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Martin7: a reference database of DNA barcodes for European epiphytic lichens and its taxonomic implications

Jan Vondrák[1,2], Stanislav Svoboda[1,2], Jiří Košnar[2], Jiří Malíček[1], Jaroslav Šoun[3,*], Ivan Frolov[4], Måns Svensson[5], Petr Novotný[6] & Zdeněk Palice[1]

[1]Institute of Botany of the Czech Academy of Sciences, CZ-25243 Průhonice, Czech Republic; [2]Department of Botany, Faculty of Science, University of South Bohemia, CZ-37005 České Budějovice, Czech Republic; [3]Museum of Dr. Bohuslav Horák in Rokycany, branch of Museum of West Bohemia, nám. J. Urbana 141, CZ-33701 Rokycany, Czech Republic; [4]Lenina Str. 50, Yekaterinburg, RU-620075 Russia; [5]Museum of Evolution, Uppsala University, Norbyvägen 16, SE-75236 Uppsala, Sweden; [6]Department of Biology Education, Faculty of Science, Charles University, Viničná 7, CZ-12844 Prague, Czech Republic
*corresponding author: jasoun@centrum.cz

Abstract: Molecular identification of organisms is now a common practice and, increasingly, species are identified from environmental samples. However, for most organisms, we still lack comprehensive reference databases of DNA barcodes to identify the sequences produced. We present a near-complete database of ITS and mtSSU barcodes, named Martin7, for accurate molecular identification of epiphytic lichens (mycobionts) of central Europe. New data were obtained by Sanger and PacBio sequencing. We obtained 907 ITS sequences from 603 species and 844 mtSSU sequences from 546 species and supplemented our dataset with sequences from other reliable sources. In total, 1,172 species are included in the database, 1,004 for the ITS barcode and 906 for mtSSU. ITS was newly sequenced for 224 species and mtSSU for 234 species. For 45 genera these are the first ITS or mtSSU (or both) barcodes ever obtained. In most cases, these barcodes distinguish species as currently circumscribed, but we detected 82 groups or pairs of species where at least one of the barcodes (mostly mtSSU) does not clearly discriminate between species. We revealed diverging genotypes, possibly representing cryptic taxa, within 37 traditionally conceived species. By sequencing phenotypically unidentifiable lichens, we detected numerous "known-unknowns" (presumed undescribed species), especially in the genera *Bacidina* and *Micarea*. Five species of sorediate crustose lichens are newly described in the genera *Bacidina* (two species), *Chrysothrix*, *Japewia* and *Lecanora*. We provide a number of taxonomic novelties, for example that *Lecidea betulicola* and *L. coriacea* are teleomorphs of *Cheiromycina*, and *Dictyocatenulata* is an anamorph of *Thelenella*.

Keywords: fungi, ITS barcode, mtSSU, PacBio, taxonomy

Introduction

Biodiversity inventories will increasingly use DNA metabarcoding through next generation sequencing (Taberlet et al. 2012). Its potential is obvious in various groups of organisms (e.g. algae, bacteria and fungi) as it repeatedly reveals significantly higher species richness than traditional voucher-based taxonomic approaches. However, the use of environmental

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DNA metabarcoding is still limited by insufficient species-level identification of the DNA sequences. Recent studies may describe the phylogenetic diversity in environmental samples, but the DNA sequences cannot be assigned to the names of species, which limits the usefulness of the data. A major difficulty is the lack of verified reference sequences – DNA barcodes – for numerous species. This problem can be solved by creating as complete a DNA barcode database as possible. In theory, this is a simple task, but in practice it is a long-term project and there are many obstacles. Consequently, such databases are still lacking for the vast majority of small organisms (including lichenized fungi).

The easiest transformation of DNA metabarcode data into a list of species is obtained by assigning the sequences (or more precisely their clusters) by the NCBI BLAST (2022) search to the best matching GenBank names. However, in its current form, this approach leads to species lists with many suspicious names and, conversely, the names of some species known to be present (and sometimes abundant) in the samples may be missing. The reason for the missing names is often the absence of reference sequences for specific species in the NCBI (2022). Incorrect names are assigned when the NCBI sequence is incorrectly named and when the NCBI has only a sequence for a closely related species, even if the ecology of that species excludes it from the sampled area. Comparison with databases including verified DNA sequences can help, but such databases are either very local (Kerr & Leavitt 2023) or cover an insufficient number of taxa (Ratnasingham & Hebert 2007, Abarenkov et al. 2010, Keepers et al. 2019, Marthinsen et al. 2019).

Epiphytic lichens are known to be an important component of the epiphytic biota and excellent bioindicators (Johansson & Gustafsson 2001, Paillet et al. 2010) and the lack of reference DNA databases seriously handicaps their modern research. Reference sequences are still lacking for many species, and even some genera. We have worked, and continue to work, towards achieving the most complete and reliable reference database for the two most common DNA barcodes: the ITS nrDNA (hereafter ITS) and the mitochondrial SSU (hereafter mtSSU). We report here on progress with our database for central-European epiphytic lichens, many of which are widespread in Europe. The database is named Martin7 and publicly available on the Atlas of Czech lichens (Malíček et al. 2023; https://dalib.cz/data/martin7).

The name of Martin7 is derived from the middle name of Karl Martin Redinger, the eminent Austrian-born lichenologist, who died in 1940 at the young age of 33 and created an important legacy of lichenology within a seven-year period. His *Arthoniaceae* monograph (Redinger 1937), for example, is still unsurpassed.

Material and methods

Sources of data

Epiphytic lichens are understood here as a functional group of lichenized fungi (mycobionts) occurring on a broad scale of organic substrates composed of living or dead plants. Lichens occurring on organic surfaces covering soil and rock substrate (i.e. epilithic/epigeic bryophytes and humus) are considered to be non-epiphytic. Some lichen species are both epiphytic and non-epiphytic; they are included if their epiphytic occurrences are not too rare.

When creating Martin7, we sequenced as many European epiphytic lichens (and semilichens sensu Vondrák et al. 2022) as we could, especially species present, or likely to be present, in central Europe. Specimens selected for sequencing were primarily determined to species from phenotypic characters. Their morphology and anatomy were examined by light microscopy and secondary substances were detected by TLC (thin layer chromatography; Orange et al. 2001).

All sequences were taken from herbarium material, mainly recently collected, but sometimes up to 10 years old. For newly sequenced genera with uncertain taxonomic placement (e.g. *Arthopyrenia*, *Biatoridium*, *Mycoporum*, *Vezdaea*, etc.), we attempted to sequence multiple specimens of each species to reduce the risk of incorrectly assigning sequences of contaminants (e.g. lichenicolous fungi) to target species. As our previous attempts with direct Sanger sequencing often gave contaminated results, we used PacBio sequencing to overcome the presence of mixed DNA templates. PacBio sequencing was successfully used for the majority of species, but in the case of species with unclear taxonomic position, it was difficult to identify target sequences, which were sometimes outnumbered by other fungi, e.g. lichenicolous fungi *Kockovaella* spp. (*Tremellales*). Target sequences were subsequently verified by repeated Sanger sequencing of these species. For both types of sequencing (Sanger and PacBio) we aimed to take material from well-developed thalli and fruiting bodies, without signs of fungal infections.

Our sequences were supplemented with data from NCBI (https://www.ncbi.nlm.nih.gov/genbank), especially for species unsequenced by us. For an overview of the barcode sequences and the respective specimens see Supplementary Table S1. Comments on the sequenced species including NCBI BLAST results of the newly obtained sequences are in Supplementary Data S1.

Laboratory work, sequencing, sequence-processing

Genomic DNA was extracted using CTAB protocol (Doyle & Doyle 1987) or DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands). Polymerase chain reactions were performed in a reaction mixture containing 2.5 mmol/l $MgCl_2$, 0.2 mmol/l of each dNTP, 0.3 µmol/l of each primer, 0.5 U Combi Taq polymerase in the manufacturer's reaction buffer (Top-Bio, Prague, Czech Republic), and PCR water to make up a final volume of 10 µl. The primers used for PCR and the cycling conditions are summarized in Supplementary Table S2. Both forward and reverse primers were fused with unique multiplex identifier (MID) sequences adopted from Roche Extended Set MIDs (454 Sequencing Technical Bulletin No. 005-2009; Roche, Basel, Switzerland). A set of 25 forward and 20 reverse uniquely MID-tagged primers providing up to 500 possible unique dual combinations was used for both barcode loci. Successful amplifications were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel, Düren, Germany). Individual MID-tagged samples from both barcode loci were pooled together in equimolar amounts and the resulting pool was further purified using Agencourt AMPure XP beads (Beckman Coulter Brea, California, USA). The final sample pool was used for PacBio library preparation and subjected to sequencing run using Sequel II System and SMRT 8M cell (Pacific Bioscience, Menlo Park, California, USA) for 8 hrs movie performed at SEQme Company (Dobříš, Czech Republic). Two sequencing runs involving pools of 384 and 932 MID-tagged samples were carried out.

High-fidelity PacBio reads were processed using software tools implemented in SEED v. 2.0 (Větrovský et al. 2018). The reads were demultiplexed into individual samples using MID sequences (no mismatch in MID sequence allowed). The reads were subsequently sorted into the two barcode loci using sequence of locus-specific primers (sequence mismatch set to 1). MID and primer sequences were trimmed, and the most abundant sequence for each sample was picked and analysed using nucleotide BLAST search at NCBI and our custom database of verified sequences. If the BLAST hit of the most abundant sequence of a given sample revealed its non-target origin, all the sample reads were clustered using 95% similarity threshold. The most abundant sequence for each 95% similarity cluster was picked and BLAST searched to identify the putative target sequence.

Polymerase chain reactions for Sanger sequencing were performed as described above, except that standard primers were used. Successful amplifications were sent to GATC Biotech (Konstanz, Germany). Sanger sequences were edited in FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) and BioEdit 7.2.5 (Hall 1999). The identity of individual sequences was sought and verified using nucleotide BLAST search at NCBI (default settings) and by comparison with our own verified sequences.

Phylogenetic analyses

Phylogenetic positions of the newly described species were assessed using Bayesian inference (Supplementary Figs S1–S6). Sequences of our specimens were supplemented by relevant sequences from NCBI (2022). Sequences were aligned by MAFFT v.7 (Katoh & Standley 2013; available online at https://mafft.cbrc.jp/alignment/server) using the L-INS-i algorithm and adjusted manually. Gaps were coded as missing data. The best-fit model of sequence evolution was selected using the Akaike information criterion calculated in jModelTest v.0.1.1 (Posada 2008). Phylogenetic relationships were reconstructed by MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). Two runs starting with a random tree and employing four simultaneous chains each (one hot, three cold) were executed. The temperature of a hot chain was set empirically to 0.1, and every 100th tree was saved. The analysis was considered to be completed when the average standard deviation of split frequencies dropped below 0.01. The first 25% of trees were discarded as the burn-in phase, and the remaining trees were used for construction of a 50% majority consensus tree.

Results

New barcode data

The database Martin7 consists of two barcode datasets: (i) ITS sequences amplified using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) and (ii) mtSSU sequences amplified using primers mtSSU1 (Zoller et al. 1999) and MSU7 (Zhou & Stanosz 2001). It currently includes 1,172 species (in 268 genera) for which at least one barcode was obtained (ITS, mtSSU or both). ITS is available for 1,004 species in 247 genera and mtSSU for 906 species in 248 genera. Both barcodes are available for 682 species and 229 genera. Geographically, central Europe is the region best represented in the database, as that is where most of our sequences originated (Supplementary Table S1). Our contribution to NCBI is 907 ITS sequences from 603 species and 844 mtSSU sequences from 546 species.

For a substantial proportion of the sequenced species there were no previous NCBI data for the DNA barcodes studied. ITS was newly sequenced for 224 species and mtSSU for 234 species (Table 1). We have added significantly to the knowledge of barcode sequences in species of the genera *Arthonia* (ITS new to 7 species/ mtSSU to 5), *Chaenotheca* (0/11), *Lecanora* (9/12), *Lecidea* s. lat. (7/6), *Micarea* (11/9) and *Rinodina* (13/15).

Both barcodes are newly published for the genera *Biatorella*, *Biatoridium*, *Cresponea*, *Exarmidium*, *Hazslinszkya*, *Leptorhaphis*, *Naetrocymbe*, *Puttea* and *Sphaeronema*. ITS sequences are newly published for the genera *Acrocordia*, *Alyxoria*, *Arthothelium*, *Catinaria*, *Celothelium*, *Cheiromycina*, *Felipes*, *Inoderma*, *Jamesiella*, *Lopadium*, *Macentina*, *Mycomicrothelia*, *Myrionora*, *Opegrapha*, *Piccolia*, *Pseudoschismatomma*, *Reichlingia*, *Rhaphidicyrtis*, *Sporodophoron*, *Steinia*, *Strangospora*, *Thelenella*, *Thelopsis*, *Wadeana* and *Zwackhia*. MtSSU sequences are newly published for the genera *Andreiomyces*, *Bactrospora*, *Dictyocatenulata*, *Eopyrenula*, *Fellhaneropsis*, *Gassicurtia*, *Mycoporum*, *Myochroidea*, *Stenocybe*, *Tetramelas* and *Xyleborus*.

Barcode resolution

Our data mostly confirm the established view that ITS better discriminates between closely related species than does mtSSU. While closely related species are rarely more than 95% identical in ITS, they are often 99–100% identical in mtSSU (82 cases in Table 2). The mtSSU barcode was found to discriminate poorly between species in e.g. *Hypogymnia*, *Lepraria*, *Physconia*, *Ramalina*, *Usnea* and in sections of *Biatora* and *Calicium*. In some cases, mtSSU does not even distinguish between genera. It is noteworthy that some *Hypogymnia* species have identical mtSSU with some *Usnea* species.

Low discriminatory ability was observed in so-called species pairs, in which generative reproduction predominates in one species and vegetative reproduction predominates or is only known in the other. Some of many examples are *Bacidia albogranulosa* (only known vegetative, v) vs. *B. polychroa* (generative, g), *Buellia griseovirens* (v) vs. *B. erubescens* (g), *Collema furfuraceum* (v) vs. *C. subnigrescens* (g), *Lecidella flavosorediata* (v) vs. *L. achristotera* (g); see Table 2 for further examples. In most of these cases, the resolution using mtSSU fails, but ITS distinguishes nearby species convincingly. Dubious ITS-based resolution (shared identity over 99%) was observed in only a few cases: e.g. *Physconia perisidiosa* (v) vs. *P. venusta* (g) and *Mycoblastus affinis* (g) vs. *M. alpinus* (v). Earlier studies have given similar results (Cubero et al. 2004, Spribille et al. 2011). For species pairs indistinguishable by both barcodes, doubts arise as to whether morphotypes with different reproductive strategies are true species.

Some species recognized traditionally on the basis of minor morphological differences have identical barcodes. We found this situation in the trio of species *Rinodina archaea*, *R. orculata* and *R. trevisanii*. They are 100% identical in mtSSU and the latter two also in ITS. Further examples are e.g. *Pertusaria alpina* vs. *P. constricta* and *Xanthomendoza fallax* vs. *X. huculica*. For the purposes of DNA barcode identification, we consider the "species" of these pairs or triplets to be conspecific. In this paper we do not address the question of whether, for other purposes, there is any merit in recognising the individual "species".

Table 1. Genera and numbers of species in Martin7. We keep some genera in a broader sense, because their nomenclature is still unsettled. Examples are *Caloplaca* (except for *Blastenia*, *Haloplaca* and *Parvoplaca*) and *Bacidia* (except for *Toniniopsis*).

Genus	Total number of species		Species sequenced by us		Species new to NCBI	
	ITS	mtSSU	ITS	mtSSU	ITS	mtSSU
Absconditella	5	6	3	4	2	3
Absconditonia	2	2	2	2	0	0
Acolium	3	1	3	0	1	0
Acrocordia	2	2	2	2	2	2
Agonimia	6	6	6	6	3	3
Agyrium	3	3	2	2	1	1
Alectoria	1	1	0	0	0	0
Alloarthopyrenia	1	1	0	1	0	0
Allocalicium	1	1	0	0	0	0
Alyxoria	2	2	2	2	2	0
Amandinea	1	1	1	1	0	0
Anaptychia	1	1	1	1	0	0
Andreiomyces	1	1	1	1	0	1
Anisomeridium	2	2	2	2	2	2
Anzina	1	1	1	0	0	0
Aquacidia	1	1	1	1	0	0
Arctomia	0	1	0	0	0	0
Arthonia	14	19	12	16	7	5
Arthopyrenia	5	4	5	2	4	2
Arthothelium	2	4	2	2	2	2
Arthrosporum (= *Toninia* p.p.)	1	1	0	0	0	0
Aspicilia	1	1	1	1	0	0
Bacidia (s.lat.)	19	17	13	11	2	1
Bacidina	21	16	14	13	3	8
Bactrospora	3	3	2	3	2	3
Baeomyces	1	1	0	0	0	0
Belonia	1	1	1	0	0	0
Biatora	27	21	15	10	2	2
Biatorella	2	1	2	1	2	1
Biatoridium	2	2	2	2	2	2
Bilimbia	1	1	0	0	0	0
Blastenia	11	1	0	1	0	1
Bryobilimbia	1	1	1	0	0	0
Bryoria	3	3	3	2	0	0
Bryostigma	2	1	1	0	1	0
Buellia	10	8	7	7	2	4
Bunodophoron	1	0	0	0	0	0
Byssoloma	4	4	1	1	1	1
Calicium	20	15	9	8	1	3
Caloplaca (s.lat.)	39	26	11	10	2	4
Candelaria	2	1	1	1	0	0
Candelariella	13	8	7	5	5	4
Carbonicola	2	2	0	1	0	0
Carestiella	1	1	0	0	0	0
Catillaria	3	3	1	1	0	0
Catinaria	2	1	2	1	2	0
Celothelium	1	1	1	1	1	1
Cetraria	1	1	1	1	0	0
Cetrelia	4	3	2	1	0	0
Chaenotheca	21	16	16	15	0	11
Chaenothecopsis	16	6	8	6	6	6

Genus	Total number of species		Species sequenced by us		Species new to NCBI	
	ITS	mtSSU	ITS	mtSSU	ITS	mtSSU
'*Cheiromycina*' (see Data S1)	1	2	1	2	1	2
Cheiromycina s.str.	1	3	1	2	1	0
Chrysothrix	2	5	2	1	2	1
Cladonia	23	18	12	11	2	8
Cliostomum	4	4	3	2	1	2
Coenogonium	4	4	3	3	1	1
Collema	0	4	0	0	0	0
Coniocarpon	2	2	0	0	0	0
Cresponea	1	1	1	1	1	1
Crutarndina	0	1	0	0	0	0
Cryptodiscus	8	8	4	5	1	1
Dendriscosticta	1	1	0	0	0	0
Dendrographa	1	1	1	1	0	0
Dictyocatenulata	1	1	0	1	0	1
Dichoporis	0	1	0	0	0	0
Diploschistes	1	1	0	0	0	0
Diplotomma	4	2	3	2	2	1
Dirina	1	0	0	0	0	0
Elixia	1	2	1	1	0	0
Enchylium	0	1	0	0	0	0
Enterographa	1	3	1	2	1	0
Eopyrenula	2	2	2	2	1	2
Epigloea	1	1	0	0	0	0
Evernia	3	3	3	2	0	0
Exarmidium	1	1	1	1	1	1
Felipes	1	1	1	1	1	0
Fellhanera	5	4	4	4	2	3
Fellhaneropsis	3	3	3	3	2	3
Flavoparmelia	2	2	1	1	0	0
Flavopunctelia	2	2	1	1	0	0
Francisrosea	0	1	0	0	0	0
Frutidella	1	1	1	1	0	1
Fuscidea	4	4	2	2	1	1
Fuscopannaria	6	5	0	0	0	0
Gabura	0	1	0	0	0	0
Gassicurtia	0	1	0	1	0	1
Glyphis	1	1	0	0	0	0
Gomphillus	1	0	0	0	0	0
Graphis	4	2	4	1	2	0
Gyalecta	9	10	6	6	4	2
Gyalidea	3	0	3	0	3	0
Gyalideopsis	3	1	3	1	3	1
Haematomma	1	1	0	0	0	0
Halecania	1	1	1	1	1	1
Haloplaca	2	0	2	0	1	0
Hazslinszkya	1	0	1	0	1	0
Hertelidea	1	1	1	1	0	0
Heterodermia	2	2	1	1	0	0
Hyperphyscia	1	1	0	0	0	0
Hypocenomyce	1	1	1	1	0	0
Hypogymnia	8	8	4	3	0	0
Hypotrachyna	6	5	2	2	0	0
Icmadophila	1	1	1	0	0	0
Ikaeria	2	0	1	0	0	0

Genus	Total number of species		Species sequenced by us		Species new to NCBI	
	ITS	mtSSU	ITS	mtSSU	ITS	mtSSU
Imshaugia	1	1	0	0	0	0
Inoderma	1	3	1	2	1	0
Jamesiella	1	1	1	1	1	0
Japewia	4	4	3	3	1	2
Karschia	0	1	0	0	0	0
Karstenia	2	3	0	1	0	1
Lambiella	1	1	0	0	0	0
Lecanactis	1	1	1	1	0	0
Lecania	9	10	5	8	0	2
Lecanographa	2	2	2	2	1	0
Lecanora	55	47	35	31	9	12
Lecanoromycetidae incertae sedis	1	0	1	0	1	0
Lecidea	14	15	12	12	7	6
Lecidella	9	6	7	5	4	2
Lepra	6	4	5	3	3	1
Lepraria	8	8	5	2	0	2
Leprocaulon	2	2	1	0	0	0
Leptogium	5	7	1	0	0	0
Leptorhaphis	3	2	3	2	3	2
Letharia	1	0	0	0	0	0
Lichenomphalia	1	0	0	0	0	0
Lithothelium	3	3	2	3	1	1
Lobaria	2	2	1	1	0	0
Lobarina	1	1	0	0	0	0
Lopadium	1	1	1	1	1	0
Loxospora	3	3	3	3	0	0
Macentina	1	1	1	1	1	0
Maronea	1	1	0	0	0	0
Megalaria	3	2	1	0	0	0
Megalospora	2	2	0	0	0	0
Megaspora	2	1	0	0	0	0
Melanelixia	5	5	4	4	0	0
Melanohalea	6	6	2	2	0	0
Melaspilea	1	1	1	1	1	1
Melaspileella	1	1	0	0	0	0
Menegazzia	2	1	1	0	0	0
Micarea	42	54	22	33	11	9
Microcalicium	4	5	1	1	0	0
Miriquidica	0	1	0	1	0	1
Multiclavula	1	0	0	0	0	0
Mycobilimbia	4	4	4	3	0	0
Mycoblastus	4	4	4	4	0	1
Mycocalicium	2	2	2	2	1	1
Mycomicrothelia	2	1	2	1	2	1
Mycoporum	0	1	0	1	0	1
Myelochroa	2	1	0	0	0	0
Myochroidea	0	1	0	1	0	1
Myriolecis	3	3	3	3	1	3
Myrionora	1	1	1	1	1	0
Naetrocymbe	1	1	1	1	1	1
Nephroma	7	7	2	1	0	0
Nephromopsis	1	1	1	1	0	1
Nevesia	1	1	0	0	0	0
Normandina	3	3	1	1	0	0

Genus	Total number of species		Species sequenced by us		Species new to NCBI	
	ITS	mtSSU	ITS	mtSSU	ITS	mtSSU
Ocellomma	1	0	0	0	0	0
Ochrolechia	11	11	9	7	2	5
Opegrapha	5	5	5	4	5	2
Pachnolepia	0	1	0	0	0	0
Palicella	1	1	1	1	0	0
Pannaria	2	2	0	0	0	0
Parmelia	7	6	6	5	0	0
Parmeliella	2	2	1	0	0	0
Parmelina	4	4	2	2	0	0
Parmeliopsis	2	2	2	2	0	0
Parmotrema	5	4	1	1	0	0
Parvoplaca	4	4	1	1	0	0
Patellaria	1	1	1	0	0	0
Pectenia	2	0	0	0	0	0
Peltigera	6	6	6	2	0	1
Pertusaria	11	12	8	8	5	3
Phaeocalicium	3	0	0	0	0	0
Phaeographis	1	1	0	0	0	0
Phaeophyscia	9	7	6	6	1	3
Phlyctis	2	2	2	2	0	0
Phyllopsora	1	1	0	0	0	0
Physcia	9	7	4	4	0	0
Physconia	9	6	2	2	0	0
Piccolia	1	0	1	0	1	0
Placynthiella	3	3	3	3	0	1
Platismatia	1	1	1	1	0	0
Pleurosticta	1	1	1	1	0	0
Polychidium	1	1	0	0	0	0
Porina	7	8	5	5	2	1
Protopannaria	1	1	0	0	0	0
Protoparmelia	2	3	0	1	0	1
Pseudevernia	1	1	1	1	0	0
Pseudocyphellaria	5	3	0	0	0	0
Pseudographis	1	1	1	1	0	0
Pseudoschismatomma	1	1	1	1	1	0
Pseudothelomma	1	1	1	1	0	0
Psilolechia	2	1	2	1	1	1
Psoroglaena	2	2	2	2	2	2
Psoroma	1	1	0	0	0	0
Ptychographa	1	1	0	0	0	0
Punctelia	4	4	3	3	0	1
Puttea	2	2	2	2	2	2
Pycnora	3	3	2	2	1	0
Pyrenula	9	6	5	4	2	0
Pyrgidium	1	1	0	0	0	0
Pyrrhospora	1	1	1	0	0	0
Pyxine	1	1	0	0	0	0
Ramalina	12	10	6	7	0	1
Ramboldia	3	2	0	0	0	0
Ramonia	0	1	0	1	0	1
Reichlingia	2	3	2	2	2	0
Rhaphidicyrtis	1	0	1	0	1	0
Ricasolia	2	2	0	1	0	0
Rinodina	29	27	24	23	13	15
Ropalospora	1	1	1	1	0	1

Genus	Total number of species		Species sequenced by us		Species new to NCBI	
	ITS	mtSSU	ITS	mtSSU	ITS	mtSSU
Rostania	0	4	0	1	0	0
Sarcosagium	1	0	1	0	1	0
Sarea	3	3	0	0	0	0
Sclerophora	4	5	4	5	2	3
Scoliciosporum	6	6	6	5	4	4
Scytinium	6	6	3	5	1	2
Schaereria	0	1	0	0	0	0
Schismatomma	1	1	1	1	0	0
Schizotrema	0	1	0	0	0	0
Schizoxylon	3	3	0	0	0	0
Snippocia	0	1	0	0	0	0
Sphaeronema	1	1	1	1	1	1
Sphaerophorus	1	1	1	1	0	0
Sphinctrina	4	3	3	3	2	2
Sporodophoron	1	2	1	1	1	1
Staurolemma	1	1	0	0	0	0
Steinia	1	1	1	0	1	0
Stenocybe	2	2	1	2	1	2
Sticta	7	7	1	0	0	0
Stictis	6	6	0	0	0	0
Strangospora	2	2	2	2	2	1
Swinscowia	3	2	3	2	2	0
Synarthonia	1	2	0	0	0	0
Syncesia	1	0	0	0	0	0
Szczawinskia	1	2	0	0	0	0
Teloschistes	1	1	0	0	0	0
Tetramelas	3	1	1	1	1	1
Thelenella	3	3	3	3	3	2
Thelocarpon	2	4	1	2	1	2
Thelopsis	1	1	1	1	1	0
Thelotrema	3	3	2	2	0	0
Tholurna	1	1	0	0	0	0
Toensbergia	1	1	1	1	0	0
Toniniopsis	3	2	2	1	1	0
Tornabea	1	1	0	0	0	0
Trapelia	1	1	1	1	0	0
Trapeliopsis	6	6	4	3	0	0
Tuckermannopsis	1	1	1	1	0	0
Tylophoron	0	1	0	0	0	0
Usnea	20	7	11	7	1	7
Usnocetraria	1	1	0	0	0	0
Vahliella	0	1	0	0	0	0
Varicellaria	3	3	2	2	0	0
Verrucaria	9	7	8	5	3	3
Vezdaea	3	1	3	1	3	1
Violella	1	0	1	0	0	0
Vulpicida	1	1	0	1	0	0
Wadeana	1	0	1	0	1	0
Xanthomendoza	4	3	3	1	0	0
Xanthoria (incl. *Polycauliona*)	3	3	3	3	0	0
Xyleborus	0	1	0	1	0	1
Xylographa	6	6	3	4	0	0
Xylopsora	3	3	3	3	1	2
Zwackhia	1	2	1	1	1	0

Table 2. Species pairs or groups more than 99% identical in at least one of the barcodes.

Species pairs / groups	Identity in ITS	Identity in mtSSU
Anaptychia ciliaris & *A. crinalis*	98.5%	> 99.5%
Bacidia albogranulosa & *B. polychroa*	97%	> 99.5%
Bacidia fraxinea & *B. rubella*	98–99%	> 99.5%
Biatora chrysantha & *B. vernalis*	> 99%	> 99.5%
Biatora chrysantha, *B. fallax* & *B. subduplex*	92–94%	100%
Biatora efflorescens & *B. helvola*	97.5%	99.5%
Buellia erubescens & *B. griseovirens*	92%	99%
Calicium episcalaris, *C. montanum* & *C. pinastri*	95.5–96.5%	> 99.5%
Calicium notarisii & *C. tigillare*	93%	> 99%
Caloplaca cerinelloides & *C. holocarpa*	96.5%	100%
Caloplaca cerinelloides & *C. pyracea*	94.5%	99.5%
Caloplaca chlorina & *C. turkuensis*	94.5%	> 99%
Candelariella boleana & *C. xanthostigma*	> 99%	NA
Cheiromycina petri & *Lecidea coriacea*	> 99.5%	100%
Chrysothrix flavovirens & *C. chrysophthalma*	NA	100%
Cladonia cenotea & *C. squamosa*	> 99%	NA
Cladonia digitata, *C. floerkeana* & *C. polydactyla*	98–99%	99.5–100%
Collema furfuraceum & *C. subnigrescens*	NA	100%
Evernia divaricata & *E. mesomorpha*	98%	100%
Hypogymnia austerodes, *H. bitteri* & *H. incurvoides*	94–98.5%	> 99%
Hypogymnia farinacea, *H. tubulosa*, *Usnea barbata* & *U. substerilis*	< 95% (*Hypogymnia*/*Usnea*)	100%
Hypogymnia physodes, *Usnea glabrata*, *U. hirta*, *U. subfloridana* & *U. viktoriana*	< 95% (*Hypogymnia*/*Usnea*)	100%
Japewia gyrophorica & *J. tornoensis*	97–98.5%	98.3–99.8%
Lecanographa amylacea & *L. lyncea*	97%	99.5%
Lecanora allophana & *L. impudens*	98.5%	100%
Lecanora carpinea & *L. subcarpinea*	95%	99.5%
Lecanora circumborealis & *L. pulicaris*	99%	100%
Lecanora excludens & *L. intumescens*	93%	> 99%
Lecanora praesistens & *L. sinuosa*	NA	100%
Lecanora stanislai & *L. strobilina*	> 99%	100%
Lecidella achristotera & *L. flavosorediata*	95–96%	99.5–100%
Lepraria eburnea, *L. elobata*, *L. incana*, *L. jackii*, *L. rigidula* & *L. umbricola*	~ 92–98%	> 99%
Melanelixia epilosa & *M. glabra*	96%	> 99%
Melanelixia epilosa & *M. subargentifera*	98.5%	> 99.5%
Melanohalea elegantula & *M. exasperata*	94.5%	> 99%
Melanohalea elegantula & *M. laciniatula*	99.5–100%	100%
Micarea melaeniza & *M. nigella*	92%	100%
Mycoblastus affinis & *M. alpinus*	> 99%	> 99.5%
Myriolecis persimilis & *M. sambuci*	97.5%	100%
Nephromopsis laureri & *Vulpicida pinastri*	94.5%	> 99%
Pannaria conoplea & *P. rubiginosa*	95.5%	99.5%
Parmelia encryptata & *P. sulcata*	97–98%	> 99.5%
Parmelia ernstiae & *P. submontana*	~ 98%	100%
Parmelia serrana & *P. saxatilis*	99.5%	99.5%
Parmelina pastillifera & *P. tiliacea*	> 99%	99.5%
Parmeliopsis ambigua & *P. hyperopta*	97%	> 99%
Parvoplaca (all included species)	94–97.5%	> 99%
Peltigera collina & *P. degenii*	~ 88%	> 99%
Pertusaria alpina & *P. constricta*	> 99.5%	NA
Pertusaria alpina & *P. leioplaca*	94–95%	> 99%
Pertusaria coronata & *P. pertusa*	NA	> 99.5%

Species pairs / groups	Identity in ITS	Identity in mtSSU
Pertusaria flavida & *P. hymenea*	95%	99.5%
Pertusaria macounii & *P. pertusa*	95–100%	> 99.5%
Phaeophyscia ciliata & *P. orbicularis*	99–100%	99–100%
Phlyctis agelaea & *P. argena*	< 92.5%	100%
Physcia adscendens, *P. tenella* & *P. leptalea*	> 99.5%	> 99.5%
Physcia biziana & *P. stellaris*	> 98.5%	100%
Physcia spp. (most species)	95–100%	> 99%
Physconia spp. (all included species)	~ 92–98%	(99.5–)100%
Physconia perisidiosa & *P. venusta*	> 99%	100%
Placynthiella dasaea & *P. icmalea*	99–100%	100%
Protoparmelia hypotremella & *P. oleagina*	92%	> 99%
Pycnora praestabilis & *P. sorophora*	99–100%	99.5–100%
Pyrenula chlorospila & *P. macrospora*	96.5%	> 99.5%
Ramalina spp. (the majority of species)	mostly < 97%	99.5–100%
Rinodina archaea, R. orculata & *R. trevisanii*	NA	100%
Rinodina capensis & *R. subpariata*	92%	> 99%
Rinodina freyi & *R. sophodes*	91%	99.5%
Rinodina orculata & *R. trevisanii*	100%	100%
Rinodina tenuis & *R. willeyi*	95–96%	> 99%
Rostania effusa & *R. populina*	NA	> 99%
Scoliciosporum gallurae & *S. sarothamni*	100%	NA
Scytinium aragonii & *S. magnussonii*	91%	99–99.5%
Scytinium fragrans & *S. magnussonii*	NA	99.5–100%
Toniniopsis dissimilis & *T. separabilis*	97%	> 99%
Usnea barbata, *U. perplexans* & *U. substerilis*	99.5–100%	100%
Usnea glabrata, *U. hirta*, *U. subfloridana* & *U. viktoriana*	100% (*U. viktoriana* 99.5%)	100%
Xanthomendoza fallax & *X. huculica*	> 98.5%	100%
Xanthoria candelaria & *X. polycarpa*	99.5%	> 99.5%
Xylographa pallens & *X. rubescens*	~ 99%	100%
Xylographa parallela & *X. soralifera*	92%	> 99%
Xylopsora caradocensis, *X. friesii* & *Xylopsora* sp.1 (sorediate)	~ 98%	> 99%

Diverging genotypes within traditionally understood species

In many cases, we sequenced the same traditionally recognized species two or more times and usually obtained either identical or very close sequences (with more than 97% identity in ITS and 99% in mtSSU). However, we also discovered a number of species within which two or more diverging genotypes occur in one or both barcodes (37 cases in Table 3). In some cases, phenotypic characters distinguishing diverging genotypes have been found, supporting the existence of two or more previously undifferentiated species within traditionally defined species. For example, within the species *Buellia disciformis*, characterized by, among other things, an inspersed hymenium, individuals with a clear hymenium are included and these individuals are also genotypically distinct. In other cases, individual genotypes differ ecologically, such as lowland vs. upland populations of *Micarea globulosella*. More complex cases are known where more than two diverging genotypes have been found within a traditional species. A typical and well-known example is *Graphis scripta*, where four morphologically slightly different individuals yielded four significantly different ITS barcode variants.

Table 3. Traditionally understood species that include two or more diverging genotypes identical < 96% in at least one of the barcodes. Empty cells indicate that the information is not available.

Original species	No. of distinct genotypes	Shared identity in ITS	Shared identity in mtSSU	Differences in phenotype/ecology
Anisomeridium polypori	at least 2	90%		
Arthonia mediella	3	90–95%		
Bacidia absistens	2	92.5%		
Bacidina chloroticula	2	94.5%		
Bryostigma muscigenum	2	89%		
Buellia disciformis	2	88–89%		Yes
Calicium salicinum	2	93%	98%	
Catinaria atropurpurea	at least 2	89%	94%	Yes
Coenogonium luteum	at least 3	82–90%	90–95%	
Coenogonium pineti	2	83%	94%	
Dictyocatenulata alba	2		91%	Yes
Graphis scripta	at least 4	80–90%		Yes
Ikaeria aurantiellina	2	91–92%		
Japewia aliphatica	2	93%		
Lecania croatica	2	94–95%	98.5%	
Lecanora phaeostigma	2	91–92%	99%	
Lecanora thysanophora	2	93–95%	99.5–100%	
Lecidea betulicola	2		95%	
Lecidella elaeochroma	at least 2	94%	99%	
Micarea globulosella	2	84%		Yes
Micarea isidioprasina	2	95%		
Micarea nowakii	2	95%	98%	
Micarea prasina	2	95%	98%	
Mycobilimbia epixanthoides	2	92%	97%	
Mycoblastus caesius	2 (+ intermediate)	94%	97%	
Mycoblastus sanguinarius	2	90–93%	96–97%	
Normandina acroglypta	2	89%	98.5%	Yes
Opegrapha niveoatra	2	90%	96%	
Pertusaria pupillaris	2	93%	>99%	
Pyrenula laevigata	2		92%	
Sclerophora pallida	2		95.5%	
Scoliciosporum chlorococcum	2	~ 80%	~ 90%	Yes
Scoliciosporum umbrinum	at least 3	95%, 95%, 94%		
Scytinium subtile	2	88%	97.5%	Yes
Swinscowia jamesii	2	86%		
Trapeliopsis glaucolepidea	2	94–96%		
Violella fucata	2	91%		

Known-unknowns

For the identification of lichens from environmental samples, our aim was to include in the reference database not only sequences from known species, but also sequences from samples that could not be linked to known names. We obtained barcode sequences for a number of currently unknown (and possibly undescribed) species. Highest numbers were in the genera *Bacidina* (6) and *Micarea* (8). Single hitherto unknown species have been recognized in other genera, e.g. *Biatorella*, *Cryptodiscus*, *Psilolechia* and *Solitaria* (= *Caloplaca* s. lat.). Some sequenced taxa with unknown generic position were only

assigned to family (*Collemataceae*, *Verrucariaceae*), or subclass (*Lecanoromycetidae*). We have also discovered five previously undescribed species of sterile sorediate crusts, which we describe formally below.

Discussion

Need for a reference barcode database

Accurate reference data are of fundamental importance to any molecular identification tool. Many theoretical works deal with molecularly defined taxa that are not assigned to organism names, often referred to as operational taxonomic units, i.e. OTUs (e.g. Brunbjerg et al. 2019). In many cases it is useful to go beyond OTU's and identify the DNA sequences with existing organism names. For example, we may be interested in knowing which endangered species are present in the sample. Several lichenological studies have attempted to identify sequences from environmental samples, but all have encountered the problem of insufficient reference data. Wright et al. (2019) used the UNITE database (https://unite.ut.ee; Abarenkov et al. 2010) for the identification, which contains some taxonomically screened sequences from NCBI (GenBank). This is undoubtedly a useful resource for assigning names to sequences, but its major drawback is its insufficient taxonomic coverage. Keepers et al. (2019) created their own reference database of whole nuclear ribosomal DNA (rDNA) complexes for 273 species, a significant feat, but their database is still very incomplete, even for local use in their area (Appalachian Mountains, USA). Henrie et al. (2022) even limited themselves to assigning OTU's to families only.

The NCBI database is undoubtedly the most extensive genotype database in existence. Therefore, it is possible to use it for assigning names to sequences from environmental samples. However, there are several fundamental problems. (i) This database, although the most extensive, is still very incomplete, and this is also true for the most commonly used barcodes: ITS and mtSSU. (ii) A significant number of sequences are incorrectly named. (iii) Some sequences are incorrectly edited (i.e. contain erroneous characters), some are too truncated, and some have had difficult-to-align sections cut out.

As regards point (i) above, there are still significant gaps in the sequencing of some genera, but also of larger taxonomic groups. For example, the ITS barcode is very poorly represented within the entire class *Arthoniomycetes*. The same is true for the large families *Collemataceae* and *Malmideaceae* and for the large genera *Candelariella*, *Lecidea* s. lat. and *Rinodina*.

As regards (ii), we commonly encounter incorrectly assigned sequences in GenBank (cf. Nilsson et al. 2012). In some cases, these errors are caused by an incomplete knowledge of taxonomy and sequences are assigned names of closely related but distinct taxa. In other cases, we encounter assignments to completely unrelated taxa (e.g. comments on *Brownlielloideae* in Wilk et al. 2021). ITS sequences of lichenicolous fungi are sometimes assigned to lichens, for example members of *Leotiales* were assigned to *Micarea* (Andersen & Ekman 2004) or *Tremellales* to *Gyrographa gyrocarpa*, *Opegrapha vermicellifera* and *Pertusaria pertusa* (Resl et al. 2015, Marthinsen et al. 2019).

The situation is exemplified by the incorrect annotation of NCBI sequences to the species *Arthopyrenia salicis*. Lumbsch et al. (2005) and Schmitt et al. (2005) provided

sequences of three loci of this species for the first time. Based on these sequences, Nelsen et al. (2011) placed *A. salicis* in *Pleosporales*. Ecologically diverse fungi were assigned to the name *A. salicis* based on similarities to original sequences. For example, Gnavi et al. (2017) assigned it to a fungus associated with seaweeds. However, the real sequences (specifically ITS) of *A. salicis* were only obtained by Marthinsen et al. (2019), and we have repeatedly obtained both ITS and mtSSU target sequences. According to our data, *A. salicis* belongs in the vicinity of *Naetrocymbe punctiformis*, i.e. to *Capnodiales*, where a few other lichenized fungi also belong (e.g. Muggia et al. 2008).

As regards point (iii), it is difficult to prove incorrect editing, but the strikingly frequent unique nucleotides at the beginnings and ends of the sequences may suggest this (Nilsson et al. 2017). Easier to demonstrate is the excision of difficult-to-align sections that has been done in some classical taxonomic works. For example, Andersen & Ekman (2005) provided the mtSSU dataset to the genus *Micarea*, which includes a number of valuable sequences for rare species, but all sequences are excised in several places.

A relatively successful attempt to obtain a comprehensive database of ITS barcodes for Nordic lichens was presented by Marthinsen et al. (2019). They obtained sequences for 507 species which were, however, only 20–21% of the then accepted Nordic lichenized species (Marthinsen et al. 2019). In terms of European epiphytic lichens, this database is still very incomplete. We have taken the next step and compiled a robust basis for the ITS and mtSSU barcodes of European epiphytic lichens, which currently covers more than 90% of the epiphytic species known in central Europe, but lower coverage is expected for the Mediterranean, Euoceanic and Nordic regions.

Limitations of ITS and mtSSU barcodes

Schoch et al. (2012) proposed ITS as a universal fungal DNA barcode because of its ease of amplification and its considerable sensitivity to distinguish closely related species. Keepers et al. (2019) proposed the use of a whole genome shotgun to obtain a nuclear ribosomal DNA (rDNA) complex (also containing ITS) and use this entire section as a barcode. We opted for classical amplicon-based sequencing of the ITS for reasons of significantly lower cost, less methodological complexity and above all for easier control of correct assignment of target species to specific sequences.

ITS is a fairly universal barcode for lichenized fungi, but it has several shortcomings. One is the low sequencing success in some groups, probably due to the poor amplifiability of the target species (e.g. Kelly et al. 2011). For example, our numerous attempts to sequence members of *Arthoniomycetes* resulted in obtaining sequences of lichenicolous *Tremellales* (*Kockovaella* spp.) when using Sanger sequencing. In the case of NGS sequencing (PacBio), we ended up with a mixture of fungi, none of which matched the target lichen. Even after considerable effort, we were unable to obtain required sequences for some Arthoniomycetes. Another feature of ITS as a DNA barcode is the substantial infraspecific variability in some species (see below).

Mitochondrial SSU is the second most frequently sequenced DNA locus in lichenized fungi after ITS. It can be easily obtained in some groups where ITS is difficult to amplify (*Arthoniomycetes*, *Collemataceae*, *Malmideaceae*, etc.). We chose mtSSU as a complementary DNA barcode, as it enables detection of some species without sequenced ITS, however, it is definitely not advisable to use mtSSU as an exclusive DNA barcode because it has a low sensitivity to discriminate closely related species (Table 2).

Diverging genotypes within traditional species and their impact on metabarcoding

Our data, as well as a number of previous studies (e.g. Palice & Printzen 2004, Vondrák et al. 2020), revealed a number of traditionally conceived species comprising two or more widely divergent genotypes. Many contemporary taxonomic works even place such genotypes at the level of species and thus a number of cryptic and semicryptic species are described within traditional morphospecies (e.g. Leavitt et al. 2013, 2016, Magain & Sérusiaux 2015, Alors et al. 2016, Divakar et al. 2016, Guzow-Krzemińska et al. 2019, Launis et al. 2019, Košuthová et al. 2022). However, describing new cryptic species solely on the basis of distinct ITS genotypes is risky because ITS can exhibit considerable infraspecific/intragenomic variation in fungi (e.g. Lücking et al. 2020, Bradshaw et al. 2023).

Our study does not delve deeply into taxonomic issues of diverging genotypes within traditional species, but we would like to emphasize their importance for evaluating data in metabarcoding studies. The presence of only one or a few genotypes from extensive infraspecific variation in the reference database leads to underestimation or even false absence of particular species in the samples. If we use a high similarity threshold for matching to reference sequences, diverging genotypes missing in the database will not be assigned to a taxon name. If, on the other hand, we use a low threshold, there is a risk of incorrectly assigning these genotypes to other species.

Only a small part of these infraspecific genotype complexes can be revealed within the restricted time frames of our projects. For example, within the "species" *Graphis scripta*, which has already been studied both phenotypically (Neuwirth & Aptroot 2011) and genotypically (Kraichak et al. 2015), we expect many more distinct ITS genotypes than the four we have identified. To refine the identification, it is therefore necessary to continue adding newly identified genotypes to the Martin7 database.

Martin7: towards a database of DNA barcodes for European epiphytic lichens

We are now at the stage where we have at least one of the two selected DNA barcodes for the vast majority of central-European epiphytic lichens. For example, more than 98% of the epiphytic lichen species listed in the Atlas of Czech lichens (Malíček et al. 2023) are represented in the database. Only a few true rarities (e.g. *Micarea vulpinaris* and *Waynea giraltiae*) and little-known species (e.g. *Arthonia reniformis* and *Lecania koerberiana*) are missing. Our intention is to develop Martin7 to cover the whole of Europe in the future, by adding lichens especially from Mediterranean and Euoceanic regions. European species without ITS and mtSSU sequences in NCBI are listed in Supplementary Table S3.

Taxonomic insights and novelties

Large-scale sequencing of epiphytic lichens across the taxonomic system has yielded many results either refining or fundamentally changing the taxonomic status of many species and genera. The taxonomic notes are listed below in alphabetical order. This paper is not primarily taxonomic and therefore we do not draw formal nomenclatural conclusions here, but merely point out interesting differences from current taxonomic views that may serve as clues for further research.

Andreiomyces obtusaticus may contain anthraquinones in varying amounts

Hodkinson & Lendemer (2013) distinguished the European *Andreiomyces obtusaticus* from the North American *A. morozianus* by its lack of isousnic acid and anthraquinones in the thallus. Their claim was based on research by Tønsberg (1992) who explicitly mentioned neither isousnic acid nor anthraquinones in his description of *Lepraria obtusatica*, as well as on their own study of one of the isotypes of *L. obtusatica*. However, Tønsberg (1992) noticed a non-constant low content of an unidentified yellow pigment revealed by TLC, that may eventually prove to be an anthraquinone. We have sequenced two unidentified specimens of *Andreiomyces*, one with a distinct anthraquinone pigmentation in spots, and one with anthraquinones restricted to the hypothallus. They have almost identical ITS sequences (> 99% identity) and match *A. obtusaticus* (AF517896, sub *Lepraria obtusatica* in Ekman & Tønsberg 2002) with identities close to 100%. ITS sequences of *Andreiomyces morozianus* are distinct from *A. obtusaticus* with shared identities about 97%. The chemical differences used to distinguish the two species are not as obvious as previously thought.

Arthonia apatetica, *A. faginea* and *A. tenellula* belong to *Bryostigma*

These species are closely related to *Bryostigma biatoricola* and *B. muscigenum* and in the Bayesian trees for both loci studied, they form a distinct supported clade within the *Arthoniomycetes*. *Arthonia faginea* is a little-known epiphytic species with 2-septate ascospores, which is common on smooth bark of deciduous trees in montane forests of the Caucasus. It is very close to the saxicolous *B. lapidicola* in NCBI (KJ850997; 99% identity, 69% cover in mtSSU).

Arthopyrenia salicis: supposed classification in *Capnodiales*

The type species of *Arthopyrenia*, *A. cerasi*, was sequenced and, together with a few other *Arthopyrenia* species, belongs to *Trypetheliales* (Thiyagaraja et al. 2021). In contrast, *A. salicis* has long been considered a representative of *Pleosporales* (Nelsen et al. 2011, Thiyagaraja et al. 2021), but this taxonomic concept is based on erroneously assigned DNA data (see the fifth paragraph of the discussion). Our identical ITS sequences of three specimens match *A. salicis* sequences provided by Marthinsen et al. (2019) with 98–99% identities. Other relatives are *Capnodiales* spp. with shared identities up to 88%. Our mtSSU also has the closest NCBI BLAST matches to *Capnodiales* spp. (e.g. *Mycosphaerella* and *Racodium*) with shared identity up to 90%. According to our data, the morphologically similar *Naetrocymbe punctiformis* is related to *A. salicis*, with identities 85% in ITS and 91% in mtSSU. Only a few orders of *Dothideomycetes* include lichenized fungi and semilichens, so the placement in *Capnodiales*, comprising several lichen genera (e.g. Muggia et al. 2008, Lücking et al. 2016), is not surprising.

Biatoridium: supposed classification in *Lichinomycetes*

Díaz-Escandón et al. (2022) expanded the previously narrowly defined class *Lichinomycetes* to include a number of previously orphaned lichen lineages, such as those from the *Candelariomycetes* and *Coniocybomycetes*. In the current understanding, this class includes previously unclassifiable lichen genera, such as *Piccolia*, *Sarcosagium*,

Thelocarpon and *Vezdaea*. According to our data, these genera are not very similar in their ITS and mtSSU sequences, but they share the common feature that their closest NCBI BLAST generally matches those of orphan genera, such as *Candelariella*, *Pycnora*, *Sarea* and *Umbilicaria*. We obtained ITS and mtSSU from *Biatoridium delitescens* and *B. monasteriense*, and their sequences also have the closest relatives in the orphan genera of *Lichinomycetes*, namely *Candelariella*, *Pycnora* and *Sarea* in ITS with identities up to 90%, and e.g. *Candelariella*, *Myriospora*, *Peltula*, *Sarcosagium* and *Thyrea* in mtSSU with identities up to 86%. Similar BLAST results are shown in the genus *Strangospora*, in which, however, some *Acarosporomycetidae* are also close and therefore the taxonomic placement is unclear.

Dictyocatenulata is an anamorphic morphotype in *Thelenella*

ITS sequences of *Dictyocatenulata alba* had already been obtained by An et al. (2012), who placed this sporodochia-forming lichen in *Ostropomycetidae*. We newly provide mtSSU sequences from nine *Dictyocatenulata* specimens and both ITS and mtSSU have the most similar sequences in the genus *Thelenella*, including the type species, *T. modesta* (our data). MtSSU sequences of *Dictyocatenulata alba* include two diverging genotypes. The more common genotype is 95% identical to our sequences of *Thelenella muscorum* subsp. *muscorum*. The other genotype, obtained from a single specimen, was 98% identical to *T. vezdae*. We hypothesise that *Dictyocatenulata* represents anamorphic stages of unknown (or unsequenced) species of *Thelenella*.

Eopyrenula belongs to *Ostropomycetidae*

Eopyrenula was an unassigned genus within the subphylum *Pezizomycotina* in Lücking et al. (2016). It was subsequently placed in *Dacampiaceae*, *Dothideomycetes*, based on morphological characters (Doilom et al. 2018). Our mtSSU and ITS sequences of the species *E. avellanae* (both anamorph and teleomorph sequenced) and *E. leucoplaca* demonstrate that these species belong to *Ostropomycetidae*, close to *Ostropales* (e.g. *Stictis*).

Exarmidium inclusum is a semilichen from *Ostropomycetidae*

Traditionally considered to be a non-lichenized wood-dwelling fungus, which explains the absence of this species from the lichenological literature. We, however, consider it a semilichen (sensu Vondrák et al. 2022), as it is frequently associated with *Stichococcus*-like algae that form greenish patches surrounding perithecia. On the basis of a blue reaction of ascal tips with iodine, Barr & Boise (1985) and Aptroot (1998) placed *Exarmidium* in *Hyponectriaceae* (*Sordariomycetes*, *Xylariales*). *Exarmidium* may be heterogeneous, but *E. inclusum* most probably belongs to *Ostropomycetidae*. Both ITS and mtSSU sequences have the closest NCBI BLAST hits to *Cryptodiscus* and *Xylographa* with identities up to 83% (ITS) and 90% (mtSSU).

Lecanora cadubriae is closely related to *Myochroidea porphyrospoda*

Myochroidea was created to accommodate species from the *Lecidea leprosula* group (Printzen et al. 2008). The genus is placed in *Lecanorales* without closer (family) affiliation (Lücking et al. 2016), and DNA sequence data for it are still lacking in NCBI. We

obtained mtSSU from *M. porphyrospoda*, which turned out to be close to three other species in our dataset. It shares about 95% identity with *Lecidea nylanderi* and *Miriquidica majae* and even 98.5% with *Lecanora cadubriae*. If a close relationship of *M. porphyrospoda* to the type species of *Myochroidea* (*M. rufofusca*) is demonstrated by further molecular studies, then *Lecanora cadubriae* is also likely to belong to this genus, which appears to be related to *Miriquidica* (*Lecanoraceae*).

Lecidea betulicola and *Lecidea coriacea* are apothecial morphotypes of *Cheiromycina*

On the basis of nuLSU and mtSSU sequences, Muggia et al. (2017) placed the genus *Cheiromycina* in the predominantly tropical family *Malmideaceae*, but they did not reveal any temperate relatives. According to our mtSSU and ITS data, some temperate species of *Lecidea* s. lat. also tend to be related to *Cheiromycina*, and two of them, *L. betulicola* and *L. coriacea*, are apparently congeneric with *Cheiromycina* (Supplementary Data S2). Sequences of mtSSU from *L. betulicola* represent two genotypes sharing 95% identity and do not have the exact match to any sequences of *Cheiromycina*, but ITS and mtSSU of *L. coriacea* are identical to some of *C. petri*. Moreover, several times we have observed thalli of *L. coriacea* with apothecia and also with sporodochia of *C. petri*.

Leptorhaphis and *Rhaphidicyrtis* are related genera in *Phaeomoniellales*

Leptorhaphis has recently been assigned to *Pleosporales*, *Dothideomycetes* (Harris 1995) and *Rhaphidicyrtis* to *Pyrenulales*, *Chaetothyriomycetidae* (Lücking et al. 2016). DNA data for both genera have not yet been available in NCBI. We obtained identical ITS sequences from three samples of *R. trichosporella* as well as ITS sequences for *Leptorhaphis atomaria*, *L. epidermidis* and *L. maggiana*. The two genera have similar sequences sharing approximately 91% identity, and, according to NCBI BLAST results, both genera are close to various non-lichenized representatives of *Phaeomoniellales* (*Chaetothyriomycetidae*) with identities up to 98%. *Phaeomoniellales* includes mainly endophytes and plant pathogens, but also lichens of the genus *Celothelium* (Chen et al. 2015). The mtSSU sequences of *Leptorhaphis atomaria* and *L. maggiana* also indicate affiliation with *Chaetothyriomycetidae*, *incertae sedis*, but not with *Pyrenulales*, where *Rhaphidicyrtis* was assigned.

Leptorhaphis is probably congeneric with *Xenocylindrosporium* involving alleged plant pathogens. ITS sequences of *Xenocylindrosporium* spp. are more than 90% identical with our sequences of three sequenced *Leptorhaphis* species. Moreover, *X. margaritatum* has ITS about 98% identical with *L. atomaria*. Morphological description of *Xenocylindrosporium* is based on cultures producing cylindrical and curved conidia (Crous et al. 2009) identical in shape to those of *Leptorhaphis* (Aguirre-Hudson 2009). Conidia of *X. margaritatum* (Spies et al. 2020) are even nearly identical in size to the closely related *L. atomaria*.

Mycoblastus caesius has an unsettled position in *Lecanorales*

Mycoblastus caesius does not belong to *Mycoblastus* s. str. (*Mycoblastaceae*). We have obtained ITS and mtSSU sequences from a number of specimens and it appears that *M. caesius* should be placed in *Lecanorales*, but its family affiliation is not clear. Its highest

NCBI BLAST hits are only around 86% in both mtSSU and ITS with e.g. *Pilocarpaceae*, *Psoraceae* and *Ramalinaceae*, but these results are rather irrelevant.

Mycoporum antecellens: supposed classification in *Capnodiales*

The genus *Mycoporum* is traditionally placed in *Pleosporales* (e.g. Lücking et al. 2016), but has so far not been sequenced. We obtained nearly identical mtSSU sequences from two samples and these have the closest NCBI BLAST matches to *Capnodiales* (up to 88% identity). Our data indicated a close relationship to *Naetrocymbe punctiformis*, whose sequences have ~ 92% identity with *M. antecellens*. It is still not clear whether other *Mycoporum* species are related to *M. antecellens* or also belong in *Capnodiales*.

Naetrocymbe punctiformis: supposed classification in *Capnodiales*

Naetrocymbe is an old genus recently placed in *Naetrocymbaceae*, *Pleosporales* (Harris 1995). According to Harris (1995), it is distinguished from the similar genus *Arthopyrenia* by anatomical differences in pseudoparaphyses, asci and conidia. Its representatives had not yet been sequenced and thus their phylogenetic position is not yet known. We sequenced *N. punctiformis* and obtained identical ITS from two specimens. Their closest NCBI BLAST matches are to *Capnodiales* spp. and *Mycosphaerellales* spp. We obtained identical mtSSU sequences from five specimens; they are closest to the lichenized *Cystocoleus ebeneus* (*Capnodiales*) with shared identities 92.5–93.5%.

Harris (1995) synonymized *Sporoschizon petrakianum*, a species characterized by ascospore fragmentation, with *N. punctiformis*, noting that he did not observe this fragmentation in the studied isotype. In some specimens collected in central Europe, we observed this disintegration of originally two-celled ascospores into single-celled fragments, leading us to conjecture that this may be a species of the heterogeneous genus *Strigula* (*Strigula* sp. 1 in Vondrák et al. 2022). The mtSSU sequences of these specimens are identical to sequences from typical *N. punctiformis* without disintegrating ascospores, confirming the statement of Harris and disproving the conjecture about *Strigula*.

Sphaeronema truncatum is a common European semilichen from *Ostropomycetidae*

We have frequently observed a semilichen long-recognized by European lichenologists, but unnamed (known-unknown). It has tall black and "chimney-shaped" pycnidia, typically with a white drop of released conidia at their top, which slightly resemble overgrown pycnidia of *Micarea misella*. It grows on soaked, slowly decaying wood of conifers and is associated with *Stichococcus*-like algae that usually form greenish spots surrounding pycnidia. According to our DNA data, it appears to be a member of *Ostropomycetidae*. Our ITS sequences from seven specimens share the identity over 99.5% and they have the closest NCBI BLAST to *Mulderomyces* (~ 87% identity) and *Elongaticonidia* (~ 86.5% identity); both genera are classified in *Ostropales*. Our mtSSU has the closest BLAST to lichens from *Ostropales* (*Thrombium*) and *Trapeliales* (*Rimularia*) with shared identities up to 87.5%.

We found a plausible old name, *Sphaeronema truncatum*, for this taxon, and then we compared a syntype (Fries: Scleromyceti Sueciae 105; PRM773237) with recent material and found that they match morphologically. *Sphaeronema* (also as '*Sphaeronaema*') is

a long-forgotten genus including *Coelomycetes* with raised pycnidia and with a white drop of released conidia at their top (Fries 1818). The genus is likely heterogeneous and its correct placement is currently unclear.

Wadeana: supposed classification in *Acarosporomycetidae*

Wadeana was considered an unassigned genus within the subphylum *Pezizomycotina* in Lücking et al. (2016), but our ITS of *W. dendrographa* has the closest relatives in *Acarosporaceae* with the shared identities up to 85%. Its placement in *Acarosporomycetidae* is supported by the phenotype (polysporic asci). *Wadeana* would be the first reported lichen in *Acarosporomycetidae* with trentepohlioid photobiont.

New species

Bacidina acerina Vondrák, Svoboda et Malíček, spec. nova

MycoBank: MB#847418; Fig. 1A
Etymology: Named after its common occurrences on *Acer campestre* in central-European lowlands.
Type: **Czech Republic**. Central Bohemia: district Rakovník, Skryje, Týřovické skály rock, alt. 280 m, 49°58'53.8"N, 13°47'37.0"E, on bark of *Acer campestre*, 21 Oct. 2020, J. Vondrák 24355, PRA, **holotype**.
Type sequences: ITS (OK332880); mtSSU (OK465494).

Diagnostic characters: Extensive green sorediate crust with diffused soralia, soon merged into a uniform green coat. Apothecia very rare and pycnidia unknown. Morphological differentiation from similar species (e.g. *Bacidina* spp.) is not always possible with certainty.

Morphology-anatomy: Green sorediate crust, fading rapidly into pale grey in herbarium. Formed of diffused soralia, soon merging into extensive patches, up to several cm diam. (But delimited punctiform soralia present on the single fertile specimen.) Soredia farinose, 15–25 µm in diam., occasionally joining into consoredia, up to 40 µm in diam. Thallus surrounding soralia usually invisible, endophloedal or very thin epiphloedal, however, patches of thicker (up to 0.1 mm), well-developed and almost subsquamulose thallus observed in the fertile specimen. Photobiont trebouxioid, globose, 4–11 µm in diam. Apothecia known from a single specimen (Malíček 6441), biatorine, 0.2–0.4 mm in diam., convex, pale beige, without internal pigmentation. Slightly raised apothecial margin ± distinct only in young apothecia, sometimes slightly paler than the discs. Proper exciple colourless, without crystals, composed of radiating branched ± thin-walled hyphae; with cell lumina up to 10 µm long and up to 3 µm wide. Hypothecium colourless. Hymenium colourless, 35–40 µm high. Paraphyses 1–2 µm thick, unbranched. Epihymenium colourless. Asci: clavate, 8-spored. Ascospores bacilliform with rounded apices, 3-septate, colourless, 20–30 × 2–3 µm. Pycnidia unknown.

Chemistry: No substances detected by TLC. Spot tests negative, UV–.

Ecology and distribution: Epiphytic on nutrient-rich bark of a broad spectrum of deciduous trees and shrubs. Recorded with certainty on *Acer campestre*, *A. platanoides*, *Alnus glutinosa*, *Carpinus orientalis*, *Sambucus nigra*, *Tilia* sp. and *Ulmus laevis*. It is frequently a predominant species in lowland forests of central Europe, often covering large patches of bark in shaded sites. We expect it to be broadly distributed in Europe, as it is common in central Europe and also occurs in warm areas of the Caucasus.

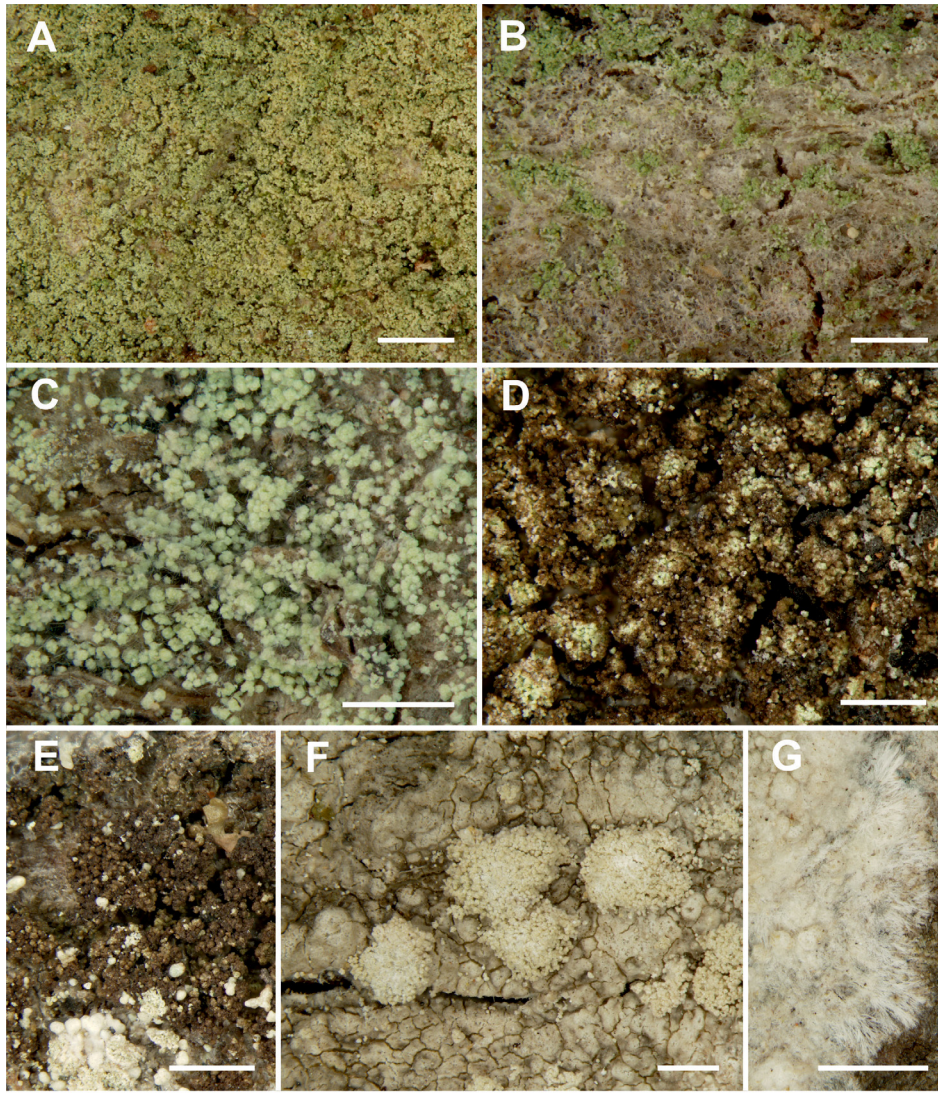

Fig. 1. New species: (A) *Bacidina acerina*, Vondrák 24353, PRA, sorediate thallus. (B) *Bacidina paradoxa*, Palice 33646, PRA, scattered soralia. (C) *Chrysothrix fagicola*, holotype, sorediate thallus with needle-like crystals of zeorin. (D, E) *Japewia gyrophorica*, Palice 32415, PRA, older thallus with eroded soralia (D), holotype, young thallus (E). (F, G) *Lecanora arachnoidea*, Vondrák 13908, PRA, thallus with soralia (F), fibrillous prothallus (G). All scales: 0.5 mm.

Similar species: *Bacidina acerina* is mostly known sterile and thus the thallus can be mistaken for other green sorediate lichens; however, the diffuse character of the poorly delimited soralia is usually characteristic. *Bacidia hyalina*, *Bacidina* spp. (sorediate species), and *Lecania croatica* tend to be similar, but usually have discrete, at least partly delimited soralia. The only fertile specimen from Ukraine (Malíček 6441) is quite distinct from the numerous sterile specimens. It has scattered punctiform soralia and resembles

the recently described *B. maculans* (Ekman 2023), which is known from warmer areas of Scandinavia and its ecology corresponds to *B. acerina*. *Bacidina maculans* has apparently better developed thallus, smaller photobiont cells and thinner ascospores with frequently more than 3 septa. Unfortunately, *B. maculans* was described without providing DNA barcode data demonstrating its relationship to *B. acerina*.

DNA data: The ITS sequences obtained from seven specimens are 97% identical and have the closest NCBI BLAST to *Bacidina varia* and *B. friesiana* which are up to 93% identical. In the Bayesian tree, *B. acerina* belongs to a clade including *B. friesiana*, *B. phacodes* and *B. piceae* (Supplementary Fig. S1). The mtSSU sequences from the nine specimens are 99.5% identical, and the closest NCBI BLAST results to them are representatives of *Bacidina*, *Toniniopsis* and *Waynea* which are up to 95% identical. However, the position in the Bayesian mtSSU tree places *Bacidina acerina* in the clade with *B. phacodes* and *B. piceae* (Supplementary Fig. S2).

Paratypes: **Austria.** Lower Austria: national park Thayatal, Hardegg, in valley of Fugnitz stream, altitude 340 m, 48°50'28.4"N, 15°50'49.2"E, on roots of *Alnus glutinosa* at stream, 23 Mar. 2022, J. Vondrák 25954, PRA. **Czech Republic.** Central Bohemia: district Rakovník, Skryje, Týřov Protected Area, ruin of castle Týřov, altitude 320 m, 49°58'24.5"N, 13°47'24.5"E, on bark of *Ulmus*, 12 Aug. 2018, J. Vondrák 20656, PRA. Ibid.: Týřovické skály rock, alt. 290 m, 49°58'53.9"N, 13°47'40.8"E, on bark of *Acer campestre*, 21 Oct. 2020, J. Vondrák 24353, PRA. Ibid.: well-lit forest on rocky, steep S–SSW-facing slope of the hill Průhonek (472 m), alt. 310 m, 49°57'52.5"N, 13°48'41.9"E, on bark of *Tilia*, 6 Oct. 2020, Z. Palice 30104, PRA. Ibid.: Týřovice, Velká pleš National Nature Reserve, natural deciduous forests in valley of a brook SSE of U Rozvědčíka pub, 49°59'51.7"N, 13°48'53.2"E, alt. 250–350 m, on bark of *Acer platanoides*, 5 Oct. 2022, J. Malíček 15877, herb. Malíček. Southern Moravia, distr. Břeclav: Ranšpurk Nature Reserve, a flood-plain forest around the largest blind arm, alt. 153 m, 48°40'46.0"N, 16°56'46.0"E, on bark of *Sambucus nigra*, 17 Sep. 2020, Z. Palice 29823, PRA. **Russia.** Caucasus: Adler, Kazachiy Brod, forest on limestone cliff above river Mzymta, alt. 290 m, 43°31'41.7"N, 40°0'9.7"E, on bark of *Carpinus orientalis*, 29 Jun. 2019, J. Vondrák 22463, PRA. **Ukraine.** Zakarpattia Oblast Province, Berehovo, Kvasovo: flood-plain forest Otok 1.5 km N of village, close to Mala Borzhava River, 48°12'35"N, 22°46'08"E, alt. 120 m, on bark of *Acer campestre*, 22 Oct. 2013. J. Malíček 6441 & J. Vondrák, herb. Malíček (fertile! & confirmed by ITS and mtSSU sequence).

Bacidina paradoxa Palice, spec. nova

MycoBank: MB#847420; Fig. 1B

Etymology: Derived from the paradox that, although it is usually the predominant lichen species in its sites of occurrence, it remained unnoticed by lichenologists until recently, even though identifiable by TLC. Moreover, it is unique among representatives of the genus *Bacidina* in possessing secondary lichen substances.

Type: **Czech Republic**. Central Bohemia, Kokořínsko Protected Landscape Area, Deštná, Vrabcov, Mokřady horní Liběchovky Nature Reserve, alt. 245 m, 50°31'32.4"N, 14°31'38.5"E, on bark of *Sambucus nigra*, 7 Oct. 2018, Z. Palice 26142, PRA, **holotype**; UPS L-941465, isotype.

Type sequences: ITS (OQ717326); mtSSU (OQ682876).

Diagnostic characters: Sterile green sorediate species forming extensive patches on roughened bark with soft weathered surface. The soralia producing small farinose soredia are first delimited and permanently discrete or later merging into a more or less uniform sorediate crust. Its unique chemistry, including an unknown depside/depsidone with a high Rf value and zeorin, is diagnostic.

Morphology-anatomy: Thallus crustose, thin, completely immersed, semi-immersed to indistinctly areolate, greyish green, sorediate. Soralia pale pea green, sometimes with a faint yellowish or bluish tint (colours fading in herbarium), irregularly orbicular to prolonged, 0.1–0.5 mm in diam., permanently discrete or later sometimes patchily merging. Soredia farinose, 10–30 (–35) µm in diam. Photobiont small, chlorococcoid, usually 4–10 µm, individually up to 12 µm in diam. Apothecia and pycnidia not seen.

Chemistry: The dominant substance is an unknown depside/depsidone with a high Rf value, slightly above the level of atranorin in all standard solvent systems (A, B', C). Zeorin is present as a minor substance or in traces, sometimes not visible on TLC plates. Additional terpenoids (Rf 5–6 in all three systems) revealed in traces in some specimens but it cannot be completely ruled out that these were contaminant terpenoids from bark. Spot tests negative, UV–.

Ecology and distribution: A dominant species on nutrient-enriched, roughened and often superficially soft bark of deciduous phorophytes. So far recorded on *Acer platanoides*, *Fagus sylvatica*, *Pyrus communis*, *Sambucus nigra, S. racemosa*, *Sorbus aucuparia*, *Quercus petraea*, *Q. pubescens* and *Q. robur*. We expect it to occur also on a broader range of tree and shrub species, and possibly also other substrates since the thalli of studied specimens also overgrow neighbouring epiphytic bryophytes. Usually only a few nitrophilic or nitrogen-tolerant species are associated (e.g. *Anisomeridium polypori*, *Biatoridium monasteriense*, *Candelariella efflorescens* agg., *Lecania cyrtella, L. naegelii*, *Phaeophyscia* spp., *Physcia* spp. and *Piccolia ochrophora*). It prefers fairly well-lit sites, e.g. gaps in forests, but it has also been recorded in more or less shaded places. Its habitats are disturbed sites in natural forests as well as semi-native and secondary woodlands, e.g. forests spontaneously disseminated in areas of former land-use.

Similar species: Epiphytic species with similar appearance include: *Bacidia hyalina*, *Bacidina* spp., *Fellhanera viridisorediata*, *Lecania croatica*, *Halecania viridescens*, *Mycobilimbia epixanthoides* and *Biatora* spp. The unknown substance, which is diagnostic for *B. paradoxa*, shows a similar position to argopsin (slightly above the level of atranorin on TLC plates in three standard solvent systems; after H_2SO_4 application and charring forming a faint greenish-grey spot, LW UV+ yellowish-green), which may lead to confusion with argopsin-containing (Pd+ red) species (e.g. *Biatora efflorescens*, *Halecania viridescens*), when spot reactions are not performed. TLC is recommended for separating *B. paradoxa* from similar species with negative spot reactions, especially sorediate/finely granulose species of *Bacidina* like e.g. newly or recently described *B. acerina* (this paper) or *B. maculans* (Ekman 2023).

DNA data: Identical ITS sequences obtained from three specimens of *B. paradoxa* are about 97% identical with *B. flavoleprosa* and *B. terricola* in NCBI. In the ITS Bayesian tree, *B. paradoxa* is in a polytomy with many species of the genus (Supplementary Fig. S1). Identical mtSSU sequences from the three specimens have no close relatives in NCBI. The closest BLAST hits are *Bacidina* spp. with identities up to 92%. In the mtSSU Bayesian tree, *B. paradoxa* stands in polytomy with e.g. *B. flavoleprosa* and *B. neosquamulosa* (Supplementary Fig. S2).

Paratypes: **Austria**. Lower Austria, Ybbstal Alps, Wilderness Area Dürrenstein, Rothwald – Kleiner Urwald, primeval beech dominated forest on a crest above the valley of Moderbach brook, alt. 1010 m, 47°46'31.0"N, 15°6'11.0"E, on dead branch near the ground of decaying log of *Fagus*, 6 Jul. 2022, Z. Palice 34621, PRA. **Czech Republic**. Northern Bohemia, Jablonec nad Nisou, Jizerské hory Mts, Desná – Jizerka: Rašeliniště Jizerky Nature Reserve, along educational path near the bridge over Jizerka river, alt. 865 m, 50°49'40"N, 15°19'57.0"E, on bark of *Sorbus aucuparia*, 30 Aug. 2013, J. Malíček 5999 & J. Vondrák, herb. Malíček. Southern Bohemia, Šumava Mts, Frymburk, Otovský potok Nature Reserve, boggy meadow/pasture, near the navigational canal Schwarzenberský kanál, alt. 781 m, 48°38'32.2"N, 14°2'58.0"E, on bark of *Sambucus* on open place, 6 Nov. 2021, Z. Palice 32056, 32994, PRA. Ibid.: Prachatice, Záblatí: Saladínská olšina Nature Reserve, the valley of Cikánský potok, alluvial forest, alt. 585 m, 49°0'23"N, 13°55'30.0"E, on bark of *Sambucus racemosa*, 11 Jun. 2022, Z. Palice 33646, PRA. Southern Bohemia, Třeboň area: Stará a Nová řeka Nature Reserve, young forest with veteran trees not far from Novořecká bašta, alt. 435 m, 49°0'3.5"N,

14°50'48.1"E, on bark of dead *Quercus robur*, 22 Oct. 2021, Z. Palice 32993, S. Svoboda & J. Vondrák, PRA. Central Bohemia, Bohemian Karst, Karlštejn – Budňany, Karlštejn Nature Reserve, Mt Prostřední hora (383 m), well-lit oak dominated forest (*Quercus pubescens*) on SSW–SW-facing slope, alt. 343 m, 49°56'15.0"N, 14°10'6.2"E, on bark of *Quercus pubescens*, 7 Oct. 2021, Z. Palice 33292, PRA. Ibid.: Karlštejn – Srbsko, Karlštejn Nature Reserve, a deciduous forest (*Tilia cordata* and *Quercus petraea* dominating) at SSE-facing slope of Mt Doutnáč (433 m), 0.8 km NNW of Kubrychtova bouda, alt. 354 m, 49°57'13.9"N, 14°9'17.3"E, on bark of *Quercus petraea*, 2 Oct. 2021, Z. Palice 33276, PRA. Ibid.: Vinařice, Mt Šamor (481 m), a mixed deciduous forest at SSE-facing slope, ~ 200 m SSE of the top, alt. 463 m, 49°53'26.9"N, 14°7'2.5"E, on bark of *Pyrus communis*, 14 Sep. 2021, Z. Palice 33574, PRA. Ibid.: Beroun, Tetín, Koda Nature Reserve, beech-dominated forest on steep ENE- facing slope, 0.3–0.4 km SW of the Srbsko railway stop, alt. 347 m, 49°56'6.6"N, 14°7'38.6"E, on bark of *Acer platanoides* (foot), 1 Oct. 2021, Z. Palice 35202, PRA. **Slovakia**. W Carpathians, Muránska planina plateau, Šarkanica Nature Reserve: SSE foothill of Mt Zadná Šajba (958 m), deciduous forest just W of the saddle Dielik, 48°42'3.0"N, 19°58'34.1"E, alt. 717 m, on bark at foot of *Quercus* snag, 24 Nov. 2016, Z. Palice 22415, PRA.

Chrysothrix fagicola Malíček et Vondrák, spec. nova

MycoBank: MB#847636; Fig. 1C
Etymology: Named after its exclusive occurrence on *Fagus sylvatica*.
Type: **Czech Republic**. Southern Bohemia: distr. Český Krumlov, Novohradské hory Mts, Hojná Voda, fragment of old-growth beech-spruce mixed forest 0.5 km NE of Zlatá Ktiš pond, 48°40'58.2"N, 14°42'56.8"E, alt. 835 m, on bark of old *Fagus sylvatica*, 10 Aug. 2020, J. Malíček 14013, PRA, **holotype**.
Type sequences: ITS (OQ717370); mtSSU (OQ682926).

Diagnostic characters: Sterile sorediate lichen, formed by the ± immersed hypothallus and relatively sparse yellowish soredia. Similar to *C. caesia*, but differing in the thallus and molecular characters, and ecology.

Morphology-anatomy: Thallus immersed to semi-immersed, formed by a hypothallus and visible as a pale film, covered by relatively sparse soredia, or groups of soredia, or locally soredia crowded in patches; prothallus and soralia absent. Soredia yellow-white to white-grey, usually with yellow or bluish tinge, finely granular, 25–50 µm in diam., often in consoredia up to 80 µm; soredia wall ± compact, without projecting hyphae, colourless. Photobiont chlorococcoid, globose cells 5–15 µm in diam. Apothecia and pycnidia unknown.

Chemistry: usnic acid and zeorin detected by TLC in three analysed specimens. In one collection, zeorin present only in a trace amount. After several years in herbarium, needle-like crystals of zeorin visible on the thallus surface. Spot tests: K+ yellowish, C–, KC+ yellow, P–, UV–.

Ecology and distribution: The new species is so far known only from the bark on trunks of old *Fagus sylvatica*, rarely also overgrowing mosses and lichens on the bark. It prefers lichen-poor communities in more or less shady microhabitats, in communities with *Lepraria elobata*, *L. finkii*, *Micarea micrococca* agg., rarely also with *Fellhaneropsis vezdae*, *Melanelixia glabratula* and *Pertusaria pupillaris*. *Chrysothrix faginea* is known only from five localities at middle and submontane elevations (545–835 m a.s.l.) with natural occurrence of beech in Bohemia, the Czech Republic. Three of them are situated in old-growth forests, two on old beech trees left in mature coniferous plantations.

Similar species: *Chrysothrix fagicola* is an inconspicuous species, which may be easily overlooked. Macroscopically it resembles some *Lepraria* species, *Lecanora expallens* or *L. stanislai*. The new species differs mainly in the density of soredia, which are usually sparse and do not form a continuous crust. This character is visible in the field. Young thalli of both *Lecanora* species are usually clearly delimited by a prothallus, but no

prothallus has been observed in *C. fagicola*. Due to its sparse soredia, the species is very similar to *Bacidia albogranulosa*, which produces atranorin and occurs on deciduous trees with higher bark pH (Malíček et al. 2018).

The closely related and similar *Chrysothrix caesia* often forms bluish-grey pruinose apothecia, its thallus is usually distinct, continuous, granular to leprose or locally ± areolate. Leprose parts are continuously sorediate, which is the important difference from *C. faginea*. *Chrysothrix caesia* occurs on bark of various deciduous trees (e.g. Redinger 1937, Brodo et al. 2001), not rarely in ± pioneer communities. It is widespread in eastern North America (see the GBIF database), but very rare in Europe (e.g. Redinger 1937, Nimis et al. 2018), from where it was described (Koerber 1855).

DNA data: We obtained ITS sequences from five specimens which are > 99.5% identical. The sister lineage consists of *Chrysothrix* sequences (*C. candelaris*, *C. xanthina* and *C.* sp.) which are 85–93% identical to *C. fagicola*. These species together with *Arthonia mediella* form a supported clade of *Chrysotrichaceae* which is sister to *Andreiomycetaceae* (Supplementary Fig. S3). The two almost identical mtSSU sequences from *C. fagicola* are 86.5% identical with *C. caesia*. Both species then form a sister group to the clade *C. candelaris* and *C. xanthina*, and all these species together with other members of *Chrysothrix* and with *Arthonia mediella* form a clade corresponding to *Chrysotrichaceae*, a sister group to *Andreiomycetaceae* (Supplementary Fig. S4).

Paratypes: **Czech Republic**. Central Bohemia: Rakovník, Jesenice, mixed managed forest 0.8 km E of Svatý Hubert castle, 50°04'19.9"N, 13°31'22.2"E, alt. 545 m, on bark of *Fagus sylvatica*, 22 Nov. 2019, J. Malíček 13296 & Z. Sejfová, herb. Malíček. Western Bohemia: Český les Mts, Tachov, fragment of old-growth beech forest 3.7 km NW of Lesná, 49°46'18.7"N, 12°29'42.1"E, alt. 730 m, on bark of *Fagus sylvatica*, 25 Oct. 2019, J. Malíček 13300 & J. Rydlo, herb. Malíček. Rokycany, Březina, managed spruce forest with a few beech trees 1.2 km S of Skelná Huť, 49°48'31.6"N, 13°38'05.8"E, alt. 610 m, on bark of *Fagus sylvatica*, 19 Sep. 2019, J. Malíček 13310 & E. Hodková, herb. Malíček. Southern Bohemia: Novohradské hory Mts, Pohorská ves, Žofínský prales National Nature Reserve, primeval beech forest in N part of the reserve, 48°40'07.6"N, 14°42'21.7"E, alt. 765 m, on bark of *Fagus sylvatica*, 15 Sep. 2020, J. Malíček 14081 & Z. Sejfová, herb. Malíček.

Japewia gyrophorica Palice, Malíček et Vondrák, spec. nova

MycoBank: MB#847426; Fig. 1D, E

Etymology: The name is derived from its characteristic compound: gyrophoric acid.

Type: **Czech Republic**. W Bohemia, Šumava Mts, Mt Smrkový vrch (1112 m), spruce-beech forest at road 0.5 km E of the top, 49°1'50.0"N, 13°25'51.0"E, alt. 1090 m, on bark of *Fagus sylvatica*, 20 Oct. 2020, J. Malíček 14469, PRA, **holotype**.

Type sequences: ITS (OQ717875); mtSSU (OQ646254).

Diagnostic characters: Characterized by the brown sorediate-blastidiate thallus, with a dark coloured exterior and a contrasting bright pale, almost purely greenish-white interior of soralia. It resembles *Japewia aliphatica* and *J. subaurifera*, but is easily distinguishable by the presence of gyrophoric acid. The similar, chemically identical, ubiquitous *Placynthiella dasaea* differs in having less contrasting external and internal soredia and in having a different pattern of the soredial surface and a different photobiont (more details below).

Morphology-anatomy: Thallus sorediate-blastidiate, greyish-, reddish- to dark chocolate brown (olive- or reddish brown coloured when wet), forming small irregular or orbicular thalli of few mm^2 intermingled among other crustose lichens, or forming uniform extensive crust up to several dm^2. Initially the thallus is formed by dispersed brownish areoles

or granules up to 0.15–0.2 mm in diam. (observed only in small part of the studied material), which rather soon proliferate to forming delimited soralia. These subsequently merge into a more-less continuous cracked crust usually up to 0.2–0.3 mm thick, but in extremely well-developed specimens soredia can produce a thick crust reaching almost 1 mm in height. The sorediate-blastidiate surface often erodes, exposing the inner layer of lighter (almost white) younger soredia. Soredia 15–45 µm in diam., eventually developing consoredia up to 60–65 µm in diam. Smaller soredia may contain a single, relatively large algal cell. Algae trebouxioid, usually 5–12 µm in diam., individually reaching even 16–18 µm. Soredia enveloped by distinct fungal cover of tightly and irregularly arranged hyphae about 3 µm thick (but not forming pseudoparenchyma). Hyphae visibly septate, forming rough, somewhat bulging surface of the soredia. Mature external soredia with unevenly pigmented brown outermost wall, occassionally showing slightly enlarged (3.5–5 µm broad) dark-capped terminal hyphal cells. Apothecia and pycnidia not seen.

Chemistry: gyrophoric acid by TLC. Spot tests: K–, C+ red, fading fast (gyrophoric acid), P–, UV– or UV+ faintly bluish-white.

Ecology and distribution: Occurring in humid montane forests, with habitat and substrate requirements similar to those of the related *Japewia subaurifera* and *J. aliphatica*. It grows on acid smooth bark of deciduous trees (*Alnus incana*, *Acer pseudoplatanus*, *Betula* sp., *Fagus sylvatica*, *Populus tremula*), as well as conifers (*Abies alba*, *Picea abies*, *Pinus rotundata*, *P. sylvestris*) and was recorded also on hard, slowly decaying conifer wood. So far known from dozens of localities in the Czech Republic, Slovakia and Romania, but is probably a much more widespread species in boreal-temperate forest regions, that was not previously distinguished in the sterile state from the variable taxon *Placynthiella dasaea*.

Similar species: *Japewia aliphatica* and *J. subaurifera* form similar extensive, brown coloured blastidiate-sorediate thalli, that are distinctly brighter and paler inside after eroding or scraping the outermost soredia, but only *J. gyrophorica* contains gyrophoric acid. Sterile epiphytic specimens of chemically concordant *Placynthiella dasaea* may be very similar, but the latter species differs somewhat in having soralia that are finer and often more greenish in colour, sometimes with an ochre-brownish tint, as well as in their lesser contrast between external and internal colour. The surface of mature blastidia/isidia in *Placynthiella* shows a paraplectenchymatic cellular pattern in outer view unlike the hyphal pattern in vegetative propagules of *Japewia*. The photobiont in *Placynthiella dasaea* forms regular, densely packed colonies of pairs/tetrads of algal cells, with the youngest daughter cells often closely attached to each other and belongs to the genus *Pseudochlorella* (Voytsekhovich et al. 2011). This photobiont is different from the variously sized and less-organized *Trebouxia*-like algal partner in *Japewia gyrophorica*, where the algal daughter cells are soon visibly separated by hyphae of the mycobiont.

DNA data: On the basis of ITS and mtSSU data, *Japewia* represents a well-defined genus within the *Lecanorales* (Malíček et al. 2020). ITS sequences from four *Japewia gyrophorica* specimens are >99.5% identical and form a supported sister clade to *J. subaurifera* and *J. tornoensis* (Supplementary Fig. S5). Identical mtSSU sequences from three *Japewia gyrophorica* specimens are simultaneously more than 99.5% identical with sequences of *J. tornoensis*, and the two species are not distinguished in the Bayesian tree (Supplementary Fig. S6). The other two species, *J. aliphatica* and *J. subaurifera*, are distinct in mtSSU.

Paratypes: **Czech Republic**. W Bohemia, Šumava Mts, Prášily: Mt Ždanidla, SW–SSW-facing slope, remnant of montane mixed forest, alt. 1210 m, 49°6'3.2"N, 13°20'43.2"E, on dry wood of *Picea* snag, 12 Aug. 2021, Z. Palice 31831, PRA. Ibid.: Mt Ždanidla – E-facing slope, managed montane mixed forest, alt. 1135 m, 49°6'8.8"N, 13°21'28.1"E, on bark of *Fagus sylvatica*, 29 Sep. 2021, Z. Palice 31866, 32395, 32415, 32589, 32596 & J. Vondrák, PRA, J. Vondrák 25859, PRA. Ibid.: Srní – Povydří: trees along Vydra River in surrounding of former Hálkova chata, 450 m SSE of Klosterman's bridge, alt. 840 m, 49°4'35.1"N, 13°30'40.9"E, on bark of *Populus tremula*, 18 Jan. 2020, J. Malíček 13440, herb. Malíček. Ibid.: Modrava, Javoří Pila, remnant of old-growth mixed forest near the state border with Germany, on S-SSE facing slope, 1.7–1.8 km SW of Mt Smrkový vrch (1112 m), just NW of the peatbog Rokytecká slať, alt. 1147 m, 49°1'17.8"N, 13°24'18.5"E, on bark of *Fagus sylvatica*, 20 Oct. 2020, Z. Palice 32138, PRA. Ibid.: Modrava: margin of spruce-beech forest on E-facing slope of Mt Smrkový vrch (1112 m), alt. 1080 m, 49°1'50.0"N, 13°25'57.5"E, on bark of *Fagus sylvatica*, 25 Apr. 2023, Z. Palice 35554, PRA. Ibid.: Modrava, Javoří Pila: Mt Nad Roklanským potokem (1133 m) – remnants of spruce-beech forest on NE facing slope below the top, alt. 1124 m, 49°0'56.6"N, 13°26'32.5"E, on bark of *Fagus sylvatica*, 26 Apr. 2023, Z. Palice 36610, PRA. S Bohemia, Šumava Mts, Volary: boggy, taiga-like forest with *Pinus* dominating near the channel of the brook Hučina, 0.5–0.6 km ESE from the railway-stop Černý Kříž, alt. 737 m, 48°51'32.0"N, 13°52'4.0"E, on bark of *Pinus rotundata* and *P. sylvestris*, 4 Apr. 2010, J. Halda & Z. Palice 13356, 14050, PRA. Ibid.: Volary, Černý Kříž: alder wood at the confluence of Lesní potok and Hučina creeks, alt. 740 m, 48°51'10.9"N, 13°51'47.2"E, on bark of *Alnus incana*, 24 Apr. 2011, 26 Feb. 2023, Z. Palice 14805, 35206, PRA. Ibid.: Volary – Dobrá: old-growth beech and scree forest on steep NE-facing slope of Mt Stožec (1065 m), alt. 890–910 m, 48°52'49.0"N, 13°50'19.0"E, on bark of *Fagus sylvatica*, 17 Oct. 2016, J. Malíček 10081, 10082, Z. Palice & J. Vondrák, herb. Malíček. Ibid.: Stožec: Mt Stožec (1065 m) – managed beech-spruce forest with some sycamore, just below of forest road, on NE-facing slope, alt. 875 m, 48°53'3.5"N, 13°49'57.0"E, on bark of *Fagus*, 18 Oct. 2016, Z. Palice 24261, PRA. Ibid.: Volary – České Žleby: old-growth forest predominated by beeches in upper part of Mt Spáleniště (960 m), alt. 930–940 m, 48°52'38.0"N, 13°47'38.0"E, on bark of *Fagus sylvatica*, 19 Oct. 2016, J. Malíček 9953, Z. Palice & J. Vondrák, herb. Malíček. Ibid.: Volary: Mt Trojmezná, ca 0.5 km NW of the top, dead climatic spruce forest on N-facing slopes, alt. 1295 m, 48°46'32.0"N, 13°49'24.5"E, on bark of dead *Picea abies*, 14 May 2011, Z. Palice 15818, V. Pouska & J. Vondrák, PRA. Ibid.: Kubova Huť – Boubínský prales, old-growth beech-spruce forest in central part of reserve, alt. 950–1000 m, 48°58'30.0"N, 13°48'54.0"E, on bark of *Fagus sylvatica*, 9 Jun. 2011, J. Malíček 3565, herb. Malíček. Ibid.: Volary: Mt Trojmezná, 130–150 m NNW of the top, dead climatic spruce forest on N-facing slope, alt. 1340 m, 48°46'22.0"N, 13°49'33.5"E, on wood and bark of dead standing *Picea abies*, 14 Nov. 2012, I. Frolov, Z. Palice 15813, 15818, PRA. Ibid.: Mt Plechý (1378 m), dead climatic spruce forest ~0.6 km NW of the top, just N of the point Rakouská louka, a spring area of Stocký potok, alt. 1325 m, 48°46'29.5"N, 13°50'59.5"E, on bark of young but dead *Picea abies*, 14 May 2011, Z. Palice 14382 & V. Pouska, PRA. Ibid.: Nová Pec: Mt Plechý, E-ENE slope of the hill Steinwand (1054 m), U Rakouské cesty Nature Reserve, 1.8–1.9 km ESE of the top of Mt Plechý, remnants of montane old-growth beech-dominated forest, alt. 1025 m, 48°45'57.2"N, 13°52'52.3"E, on bark of *Fagus sylvatica*, 4 Jul. 2023, Z. Palice 35962, PRA. Ibid.: Nová Pec – upper border of virgin spruce-beech forest on NE slope of Mt Hraničník (1281 m), 0.25 km ENE of top, alt. 1220 m, 48°45'1.0"N, 13°54'29.0"E, on bark of *Fagus sylvatica*, 25 Sep. 2012, J. Malíček 4734, F. Bouda, O. Peksa, D. Svoboda & L. Syrovátková, herb. Malíček. Ibid.: Nová Pec – old-growth beech-spruce forest on N-facing slope of Mt Hraničník (1281 m), alt. 1170 m, 48°45'13.0"N, 13°54'17.0"E, on bark of *Fagus sylvatica*, 15 Jun. 2017, J. Malíček 11283, herb. Malíček. Ibid.: Nová Pec: Mt Hraničník – NE slope, managed forest with beech predominant, alt. 1130 m, 48°45'24.0"N, 13°54'10.0"E, on bark of young *Fagus*, 2 Aug. 2017, Z. Palice 24372, PRA. Ibid.: Nová Pec – managed beech forest on NE-facing slope of Mt Studničná (1160 m), alt. 1130 m, 48°45'24.0"N, 13°54'10.0"E, on bark of *Fagus sylvatica*, 15 Jun. 2017, J. Malíček 11267 & J. Vondrák, herb. Malíček. Ibid.: Prachatice, Záblatí: the canyon-like valley of Blanice, alluvial forest on the right bank of the rivulet, NNW foothill of Mt Panský vrch (834 m), 1.4 km SW of the settlement Hlásná Lhota, alt. 658 m, 48°58'17.5"N, 13°55'29.3"E, on bark of *Acer pseudoplatanus*, 12 Jul. 2022, Z. Palice 33915, PRA. Ibid.: Prachatice, Záblatí: Kaňon Blanice Nature Reserve – the westernmost part, the valley of Blanice, alt. 617 m, 48°59'7.8"N, 13°54'42.1"E, on bark of *Betula*, 17 Jun. 2022, Z. Palice 34475, PRA. S Bohemia, Novohradské hory Mts, Pohorská Ves: virgin forest in the SE part of the Žofínský prales National Nature Reserve, ENE-NE-facing slope, 1.7–1.8 km SE of Žofín settlement, alt. 830 m, 48°39'48.3"N, 14°42'34.5"E, on bark of *Fagus*, 13 Oct. 2010, I. Černajová, J. Malíček & Z. Palice 13996, PRA. Ibid.: virgin forest in the E part of the Žofínský prales National Nature Reserve, alt. 785 m, 48°39'59.7"N, 14°42'38.2"E, on bark of *Fagus sylvatica*, 25 May 2010, J. Malíček 2669 & Z. Palice, herb. Malíček, Palice 13802, PRA. Ibid.: virgin forest in the S part of the Žofínský prales National Nature Reserve, alt. 815 m, 48°39'36.7"N, 14°42'15.3"E, on bark of *Fagus sylvatica*, 24 May 2011, J. Malíček 3596 & Z. Palice, herb. Malíček, Palice

13996, PRA. Ibid.: old, well-lit managed spruce forest with dispersed beeches and thick undergrowth on S-facing slope of Mt Smrčina (910 m), just SE of the top, 1.9 km SW of Žofín settlement, alt. 900 m, 48°39'43.9"N, 14°40'37.6"E, on bark of *Fagus*, 15 Oct. 2010, I. Černajová, J. Malíček & Z. Palice 13913, PRA. Ibid.: beech forest 1,2 km NE of the settlement Žofín, alt. 800 m, 48°41'3.0"N, 14°42'11.0"E, on bark of *Fagus sylvatica*, 26 Jul. 2010, J. Malíček 2798 & L. Syrovátková, herb. Malíček. E Moravia, Beskydy Protected Landscape Area, Frenštát pod Radhoštěm – Kněhyně-Čertův mlýn Nature Reserve, NW-facing slope of Mt Čertův mlýn (1206 m), old-growth beech-spruce forest, alt. 1150 m, 49°29'14.0"N, 18°18'5.0"E, on bark of *Acer pseudoplatanus*, 28 Sep. 2013, J. Malíček 6109 & J. Vondrák, herb. Malíček. Ibid.: Karolinka – Malý Javorník Nature Reserve, old-growth spruce-beech forest, alt. 900–960 m, 49°18'20.9"N, 18°17'18.6"E, on bark of *Fagus sylvatica*, 10 Aug. 2023, J. Malíček 16216 & I. Černajová, herb. Malíček. **Romania**. Southern Carpathians: distr. Braşov, Piatra Craiului Mts, Zărneşti, rocks and spruce-silver fir forest on limestone along tourist path 0.5 km E of Mt Vf. Padina Popii (1970 m), alt. 1680 m, 45°33'10.5"N, 25°14'57.5"E, on bark of *Picea abies*, 23 Jul. 2021, J. Malíček 15304 & J. Steinová, herb. Malíček. **Slovakia**. Western Carpathians, Muránska planina plateau: Malá Stožka (1204 m), fir-beech forest on steep WNW facing slope, SSW from the top, alt. 1068 m, 48°46'27.2"N, 19°55'15.2"E, on bark of *Abies alba*, 9 Oct. 2019, A. Guttová & Z. Palice 27792, PRA.

Lecanora arachnoidea Vondrák, Malíček et Svoboda, spec. nova

MycoBank: MB#847427; Fig. 1F, G
Etymology: Named after its prothallus, which is formed of thin fibres, resembling a spider's web.
Type: **Czech Republic**. Central Bohemia: district Rakovník, Týřov National Nature Reserve, in valley of Úpořský potok stream, alt. 340 m, 49°57'57.2"N, 13°49'49.6"E, on bark of *Carpinus betulus*, 5 Sep. 2020, J. Vondrák 24028, PRA, **holotype**.
Type sequences: ITS (OL457932); mtSSU (OK465506).

Diagnostic characters: The combination of thallus chemistry (atranorin and perlatolic acid), pale grey, sorediate thallus and white fibrillous prothallus is diagnostic.

Morphology-anatomy: Thallus crustose, pale grey to white, epiphloedal, up to 100 µm thick, sorediate. Young soredia green-grey, pustulate, 0.2–0.5 mm diam. Well-developed soralia rarely present, convex, punctiform, up to 1 mm diam. Soredia ~ 20–40 µm diam. White prothallus distinct, often fibrillous (i.e. formed of thin fibres). Apothecia and pycnidia absent in studied specimens.

Chemistry: Atranorin and perlatolic acid detected by TLC in four analysed specimens. Spot tests: K+ yellowish (atranorin), C–, P–, UV+ white (perlatolic acid).

Ecology and distribution: Occurring on smooth bark of deciduous trees (most collections are from *Carpinus betulus*), typically in communities with *Arthonia radiata*, *Lecanora argentata* and *Lecidella elaeochroma*. We expect it to be broadly distributed in Europe, but so far it is only known from lowland forests in the Czech Republic and Ukraine.

Similar species: Mature thalli resemble e.g. *Lecanora substerilis*, which also has similar ecology, but has different chemistry (lacking perlatolic acid and thus UV–) and does not have the fibrillous (cottony) prothallus. The characteristic prothallus is similar to *Haematomma ochroleucum*, *Lecanora thysanophora* and *Phlyctis argena*, but these species differ in thallus appearance and chemistry (also lacking perlatolic acid and thus UV–). The following species have similar chemistry and UV+ white medulla and soralia: *Cliostomum haematommatis* and *Loxospora cristinae* with 2'-*O*-methylperlatolic acid, and *Mycoblastus caesius* with perlatolic acid. None of these three species forms a fibrillous prothallus. On the basis of ITS and mtSSU sequences, all mentioned species are distinct and not closely related to *Lecanora arachnoidea*.

DNA data: ITS sequences from two specimens (type & Vondrák 25905) sharing > 99.5% identity, and identical mtSSU sequences from the same two specimens were obtained. Short identical mtSSU sequences (of 380 BP length) with 100% match to the

type sequence were also generated from the three Ukrainian specimens (Vondrák 13908, 13946, 13988). The closest NCBI BLAST matches are to *Lecanorales* spp. with identities up to 84% (ITS) and 95% (mtSSU). Bayesian trees, including the closest known species in the NCBI, show the indistinct relationships of the newly described species with members of *Lecanorales* for both ITS and mtSSU loci (Supplementary Figs S7, S8). *Lecanora arachnoidea* apparently does not belong to the core group of the genus *Lecanora* and therefore its current generic placement may have to be changed eventually.

Paratypes: **Czech Republic**. Southern Bohemia: České Budějovice, Hluboká nad Vltavou, valley of Vltava river N of village, alt. 380 m, 49°4'50.6"N, 14°27'25.2"E, on bark of *Carpinus betulus*, 19 Jan. 2022, J. Vondrák 25905, PRA, herb. Malíček. **Ukraine**. Eastern Carpathians: Khust, Uglya, Velika Ugolka, in valley of Velika Ugolka brook above village, alt. 420–440 m, 48°14'41.6"N, 23°41'40.7"E, on bark of *Carpinus betulus*, 19 May 2015, J. Vondrák 13908, PRA. Ibid.: on bark of *Fagus sylvatica*, Vondrák 13946, PRA. Ibid.: alt. 500–520 m, 48°15'3.0"N, 23°41'47.2"E, on bark of *Fagus sylvatica*, 13 May 2015, J. Vondrák 13988, PRA.

Supplementary materials

Data S1. Comments to identities of sequences in Martin7.
Data S2. Phylogenetic analysis, *Cheiromycina*.
Fig. S1. ITS tree with *Bacidina acerina* and *B. paradoxa*.
Fig. S2. MtSSU tree with *Bacidina acerina* and *B. paradoxa*.
Fig. S3. ITS tree with *Chrysothrix fagicola*.
Fig. S4. MtSSU tree with *Chrysothrix fagicola.*
Fig. S5. ITS tree with *Japewia gyrophorica*.
Fig. S6. MtSSU tree with *Japewia gyrophorica*.
Fig. S7. ITS tree with *Lecanora arachnoidea*.
Fig. S8. MtSSU tree with *Lecanora arachnoidea*.
Table S1. Specimens involved in Martin7.
Table S2. PCR primers and cycling conditions.
Table S3. European epiphytic lichen species without ITS and mtSSU in Martin7.

Supplementary materials are available at www.preslia.cz

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References

Abarenkov K., Nilsson R. H., Larsson K.-H., Alexander I. J., Eberhardt U., Erland S., Høiland K., Kjøller R., Larsson E., Pennanen T., Sen R., Taylor A. F. S., Tedersoo L., Ursing B. M., Vrålstad T., Liimatainen K., Peintner U. & Kõljalg U. (2010) The UNITE database for molecular identification of fungi – recent updates and future perspectives. – New Phytologist 186: 281–285.

Aguirre-Hudson B. (2009) *Leptorhaphis* Minks (1876). – In: Smith C. W., Aptroot A., Coppins B. J., Fletcher A., Gilbert O. L., James P. W. & Wolseley P. A. (eds), The lichens of Great Britain and Ireland, p. 368–369, The British Lichen Society, London.

Alors D., Lumbsch H. T., Divakar P. K., Leavitt S. D. & Crespo A. (2016) An integrative approach for understanding diversity in the *Punctelia rudecta* species complex (*Parmeliaceae*, *Ascomycota*). – PLoS ONE 11: e0146537.

An K.-D., Degawa Y., Fujihara E., Mikawa T., Ohkuma M. & Okada G. (2012) Molecular phylogenetic analyses based on the nuclear rRNA genes and the intron-exon structures of the nuSSU rRNA gene in *Dictyocatenulata alba* (anamorphic *Ascomycota*). – Fungal Biology 116: 1134–1145.

Andersen H. L. & Ekman S. (2004) Phylogeny of the *Micareaceae* inferred from nrSSU DNA sequences. – Lichenologist 36: 27–35.

Andersen H. L. & Ekman S. (2005) Disintegration of the *Micareaceae* (lichenized *Ascomycota*): a molecular phylogeny based on mitochondrial rDNA sequences. – Mycological Research 109: 21–30.

Aptroot A. (1998) A world revision of *Massarina* (*Ascomycota*). – Nova Hedwigia 66: 89–162.

Barr M. E. & Boise J. (1985) A revision of *Exarmidium* (*Ascomycetes*). – Mycotaxon 23: 233–240.

Bradshaw M. J., Aime M. C., Rokas A., Maust A., Moparthi S., Jellings K., Pane A. M., Hendricks D., Pandey B., Li Y. & Pfister D. H. (2023) Extensive intragenomic variation in the internal transcribed spacer region of fungi. – iScience 26: 107317.

Brodo I. M., Sharnoff S. D. & Sharnoff S. (2001) Lichens of North America. – Yale University Press, New Haven and London.

Brunbjerg A. K., Bruun H. H., Brøndum L., Classen A. T., Dalby L., Fog K., Frøslev T. G., Goldberg I., Hansen A. J., Hansen M. D. D., Høye T. T., Illum A. A., Læssøe T., Newman G. S., Skipper L., Søchting U. & Ejrnæs R. (2019) A systematic survey of regional multi-taxon biodiversity: evaluating strategies and coverage. – BMC Ecology 19: 43.

Chen K.-H., Miadlikowska J., Molnár K., Arnold E., U'Ren J. M., Gaya E., Gueidan C. & Lutzoni F. (2015) Phylogenetic analyses of eurotiomycetous endophytes reveal their close affinities to *Chaetothyriales*, *Eurotiales*, and a new order – *Phaeomoniellales*. – Molecular Phylogenetics and Evolution 85: 117–130.

Crous P. W., Verkley G. J. M. & Groenewald J. Z. (2009) *Xenocylindrosporium kirstenboschense*. Fungal Planet 44. – Persoonia 23: 200–201.

Cubero O. F., Crespo A., Esslinger T. L. & Lumbsch H. T. (2004) Molecular phylogeny of the genus *Physconia* (*Ascomycota*, *Lecanorales*) inferred from a Bayesian analysis of nuclear ITS rDNA sequences. – Mycological Research 108: 498–505.

Díaz-Escandón D., Tagirdzhanova G., Vanderpool D., Allen C. C. G., Aptroot A., Češka O., Hawksworth D. L., Huereca A., Knudsen K., Kocourková J., Lücking R., Resl P. & Spribille T. (2022) Genome-level analyses resolve an ancient lineage of symbiotic ascomycetes. – Current Biology 32: 5209–5218.

Divakar P. K., Leavitt S. D., Molina M. C., Del-Prado R., Lumbsch H. T. & Crespo A. (2016) A DNA barcoding approach for identification of hidden diversity in *Parmeliaceae* (*Ascomycota*): *Parmelia* sensu stricto as a case study. – Botanical Journal of the Linnean Society 180: 21–29.

Doilom M., Hyde K. D., Phookamsak R., Dai D. Q., Tang L. Z., Hongsanan S., Chomnunti P., Boonmee S., Dayarathne M. C., Li W. J., Thambugala K. M., Perera R. H., Daranagama D. A., Norphanphoun C., Konta S., Dong W., Ertz D., Phillips A. J. L., McKenzie E. H. C., Vinit K., Ariyawansa H. A., Jones E. B. G., Mortimer P. E., Xu J. C. & Promputtha I. (2018) Mycosphere Notes 225–274: types and other specimens of some genera of *Ascomycota*. – Mycosphere 9: 647–754.

Doyle J. J. & Doyle J. L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – Phytochemical Bulletin 19: 11–15.

Ekman S. (2023) Four new and two resurrected species of *Bacidina* from Sweden, with notes and a preliminary key to the known Scandinavian species. – Nordic Journal of Botany 2023: e03846.

Ekman S. & Tønsberg T. (2002) Most species of *Lepraria* and *Leproloma* form a monophyletic group closely related to *Stereocaulon*. – Mycological Research 106: 1262–1276.

Fries E. (1818) Uppställning af de i Sverige funne Vårtsvampar (Scleromyci) [List of wart-fungi (Scleromyci) found in Sweden]. – Kungliga Svenska Vetenskapsakademiens Handlingar 37 (1816–1819): 126–157.

Gardes M. & Bruns T. D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. – Molecular Ecology 2: 113–118.

Gnavi G., Garzoli L., Poli A., Prigione V., Burgaud G. & Varese G. C. (2017) The culturable mycobiota of *Flabellia petiolata*: first survey of marine fungi associated to a Mediterranean green alga. – PLoS ONE 12: e0175941.

Guzow-Krzemińska B., Sérusiaux E., van den Boom P. P. G., Brand A. M., Launis A., Łubek A. & Kukwa M. (2019) Understanding the evolution of phenotypical characters in the *Micarea prasina* group (*Pilocarpaceae*) and descriptions of six new species within the group. – MycoKeys 57: 1–30.

Hall T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series 41: 95–98.

Harris R. C. (1995) More Florida lichens, including the 10¢ tour of the pyrenolichens. – Privately Published, New York.

Henrie J. R., Thomson B. M., Yungfleisch A. A., Kerr M. & Leavitt S. D. (2022) Characterizing crustose lichen communities – DNA metabarcoding reveals more than meets the eye. – Diversity 14: 766.

Hodkinson B. P. & Lendemer J. C. (2013) Next-generation sequencing reveals sterile crustose lichen phylogeny. – Mycosphere 4: 1028–1039.

Huelsenbeck J. P. & Ronquist F. (2001) MRBAYES: Bayesian inference of phylogeny. – Bioinformatics 17: 754–755.

Johansson P. & Gustafsson L. (2001) Red-listed and indicator lichens in woodland key habitats and production forests in Sweden. – Canadian Journal of Forest Research 31: 1617–1628.

Katoh K. & Standley D. M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. – Molecular Biology and Evolution 30: 772–780.

Keepers K. G., Pogoda C. S., White K. H., Anderson-Stewart C. R., Hoffman J. R., Ruiz A. M., McCain C. M., Lendemer J. C., Kane N. C. & Tripp E. A. (2019) Whole genome shotgun sequencing detects greater lichen fungal diversity than amplicon-based methods in environmental samples. – Frontiers in Ecology and Evolution 7: 484.

Kelly L. J., Hollingsworth P. M., Coppins B. J., Ellis C. J., Harrold P., Tosh J. & Yahr R. (2011) DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context. – New Phytologist 191: 288–300.

Kerr M. & Leavitt S. D. (2023) A custom regional DNA barcode reference library for lichen-forming fungi of the Intermountain West, USA, increases successful specimen identification. – Journal of Fungi 9: 741.

Koerber G. W. (1855) Systema Lichenum Germaniae. – Trewendt & Granier, Breslau.

Košuthová A., Westberg M. & Wedin M. (2022) A revision of the *Rostania occultata* (*Collemataceae*) complex in Fennoscandia. – Lichenologist 54: 13–24.

Kraichak E., Lücking R., Aptroot A., Beck A., Dornes P., John V., Lendemer J. C., Nelsen M. P., Neuwirth G., Nutakki A., Parnmen S., Sohrabi M., Tønsberg T. & Lumbsch H. T. (2015) Hidden diversity in the morphologically variable script lichen (*Graphis scripta*) complex (*Ascomycota*, *Ostropales*, *Graphidaceae*). – Organisms Diversity and Evolution 15: 447–458.

Launis A., Malíček J., Svensson M., Tsurykau A., Sérusiaux E. & Myllys L. (2019) Sharpening species boundaries in the *Micarea prasina* group, with a new circumscription of the type species *M. prasina*. – Mycologia 111: 574–592.

Leavitt S. D., Esslinger T. L., Divakar P. K., Crespo A. & Lumbsch H. T. (2016) Hidden diversity before our eyes: delimiting and describing cryptic lichen-forming fungal species in camouflage lichens (*Parmeliaceae*, *Ascomycota*). – Fungal Biology 120: 1374–1391.

Leavitt S. D., Fernández-Mendoza F., Pérez-Ortega S., Sohrabi M., Divakar P. K., Lumbsch H. T. & St. Clair L. L. (2013) DNA barcode identification of lichen-forming fungal species in the *Rhizoplaca melanophthalma* species-complex (*Lecanorales*, *Lecanoraceae*), including five new species. – MycoKeys 7: 1–22.

Lücking R., Aime M. C., Robbertse B., Miller A. N., Ariyawansa H. A., Aoki T., Cardinali G., Crous P. W., Druzhinina I. S., Geiser D. M., Hawksworth D. L., Hyde K. D., Irinyi L., Jeewon R., Johnston P. R., Kirk P. M., Malosso E., May T. W., Meyer W., Öpik M., Robert V., Stadler M., Thines M., Vu D., Yurkov A. M., Zhang N. & Schoch C. L. (2020) Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? – IMA Fungus 11: 14.

Lücking R., Hodkinson B. P. & Leavitt S. D. (2016) The 2016 classification of lichenized fungi in the *Ascomycota* and *Basidiomycota* – Approaching one thousand genera. – Bryologist 119: 361–416.

Lumbsch H. T., Schmitt I., Lindemuth R., Miller A., Mangold A., Fernandez F. & Huhndorf S. (2005) Performance of four ribosomal DNA regions to infer higher-level phylogenetic relationships of inoperculate euascomycetes (*Leotiomycota*). – Molecular Phylogenetics and Evolution 34: 512–524.

Magain N. & Sérusiaux E. (2015) Dismantling the treasured flagship lichen *Sticta fuliginosa* (*Peltigerales*) into four species in Western Europe. – Mycological Progress 14: 97.

Malíček J., Palice Z., Bouda F., Knudsen K., Šoun J., Vondrák J. & Novotný P. (2023) Atlas českých lišejníků [Atlas of Czech lichens]. – URL: https://dalib.cz/ (accessed on 4 January 2023).

Malíček J., Palice Z., Vondrák J., Łubek A. & Kukwa M. (2018) *Bacidia albogranulosa* (*Ramalinaceae*, lichenized *Ascomycota*), a new sorediate lichen from European old-growth forests. – MycoKeys 44: 51–62.

Malíček J., Palice Z., Vondrák J. & Tønsberg T. (2020) *Japewia aliphatica* (*Lecanoraceae*, lichenized *Ascomycota*), a new acidophilous, sorediate-blastidiate lichen from Europe. – Phytotaxa 461: 21–30.

Marthinsen G., Rui S. & Timdal E. (2019) OLICH: a reference library of DNA barcodes for Nordic lichens. – Biodiversity Data Journal 7: e36252.

Muggia L., Hafellner J., Wirtz N., Hawksworth D. L. & Grube M. (2008) The sterile microfilamentous lichenized fungi *Cystocoleus ebeneus* and *Racodium rupestre* are relatives of plant pathogens and clinically important dothidealean fungi. – Mycological Research 112: 50–56.

Muggia L., Mancinelli R., Tønsberg T., Jablonska A., Kukwa M. & Palice Z. (2017) Molecular analyses uncover the phylogenetic placement of the lichenized hyphomycetous genus *Cheiromycina*. – Mycologia 109: 588–600.

National Library of Medicine, National Center for Biotechnology Information (NCBI) (2022) URL: https://www.ncbi.nlm.nih.gov/ (accessed on 4 January 2023).

National Library of Medicine, National Center for Biotechnology Information, Basic Local Alignment Search Tool (NCBI BLAST) (2022) URL: https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 4 January 2023).

Nelsen M. P., Lücking R., Mbatchou J. S., Andrew C. J., Spielmann A. A. & Lumbsch H. T. (2011) New insights into relationships of lichen-forming *Dothideomycetes*. – Fungal Diversity 51: 155–162.

Neuwirth G. & Aptroot A. (2011) Recognition of four morphologically distinct species in the *Graphis scripta* complex in Europe. – Herzogia 24: 207–230.

Nilsson R. H., Sánchez-García M., Ryberg M., Abarenkov K., Wurzbacher C. & Kristiansson E. (2017) Read quality-based trimming of the distal ends of public fungal DNA sequences is nowhere near satisfactory. – MycoKeys 26: 13–24.

Nilsson R. H., Tedersoo L., Abarenkov K., Ryberg M., Kristiansson E., Hartmann M., Schoch C., Nylander J., Bergsten J., Porter T., Jumpponen A., Vaishampayan P., Ovaskainen O., Hallenberg N., Bengtsson-Palme J., Eriksson K. M., Larsson K., Larsson E. & Kõljalg U. (2012) Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. – MycoKeys 4: 37–63.

Nimis P. L., Hafellner J., Roux C., Clerc P., Mayrhofer H., Martellos S. & Bilovitz P. O. (2018) The lichens of the Alps – an annotated checklist. – Mycokeys 31: 1–634.

Orange A., James P. W. & White F. J. (2001) Microchemical methods for the identification of lichens. – British Lichen Society.

Paillet Y., Bergès L., Hjältén J., Ódor P., Avon C., Bernhardt-Römermann M., Bijlsma R.-J., De Bruyn L., Fuhr M., Grandin U., Kanka R., Lundin L., Luque S., Magura T., Matesanz S., Mészáros I., Sebastià M. T., Schmidt W., Standovár T., Tóthmérész B., Uotila A., Valladares F., Vellak K. & Virtanen R. (2010) Biodiversity differences between managed and unmanaged forests: meta-analysis of species richness in Europe. – Conservation Biology 24: 101–112.

Palice Z. & Printzen C. (2004) Genetic variability in tropical and temperate populations of *Trapeliopsis glaucolepidea*: evidence against long-range dispersal in a lichen with disjunct distribution. – Mycotaxon 90: 43–54.

Posada D. (2008) jModelTest: phylogenetic model averaging. – Molecular Biology and Evolution 25: 1253–1256.

Printzen C., Spribille T. & Tønsberg T. (2008) *Myochroidea*, a new genus of corticolous, crustose lichens to accommodate the *Lecidea leprosula* group. – Lichenologist 40: 195–207.

Ratnasingham S. & Hebert P. D. N. (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). – Molecular Ecology Notes 7: 355–364.

Redinger K. (1937) *Arthoniaceae*. – In: Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Band IX. Abt. 2(1), p. 1–180, Borntraeger, Leipzig.

Resl P., Schneider K., Westberg M., Printzen C., Palice Z., Thor G., Fryday A., Mayrhofer H. & Spribille T. (2015) Diagnostics for a troubled backbone: testing topological hypotheses of trapelioid lichenized fungi in a large-scale phylogeny of *Ostropomycetidae* (*Lecanoromycetes*). – Fungal Diversity 73: 239–258.

Schmitt I., Mueller G. & Lumbsch H. T. (2005) Ascoma morphology is homoplaseous and phylogenetically misleading in some pyrenocarpous lichens. – Mycologia 97: 362–374.

Schoch C. L., Seifert K. A., Huhndorf S., Robert V., Spouge J. L., Levesque C. A., Chen W. & Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. – Proceedings of the National Academy of Sciences of the United States of America 109: 6241–6246.

Spies C. F. J., Mostert L., Carlucci A., Moyo P., van Jaarsveld W. J., du Plessis I. L., van Dyk M. & Halleen F. (2020) Dieback and decline pathogens of olive trees in South Africa. – Persoonia 45: 196–220.

Spribille T., Klug B. & Mayrhofer H. (2011) A phylogenetic analysis of the boreal lichen *Mycoblastus sanguinarius* (*Mycoblastaceae*, lichenized *Ascomycota*) reveals cryptic clades correlated with fatty acid profiles. – Molecular Phylogenetics and Evolution 59: 603–614.

Taberlet P., Coissac E., Pompanon F., Brochmann C. & Willerslev E. (2012) Towards next-generation biodiversity assessment using DNA metabarcoding. – Molecular Ecology 21: 2045–2050.

Thiyagaraja V., Lücking R., Ertz D., Coppins B. J., Wanasinghe D. N., Karunarathna S. C., Suwannarach N., To-Anun C., Cheewangkoon R. & Hyde K. D. (2021) Sequencing of the type species of *Arthopyrenia* places *Arthopyreniaceae* as a synonym of *Trypetheliaceae*. – Mycosphere 12: 993–1011.

Tønsberg T. (1992) The sorediate and isidiate, corticolous, crustose lichens in Norway. – Sommerfeltia 14: 1–331.

Větrovský T., Baldrian P. & Morais D. (2018) SEED 2: a user-friendly platform for amplicon high-throughput sequencing data analyses. – Bioinformatics 34: 2292–2294.

Vondrák J., Frolov I., Košnar J., Arup U., Veselská T., Halıcı G., Malíček J. & Søchting U. (2020) Substrate switches, phenotypic innovations and allopatric speciation formed taxonomic diversity within the lichen genus *Blastenia*. – Journal of Systematics and Evolution 135: 295–330.

Vondrák J., Svoboda S., Malíček J., Palice Z., Kocourková J., Knudsen K., Mayrhofer H., Thüs H., Schultz M., Košnar J. & Hofmeister J. (2022) From Cinderella to Princess: an exceptional hotspot of lichen diversity in a long-inhabited central-European landscape. – Preslia 94: 143–181.

Voytsekhovich A., Dymytrova L. & Nadyeina O. (2011) Photobiont composition of some taxa of the genera *Micarea* and *Placynthiella* (*Lecanoromycetes*, lichenized *Ascomycota*) from Ukraine. – Folia Cryptogamica Estonica 48: 135–148.

White T. J., Bruns T. D., Lee S. B. & Taylor J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J. (eds), PCR protocols: a guide to methods and applications, p. 315–322, Academic Press, New York.

Wilk K., Pabijan M., Saługa M., Gaya E. & Lücking R. (2021) Phylogenetic revision of South American *Teloschistaceae* (lichenized *Ascomycota*, *Teloschistales*) reveals three new genera and species. – Mycologia 113: 278–299.

Wright B., St. Clair L. L. & Leavitt S. D. (2019) Is targeted community DNA metabarcoding suitable for biodiversity inventories of lichen-forming fungi? – Ecological Indicators 98: 812–820.

Zhou S. & Stanosz G. R. (2001) Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. – Mycological Research 105: 1033–1044.

Zoller S., Scheidegger C. & Sperisen C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. – Lichenologist 31: 511–516.

Martin7 – referenční databáze pro spolehlivou DNA identifikaci evropských epifytických lišejníků a její taxonomický přínos

Identifikace organismů pomocí DNA sekvencí je dnes běžnou praxí a stále častěji se druhy identifikují z environmentálních vzorků (tj. ze směsného biologického materiálu obsahujícího často velké množství nejrůznějších organismů). U většiny organismů však stále postrádáme komplexní referenční DNA databáze, které by umožnily identifikovat získané sekvence. Vytvořili jsme referenční databázi pro přesnou molekulární identifikaci epifytických lišejníků (mykobiontů) střední Evropy. Naše databáze s názvem Martin7 zahrnuje sekvence nejčastějších lišejníkových „DNA barkódů", jaderný úsek ITS nrDNA (ITS) a mitochondriální SSU (mtSSU). Pomocí Sangerova sekvenování a NGS (PacBio) jsme získali 907 sekvencí ITS od 603 druhů a 844 sekvencí mtSSU od 546 druhů a doplnili jsme náš soubor dat o sekvence z dalších spolehlivých zdrojů. Celkem je v databázi zahrnuto 1172 druhů, 1004 pro ITS a 906 pro mtSSU. ITS bylo nově sekvenováno u 224 druhů a mtSSU u 234 druhů. DNA sekvence byly zcela nově získány pro 45 rodů. Ve většině případů tyto barkódy umožňují rozlišit druhy tak, jak jsou v současné době vymezeny, ale zjistili jsme 82 skupin nebo dvojic druhů, kde alespoň jeden z barkódů (většinou mtSSU) jednoznačně nerozlišuje morfologicky rozlišované druhy. V rámci 37 tradičně morfologicky pojatých druhů jsme odhalili přítomnost odlišných genotypů, které v některých případech představují kryptické taxony. Sekvenováním fenotypově neidentifikovatelných lišejníků jsme prokázali existenci mnoha předpokládaných nepopsaných druhů, zejména v rodech *Bacidina* a *Micarea*. Nově bylo popsáno pět druhů sorediózních korovitých lišejníků z rodů *Bacidina* (2 druhy), *Chrysothrix*, *Japewia* a *Lecanora*. Uvádíme řadu taxonomických novinek, např. že *Lecidea betulicola* a *L. coriacea* jsou teleomorfy rodu *Cheiromycina* a *Dictyocatenulata* je anamorfou rodu *Thelenella*. Název databáze Martin7 je odvozen od druhého jména Karla Martina Redingera, významného rakouského lichenologa, který zemřel v roce 1940 ve věku 33 let a během sedmi let vytvořil významná lichenologická díla. Například jeho monografie *Arthoniaceae*, vydaná v roce 1937, je dodnes nepřekonána.

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