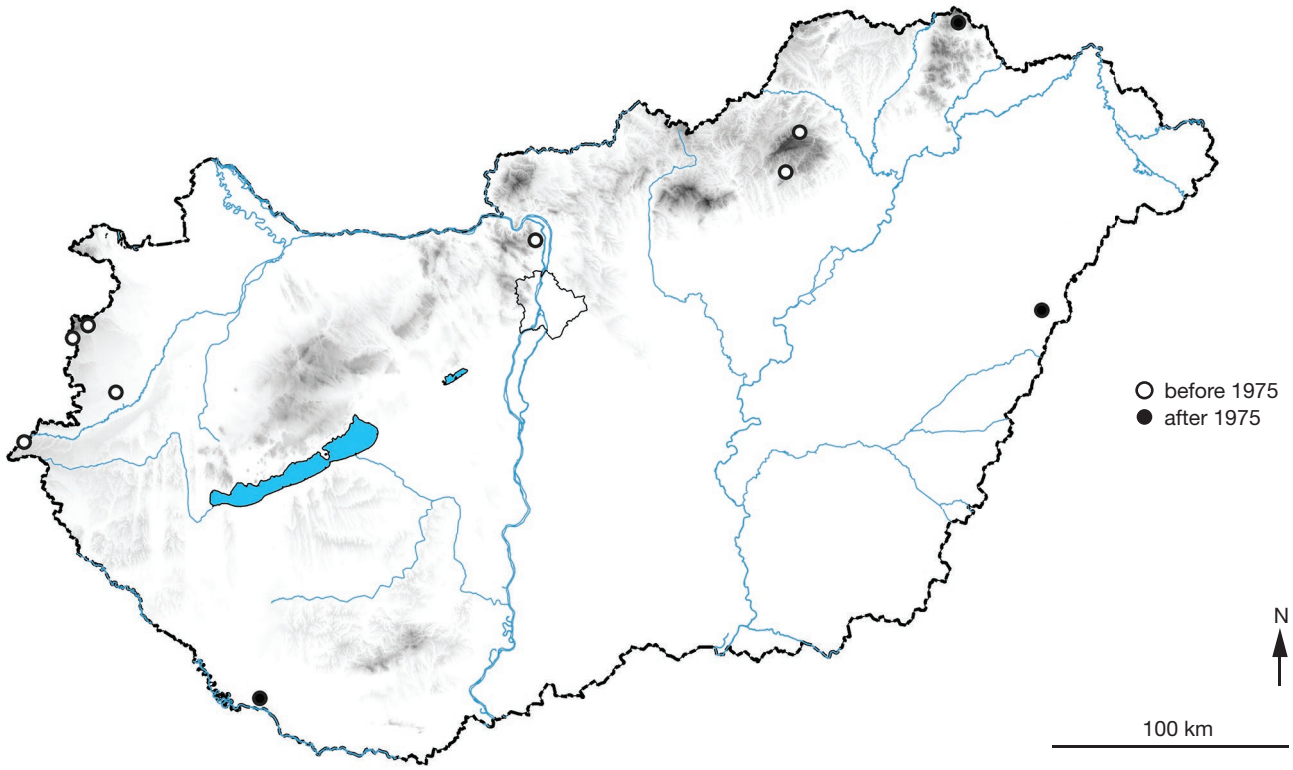

Fig. 7. — Distribution of *Cetrelia cetrarioides* (Delise) W.L. Culb. & C.F. Culb. in Hungary after revision.

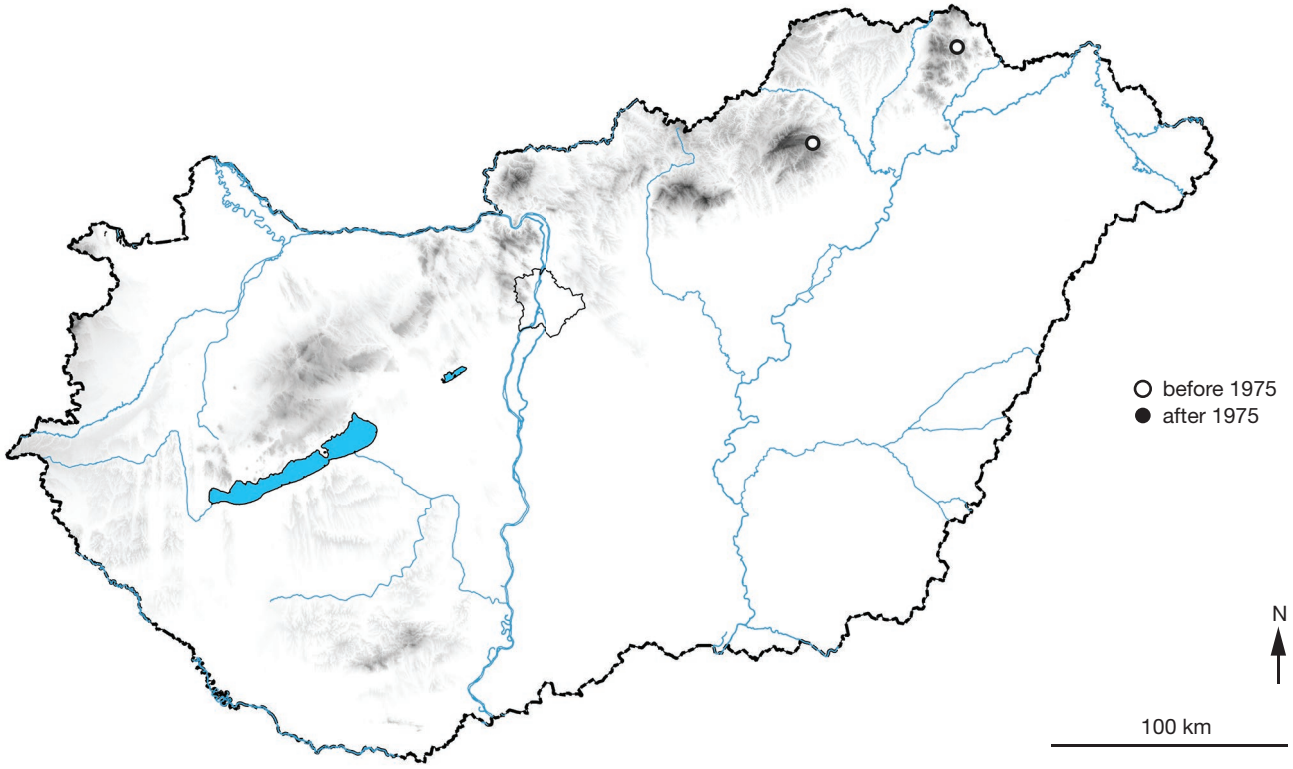

Fig. 8. — Distribution of *Cetrelia chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb. in Hungary.

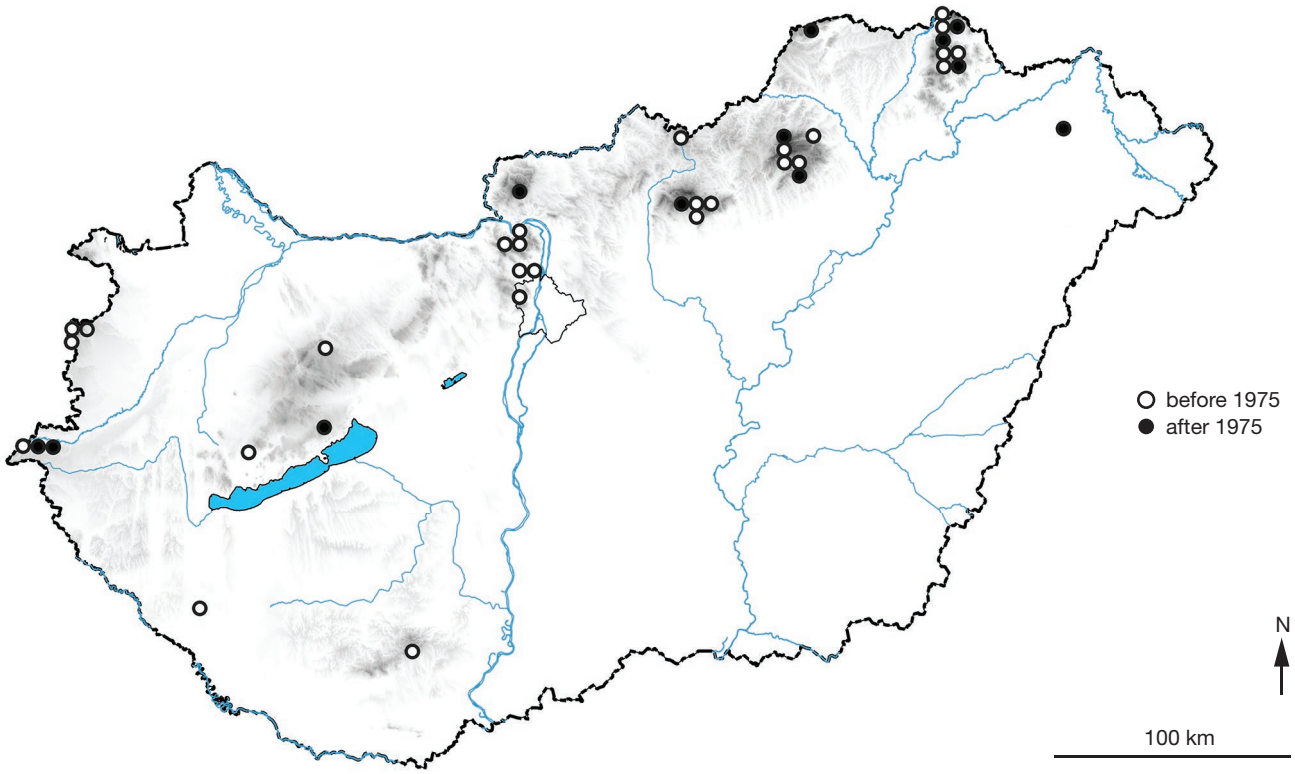

Fig. 9. — Distribution of *Cetrelia monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. in Hungary.

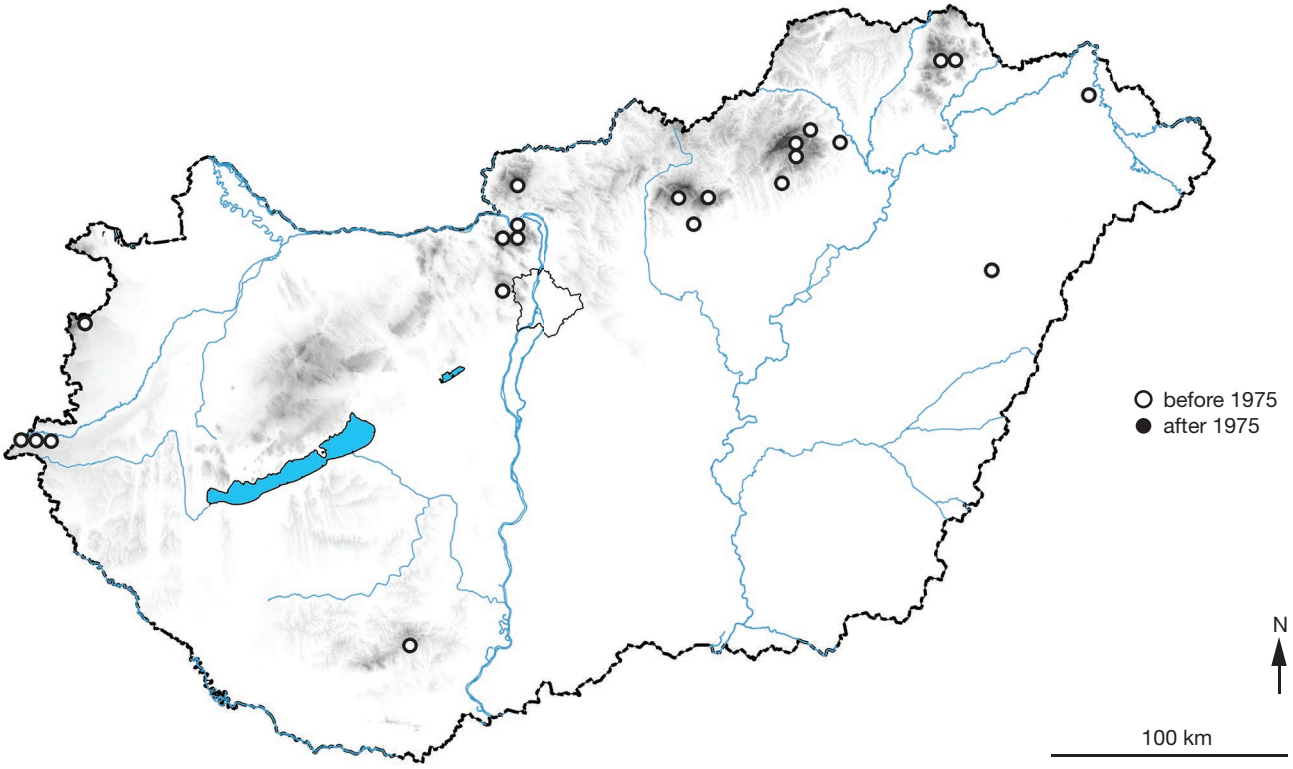

Fig. 10. — Distribution *Cetrelia olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. in Hungary before revision.

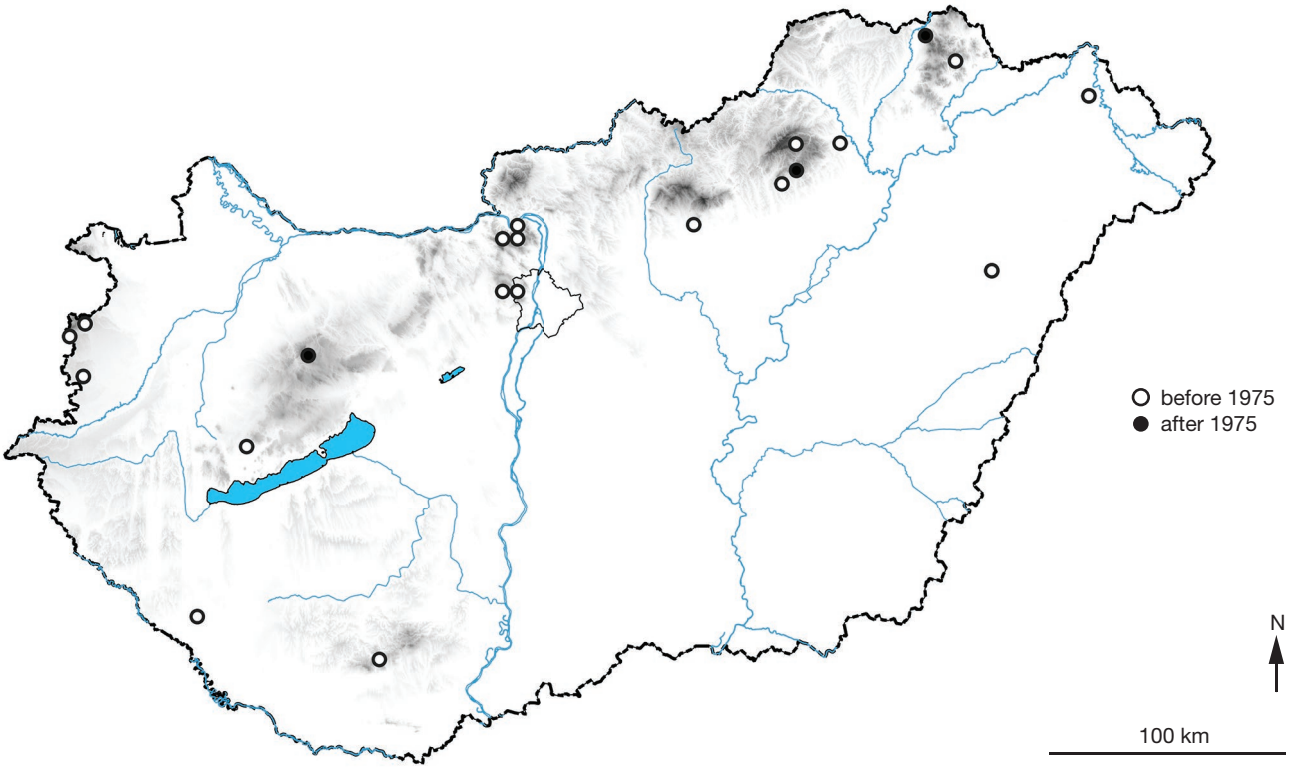

Fig. 11. — Distribution of *Cetrelia olivetorum* (Nyl.) W.L. Culb. & C.F. Culb. in Hungary after revision.

from fine to coarse, 36.4 ± 9.5 µm in Hungarian samples [versus 25-55 µm diam. in Obermayer & Mayrhofer (2007)], while pseudocyphellae – if any – are rare on both upper and lower cortex and small (20-50 µm).

This species was collected from rocks (39%), but was mostly found on bark of *Quercus* (13%), *Fagus* (9%) and various unidentified trees (39%) at 100-800 m a.s.l. It is the second most frequent species of *Cetrelia*, known from a moderate 26 collections (Figs 10; 11). *Cetrelia olivetorum* is proposed as vulnerable (VU) in the Hungarian red-list.

Taxonomic remarks

Lectotypification of Parmelia cetrarioides Delise f. pseudofallax

All three specimens (BP 21541 from the Caucasus, BP 21492 from Hungary, BP 21540 from Croatia) served as type material of *Pseudoparmelia pseudofallax* cited in the protologue (Gyelnik 1933) were localised and revised. After our revision one of them proved to be *Cetrelia cetrarioides* (BP 21541), and the other two were determined as *C. monachorum* (BP 21492, BP 21540). Unfortunately, the species *Pseudoparmelia pseudofallax* was never validly described. Instead, Gyelnik (1935) published a later description of the taxon where he treated it as a form of *Parmelia cetrarioides,* referring to the former publication with the three specimens. In 1937 he issued an exsiccatum of the same taxon (as *Parmelia cetrarioides* f. *pseudofallax*) from another Hungarian locality (Haláp), which was not cited in the protologue (Gyelnik 1937). One specimen of this set was selected as syntype and revised as *Parmelia cetrarioides* by Ödön Szatala in 1956, later confirmed by Mason E. Hale in 1961. This syntype specimen was included in the type catalogue of Verseghy (1964) as syntype of *Pseudoparmelia pseudofallax* and it was later revised and listed as *Cetrelia olivetorum* var. *cetrarioides* by Verseghy (1988) as *Parmelia pseudofallax*). According to our revision the syntype specimen and 12 available duplicates also proved to be *C. monachorum,* except for one *C. olivetorum*. *Parmelia cetrarioides* f. *pseudofallax* is lectotypified here based on the most typical Hungarian specimen corresponding well with Gyelnik's concept:

Parmelia cetrarioides Delise f. ***pseudofallax*** Gyelnik

Revue bryologique et lichénologique 7: 220 (1935).

MycoBank No. — MB435214.

Cetrelia monachorum (Zahlbr.) W.L. Culb. & C.F. Culb, *Systematic Botany* 1 (4): 326 (1977).

Lectotype. — (designated here under no. MBT392402): **Hungary**. Mohás trachyttufa sziklán. "Keserűs-hegy" *c.* 500 m. Dömös mel., Esztergom vm.; leg.: Timkó Gy., 12.X.1913. (BP 21492 sub *Parmelia cetrarioides*).

Identity of the type materials for *Parmelia olivaria* var. *subvenosa* Gyeln.

Type specimens (holotype, isotype and one additional authentic specimen) of *Parmelia olivaria* var. *subvenosa* Gyeln. were also checked and evaluated. The holotype (BP 22803, T 636/a) specimen was confirmed as *Cetrelia olivetorum* (Culberson & Culberson 1968), however the isotype (BP 22802, T 636/b) and an additional specimen from the same place and date (BP 21493) proved to be *C. monachorum* after the revision.

CONCLUSIONS

The characteristic compositon of Lichen Secondary Metabolites (LSMs) described in literature was supported by HPTLC and TLC analysis of 166 specimens of *Cetrelia* from Hungary; an additional nine specimens of *C. chicitae* were investigated from United States (3) and Europe (6). The specimens showed homogeneous secondary chemistry on the basis of 7 LSMs (including two substances as newly identified from this species). Thus the presence of *C. chicitae* (described from America) in Europe was confirmed, based on chemistry. Although in *C. monachorum* slightly larger soredia were measured in Hungarian specimens than given by Obermayer & Mayrhofer (2007), the subtle differences in soredium sizes of the four species was generally confirmed by our measurements. With the relatively wide range in diameter of the soredia, the large standard deviation in *C. olivetorum* seems to be an important feature of the species.

The current knowledge on the frequency of species considerably changed after the chemotaxonomic revision. *Cetrelia* species are predominantly distributed in the mountainous woodland areas of Hungary, especially the Inner Western Carpathians (Zemplén Mts, Bükk Mts, Mátra Mts, Börzsöny Mts, etc.), the Transdanubian Mountains and the eastern foothills of the Alps. *Cetrelia chicitae* was found only in the NE part of Hungary, however only slight differences or tendency in distribution, ecology or habitat preference were recognised in Hungary between the four species. While in Cental Europe frequency maxima of *Cetrelia* species are around 600 and 1000 m a.s.l., it is at 300 and 500 m in case of the same species in Hungary (Fig. 12; cf. Obermayer & Mayrhofer 2007). These lichens most probably find their adequate habitat conditions also at lower elevations due to various micrcolimatic effects. Consequently, the analysis of Hungarian specimens largely contributed to the knowledge on ecological requirements of these species.

The increased number of species in different parts of Hungary after the revision is shown in a map (Fig. 13). Before the revision, the genus *Cetrelia* was thought to be represented exclusively by varieties of *C. olivetorum* in Hungary, the analysis of LSMs revealed the chemical diversity of this taxonomic group and the hidden chemical diversity, together with morphological characters, confirmed presence of four different species. However, the current status of *C. chicitae* in Hungary is uncertain, since it was collected only twice, in 1933 and 1961, and has not been observed since then. Global climatic change is a major risk for species extinction also in Hungary (cf. Thomas *et al.* 2004; Thuiller *et al.* 2005; UNFCCC 2013; Woodruff 2001). All existing *Cetrelia* species are rare in Hungary. There is a limited number of recent collections. Since there is only a low number of recently collected

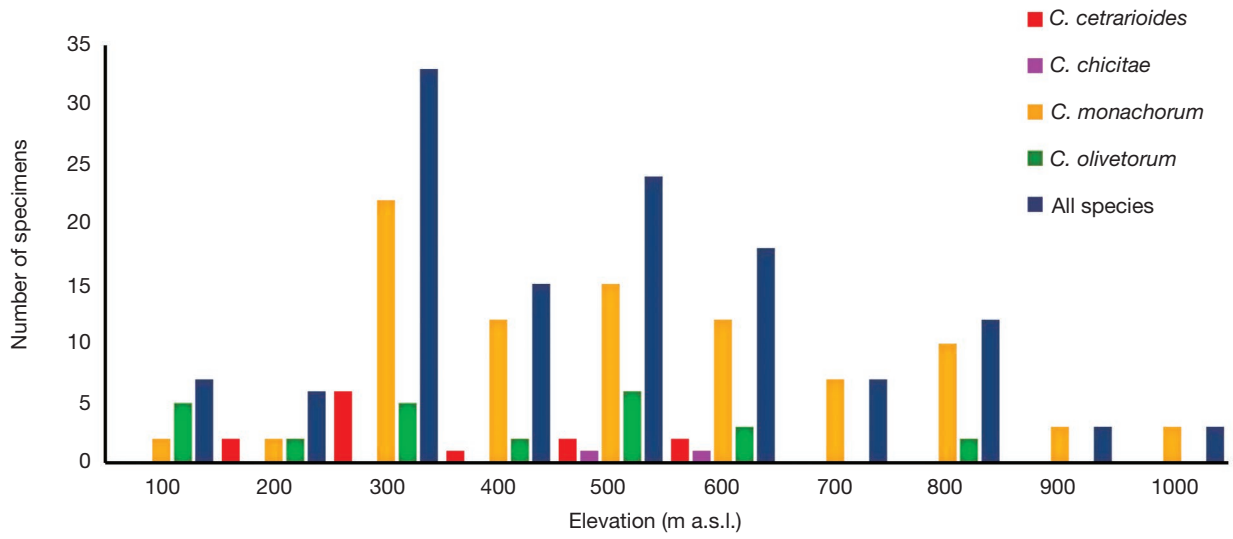

Fig. 12. — Altitudinal distribution of *Cetrelia* W.L. Culb. & C.F. Culb. species in Hungary.

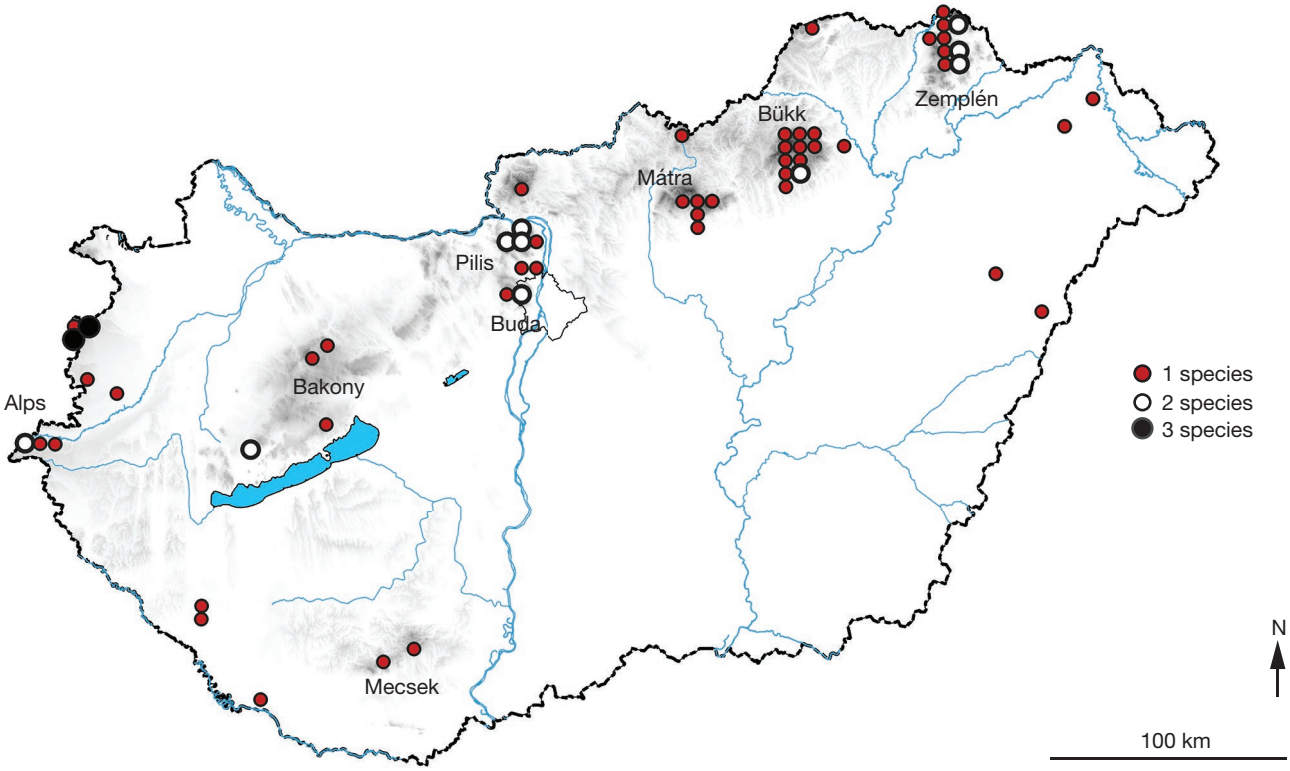

Fig. 13. — The number of *Cetrelia* W.L. Culb. & C.F. Culb. species after revision in various parts of Hungary.

specimens for *C. cetrarioides*, it is worthy of legal protection, together with *C. chicitae*. Current populations of all species of the genus should be further studied to be able to estimate their size and establish their IUCN Red List categories more precisely (IUCN 2018).

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REFERENCES

Armaleo D., Zhang Y. & Cheung S. 2008. — Light might regulate divergently depside and depsidone accumulation in the lichen *Parmotrema hypotropum* by affecting thallus temperature and water potential. *Mycologia* 100 (4): 565-576. https://doi.org/10.3852/07-162R

Arup U., Ekman S., Lindblom L. & Mattsson J.-E. 1993. — High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. *The Lichenologist* 25 (1): 61-71. https://doi.org/10.1006/lich.1993.1018

Bely P., Golubkov V., Tsurykau A. & Sidorovich E. 2014. — The lichen genus *Cetrelia* in Belarus: distribution, ecology and conservation. *Botanica Lithuanica* 20 (2): 69-76. https://doi.org/10.2478/botlit-2014-0010

Biró B., Lőkös L. & Farkas E. 2015. — HPTLC chromatographic analysis of *Cetrelia* species, *in* Farkas E. & Varga N. (eds), *Information, Program and Abstracts. 2nd Young Lichenologists' Workshop in Hungary*, Budapest. – Institute of Ecology and Botany, MTA Centre for Ecological Research, Hungarian Academy of Sciences, Vácrátót: 7. www.lichen.hu/images/Farkas_Varga_eds_YLW_2015.pdf

Bjerke J. W., Elvebakk A., Dominguez E. & Dahlback A. 2005. — Seasonal trends in usnic acid concentrations of Arctic, alpine and Patagonian populations of the lichen *Flavocetraria nivalis*. *Phytochemistry* 66: 337-344. https://doi.org/10.1016/j.phytochem.2004.12.007

Borbás V. 1879. — *Budapestnek és környékének növényzete*. Királyi Magyar Egyetemi Nyomda, Budapest: 34-35.

Borhidi A. 1984. — Role of mapping the flora of Europe in nature conservation. *Norrlinia* 2: 87-98.

Boros Á. 1925 (1924). — A drávabalparti sikság Flórájának alapvonásai, különös tekintettel a lápokra. (Grundzüge der Flora der linken Drauebene mit besonderer Berücksichtigung der Moore). *Magyar Botanikai Lapok* 23: 1-56.

Boros Á. 1929. — A Nyírség flórája és növényföldrajza. (Die Flora und die pflanzengeographischen Verhältnisse des Nyírség). *Matematikai és Természettudományi Értesítő* 46: 48-59.

Boros Á. 1931. — A Nyírség flórája és növényföldrajza. Lichenes. *Debreceni Tisza István Tudományos Társaság kiadványai* 7: 25-29.

Brakni R., Ahmed. M. A., Burger P., Schwing A., Michel G., Pomares C., Hasseine L., Boyer L., Fernander X., Landreau A. & Michel T. 2018. — UHPLC-HRMS/MS based profiling of Algerian lichens and their antimicrobial activities. *Chemistry and Biodiversity* 15 (4): e1800031. https://doi.org/10.1002/cbdv.201800031

Carlin G. 1987. — On the use of chemical characters in lichen taxonomy. *Graphis Scripta* 1: 72-76.

Crespo A. & Lumbsch H. T. 2010. — Cryptic species in lichen-forming fungi. *IMA Fungus* 1 (2): 167-170. https://doi.org/10.5598/imafungus.2010.01.02.09

Culberson C. F. 1972. — Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* 72: 113-125. https://doi.org/10.1016/0021-9673(72)80013-x

Culberson C. F. 1974. — Conditions for the use of Merck silica gel 60 F254 plates in the standardized thin-layer chromatographic technique for lichen products. *Journal of Chromatography* 97 (2): 107-108. https://doi.org/10.1016/s0021-9673(01)97595-8

Culberson C. F. 1986a. — Biogenetic relationships of the lichen substances in the framework of systematics. *The Bryologist* 89 (2): 91-98. https://doi.org/10.2307/3242749

Culberson W. L. 1969. — The use of chemistry in the systematics of the lichens. *Taxon* 18: 152-166. https://doi.org/10.2307/1218673

Culberson W. L. 1970. — Chemosystematics and ecology of lichen-forming fungi. *Annual Review of Ecology and Systematics* 1: 153-170. https://doi.org/10.1146/annurev.es.01.110170.001101

Culberson W. L. 1986b. — Chemistry and sibling speciation in the lichen-forming fungi: ecological and biological considerations. *The Bryologist* 89 (2): 123-131. https://doi.org/10.2307/3242752

Culberson W. L. & Culberson C. F. 1968. — The lichen genera *Cetrelia* and *Platismatia* (Parmeliaceae). *Contributions from the United States National Herbarium* 34 (7): 449-558.

Culberson C. F. & Culberson W. L. 1977. — Chemosyndromic variation in lichens. *Systematic Botany* 1 (4): 325-339. https://doi.org/10.2307/2418700

Culberson C. F. & Culberson W. L. 2001. — Future directions in lichen chemistry. *The Bryologist* 104 (2): 230-234. https://doi.org/10.1639/0007-2745(2001)104[0230:fdilc]2.0.co;2

Culberson C. F. & Kristinsson H. 1970. — A standardized method for the identification of lichen products. *Journal of Chromatography* 46: 85-93. https://doi.org/10.1016/s0021-9673(00)83967-9

Degtjarenko P., Jüriado I. & Lõhmus P. 2018. — New Estonian records. Lichenized fungi. *Folia Cryptog. Estonica* 55: 151-154. https://doi.org/10.12697/fce.2018.55.15

Duong T. H., Huynh B. L. C., Chavasiri W., Chollet-Krugler M., Nguyen V. K., Nguyen T. H. T., Hansen P. E., Le Pogam P., Thüs H., Boustie J. & Nguyen K. P. P. 2017. — New erythritol derivatives from the fertile form of *Roccella montagnei*. *Phytochemistry* 137: 156-164. https://doi.org/10.1016/j.phytochem.2017.02.012

Egan R. S. 1986. — Correlations and non-correlations of chemical variation patterns with lichen morphology and geography. *The Bryologist* 89: 99-110. https://doi.org/10.2307/3242750

Elix J. A. 1996. — Biochemistry and secondary metabolites, *in* Nash T. H. III (ed.), *Lichen Biology*. First edition. Cambridge University Press, Cambridge: 155-180.

Elix J. A. 2014. — *A Catalogue of Standardized Chromatographic Data and Biosynthetic Relationships for Lichen Substances*. Third edition. Published by the author, Canberra, 322 p.

Farkas E. 1990. — Lichenológiai vizsgálatok Budapesten és a Pilis Bioszféra Rezervátumban – elterjedés, bioindikáció. (Investigation of the lichen flora in Budapest and in the Pilis Biosphere Reservation – distribution and bioindication.) (In Hungarian). Kand. dissz., Vácrátót, 121 + 30 p.

Farkas E. & Biró B. 2015. — A hazai *Cetrelia* zuzmófajok elterjedési és élőhely-ökológiai viszonyai, *in:* Padisák J., Liker A. & Stenger-Kovács C. S. (eds), *X. Magyar Ökológus Kongresszus*. Veszprém, Magyarország. Pannon Egyetem, Veszprém, 52 p.

Farkas E., Biró B., Sinigla M. & Lőkös L. 2016. — Aspects of biodiversity and chemical diversity studied in Hungarian lichen herbaria. *British Lichen Society Bulletin* 119: 105-106.

Farkas E., Lőkös L. & Mázsa K. 1998. — HPTLC-vizsgálatok magyarországi *Umbilicaria* zuzmófajokon. *Kitaibelia* 3: 349-351.

Fóriss F. 1938. — Lichenes Bükkenses exsiccati. Fasc. III. Miskolc, 4 p.

Fóriss F. 1940. — Megjegyzések F. Fóriss: Lichenes Bükkenses exsiccati Fasc. I–V. (No 1-100)-hoz. (Bemerkungen zu F. Fóriss: Lichenes Bükkenses exsiccati Fasc. I-V. (No 1-100)). *Borbásia* 2 (3-10): 71-95.

Gallé L. 1975. — A Mátra-hegység zuzmócönózisai. (Die Flechtencönosen im Mátra-Gebirge). *Botanikai Közlemények* 62 (3): 179-189.

Gyelnik V. 1926. — Adatok Magyarország zuzmó vegetációjához I. (Beiträge zur Flechtenvegetation Ungarns I. Com. Szabolcs). *Folia Cryptogamica* I (4): 237-242.

Gyelnik V. 1928. — Adatok Magyarország zuzmó vegetációjához. II. (Beiträge zur Flechtenvegetation Ungarns. II). *Folia Cryptogamica* I (6): 577-604.

Gyelnik V. 1931. — Additamenta ad cognitionem Parmeliarum. 1. *Feddes Repertorium* 29: 149-157/(389-397).

Gyelnik V. 1933. — Lichenes varii novi criticique. Acta pro Fauna et Flora Universali Bucureşti, Seria II, Botanica 1 (5-6): 3-10.

Gyelnik V. 1934. — Additamenta ad cognitionem Parmeliarum. 6. *Feddes Repertorium* 36: 299-302 (315-318).

Gyelnik V. 1935. — Additamenta lichenologica I. *Revue Bryologique et Lichénologique* 7: 219-222.

Gyelnik V. 1937. — Lichenotheca. Fasc. 5-10. No. 81-200. Budapest.

Hauck M. 2011. — Eutrophication threatens the biochemical diversity in lichens. *The Lichenologist* 43: 147-154. https://doi.org/10.1017/s0024282910000654

Hauck M., Willenbruch K. & Leuschner C. 2009. — Lichen substances prevent lichens from nutrient deficiency. *Journal of Chemical Ecology* 35: 71-73. https://doi.org/10.1007/s10886-008-9584-2

Hawksworth D. L. 1976. — Lichen chemotaxonomy, *in* Brown D. H., Hawksworth D. L. & Bailey R. H. (eds), *Lichenology: Progress and Problems*. The Systematics Association Special Volume, No. 8, Academic Press, London and New York: 139-184.

Hazslinszky F. 1868. — Néhány adat a Bükkhegység kryptogamjai megismertetéséhez. *A magyar orvosok és természetvizsgálók nagygyűlésének munkálatai* 13: 227-229.

Hazslinszky F. 1884. — *A Magyar Birodalom zuzmó-flórája*. Királyi Magyar Természetudományi Társulat, Budapest, 304 p.

Huneck S. 1999. — The significance of lichens and their metabolites. *Naturwissenschaften* 86: 559-570. https://doi.org/10.1007/s001140050676

Huneck S. & Yoshimura I. 1996. — *Identification of lichen substances.* Springer Verlag, Berlin, Heidelberg, 493 p. https://doi.org/10.1007/978-3-642-85243-5

IUCN 2018. — *The IUCN Red List of Threatened Species*. Version 2018-2. http://www.iucnredlist.org.

Kiszely Gy. 1968. — Adatok a Mátra-hegység zuzmóflórájához. *Acta Academiae Paedagogicae Agriensis* (*Egri Tanárképző Főiskola Füzetei 463*), ser. nov. 6: 391-394.

Kiszelyné-Vámosi A. 1983 — A Mátra-hegység zuzmóflórája II. (Lichen flora of the Mátra Mountains, Part II). *Folia Historico Nauralia Musei Matraensis* 8: 63-76 (1982-1983).

Kukwa M. & Motiejūnaitė J. 2012. — Revision of the lichen genera *Cetrelia* and *Punctelia* (Lecanorales, Ascomycota) in Lithuania with implications for their conservation. *Herzogia* 25: 5-14. http://www.bioone.org/doi/abs/10.13158/heia.25.1.2010.15

Kukwa M., Pietnoczko M. & Czyżewska K. 2012. — The lichen family *Parmeliaceae* in Poland. II. The genus *Cetrelia*. *Acta Societatis Botanicorum Poloniae* 81 (1): 43-52. https://doi.org/10.5586/asbp.2012.007

Leavitt S. D., Johnson L. A., Goward T. & St. Clair L. L. 2011. — Species delimitation in taxonomically difficult lichen-forming fungi: an example from morphologically and chemically diverse *Xanthoparmelia* (Parmeliaceae) in North America. *Molecular Phylogenetics and Evolution* 60 (3): 317-332. https://doi.org/10.1016/j.ympev.2011.05.012

Lendemer J. C. 2012. — Perspectives on chemotaxonomy: molecular data confirm the existence of two morphologically distinct species within a chemically defined *Lepraria caesiella* (Stereocaulaceae). *Castanea* 77 (1): 89-105. https://doi.org/10.2179/11-042

Lőkös L. 2009. — The lichen-forming fungi of the Aggtelek National Park (NE Hungary), *in* Papp B. (ed.), *Flora of the Aggtelek National Park. Cryptogams.* Hungarian Natural History Museum, Budapest: 109-174.

Lőkös L. 2010. — A Mecsek zuzmóflórája. (The lichen flora of the Mecsek Mts (SW Hungary)). *Dunántúli dolgozatok, (A) Természettudományi Sorozat* 12: 20-41.

Lőkös L. & Balogh L. 2016. — Herbarium Piersianum 2. Piers Vilmos herbáriumának zuzmói a szombathelyi Savaria Múzeumban. (Herbarium Piersianum 2. Lichens from Vilmos Piers' herbarium in the Szombathely Savaria Museum). *Savaria, a Vas megyei Múzeumok Értesítője* 38: 7-49.

Lőkös L. & Farkas E. 1997. — Az Őrség zuzmóflórájának alapvetése. *Savaria, a Vas megyei múzeumok értesítője* 24 (2): 127-160.

Lőkös L. & Tóth E. 1997. — Red list of lichens of Hungary (a proposal), *in* Tóth E. & Horváth R. (eds), *Proceedings of the "Research, Conservation, Management" Conference*, Aggtelek, Vol. I: 337-343.

Lőkös L., Tóth Z. & Balogh L. 1997. — A Kőszegi-hegység zuzmóflórája. *Tilia* 5: 7-91.

Mark M., Randlane T., Thor G., Hur J.-S., Obermayer W. & Saag A. 2018. — Lichen chemistry is concordant with multilocus gene genealogy in the genus *Cetrelia* (Parmeliaceae, Ascomycota). *Fungal Biology*: 123 (2): 125-139. https://doi.org/10.1016/j.funbio.2018.11.013

Matteucci E., Occhipinti A., Piervittori R., Maffei M. E. & Favero-Longo S. E. 2017. — Morphological, secondary metabolite and ITS (rDNA) variability within usnic acid-containing lichen thalli of *Xanthoparmelia* explored at the local scale of rock outcrop in W-Alps. *Chemistry and Biodiversity* 14 (6): e1600483. https://doi.org/10.1002/cbdv.201600483

Mietzsch E., Lumbsch, H. T. & Elix, J. A. 1994. — *Wintabolites (Mactabolites for Windows) users manual.* 2nd edition. Universität Essen, Essen.

Mishra G. K. & Upreti D. K. 2015. — The lichen genus *Cetrelia* (Parmeliaceae, Ascomycota) in India. *Phytotaxa* 236 (3): 201-214. https://doi.org/10.11646/phytotaxa.236.3.1

Molnár K. & Farkas E. 2010. — Current results on biological activities of lichen secondary metabolites: a review. *Zeitschrift für Naturforschung, Sect. C* 65 (3-4): 157-173. https://doi.org/10.1515/znc-2010-3-401

Molnár K. & Farkas E. 2011. — Depsides and depsidones in populations of the lichen *Hypogymnia physode*s and its genetic diversity. *Annales Botanici Fennici* 48: 473-482. https://doi.org/10.5735/085.048.0605

Nelsen M. P. & Gargas A. 2008. — Phylogenetic distribution and evolution of secondary metabolites in the lichenized fungal genus *Lepraria* (Lecanorales: Stereocaulaceae). *Nova Hedwigia* 86: 115-131. https://doi.org/10.1127/0029-5035/2008/0086-0115

Nelsen M. P., Chavez N., Hermann E. S., Thell A., Randlane T., Divakar P. K., Rico V. J. & Lumbsch T. H. 2011. — The cetrarioid core group revisited (Lecanorales: Parmeliaceae). *The Lichenologist* 43 (6): 537-551. https://doi.org/10.1017/S0024282911000508

Niklfeld H. 1971. — Bericht über die Kartierung der Flora Mitteleuropa. *Taxon* 20 (4): 545-571. https://doi.org/10.2307/1218258

Nourish R. & Oliver W. A. 1976. — Chemotaxonomic studies on the *Cladonia chlorophaea-pyxidata* complex and some allied species in Britain, *in* Brown D. H., Hawksworth D. L. & Bailey R. (eds), *Lichenology: Progress and Problems.* The Systematics Association, Special Vol. 8. Academic Press, London and

New York: 185-214.
Nylander W. 1866a. — Circa novum in studio lichenum criterium chemicum. *Flora (Jena)* 49: 198-201.
Nylander W. 1866b. — Quaedam addenda ad nova criteria chemica in studio lichenum. *Flora (Jena)* 49: 233-234.
Obermayer W. & Mayrhofer H. 2007. — Hunting for *Cetrelia chicitae* (lichenized Ascomycetes) in the eastern European Alps (including an attempt for a morphological characterization of all taxa of the genus *Cetrelia* in Central Europe). *Phyton* (Horn, Austria) 47: 231-290.
Orange A., James P. W. & White F. J. 2010. — *Microchemical methods for the identification of lichens.* 2nd edition. British Lichen Society, London, 101 p.
Osyczka P. & Skubała K. 2011. — Chemical races of *Cladonia cariosa* and *C. symphycarpa* (lichenized Ascomycota) a Polish case study in a worldwide context. *Nova Hedwigia* 93 (3-4): 363-373. https://doi.org/10.1127/0029-5035/2011/0093-0363
Otnyukova T. N., Stepanov N. V. & Elix J. A. 2009. — Three new species of Parmeliaceae (Ascomycota) from Siberia. *Mycotaxon* 108: 249-256. https://doi.org/10.5248/108.249
Randlane T. & Saag A. 1991. — Chemical and morphological variation in the genus *Cetrelia* in the Soviet Union. *The Lichenologist* 23 (2): 113-126. https://doi.org/10.1017/s0024282991000282
Randlane T., Saag A., Thell A. & Ahti T. 2013. — Third world list of cetrarioid lichens. *Cryptogamie, Mycologie* 34 (1): 79-94. https://doi.org/10.7872/crym.v34.iss1.2013.79
Randlane T., Tõrra T., Saag A. & Saag L. 2009. — Key to European Usnea species. *Bibliotheca Lichenologica* 100: 419-462.
Roux C., Monnat J.-Y., Gonnet D., Gonnet O., Poumarat S., Esnault J., Bertrand M., Gardiennet A., Masson D., Bauvet C. *et al.* 2017. — *Catalogue des Lichens et Champignons Lichénicoles de France métropolitaine*. Editions d'Art, H des Abbayes, 2e édition revue et augmentée. Éditions Association française de Lichénologie (A. F. L.), Fontainebleau, 1581 p.
Schmitt I. & Lumbsch H. T. 2004. — Molecular phylogeny of the *Pertusariaceae* supports secondary chemistry as an important systematic character set in lichen forming ascomycetes. *Molecular Phylogenetics and Evolution* 33: 43-55. https://doi.org/10.1016/j.ympev.2004.04.014
Seaward M. R. D., Kiss T. & Bylinska E. L. 1985. — Contribution to the lichen flora of West Hungary. *Botanikai Közlemények* 72 (1-2): 159-161.
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, 1046 p.
Stenroos S., Ahti T., Lohtander K. & Myllys L. (eds) 2011. — Suomen jäkäläopas. [Lichen flora of Finland]. *Norrlinia* 21: 1-534.
Stenroos S., Velmala V., Pykälä J. & Ahti T. (eds) 2016. — Lichens of Finland. *Norrlinia* 30: 1-896.
Stocker-Wörgötter E. 2008. — Metabolic diversity of lichen-forming ascomycetous fungi: culturing, polyketide and shikimate metabolite production, and PKS genes. *Natural Product Reports* 25: 188-200. https://doi.org/10.1039/b606983p
Stocker-Wörgötter E. 2015. — Biochemical diversity and ecology of lichen-forming fungi: lichen substances, chemosyndromic variation and origin of polyketide-type metabolites (biosynthetic pathways), *in* Upreti D.K., Divakar P.K., Shukla V. & Bajpai R. (eds), *Recent Advances in Lichenology: Modern Methods and Approaches in Biomonitoring and Bioprospection.* 2. Springer: 161-180.
Szatala Ö. 1925. — Adatok Magyarország zuzmóflórájának ismeretéhez. (Beiträge zur Kenntnis der Flechtenflora Ungarns). *Magyar Botanikai Lapok* 24: 43-75.
Szatala Ö. 1926. — Adatok Magyarország zuzmóflórájának ismeretéhez. II. (Beiträge zur Kenntnis der Flechtenflora Ungarns. II). *Magyar Botanikai Lapok* 25: 201-219.
Thell A. & Kärnefelt I. 2011. — *Cetrelia*, *in* Thell A. & Moberg R. (eds), *Parmeliaceae*. Museum of Evolution, Uppsala: 45-46.
Thell A., Stenroos S., Feuerer T., Kärnefelt I., Myllys L. & Hyvönen J. 2002. — Phylogeny of cetrarioid lichens (*Parmeliaceae*) inferred from ITS and b-tubulin sequences, morphology, anatomy and secondary chemistry. *Mycological Progress* 1 (4): 335-354. https://doi.org/10.1007/s11557-006-0031-x
Thiers B. [continuously updated]. — Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/
Thomas C. D., Cameron A. & Green R. E. 2004. — Extinction risk from climate change. *Nature* 427: 145-148. https://doi.org/10.1038/nature02121
Thuiller W., Lavorel S., Araújo M. B., Martin T., Sykes M. T. & Prentice I. C. 2005. — Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences* 102 (23): 8245-8250. https://doi.org/10.1073/pnas.0409902102
Timkó Gy. 1925. — Új adatok a Budai és Szentendre-Visegrádi hegyvidék zuzmóvegetációjának ismeretéhez. (Neue Beiträge zur Kenntnis der Flechtenvegetation des Buda-Szentendre-Visegráder Gebirges). *Botanikai Közlemények* 22 (1-6): 81-104.
UNFCCC 2013. — Sixth national communication of Hungary to the United Nations framework convention on climate change (UNFCCC) Report (2014), Hungary, 358 p. http://unfccc.int/files/national_reports/annex_i_natcom/submitted_natcom/application/pdf/nc6-final_hun[1].pdf
Verseghy K. 1964. — *Typen-Verzeichnis der Flechtensammlung in der Botanischen Abteilung des Ungarischen Naturwissenschaftlichen Museums.* Természettudományi Múzeum, Budapest, 146 p.
Verseghy K. 1965. — Angaben zur Flechtenflora des Zemplén-Gebirges I. Strauch- und Laubflechten. (Adatok a Zemplén hegység zuzmóflórájához). *Fragmenta Botanica* 3 (1-4): 41-75.
Verseghy K. 1973. — A Vendvidék zuzmói. (Die Flechten des Vend-Gebietes). *Savaria, a Vas Megyei Múzeumok Értesítője* 4: 31-42. (1966-1970).
Verseghy K. 1988. — Magyarországi zuzmóflóra revíziójának eredményei. (Ergebnisse der Revision der Flechtenflora von Ungarn). *Botanikai Közlemények* 74-75 (1-2): 31-46.
Verseghy K. 1994. — *Magyarország zuzmóflórájának kézikönyve.* Magyar Természettudományi Múzeum, Budapest, 415 p.
Wang X.-N., Zhang H.J., Ren D.-M., Ji M., Yu W. T. & Lou H.-X. 2009. — Lobarialides A-C, antifungal triterpenoids from the lichen *Lobaria kurokawae*. *Chemistry and Bioidiversity* 6 (5): 746-753. https://doi.org/10.1002/chin.200938183
Wirth V. 1980. — *Flechtenflora.* Eugen Ulmer, Stuttgart, 522 p.
Wirth V., Hauck M. & Schultz M. 2013. — *Die Flechten Deutschlands.* Ulmer Verlag, Stuttgart, 1244 p.
Woodruff D. S. 2001. — Declines of biomes and biotas and the future of evolution. *Proceedings of the National Academy of Sciences* 98: 5471-5476. https://doi.org/10.1073/pnas.101093798.
Zhou Q.-M., Guo S.-Y., Huang M.-R. & Wei J.-C. 2006. — A study of the genetic variability of *Rhizoplaca chrysoleuca* using DNA sequences and secondary metabolic substances. *Mycologia* 98 (1): 57-67. https://doi.org/10.3852/mycologia.98.1.57

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