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Combining environmental DNA data and taxonomic surveys provides an unprecedented understanding of lichen diversity and accelerates the discovery of new species

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Abstract: Sequencing of environmental DNA is increasingly used to estimate biodiversity at various taxonomic and spatial levels. However, most such studies tend to deal with abstract numbers not linked to species names, which hampers evaluation and downstream use of the results. In our survey of epiphytic lichens in the Czech Republic, we managed to link sequences from environmental DNA with species names, using an existing reference database of DNA barcodes. On 1-ha sites in various types of central-European forests, we were able to compare DNA data from environmental samples with (i) results of a parallel taxonomic survey and (ii) species abundance data on a country-wide scale. In the environmental DNA data, we detected a large number of species strongly under-recorded in taxonomic surveys and in previous distributional data from the Czech Republic. Most of these species are either very small or poorly known microlichens notoriously overlooked by taxonomists. Some are rare species with specific ecological requirements, but many are relatively abundant. Numerous species apparently new to science were detected, of which 12 species and two genera are newly described here: *Allarthothelium endochlorum*, *Atrodiscus fagicola* (new genus), *Bacidina omnicola*, *Biatorella ligni-putridi*, *Cryptodiscus neglectus*, *Gyalidea gabretae*, *Karstenia dryina*, *Micarea lobarica*, *Monilibrachium splendens* (new genus), *Psoroglaena neglecta*, *Toniniopsis pruinosa* and *Xylopsora diffissa*. In the descriptions, eDNA data are, for the first time in lichenology, utilized for characterizing ecology and distribution of the new species. In addition, 43 species detected by eDNA are new to the Czech Republic (23 of them confirmed by the parallel taxonomic survey). *Absconditella amabilis* and *Chaenotheca nitidula* are new to Europe.

Keywords: DNA barcode, eDNA, epiphyte, lichenized fungi

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Introduction

Sequencing of environmental DNA (hereafter eDNA) is now quite commonly used in surveys of local or regional biodiversity of some, usually taxonomically well understood groups, e.g. fish and amphibians (Valentini et al. 2015). It is also used to determine the species richness of less known, large and species-rich groups, such as fungi (e.g. Baldrian et al. 2022), or even across whole groups of organisms (Ficetola & Taberlet 2023). In these cases, however, eDNA data can hardly be linked to classical taxonomic surveys due to the lack of reference DNA barcode data. This has also been the case with lichen mycobionts, hereafter "lichens" (Wright et al. 2019, Henrie et al. 2022).

Studies using eDNA metabarcoding consistently find that biodiversity data obtained in this way are more complete than those obtained from classical taxonomic surveys (e.g. Nørgaard et al. 2021). Most papers point to this only indirectly, as it is not yet possible to link the two independent datasets. In lichenology, however, there have been recent attempts to create comprehensive local and regional DNA-barcode databases (Marthinsen et al. 2019, Kerr & Leavitt 2023), which to some extent allow linking data from eDNA samples with data from taxonomic surveys. For European epiphytic lichens, the Martin7 database (Vondrák et al. 2023) has already been used as a reference database for eDNA samples from central-European forests (Vondrák et al. 2025).

Here we build on the latter study, this time using a more detailed approach, to reveal gaps in the knowledge of the biodiversity of epiphytic lichens in the Czech Republic. Twenty forest sites along a gradient from lowland to montane spruce forests were surveyed by (i) a classical taxonomic voucher-based method and (ii) sequencing of environmental samples. We were interested in the differences in results, and especially in species that are significantly more represented in the eDNA than in the data from taxonomic surveys. We also extracted data from the DaLiBor database for the known distribution of lichen species in the Czech Republic (Man et al. 2022) to compare abundance data from eDNA with the frequency of records in the country.

First, we note the important point that abundances of species from taxonomic surveys correlate well with abundances from eDNA: common species are usually also abundant in eDNA; rare species are usually scarce in eDNA. This may sound trivial, but it proves that the "black box" of molecular species identification from eDNA works reasonably well. For individual species, abundance based on eDNA usually also correlate with the frequency of occurrences in the Czech Republic. However, in both cases, we found some species for which these correlations do not hold, and even show the opposite trend, i.e. species more abundant in eDNA than in taxonomic surveys and frequencies of occurrence in the Czech Republic.

Second, eDNA provided data to numerous species which are new to the Czech Republic or were not found in the last 25 years. Some of them are known only from eDNA, without any "floristic" evidence, but some have been confirmed by accompanying taxonomic surveys. Some of them appear to be common throughout the country, but overlooked by lichenologists. Some were previously known to us, though unnamed; we formally describe 12 of them here.

Materials and methods

Field research and sampling

Epiphytic lichens (and semilichens in the sense of Vondrák et al. 2022) were sampled, the term "epiphytic" being used in a broad sense for species occurring on all substrates composed of living or dead plants except epilithic/epigeic bryophytes and humus. Taxonomic survey and environmental sampling were performed in 10 pairs of 1-hectare square sites in the Czech Republic, chosen to cover an altitudinal gradient (170–1270 m a.s.l.; Fig. 1) and the range of representative forest communities along this gradient. Abbreviations of sites refer to Fig. 1 and detailed information is available in Supplementary Table S1. The sites cover forests dominated by *Picea abies* (BO), *Fagus sylvatica* (OS, ZD, ZF), *Abies alba* (CS), and a montane ravine forest with *Acer platanoides* and *A. pseudoplatanus* (RD). Forests at lower altitudes are mostly dominated by *Carpinus betulus*, *Fraxinus excelsior* and *Quercus petraea* (MK, PO, TY), the lowland floodplain forest (RN) consisting of *Acer campestre*, *Carpinus betulus*, *Quercus robur* and *Tilia*. Within the pairs, one site was located in an unmanaged old-growth forest stand in a nature reserve and the other in an old managed forest less than 5 km away. All sites were selected by the search for local hotspots (Vondrák et al. 2018). When selecting the sites, we avoided the northern part of the country because of the high level of historic atmospheric pollution, which significantly impoverished the epiphytic biota. Therefore, most of the upper-altitude sites were selected in the Šumava Mts, an area with relatively low air pollution.

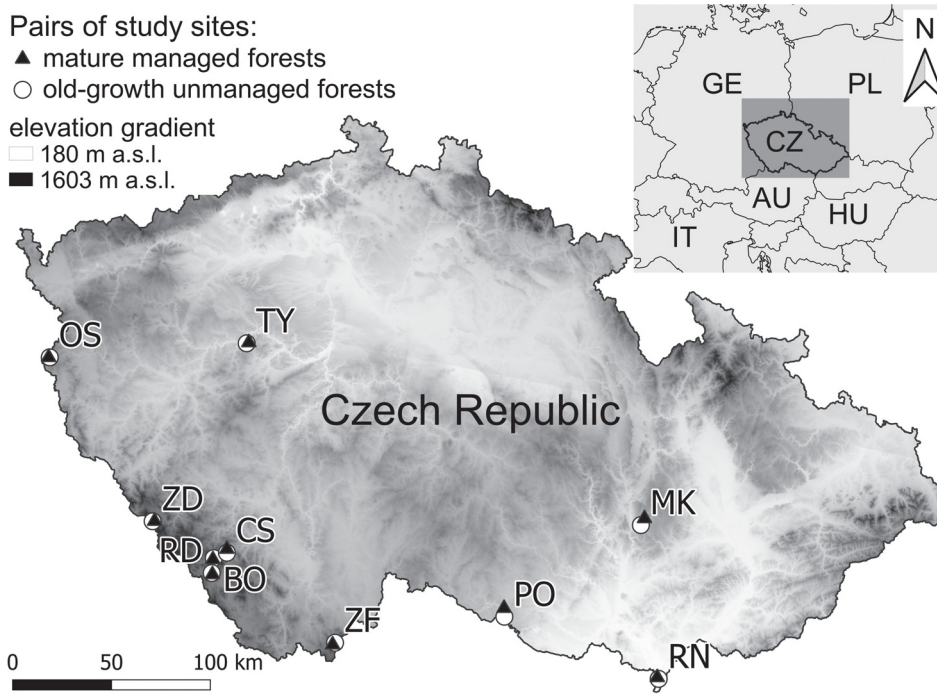

Fig. 1. Study sites. Abbreviations follow the Supplementary Table S1.

Each site was taxonomically examined in detail by three lichenologists (Palice, Šoun, Vondrák) for approximately eight hours. A minimum of five environmental samples, three by lichenologists and two by technicians (beforehand instructed about the ecological requirements of lichens), were collected from each site; in total, 126 samples were obtained from the 20 sites. Environmental samples were collected from organic substrates, i.e. the bark of trunks 0–2 m in height, the surface of branches and twigs accessible from the ground, and all types of wood (e.g. logs, stumps, and snags up to 2 m in height). Individual samples were collected on the sites for ~2 hours and were taken by scraping with a pre-cleaned knife into sterile 50 ml tubes. Each sample eventually contained about 30 ml of organic matter. Soil and inorganic substrates were avoided. The rule of thumb was to prioritize communities of small crustose lichens over the collection of biomass-rich macrolichens and to cover the full diversity of microhabitats and substrates.

DNA extraction, amplification and sequencing of eDNA

Environmental samples were ground using sterilized mortar and pestle. Approximately 10–50 ml of homemade lysis buffer (Shi et al. 2018) and 4 µl of RNase A (100 mg/ml; QIAGEN, Venlo, Netherlands) was added and the lysate was incubated at 65 °C for 5 min. Genomic DNA was extracted using DNeasy Plant Mini Kit (QIAGEN, Venlo, Netherlands) according to the manufacturer's instructions, except that 400 µl of the lysate was processed starting from step 3 of Quick-Start Protocol. The DNA isolates were purified using PowerClean Pro DNA Clean-Up Kit (QIAGEN, Venlo, Netherlands) and samples with a high DNA concentration were further diluted with sterile water when necessary (usually at 1:10 ratio).

The three barcodes (ITS1, ITS2 and mtSSU) were amplified for each sample using primers and cycling conditions summarized in Supplementary Table S2. Unique dual tagged primers were used, i.e. both forward and reverse primers were fused with sample-specific tag sequence (adopted from Roche Extended Set MIDs (454 Sequencing Technical Bulletin No. 005-2009; Roche, Basel, Switzerland; sequences no. 1–142 were used for each of the three barcodes). Polymerase chain reactions (PCRs) were performed in a reaction mixture containing 2.5 µM $MgCl_2$, 0.2 µM of each dNTP, 0.3 µM of each primer, 0.5 U Combi Taq polymerase in the manufacturer's reaction buffer (Top-Bio, Praha, Czech Republic), 0.6 µl of template DNA, and milli-Q water to make up a final volume of 10 µl. Each sample was amplified in three independent replicates. Individual tagged amplifications were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel, Düren, Germany). All amplifications of given barcode were pooled at equimolar ratio. The three resulting barcode pools were further purified using Agencourt AMPure XP beads (Beckman Coulter Brea, California, USA).

The three barcode pools were used for Illumina library preparation including ligation of indexed adapters and subjected to Illumina sequencing run for paired-end 2× 250 bp performed at SEQme Company (Dobříš, Czech Republic).

Bioinformatic analysis

As some of the sequences in reference database Martin7 are incomplete (Vondrák et al. 2023), the data were processed in order to include barcode regions with highest coverage in the database. Therefore, regions starting from reverse primers were selected for ITS1

(primer ITS2) and mtSSU (primer mrSSU3R) barcodes, whereas the region starting from forward primer (5.8S-Fun) was selected for ITS2 barcode.

The total dataset of 110.565.274 raw Illumina paired-end reads was processed using software tools implemented in SEED v.2.0 (Větrovský et al. 2018), Mothur v.1.39.5 (Schloss et al. 2009) and PipeCraft v.1.0 (Anslan et al. 2017).

For ITS1 and ITS2 barcode datasets, paired-end reads were assembled using FLASH v1.2.11 (Magoč & Salzberg 2011) with the following settings: minOverlap = 15 bp, mismatchRatio = 0.25, and maxOverlap = 250 (ITS1) or 230 (ITS2), respectively. The sequences were demultiplexed using SEED v.2.0 with no tag mismatch allowed and primer mismatch set to 1 (ITS1F and ITS2 primers), 4 (ITS4-Fun primer) or 8 (5.8S-Fun primer), respectively, retaining only the reads with identical barcodes on both ends. Quality trimming was performed using vsearch v1.11.1 (Rognes et al. 2016) with the following settings: truncqual = 0, maxee = 999, maxee_rate = 0.01, minlen = 150, maxns = 0. The sequences were oriented to start from reverse primer (ITS2) in ITS1 barcode or from forward primer (5.8S-Fun) in ITS2 barcode, respectively. All tag and primer sequences were trimmed, yielding 13.723.340 (ITS1) and 11.435.674 (ITS2) assembled sequences spanning the complete barcode length. As the assembly of short Illumina reads fails to assemble complete sequence in taxa with barcode length exceeding ca 400 bp, non-assembled reads were also taken into consideration. The non-assembled reads were demultiplexed as described above, and the resulting reads were filtered for target reads starting with desired tagged primer (ITS2 primer for ITS1, 5.8S-Fun primer for ITS2, respectively). The corresponding tagged primer sequence from the opposite paired read was extracted and added at the end of matching reads. Only the reads with identical barcodes on both ends were retained. Quality trimming was performed as described above, and all tag and primer sequences were trimmed, yielding 818.190 (ITS1) and 109.067 (ITS2) non-assembled sequences.

For the mtSSU barcode dataset, the considerable amplicon length (approx. 800–1100 bp) did not allow for read assembly. The raw reads were demultiplexed using SEED v.2.0 with no tag mismatch allowed and primer mismatch set to 1. The resulting reads were filtered for target reads starting with desired tagged mrSSU3R primer. The corresponding tagged primer sequence from the opposite paired read was extracted and added at the end of matching reads. Only the reads with identical barcodes on both ends were retained. Quality trimming was performed as described above for ITS barcodes, and all tag and primer sequences were trimmed, yielding 2.092.392 sequences up to 220 bp long.

The Illumina sequences were BLAST identified with the sequences of lichen taxa from Martin7 database extracted into three separate barcode databases (ITS1, ITS2, and mtSSU; see Supplementary Table S2 for details) covering homologous regions as processed Illumina sequences. In taxa represented by multiple identical sequences of a given barcode, only one sequence was retained for the barcode database. In taxa represented by multiple otherwise identical sequences but differing in length, the most complete (i.e. longest) sequence was selected. When two or more taxa showed identical database sequence, the taxa were labelled as a group of indistinguishable taxa (Supplementary Table S3). The sequences in ITS1 and ITS2 databases were shortened to 180 bp and 220 bp, respectively. Uniform length in both ITS barcodes was necessary to avoid false positive detections of taxa represented by longer Martin7 sequences, because our trial BLAST using non-shortened Martin7 barcode sequences revealed significant artificial detection

favouring congeners with database sequence longer than true target taxa. Sequences shorter than 180/220 bp were used only if they showed a unique genotype clearly distinguished from the others.

BLAST identification of the three separate Illumina datasets was performed using ssu pipeline (Vasar et al. 2017) supplemented with the individual barcode database. The following criteria were required for a BLAST match: sequence similarity ≥ 97%; alignment length not differing from the length of the shorter of the query and subject sequences by > 5%; and a Blast e-value < 1e–50.

Data used in this study

All rough eDNA data obtained from 126 samples is available in Dryad (doi:10.5061/dryad.fqz612k0g). Bioinformatically processed eDNA data used in this study and the data obtained from the taxonomic voucher-based survey are available as Supplementary Data S1. Taxonomic and eDNA datasets are used here in parallel to determine the occurrence (presence vs. absence) and abundance (number of records in the taxonomic overview and number of barcode sequences in the eDNA) of individual species. The focus is on species that are (i) new to science, (ii) new to the Czech Republic, (iii) poorly known, and for which our results significantly refine knowledge of their ecology and distribution.

As reference data from the Czech Republic, we extracted the records from the national database of bryophytes and lichens, DaLiBor (Man et al. 2022; data from May 2023). For each species discussed, we extracted the numbers of quadrants, ~ 6 km × 6 km, with verified occurrences in the last 25 years.

Results

Environmental sample data versus taxonomic survey

The entire dataset comprises 642 species, of which 593 were detected by eDNA and 483 by taxonomic surveys. Species that are common in floristic lists from taxonomic surveys are usually also abundant in eDNA and those that are rarely found by taxonomists usually have lower abundances in eDNA (Fig. 2). There are, however, many exceptions. These are of two types:

(i) Species under-represented in eDNA. A few species are frequent in taxonomic surveys (common species), but scarce in eDNA (e.g. *Arthonia spadicea* and *Naevia punctiformis*). This uncommon situation is probably caused by the low amplifiability of DNA barcodes for a given species. More frequently, a species rarely recorded in taxonomic surveys (rare species) is under-represented or even absent in eDNA (e.g. *Lecanora barkmaniana*, *Lecidea erythrophaea* and *Pertusaria hymenea*). These species could be scarce in the sample tubes, or by chance, not sampled in the field at all. In some cases, the absence of particular species in eDNA is caused by their indistinguishability from closely related species by DNA barcodes (Supplementary Table S3) or by the absence of reference DNA sequences (Supplementary Table S4).

(ii) Species under-represented in taxonomic surveys. Hundreds of species occur significantly more frequently in eDNA than in taxonomic surveys. Species that are abundantly represented in eDNA from at least one site, but absent or poorly represented in tax-

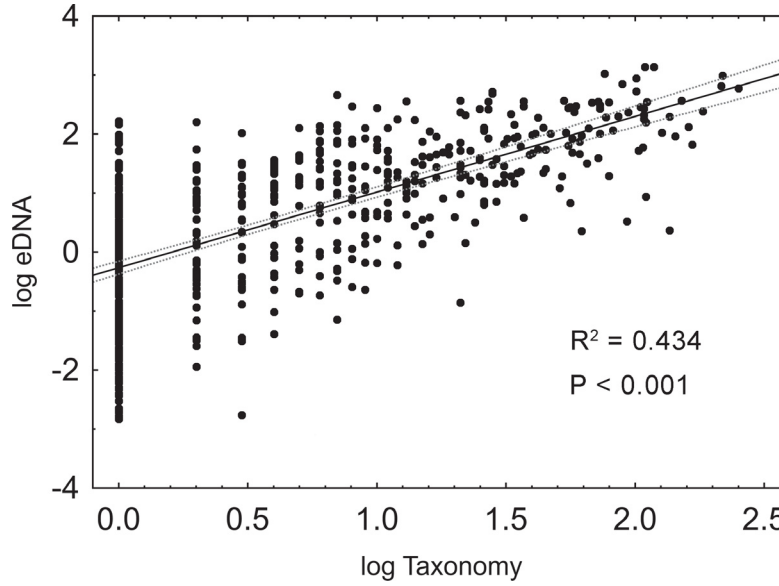

Fig. 2. Relationship between classical taxonomic survey data and eDNA data expressed by linear regression with 95% confidence interval. Points represent species detected during the survey of all study sites. Abundances in eDNA are on a relative scale between 0 and the most abundant species *Lecanora argentata*. Abundances from the taxonomic survey are expressed as the sum of individual records for each species.

onomic data, are likely to be truly abundant, but with a phenotype that is overlooked by taxonomists for various reasons. For example, many *Bacidina* spp. and *Micarea* spp., often belonging to predominant species, are overlooked as they are hardly distinguishable from some other species. There are also numerous examples of species with rather low frequencies in eDNA, but with very few or no data from taxonomy surveys. Hundred-fifty-nine species were only detected from eDNA (Supplementary Data S1). These species may not form fully developed thalli, i.e. they may occur only in immature stages or as diaspores, but we consider their presence to be proven.

Environmental sample data versus known occurrences in the Czech Republic

For the most part, our eDNA data for individual species corresponds to the frequency of their occurrence in the Czech Republic. Species abundant in the Czech Republic by conventional criteria are also abundant in eDNA; this is true almost without exception (Fig. 3A). For some species that are less abundant by conventional criteria, abundances in eDNA data are much higher than would be consistent with national data. (Fig. 3B). The last group, but for this study the most important one, consists of almost a hundred species with various abundances in eDNA, but not reported from the Czech Republic yet, or considered extinct in the country (Fig. 3C). Of these genotype-based "species", 43 are assigned to existing names (listed and commented below), while the rest are putative unnamed species (known-unknowns), mainly in taxonomically difficult or unexplored groups, i.e. *Bacidina*, *Micarea*, *Scoliciosporum* and *Verrucariaceae* (see Supplementary Data S1). Of the known unknowns, 12 are formally described below.

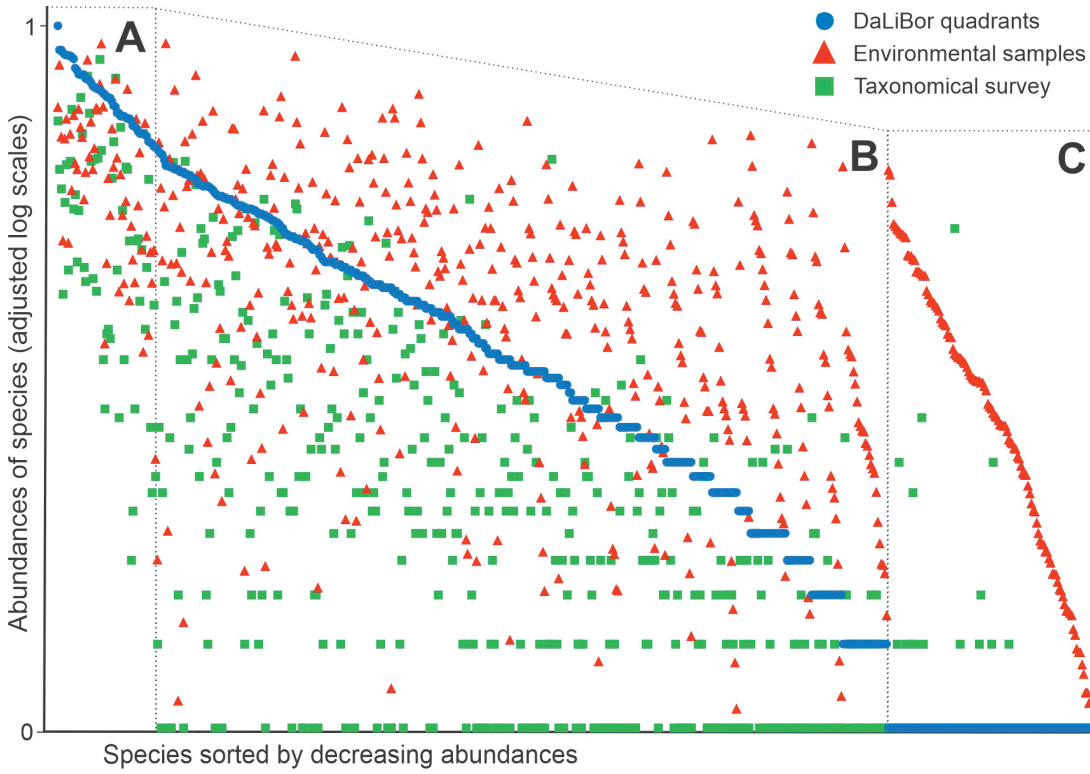

Fig. 3. Abundances of all 593 species identified from eDNA. Each species is represented by three symbols above each other, corresponding to their relative abundance in three independent datasets (legend top right). Datasets from taxonomic survey and environmental sampling were obtained from all study sites. "DaLiBor quadrants" dataset represents numbers of c. 6 × 6 km quadrants with records of particular species; data adopted from the Czech national database of bryophytes and lichens, DaLiBor (Man et al. 2022; data from May 2023). A, common species, where all datasets correspond; B, rare to common species where the datasets frequently differ; C, species new to the country or not included in the DaLiBor database. Species are sorted along the x-axis by decreasing abundance in DaLiBor quadrants (A, B) and in environmental samples (C).

Examples of species that are notably more abundant in eDNA than is consistent with known occurrences in the Czech Republic are listed in Table 1. All 39 species listed are microlichens (i.e. lichens with a crustose thallus) and some of them are extremely minute, e.g. *Absconditella celata*, *Absconditonia rubra*, *Gyalidea minuta* and *Micarea perparvula*. Our eDNA data from macrolichens (i.e. with foliose and fruticose thalli) are generally consistent with national data, except for a few mainly cryptic species (e.g. *Parmelia encryptata* and *P. serrana*) which are unsurprisingly over-represented in eDNA. This confirms that the distribution of macrolichens is much better known than that of microlichens.

Table 1. Examples of species with a noticeably high frequency of occurrence in the eDNA samples, which is contrary to the data from the Czech Republic (Man et al. 2023; data from May 2023). Full version of the table including all species is attached as Supplementary Table 5. Relative abundance in eDNA is on a scale of 0–100. where 100 represents the most abundant species *Lecanora argentata*. Occurrences in sites, altitudinal tendencies and estimated abundances are derived from eDNA from the 20 study plots. Altitudinal tendencies: lowland, < 500 m a.s.l.; upland, 500–1000 m a.s.l.; mountain, > 1000 m a.s.l.. Taxonomy – number of records in taxonomic survey. Data from the Czech Republic – numbers of occupied 36 km^2 quadrates since 1998.

Species (lichen mycobiont)	Total relative abundance in eDNA	Occurrences in sites	Altitudinal tendency	Estimated abundance	Taxonomy	Data from the Czech Republic
Absconditella celata	0.62	16	whole range	rare-abundant	3	11
Absconditonia rubra	0.44	20	whole range	rare	1	21
Arthopyrenia salicis	0.26	10	mostly upland	rare-abundant	2	8
Bacidia biatorina	0.36	5	upland	rare-abundant	1	4
Bacidia hyalina	1.21	7	lowland	rare-abundant	3	3
Bacidina acerina	26.06	8	lowland	abundant	6	3
Bacidina flavoleprosa	0.02	7	mostly upland	rare	0	1
Bacidina lignicola	6.38	18	whole range	abundant	1	1
Biatoridium delitescens	0.05	10	mostly lowland	rare	2	4
Buellia erubescens	1.10	14	mostly lowland	mostly rare	1	3
Byssoloma diederichii	0.03	13	whole range	rare	0	1
Catinaria neuschildii	0.02	6	upland	rare	0	2
Coenogonium luteum	0.04	7	mostly lowland	rare	1	2
Dichoporis taylori	7.59	9	lowland	abundant	1	2
Elixia flexella	0.67	18	whole range	mostly rare	0	5
Fuscidea arboricola	0.56	9	whole range	rare-abundant	1	7
Gyalidea minuta	0.06	5	lowland	rare	0	1
*Chaenotheca laevigata**	0.05	8	mostly lowland	rare	0	3
Chrysothrix fagicola	1.39	11	upland	rare-abundant	0	5
Japewia gyrophorica	4.66	11	mostly mountain	abundant	2	4
Karstenia idaei	0.14	7	lowland	rare	1	9
Lecanora albellula	0.15	17	whole range	rare	0	20
Lecanora compallens	3.78	9	lowland	abundant	0	6
Lecanora flavoleprosa	0.05	14	whole range	rare	0	8
Lecanora stanislai / strobilina	11.59	16	mostly lowland	abundant	1	5
Lecanora subsaligna	0.09	15	whole range	rare	1	12
Lecidella albida	0.46	7	lowland	rare-abundant	2	9
Lepraria eburnea	1.28	15	whole range	mostly rare	1	12
Leptorhaphis maggiana	0.05	13	mostly lowland	rare	2	7
Micarea contexta	1.33	8	mostly mountain	rare-abundant	1	4
Micarea czarnotae	10.59	20	whole range	abundant	0	7
Micarea deminuta	0.04	7	whole range	rare	0	9
Micarea microareolata	1.86	16	upland-mountain	rare-abundant	0	1
Micarea microsorediata	0.60	12	mostly upland	rare-abundant	0	1
Micarea perparvula	0.03	10	whole range	rare	1	2
Micarea substipitata	1.93	18	upland-mountain	rare	1	15
Psilolechia torii	0.47	5	mostly upland	rare-abundant	1	0
Rinodina poeltiana	2.87	6	lowland	abundant	3	5
*Swinscowia jamesii**	6.11	6	lowland	abundant	3	5

Discussion

Floristic data from eDNA: advantages and limitations

Detecting species richness and species composition by sequencing DNA from eDNA samples is clearly a step forward in terms of the quality of biodiversity surveys (Dickie et al. 2018, Frøslev et al. 2019, Feio et al. 2020). First of all, it provides an unprecedented amount of data. For example, if we obtain hundreds of thousands of lichen sequences per barcodes from individual environmental samples, we obtain tens of thousands of sequences (abundance units) for the more abundant species. It is about three orders of magnitude more abundance units than any taxonomic survey can obtain. Thus, with eDNA data, we can comment on species abundance at a much finer scale than with a taxonomic survey. This advantage could be skewed by the method of sampling, namely by preferring certain species (e.g. macrolichens in lichenological surveys) or certain microhabitats. In our case, we avoided these drawbacks by training the sample collectors. Results from eDNA may also be affected by different affinities of sequencing primers to sequences of different species, and thus the resulting sequence counts may not correspond to the real species abundance. In our case, this risk does not seem to play a significant role, because, for example, among the 100 most abundant species in terms of taxonomic surveys, there are no species that are significantly underrated in the eDNA data. In our case, this risk is also reduced by the synergy of the three DNA barcodes, rather than by using ITS2 region alone which is standard in lichenology and mycology (Schoch et al. 2012, Kerr & Leavitt 2023, Robison et al. 2023, Dreyling et al. 2024).

Another advantage of species detection from eDNA is its independence from taxonomic experience. All samples are evaluated in the same way, based on a single reference DNA barcode database. All taxonomists tend to overlook some species, and each taxonomist focuses on a specific range of species. In addition, there are certain groups of lichens that are regularly overlooked by most taxonomists (e.g. sorediate crustose lichens without fruiting bodies). However, objective molecular species identification from eDNA is dependent on a quality of a DNA barcode reference database and the results are inevitably biased – species missing from the database will not appear in the final dataset even though they may be abundant.

Species identification from eDNA is an extremely sensitive method for determining species richness and species composition. The sensitivity is proportional to the sequencing depth (i.e. the number of barcode sequences obtained per sample). By increasing the depth, the number of species detected increases too. In our project, we sequenced three DNA barcodes at a level of tens of thousands of sequences per barcode, and it turned out that about half of the species richness in the sites is composed of so-called "ghost species", i.e. species not recorded by taxonomic surveys (Vondrák et al. 2025). Sequencing eDNA therefore provides much more complete data on local biodiversity, but it has its pitfalls, see discussion below.

Constraints in interpretations of eDNA data

The nature of data from environmental samples is quite different from that of data collected by taxonomists in biodiversity surveys, and environmental sequencing cannot replace classical taxonomic surveys in some aspects. Therefore, we recommend combining both approaches in biodiversity research. Frøslev et al. (2019) and Dreyling et al.

(2024) reached the same conclusion with fungal and lichen inventories. The major shortcomings of eDNA data are twofold:

(i) When evaluating results from sequencing of environmental samples, we have a very limited opportunity to comment on the ecology of the species. This opportunity is further reduced when the target area increases. When sampling a 1-hectare area, we are only able to link species occurrences to rough environmental features common to the whole area (e.g. elevation and habitat). In contrast, taxonomists working on a 1-hectare site are able to characterize each record in much greater detail in relation to the substrate, microhabitat, microclimate, etc.

(ii) When studying local biodiversity only with the use of eDNA for the identification of species, we cannot comment with certainty on the phenotypic state of the species at the site. In the case of lichens, it can at least be assumed that species with high abundance of DNA barcode sequences will be abundant at a given site, probably in a state of fully developed thalli. The interpretation of data on species with lower numbers of sequences is more difficult. Here, different scenarios may arise, e.g. (i) rare occurrence of fully developed thalli, (ii) occurrence as young and poorly developed or, conversely, dying thalli, (iii) occurrence of mycobiont in the aposymbiotic stage (see Spribille et al. 2022, Pichler et al. 2023), (iv) occurrence only in the form of diaspores (e.g. soredia). The last scenario is the most problematic, as the "occurrence" of a species in this case is questionable – it may be a diaspore of a species that has no chance of long-term survival in a given habitat. In our data, with very rare exceptions, we did not find any obvious case of a lichen that is unlikely to survive in a given habitat. However, when sequencing eDNA at higher levels (i.e. millions of barcode sequences per sample), these "unlikely" species might be detected more frequently.

The use of eDNA data

When interpreting eDNA data, one must consider the much higher sensitivity in species detection compared to taxonomic surveys. From eDNA, it is possible to estimate species abundances based on the ratio of the number of obtained sequences for each species. However, it is not possible to accurately comment on the phenotypical and ecological character of individual occurrences, especially if a parallel taxonomic survey has not been carried out. In our case, eDNA reveals almost a hundred species which are new to the Czech Republic and refines our knowledge of the distribution and abundance of many lichen species. This new information will be reflected in the Atlas of Czech Lichens (Malíček et al. 2023), but individual eDNA records will not be included among the full-fledged records in the DaLiBor national database (https://dalibor.ibot.cas.cz/login) due to the lack of basic information on phenotype and ecology. Nevertheless, records based purely on eDNA data serve as motivation for attempts to confirm them by subsequent taxonomic surveys.

In this study, eDNA data were used in descriptions of new species. Some of the described species have only few voucher specimens and it is difficult to get more, mainly for two reasons – the species are either very rare (e.g. *Gyalidea gabretae*) or appear as crusts which are hardly distinguishable from other, morphologically close species (e.g. *Psoroglaena neglecta*). In these cases, eDNA data effectively provide the information on abundance, distribution and some information about ecology (at least altitudinal tendency, habitat type, etc.).

New species

In addition to morphological and chemical characters, each new species is also characterized using Sanger sequences of the mycobiont (ITS and mtSSU) and eDNA data are used to estimate the abundance and describe the distribution of the new species. Full photographic documentation will be available in the Atlas of Czech Lichens (https://dalib.cz).

Allarthothelium endochlorum Vondrák, spec. nova

MycoBank: MB#855533; Fig. 4
Etymology: Unlike most *Allarthothelium*/*Arthothelium* species, the new species has a green hymenial pigment (endochlorum = green inside).
Type: Czech Republic. Volary, Zátoň, Mt. Boubín, eastern slope between way Lukenská and hill Pažení, alt. 1100 m, 48.96563N, 13.80718E, on bark of *Acer pseudoplatanus*, 12 May 2020, J. Vondrák 23547 (PRA, holotype).
Sequences from the holotype: ITS (OQ717707); mtSSU (OQ646104); both sequences published under the name *Arthothelium scandinavicum* by Vondrák et al. (2023).
Sequence from the photobiont in the paratype: *rbc*L (PP438762).

Diagnostic characters: Thallus white, effuse, with trebouxioid photobiont. Apothecia rounded to irregularly shaped, black, usually covered by a white partly translucent membrane of dead bark cells. Hymenial pigment green. Subhymenium thin, colourless. Ascospores large, 28–50 × 13–22 µm, muriform, with a distinct gelatinous sheath (I+ orange-red, KI+ blue).

Morphology-anatomy: Thallus crustose, white, effuse, endo- or epiphloedal, up to 50 µm thick, formed of a loose proso-plectenchymatous tissue, I+ pale blue, of flexuose, ~2 µm thick hyphae. Photobiont trebouxioid (*Trebouxia* sp. according to the *rbc*L sequence), the mature cells up to 14 µm diam., dividing into several daughter cells, 4–5 µm diam.; in goniocyst-like clusters, not forming a continuous layer. Apothecia flat, arthonioid, rounded to irregularly shaped, black, but mostly covered by a ~20 µm thick membrane, of hyaline, dead bark cells that make the apothecial surface pale grey (Fig. 4B). Apothecial primordia punctiform, ~0.1 mm diam.; mature apothecia up to ~0.6 mm diam. Hymenium 60–100 µm tall, colourless in lower part, green in upper part (especially in epihymenium; Fig. 4C). Subhymenium/hypothecium up to 25 µm thick, colourless, of contorted cells. Paraphyses flexuose, richly branched and anastomosed, ~1.5–2 µm thick, without broadened tips (Fig. 4F). Asci broadly clavate to subglobose, 50–80 × 35–50 µm. Ascospores 8 in asci (Fig. 4D), muriform (Fig. 4E, G), with 6–9 transverse septa and 1–3 longitudinal septa in each transverse segment, colourless, or slightly melanized when old, ellipsoid, 28–50 × 13–22 µm, with a 1–2 µm thick gelatinous sheath (I+ orange-red, Fig. 4H; KI+ blue; swollen in KOH, Fig. 4I). Conidiomata not seen.

Chemistry: Hymenial pigment green, unchanged in KOH, HNO_3+ brown or unchanged. Hymenium hemiamyloid, I+ red, KI+ blue; asci with KI+ dark blue outer wall and often with KI+ orange content; gelatinous sheath of ascospores I+ orange-red, KI+ pale blue; thalline tissues I+ pale blue. Thallus spot tests: K–, C–, KC–, P–, UV+ white; no substances detected by TLC in the holotype.

Ecology and distribution: Only two voucher specimens are available: the holotype and another specimen collected in the type locality, both from bark of mature *Acer pseudoplatanus* trunks in a montane beech-spruce forest (Fig. 4A), at ~1100 m, in the Šumava Mts, Czech Republic. It occurs in microsites sheltered from rain, without accom-

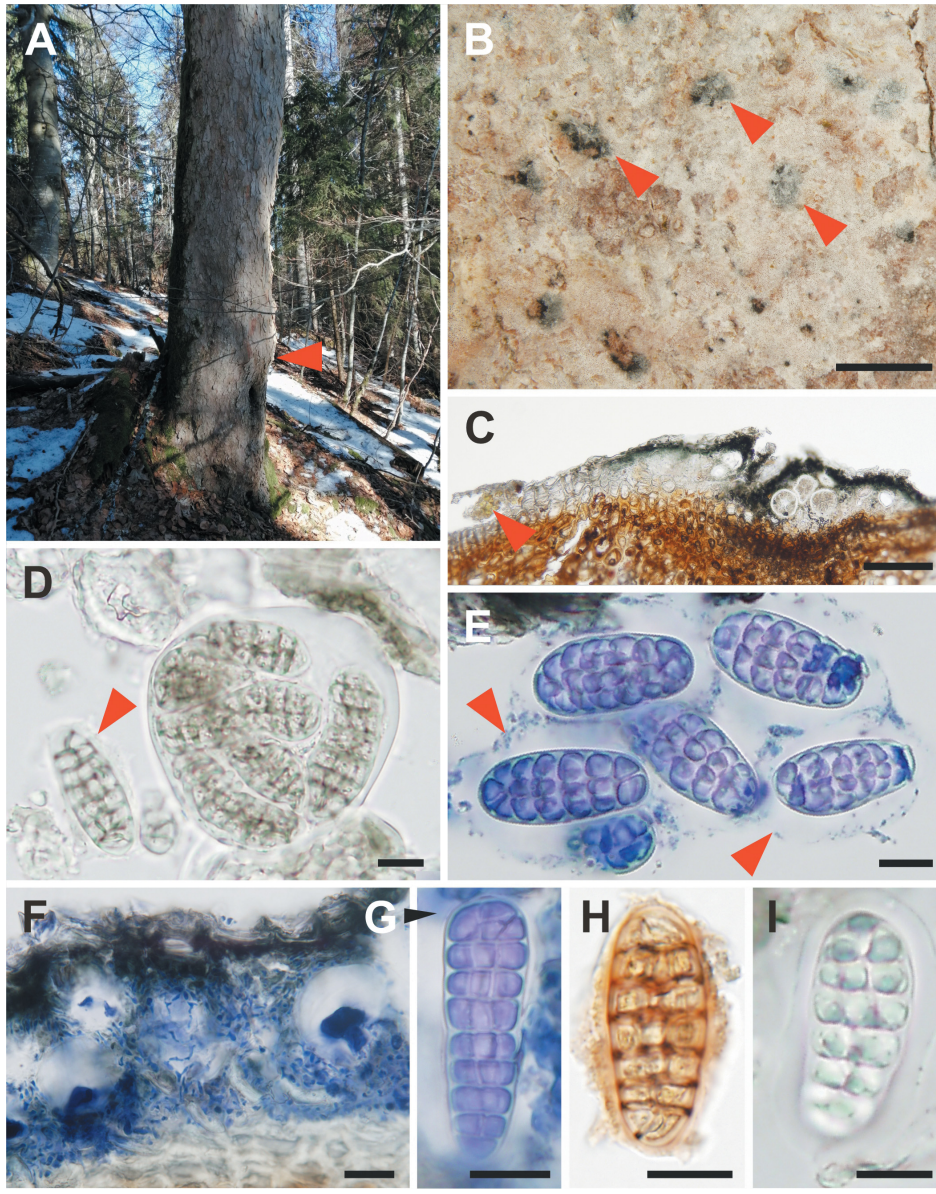

Fig. 4. *Allarthothelium endochlorum* (holotype). A, habitat and place of finding on the sycamore (arrowhead); B, thallus and apothecia (arrowheads) covered by whitish epidermis layer of the sycamore bark; C, vertical section of apothecium with spherical asci, colourless hymenium/subhymenium and green epithecium, colony of photobiont cells on the left side (arrowhead); D, ascus with eight older, slightly melanized ascospores, gelatinous sheath visible on the released ascospore (arrowhead); E, ascospores with enormously thick gelatinous sheaths (arrowheads); F, hamathecium with flexuose paraphyses; G, strongly muriform ascospore; H, ascospore with I+ orange gelatinous sheath; I, ascospore with gelatinous sheath swollen in KOH. C, D, observed in water; E–G, stained by cotton blue; H, after treatment with Lugol's solution; I, in KOH. Bars: B, 1 mm; C, 100 µm; D–H, 10 µm.

panying lichens. According to eDNA data, the species occurred in two old-growth forest sites (RD1 and ZF1) in altitudinal range 750–900 m.

Similar species: The classical system of *Arthoniaceae* by Redinger (1937) places species with muriform and submuriform ascospores into the genera *Arthothelium* (with trentepohlioid photobiont) and *Allarthothelium* (with trebouxioid photobiont). According to this concept, the new species belongs to *Allarthothelium* and we decided to employ this, recently rarely used, generic name, although we are aware that it probably does not represent a monophyletic group in the classical sense. Most *Allarthothelium* species were described in the late 19th and the early 20th century from non-European regions and they have quite distinct characters: usually smaller ascospores and different apothecial pigmentation (Eckfeldt 1889, Wainio 1896, 1915), while two species, *Allarthothelium sparsum* from China and *A. fuscoglaucum* from Guadeloupe have distinctly larger ascospores (Wainio 1915, Zahlbruckner 1930). The only European species, *Allarthothelium crenulatum*, is saxicolous and has smaller ascospores, 2–4 in elongated clavate asci (Redinger 1937). European species of *Arthonia* / *Arthothelium* with muriform ascospores usually have a brown pigment in the hymenium, a trentepohlioid photobiont and smaller ascospores. The only species lichenized with a trebouxioid photobiont is *Arthonia phlyctiformis* (Gerstmans & Ertz 2016; = *Arthothelium taediosoides* Grube & Giralt 1996). It is distinct by the typically elongate apothecia (which are not covered by the bark epidermis), smaller ascospores with fewer septa, an olivaceous-brown pigment in hymenium, and its occurrence in coastal Mediterranean sites; like the new species, it has a gelatinous sheath surrounding the ascospores (Grube & Giralt 1996). Gelatinous sheaths surrounding ascospores are also reported for *Arthonia subastroidea* (Nimis 2024), but this species has smaller ascospores, a grey epihymenium, and occurs on acidic bark of *Pinus cembra* or *Rhododendron ferrugineum* in subalpine habitats (Almquist 1880). *Arthothelium norvegicum* and *A. spectabile* have quite large ascospores (but not as large as the new species), a dark brown to red-brown hymenial pigment, and are lichenized with *Trentepohlia* (Redinger 1937, Coppins & Tønsberg 1984, Coppins 2009). *Arthothelium scandinavicum* is similar, but has smaller ascospores and a brown-black epihymenium (Almquist 1880, Fries 1865), no photobiont, and the apothecia usually have epipsamma of red crystals glowing red in polarized light and dissolving in KOH (our observations on Caucasian specimens). *Arthonia fusispora*, distinguished by Almquist (1880) from *Arthothelium scandinavicum*, differs in having ascospores with acute ends. Both of the latter species also differ in ecology, growing on bark of conifers, especially on *Picea*, mostly in boreal forests (Almquist 1880). The closest species in mtSSU is allegedly *Arthothelium puniceum* described from Australia, which has distinctly smaller ascospores and differs in the hymenial pigmentation (Müller 1893).

DNA data: ITS and mtSSU sequences were obtained from the holotype. Comparable ITS sequences are not available in the NCBI, which is due to the scarce available ITS data within the Arthoniomycetes (Vondrák et al. 2023). The closest sequences are from *Arthonia bueriana*, *A. didyma* and *A. dispersa*, but all are less than 80% identical and with cover up to 50%. In mtSSU, sequences of alleged *Arthothelium puniceum* from Korea are most similar, i.e. about 95% identical. Phylogenetic relationships of the new species and close *Arthoniaceae* in mtSSU are reconstructed (Supplementary Fig. S1).

Paratype: Czech Republic. Volary, Zátoň, Mt. Boubín, eastern slope between way Lukenská and hill Pažení, alt. 1090 m, 48.96553N, 13.80723E, on bark of *Acer pseudoplatanus*, 28 January 2024, J. Vondrák 28339 (PRA).

Atrodiscus Malíček, Palice et Vondrák, gen. nov.

MycoBank: MB#855534

Atrodiscus fagicola Malíček, Palice et Vondrák, spec. nova

MycoBank: MB#855535; Fig. 5
Etymology: The generic name describes the dark brown to black discs of the ascomata. The epithet is derived from its predominant occurrence on *Fagus sylvatica*.
Type: Czech Republic. Southern Bohemia, Chýnov, managed spruce forest with old beeches 0.7 km N of Chotčiny, alt. 675 m, 49.45431N, 14.83372E, on bark at base of *Fagus sylvatica* trunk, 3 June 2020, J. Malíček 13789 & J. Rydlo (PRA, holotype).
Sequences from the holotype: ITS (PP410486); mtSSU (PP407375).

Diagnostic characters: Saprobic fungus or semi-lichen, indistinct or visible as a pale coating on bark. Ascomata apothecia-like, black, sessile to slightly constricted at base, 0.10–0.25 mm in diam. Dark annular exciple with Hypnorum-blue pigment. Hypothecium colourless to brown; hymenium colourless or brown in upper part. Hamathecium of thin anastomosing pseudoparaphyses. Ascospores 1–3 (–4)-septate, colourless, 9–11 × 4–5 µm. It grows mostly on bark of *Fagus sylvatica* in central-European forests.

Morphology-anatomy: thallus indistinct or very thin to immersed in the substratum, sometimes visible as a pale coating, locally associated with various green algae, especially near ascomata (frequently visible in sections). According to Vondrák et al. (2022), it can be considered as a semi-lichen. Ascomata apothecia-like (Fig. 5A), 0.1–0.25 mm in diam., rounded, at first sessile to slightly immersed in the substratum, mature ascomata sessile to slightly constricted at base; discs dark brown to black, matt, flat to concave, smooth; margin distinct in all development stages, black, ±glossy, distinctly elevated above disc, ±smooth, but sometimes coarse in old ascomata. Exciple annular, but rarely patchily spreading below hypothecium, 16–35 µm thick, single cells and hyphae not visible, inner part brown, outer part or usually most of the exciple dark brown to black (Fig. 5B) with an inconspicuous violet or blue tinge, K+ blue-green. Hypothecium 15–25 (–32) µm high, almost colourless to brown, K–, sometimes with dark, K+ blue-green granules. Hymenium (35–) 50–75 (–85) µm tall, colourless or locally brownish, I+ orange to slowly red (Fig. 5C) or pale blue in low concentration of Lugol's solution (Fig. 5D), KI+ blue, rarely with K+ blue-green granules; epihymenium pale brown to brown, K–. Hamathecium formed by flexuose, branched and anastomosing, ~1 µm thick pseudoparaphyses, hardly visible in water, but observable after staining with lactophenol cotton blue (Fig. 5E). Asci unitunicate (Fig. 5F), clavate, 8-spored, non-amyloid, with an external amyloid gelatinous cap, 33–50 × 7–8.5 µm. Ascospores colourless, ellipsoid, usually 1-septate (Fig. 5J), occasionally with 2 or 3 septa (Fig. 5H, I), or exceptionally 4-septate (Fig. 5K), (8–) 9–11 (–12.5) × (3–) 4–5 (–5.5) µm. Inner cells of ±equal size; upper cell not distinctly enlarged. Conidiomata not observed.

Chemistry: The ascomata contain the Hypnorum-blue pigment (Meyer & Printzen 2000), which appears black in thicker sections due to its high concentration, but with the typical blue or violet tinge visible in thin sections. It is KOH+ blue-green (turquoise) or

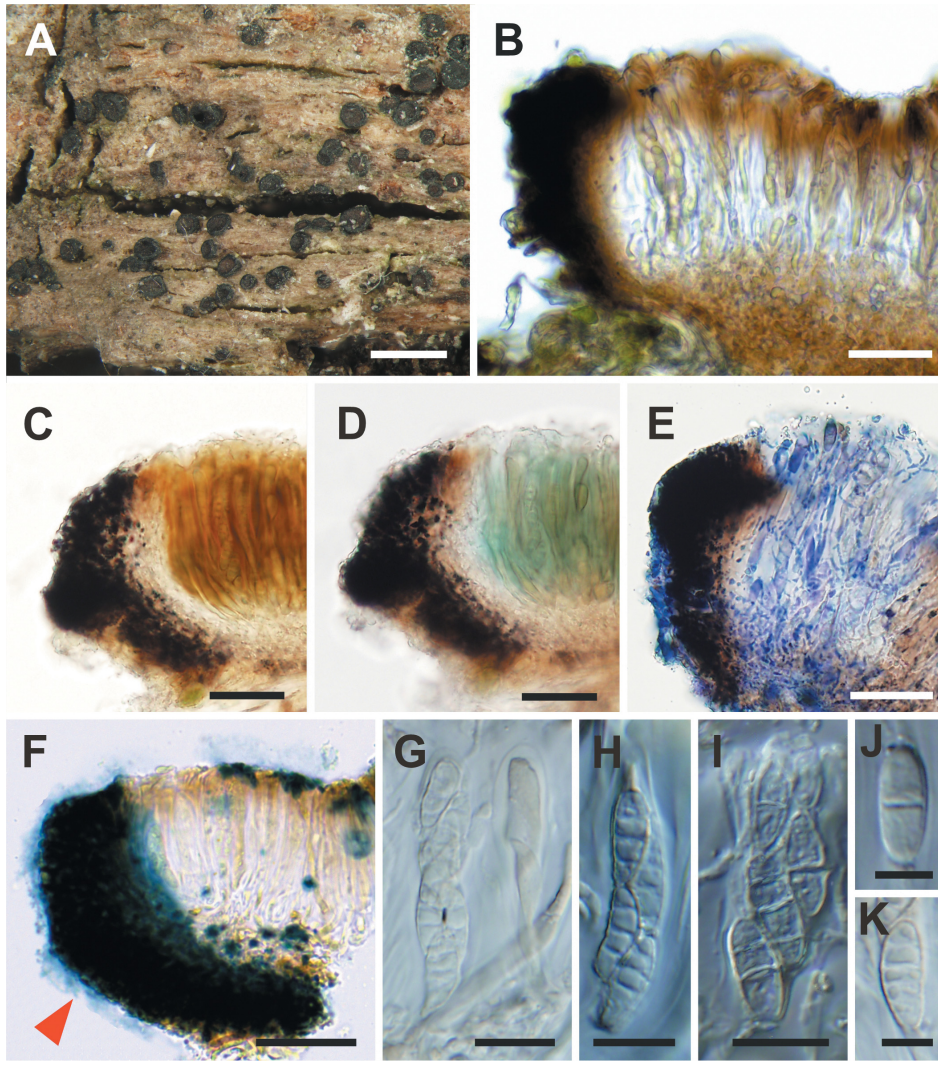

Fig. 5. *Atrodiscus fagicola* (Malíček 13433; but B, Malíček 13370; F, Malíček 14040). A, apothecium-like ascomata; B, vertical section of ascoma; C, hymenium in high concentration of the Lugol's solution; D, hymenium in low concentration of the Lugol's solution; E, hamathecium of flexuose, branched and anastomosing pseudoparaphyses; F, Hypnorum-blue pigment in exciple and hymenium, forming a blue halo (arrowhead) when concentrated; G, unitunicate asci; H–K, ascospores. B, F, G–K, observed in water; C, D, after the Lugol's solution treatment; E, stained by cotton blue; H–K, in differential interference contrast. Bars: A, 0.5 mm; B–F, 20 µm; G–I, 10 µm; J, K, 5 µm.

rarely blue, HCl+ violaceous and HNO_3–. The highest concentration occurs in the exciple, where it is present almost in the whole tissue, or at least in the outermost layer. When present in particularly high concentration, we noted formation of a blue halo around the exciple (Fig. 5F). In seven specimens (35%), we also observed this pigment in the hypothecium. It occurs there in the form of irregular granules (POL–) up to 2 µm in diam. (as in *Bryobilimbia* species). Three specimens contained sparse granules also in the

hymenium. Hypnorum-blue was not found in one specimen (Malíček 13878). Presence of secondary metabolites was not tested by TLC.

Ecology and distribution: Most of the collections come from bark of *Fagus sylvatica*, three from decaying wood of conifers, two from bark of *Ulmus glabra* and single collections from bark of *Acer pseudoplatanus* and *Picea abies*. The new species occurs in microsites poor in lichens, often at bases of trunks, but sometimes overgrowing lichen thalli (*Anisomeridium*, *Porina*) or in close association with algal colonies. *Lepraria finkii* was the most commonly associated lichen, followed by *Anisomeridium polypori* and *Coenogonium pineti*. *Agonimia repleta*, *Alyxoria ochrocheila*, *Arthonia spadicea*, *Bacidina modesta*, *Catinaria atropurpurea*, *Lepraria vouauxii*, *Micarea micrococca* agg., *M. substipitata*, *Porina aenea* and several bryophyte species were associated in single specimens. *Atrodiscus fagicola* is known from old-growth, natural and managed forests at middle to submontane altitudes (515–975 m a.s.l.). It prefers old trees, which are generally rare in managed forests, most of the records being from deciduous or mixed old-growth forests. The species is so far known from 19 localities in the Czech Republic (18 of them in Bohemia). In eDNA, it was detected from 12 of 20 sites between 360 and 1210 m a.s.l. It is probably not rare in suitable habitats and is expected to occur in surrounding countries.

Similar species: The new species is most similar to the saprobic or lichenicolous *Dactylospora aeruginosa* Holien et Ihlen [= *Sclerococcum aeruginosum* (Holien et Ihlen) Ertz et Diederich], which produces the same K+ aeruginose pigment in the exciple and hymenium (incl. the uppermost part). However, *D. aeruginosa* differs in having much larger ascomata (0.3–0.7 mm), a thicker cupulate exciple (30–80 µm) composed of polygonal lumina, hyaline to light brown epihymenium, and longer ascospores (11.0–14.5 µm) with a perispore (Ihlen et al. 2004). Macroscopically, the new species resembles members of the genera *Dactylospora* or *Melaspilea* s. lat.

DNA data: DNA sequencing was successful from six specimens extracted by Chelex (Ferencova et al. 2017), whereas extractions using CTAB and the Invitek were not successfully sequenced. The five ITS sequences are 98% identical and do not have any close relatives in NCBI. The five mtSSU sequences are 100% identical and have closest NCBI Blast matches to *Sclerococcales* sensu Réblová et al. (2017), namely to *Dactylospora* p.p., *Fusichalara*, *Rhopalophora* and *Sclerococcum*. The new species is, however, not closely related to any known species, as the closest NCBI Blast hits in mtSSU are 87% (to *Rhopalophora*). A placement in *Sclerococcales* is possible but not supported by the mtSSU phylogeny (Supplementary Fig. S2).

Paratypes: Czech Republic. Western Bohemia, Nepomuk, Chejlava National Nature Reserve, old-growth beech forest, alt. 585 m, 49.53794N, 13.55828E, on bark of snag of *Fagus sylvatica*, 1 August 2019, J. Malíček 13433 et J. Rydlo (herb. Malíček). Nepomuk, Srby: deciduous forest 100 m SW of Kámen Hill (577 m), alt. 555 m, 49.52694N, 13.57611E, on bark of *Acer pseudoplatanus*, 26 October 2018, Malíček 12158 et Z. Palice (herb. Malíček; ITS: PP410489, mtSSU: PP407372). Brdy Hills, Nové Mitrovice, Míšovské buky Nature Monument, fragment of old-growth beech-spruce forest, alt. 730 m, 49.59694N, 13.73833E, on bark of *Fagus sylvatica*, 8 July 2019, J. Malíček 13040 (herb. Malíček). Kdyně, Mezholesy: managed mixed forest 0.3 km SE of Koráb Hill (773 m), alt. 750 m, 49.39381N, 13.07892E, on bark of *Fagus sylvatica*, 24 July 2019, J. Malíček 13370 et J. Rydlo (herb. Malíček; ITS: PP410488, mtSSU: PP407373). Český les Mts, Přimda, Přimda Nature Reserve, old-growth scree and beech forest on rocky ridge of Přimda Hill (848 m), alt. 800–840 m, on bark of old *Fagus sylvatica*, 9 April 2016, J. Malíček 10140, V. Lenzová et J. Vondrák (herb. Malíček); ibid., nature reserve Diana, old-growth mixed forest with predominant beech, alt. 515 m, 49.63208N, 12.57986E, on bark of old *Fagus*, 11 April 2016, Z. Palice 21019 (PRA). Doupovské hory Mts, distr. Karlovy Vary, Valeč: mixed temperate

forest on SW facing slope, on the S-SSW foothill of Mt. Jedliny [702] above the stream of Mlýnecký potok, 0.8–0.9 km N of Valeč, alt. 587 m, 50.18214N, 13.25411E, on shaded bark of *Ulmus glabra*, 2 September 2024, Z. Palice 37486 et J. Vondrák 28481 (PRA). Eastern Bohemia, Železné hory Protect. Landscape Area, Horní Bradlo, Polom Nature Reserve, old-growth mixed forest in S part of reserve, alt. 610 m, 49.79017N, 15.75536E, on bark of old *Fagus sylvatica*, 21 August 2020, J. Malíček 13878 (herb. Malíček). Trhová Kamenice, fragment of old beech-spruce forest 0.8 km ESE of Vranovská Hájovna, alt. 595 m, 49.80764N, 15.84978E, at base of old *Fagus sylvatica*, 13 August 2020, J. Malíček 13889 et Z. Sejfová (herb. Malíček; ITS: PP410487, mtSSU: PP407374). Southern Bohemia, Šumava National Park, Volary, Dobrá: old-growth beech and scree forest on steep NE-facing slope of Mt. Stožec (1065 m), alt. 890–910 m 48.88028N, 13.83861E,, at base of *Fagus sylvatica*, 17 October 2016, J. Vondrák 26181 (PRA) et J. Malíček 10062 (herb. Malíček; ITS: PP410485, mtSSU: PP407371); ibid., Volary, Dobrá, protected virgin forest Spáleniště, alt. 940 m, 48.87724N, 13.79395E, on log, 19 October 2016, J. Vondrák 17234 (PRA); ibid., Černý Kříž: Mt. Jelení vrch (ca 3 km SSW of Černý Kříž), remnants of old-growth fir-beech forest on E slope, 270 m ENE of the peak, alt. 856 m, 48.83511N, 13.85467E, on weathered bark of old *Fagus sylvatica*, 9 August 2021, Z. Palice 31965 (PRA; ITS: PP410484, mtSSU: PP407376). Šumava Mts, Zátoň: NR Jilmová skála, old-growth mixed forest on E-ESE slope, alt. 955 m, 48.95200N, 13.79919E, on dry shaded wood of *Picea/Abies* stump, 14 July 2015, J. Malíček et Z. Palice 19121 (PRA); ibid., virgin forest Boubínský prales, 200–250 m W-WSW of Boubínské jezírko pond, humid dark beech old-growth forest with some *Picea* and *Abies*, alt. 975 m, 48.97314N, 13.81486E, on shaded wood in hollow trunk of *Abies* snag, 8 October 2015, Z. Palice 20588 (PRA). Novohradské hory Mts, nature reserve Hojná voda, old-growth beech-dominated forest at E-facing slope, alt. 873 m, 48.70539N, 14.75203E, on bark of elderly *Fagus*, 21 May 2020, J. Malíček et Z. Palice 29064 (PRA). Virgin forest Žofínský prales, NE-facing slope above the brook Tisový potok, alt. 780–790 m, 48.66983N, 14.70950E, on bark of *Ulmus glabra*, 30 July 2020, Z. Palice 30359 et J. Vondrák (PRA); ibid., 48.66939N, 14.70818E, alt. 770 m, bark at base of trunk of *Picea abies*, 18 August 2016, J. Vondrák 26182 (PRA). Central Bohemia, Brdy Protected Landscape Area, Míšov, Na Skalách Nature Reserve, low rock outcrops, beech and spruce forests in SW to middle part of the reserve, alt. 700–730 m, 49.60327N, 13.76508E, on bark of *Fagus sylvatica*, 15 June 2023, J. Malíček 16325 (herb. Malíček). Southern Moravia, Třešť: close-to-primeval forest (*Fagus sylvatica*, *Abies alba*, *Picea abies*, *Acer pseudoplatanus*) on WSW-facing slopes of Mt. Velký Špičák [734], 0.6–0.7 km SSE of the top, 2.4 km NE of Třešť, alt. 700 m, 49.30591N, 15.51417E, on bark of old *Fagus*-snag, 10 September 2010, I. Černajová, J. Malíček et Z. Palice 14040 (PRA).

Bacidina omnicola Vondrák, Palice et Malíček, spec. nova

MycoBank: MB#855536; Fig. 6

Etymology: The species occurs on a variety of organic and inorganic substrata and does not seem to have strong substrate preferences.

Type: Czech Republic. Central Bohemia, Beroun, Broumy, protected area Týřov, in valley of Úpořský potok, alt. 250 m, 49.97274N, 13.78841E, on shaded andesitic stone, 26 March 2021, J. Vondrák 24921 (PRA, holotype).

Sequences from the holotype: ITS (OK332885); mtSSU (OK465501); both sequences published under the name *Bacidina* sp. 2 by Vondrák et al. (2023).

Diagnostic characters: Frequently without pycnidia and apothecia, and then characterized by punctiform soralia producing soredia of very small size (10–20 μm diam.). The specimen with well-developed apothecia and pycnidia is further characterized by the absence of apothecial pigmentation, relatively short and thick, 0–3-septate ascospores, and rather long and thin conidia.

Morphology-anatomy: Thallus forming small to extensive irregular patches, disintegrating with age, thin, indistinctly areolate or almost membranaceous, hardly reaching 50 μm in thickness, grey-green, fading to pale grey in herbarium. Prothallus whitish, usually indistinct. Soralia tiny, punctiform, vivid green (but fading to cream colour in herbarium), usually up to 0.1 mm diam., sometimes merged into irregular, up to 0.3 mm wide sorediate patches (Fig. 6B). Soralia usually flat or only slightly convex, later eroding and becoming almost crater-like, sometimes with deep crevices. Soredia very small, 10–20 μm

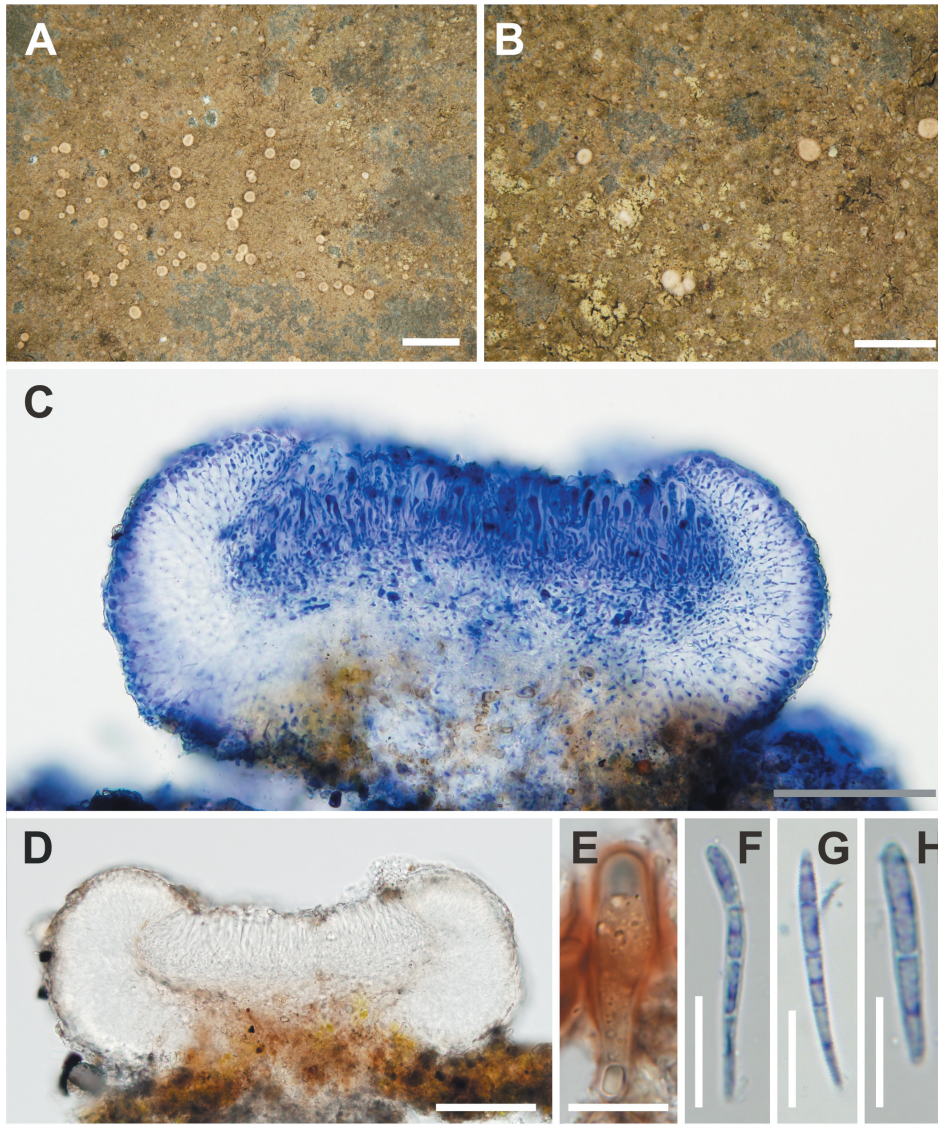

Fig. 6. *Bacidina omnicola* (holotype). A, thallus with numerous apothecia, but scarce and inconspicuous soralia; B, thallus with few apothecia and distinct soralia; C, vertical section through apothecium, highlighting cell lumina; D, vertical section through apothecium without internal pigmentation; E, ascus of *Bacidia*-type, F–H; ascospores. D, observed in water; C, F–H, stained by cotton blue; E, after KOH and Lugol's solution treatments. Bars: A, B, 1 mm; C, D, 50 µm; E–H, 10 µm.

diam., occasionally merged into up to 50 µm wide consoredia. Thallus formed by the photobiont layer, i.e. cortex and medulla absent. Photobiont green, trebouxioid; young cells spherical to ellipsoid, about 5–8 µm diam., but mature cells distinctly larger and spherical, ~9–11 µm diam., subsequently splitting into numerous daughter cells. Most specimens are richly sorediate, without apothecia and pycnidia. Numerous apothecia and

pycnidia were observed only in five specimens including the holotype. These specimens are distinct from others by scarce soralia restricted to few patches (Fig. 6A). Apothecia cream-coloured or almost white, without any pigmentation in sections (Fig. 6D), 0.1–0.5 mm diam. Apothecial margin biatorine, distinct and persistent, slightly raised above the disc, ~40–70 µm thick, consisting of short radiating hyphae with gelatinized cell walls that are almost spherical at surface, with lumina ~3–6 µm diam (Fig. 6C). Hymenium 40–60 µm tall with asci of *Bacidia*-type (Fig. 6E), ~30–40 × 8–10 µm, and with hardly distinguishable, ~2 µm thick paraphyses. Paraphyses ends sometimes widened to 3–4 µm. Ascospores acicular, straight or slightly curved, 18–33 × 1.0–2.5 µm, or clavate, thickest in upper part (sometimes up to 3 µm wide) with an obtuse end and thinning to the lower, rather pointed end; with 0–3 septa (Figs 6F–H). Pycnidia conspicuous, ~0.1 mm diam., forming white spherical formations projecting from the thallus. Conidia acicular, straight or, more frequently, slightly to strongly bow-shaped (rarely S-shaped), (25–) 35–45 (–55) × 0.5–1 µm, with obtuse ends and with 0–1 visible septum.

Chemistry: All spot test negative, UV–; apothecial sections with no substances glowing in polarized light. No secondary substances detected by TLC in thallus (specimen Vondrák 24247).

Ecology and distribution: On inorganic substrata, mainly siliceous stones and pebbles, often in damp conditions below forest canopy, or on tree bark (*Acer*, *Quercus*, *Tilia*), usually at bases of trunks. The typical habitats are lowland floodplain forests and ravine forests. It is so far known from the Czech Republic only, but our data suggest a much broader occurrence in Europe. Voucher specimen data are from low altitudes (below 500 m a.s.l.) in central and southern Bohemia and southern Moravia. According to data from eDNA, the species is frequent throughout the Czech Republic in forest localities below 500 m; present in six of eight sites and abundant in RN1 and TY2. At altitude range 500–1000 m, it occurs in two (RD2, ZF1) of eight sites. It was not detected in sites at altitudes above 1000 m.

Similar species: Sorediate species of *Bacidina* form a difficult group of hardly distinguishable species (e.g. Vondrák et al. 2022). Ekman (2023) does not use the term "sorediate" within *Bacidina*, but employs the phrase "forming spots dissolving into thallus granules". We prefer to call these spots simply "soralia", which are formed in the following epiphytic (or optionally epiphytic) European species: *B. acerina*, *B. adastra*, *B. flavoleprosa*, *B. maculans*, *B. paradoxa*, *B. piceae*, *B. tenella* and *B. violacea*. Other species, e.g. *B. arnoldiana* and *B. modesta*, form larger granules that are not produced in soralia-like spots (*B. delicata* and *B. neglecta*, both considered to be sorediate, were synonymized with non-sorediate species *B. inundata* and *B. chloroticula*; Ekman 2023). *Bacidina acerina* differs in its tendency to form an entirely leprose thallus and has slightly larger soredia (Vondrák et al. 2023). *Bacidina adastra* is distinct by its thallus not forming real "soralia", but being entirely scabrous by budding that starts from flattened thallus granules (Ekman 2023); it has longer ascospores (40–50 µm) and also usually has an aeruginose pigment in apothecia (Sparrius & Aptroot 2003). *Bacidina flavoleprosa* is hardly distinct in the sterile state, but its apothecia differ in the brownish hypothecium with Arnoldiana-brown pigment (but unpigmented apothecia mentioned in Ekman 2023) and in longer and thinner (and probably not clavate) ascospores (Czarnota & Guzow-Krzemińska 2012). *Bacidina paradoxa* is known only in sterile state and distinguishable by the production of acetone-soluble secondary substances (Vondrák et al. 2023).

Bacidina piceae is similar in the sterile state, but has pigmented apothecia, different ascospores, etc. (van den Boom 2021). *Bacidina tenella* (= *B. etayana*) has dark apothecial margin containing an olivaceous to brown pigment (van den Boom & Vězda 1996, Ekman et al. 2012). *Bacidina violacea* has distinctly larger soredia (goniocysts), 20–50 µm, and very different apothecial characters (van den Boom & Magain 2020). All the species listed above also partly differ in their ecology and all differ clearly in their ITS DNA sequences (Supplementary Fig. S3). DNA sequences are not available for the recently described *Bacidina maculans* (Ekman 2023), which, however, differs in the photobiont, which is smaller and of ellipsoid cells, and in apothecia and pycnidia, including shape, size and septation of ascospores, shape and size of conidia. The taxonomy of *Bacidina* is greatly complicated by the substrate heterogeneity of many species and by the very different phenotypes of fertile and sterile populations of the same species. Both of these are true in the case of *B. omnicola*, and its fertile occurrences (sometimes perhaps entirely without soralia) can be confused with similar non-sorediate species, especially *Bacidina chloroticula*, which may be heterogeneous even in the narrowed concept of Ekman (2023).

DNA data: The six ITS sequences obtained are more than 99% identical. Numerous related *Bacidina* species were less than 94% identical and their relationships in the ITS phylogeny to the new species are unresolved in polytomy (Supplementary Fig. S3). The six mtSSU sequences obtained were more than 99.5% identical. For relationships to other species with available mtSSU sequences see Supplementary Fig. S4.

Paratypes: Czech Republic. Central Bohemia, type locality, andesitic pebbles, 26 March 2021, J. Vondrák 24915 (PRA; ITS: OK332884, mtSSU: OK465500; with apothecia); ibid., rocks Týřovické skály, alt. ~340 m, 49.98412N, 13.79375E, bark at base of *Tilia* trunk, 13 October 2019, J. Vondrák 21138 (PRA; mtSSU: OK465498); ibid., north-east slope above valley of Úpořský potok stream, alt. 350 m, 49.96277N, 13.80933E, volcanic pebbles, 6 October, 2020, J. Vondrák 24247 (PRA; ITS: OK332883, mtSSU: OK465499); ibid., at ruin Týřov, alt. 320 m, 49.97356N, 13.79006E, andesitic pebbles, 26 March 2021, J. Vondrák 24944 (PRA; mtSSU: OK465502). Týřovice, Velká Pleš National Nature Reserve, N-NW-facing slopes between Velká and Malá Pleš Hills, natural beech and scree forests, alt. 400–490 m, 49.99306N, 13.80778E, on bark of *Tilia cordata*, 22 February 2023, J. Malíček 15981 et H. Ghlimová (herb. J. Malíček; ITS: OQ918723, mtSSU: OQ920110). Příbram, Lešetice, N side of uranium heap no. 15 between Lešetice and Brod, alt. 530 m, 49.65750N, 14.01469E, on siliceous stone, 15 March 2021, J. Malíček 14355 et I. Černajová (herb. J. Malíček; ITS: PP410490, mtSSU: PP407378). Sedlčany region, Nalžovice, Drbákov-Albertovy skály National Nature Reserve, steep rocky slopes with scree forest SW of Albertova vyhlídka view point, alt. 280 m, 49.72483N, 14.36713E, on acidic volcanic stone, 14 July 2022, J. Malíček 15462 (herb. Malíček; ITS: PP410491, mtSSU: PP407377). Northern Bohemia, Česká Lípa, Doksy: nature reserve Břehyně - Pecopala, a trail along a drainage ditch ENE of Břehyňský rybník, alt. 275 m, 50.58076N, 14.72926E, on shaded basaltic stones heaped on a bridge, 27 July 2009, J. Malíček et Z. Palice 12760 (PRA; with apothecia). České středohoří Protected Landscape Area, Raná Hill (457 m), NW-facing rock slope, alt. 350–380 m, 50.40948N, 13.77615E, on basalt pebbles, 17 November 2021, J. Malíček 14770, I. Černajová et L. Syrovátková (herb. J. Malíček). Southern Bohemia, České Budějovice, Mydlovary, Jezero pond NW of village, alt. 400 m, 49.09944N, 14.33472E, dam, on small boulder, 19 October 2007, J. Malíček 945 (herb. Malíček). Třeboň, Stará Hlína, alt. 450 m, 49.03283N, 14.84780E, bark of *Quercus robur*, coll. J. Vondrák, 1 October 2020, J. Vondrák 25004 (PRA; ITS: OQ717324). Western Bohemia, Křivoklátsko, Plzeň-sever, Chříč: Dubensko nature reserve, steep NNW-facing slope with scree forest on the right bank of the Javornice rivulet, alt. 350 m, 49.97382N, 13.68639E, on volcanite stone, 10 April 2018, J. Šoun 1710 (herb. Šoun; with apothecia). South Moravia, NP Podyjí, Vranov nad Dyjí, Čížov: Ledové sluje, scree forest on the crest, alt. 385 m, 48.88375N, 15.84250E, on bark of *Acer platanoides*, 27 August 2021, Palice 31767 (PRA; ITS: OQ717325).

Biatorella ligni-putridi Palice et Vondrák, spec. nova

MycoBank: MB#855537; Fig. 7
Etymology: "of rotten wood": the epithet describes the occurrence of the species on rotten wood, i.e. "lignum putridum".
Type: Czech Republic. Southern Bohemia, Šumava Mts, Prachatice, Včelná pod Boubínem: the valley of Cikánský potok, SW foothill of Mt. Skalní hora [836], old managed spruce dominated forest on steep WSW-facing slope, with some firs, beeches and pines, alt. 725 m, 49.02475N, 13.88119E, on strongly decayed wood of *Pinus sylvestris* stump, 19 October 2021, Z. Palice 32582 (PRA, holotype).
Sequence from the holotype: mtSSU (OQ646134, as *Biatorella* sp. in Vondrák et al. 2023).

Diagnostic characters: Thallus inapparent, endoxylic, composed of dispersed goniocysts. Algae chlorococcoid 7–16 (–20) µm in diam. Apothecia variable in size, 0.1–0.5 mm diam., dispersed, convex, emarginate, white, cream-white or with a faint ochraceous tint, translucent when wet, without pruina and without internal pigment, but sharply opaque inside (subhymenium) due to formation of numerous oily droplets. Asci thick-walled, multi-layered of *Biatorella*-type, with amyloid caps both on the surface and inside of the ascal apices (see Fig. 7 in Hafellner 1995: 103; in *Biatorella fossarum*), apically free. Ascospores > 100 in asci, small, globose, 1.5–3.0 µm diam.

Morphology-anatomy: Thallus endoxylic, inapparent, composed of dispersed goniocysts beneath apothecia or free among wood fibres. Goniocysts 10–25 (–50) µm in diam., containing often a single algal cell, or sometimes a group of up to ~24 cells. Photobiont chlorococcoid, thick-walled, of spherical cells 7–16 (–20) µm in diam., enveloped by 1–1.5 µm thick hyphae forming a dense net on the algal surface. Apothecia variable in size among specimens, white, cream-white or pale wax-coloured, translucent when wet, 0.1–0.5 mm diam., convex to hemispherical, without margin (Fig. 7A, B, E). Exciple not developed. Hymenium 40–90 µm tall, without pigments and crystals (Fig. 7C), not glowing in polarized light. Asci variable in size both within and among specimens, broadly clavate, 35–80 × 16–24 µm, with I+/KI+ dark blue layered tholus. The outermost side of ascus wall is additionally covered by a massive, I+ blue, KI+ pale blue gelatinous sheath (Fig. 7D, G). By closer examination the inner, strongly amyloid cushion of asci appears to be separated by a non- or less amyloid layer, hence the asci appear to have 3 strongly amyloid layers (2 inside and 1 outside the ascus wall) as sketched by Hafellner (1995, Fig. 8 for *Biatorella fossarum*), but this is rarely seen under the microscope, namely in young asci. Tips of asci exposed on the surface of the hymenium, giving the apothecial surface a "papillose" appearance. Paraphyses rather sparse, either reaching tips of asci or shorter, flexuose or not, ca. 1 µm thick, unthickened or only gradually slightly thickened at apices, sparingly branched and anastomosed (Fig. 7F). Ascospores > 100 in asci, colourless, globose, 1.5–3 µm diam, variable in size among specimens and among asci, but not within a single ascus. Hypothecium and subhymenium more less convex, colourless or with a faint beige tint, opaque, sharply delimited from hymenium, obscured due to the presence of tiny oil droplets (insoluble or partly slowly soluble in KOH). Subhymenium formed by intertwining thin hyphae, thicker ascogenous hyphae and primordia of asci. Conidiomata not observed.

Chemistry: No substances detected by TLC (paratype Palice 29255 examined).

Ecology and distribution: Specimens so far available only from three localities in the Czech Republic, where *B. ligni-putridi* occurs on soft rotten or hard wood (with weathered surface) of conifers (stump, log) in microsites rather sheltered from rain. In eDNA, it

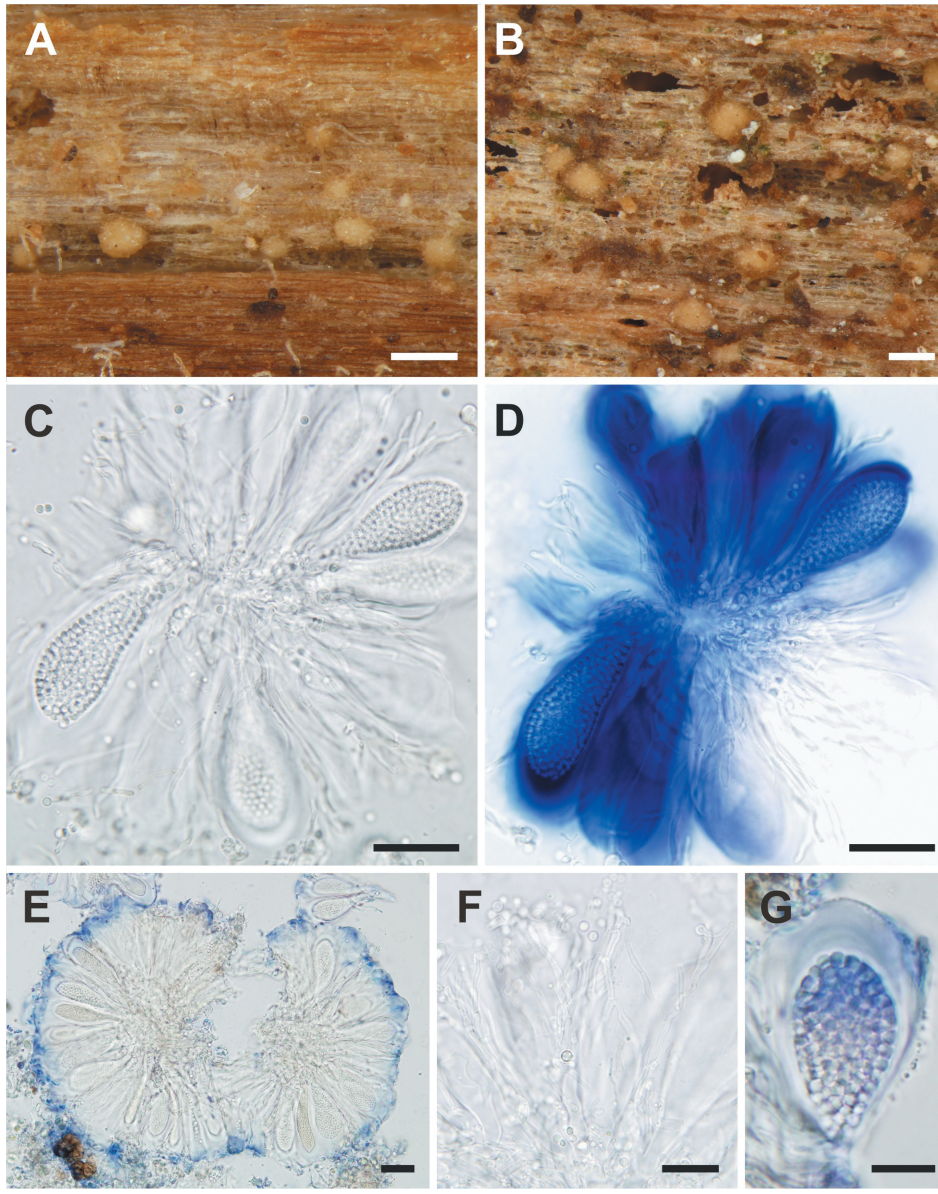

Fig. 7. *Biatorella ligni-putridi* (Palice 29255; but B, holotype). A, indistinct thallus with small apothecia; B, thallus with larger apothecia; C, hymenium with polysporic asci; D, hymenium with asci in amyloid gelatinous sheaths; E, vertical section of the hemispherical apothecium; F, detail of hamathecium; G, ascus with a gelatinous sheath. C, F, observed after the KOH treatment; D, E, G, after KOH and Lugol's solution treatments. Bars: A, B, 0.2 mm; C, D, E, 20 µm; F, G, 10 µm.

was detected from a single site below 500 m: lowland site RN1, four of eight sites at 500–1000 m (CS1, CS2, OS1, ZF1), and a single site above 1000 m (ZD1).

Phenotype variability and seasonality: *Biatorella ligni-putridi* is clearly an ephemeral, fast-growing lichen, judging from the similarity with members of the genus *Vezdaea*. It shares with most of them the thallus of dispersed goniocysts, pale, translucent, emarginate apothecia with thick-walled asci which are apically free, and giving the impression of a scabrous surface of the apothecia. The three specimens of *Biatorella ligni-putridi* so far known differ remarkably in size of apothecia (0.10–0.15 / 0.2–0.3 / 0.2–0.5 mm) and are also variable in the size of asci and ascospores. Likewise, some species of the genus *Vezdaea* (e.g. *V. aestivalis*, *V. cobria*, *V. retigera*) are known to be variable in the size of apothecia and also of ascospores within a species. The variability in size of apothecia may reflect the developmental stage of the phenotype, seasonality, and perhaps substrate conditions. Extremely small apothecia (~0.1 mm in diam.) and small ascospores (not exceeding 2 µm in diam.) were observed in an apparently juvenile lichen (specimen Palice 29255), collected in summer on soft, strongly decayed wood. On the other hand, distinctly larger apothecia (up to 0.5 mm) and larger ascospores were observed in a mature lichen (specimen Palice 36734), collected in winter on rather hard wood. Interestingly, this "winter specimen" had asci and ascospores of various sizes, but each ascus contained more or less equally-sized ascospores: either undifferentiated ascospores, or young ascospores of 1–2 µm, or ascospores of ~3 µm in diam.

Similar species: The small globose ascospores hardly exceeding 3 µm are very characteristic, only a few species having ascospores of similar dimensions, e.g. *Biatorella dryophila*, *B. flavella* and *Strangospora* spp. (Poelt & Vězda 1977). All these species are distinct in apothecial pigmentation. *Biatoridium delitescens* and *Strangospora microhaema* are somewhat similar in outer appearance, but both have distinctly larger ascospores (> 3 µm diam.). Another similar taxon, described at the intraspecific level, is *Biatorella germanica* var. *xylographoides* Vain. (Wainio 1883). Its ecology "ad lignum putridum" and small ascospores (2.5 µm) correspond to *B. ligni-putridi*, but the hypothecium described as "fulvorufescens" and apothecia up to 0.7 mm in diam. do not. This taxon was later synonymized by Magnusson (1936: 26) with *Biatorella delitescens*, but the latter has larger ascospores, 4.0–4.5 (–5.0) µm diam. (Aptroot 2009). A revision of the type specimen of *B. germanica* var. *xylographoides* is therefore desirable.

DNA data: The mtSSU sequences from two specimens are 97% identical. The closest match by the NCBI Blast search have cf. *Biatoridium delitescens* (OQ646135; Vondrák et al. 2023) and *B. monasteriense* (OQ646136, OQ682888; Vondrák et al. 2023), which are however only 86–90% identical. Other related genera are e.g. *Candelariella*, *Pycnora*, *Sarea*, *Strangospora*, *Thelocarpon* and *Watsoniomyces*, which indicates the placement of *B. ligni-putridi* into *Lichinomycetes* sensu Díaz-Escandón et al. (2022); this placement is also supported by the mtSSU phylogeny (Supplementary Fig. S5). Unfortunately, reference mtSSU data are absent from other species currently classified as *Biatorella*. We also obtained ITS sequence from the holotype (PP410492), but its identity with the target *B. ligni-putridi* is unclear.

Paratypes: Czech Republic. Southern Bohemia, Novohradské hory Mts, virgin forest Žofínský prales, NE-facing slope above the brook Tisový potok, alt. 785 m, 48.66983N, 14.70950E, on wet, decaying soft wood at a vertical side of a conifer (*Abies* or *Picea*) log, 30 July 2020, Z. Palice 29255 et J. Vondrák (PRA; mtSSU: OQ682887). Šumava Mts, Volary: nature reserve Mrtvý luh - boggy pine-birch forest, SW marginal part (lagg) of the peatbog, 0.7–0.8 km NE-ENE of the railway-station Černý Kříž, alt. 735 m, 48.86412N, 13.87033E, on dry, slowly decaying wood of high *Pinus* stump (sheltered side), 4 February 2024, Z. Palice 36734 (PRA).

Cryptodiscus neglectus Palice et Vondrák, spec. nova

MycoBank: MB#855538; Fig. 8
Etymology: "overlooked": the small apothecia initially embedded in the wood and the occurrence in sheltered niches are reasons for neglecting this species. The status of the overlooked species is also supported by eDNA data.
Type: Czech Republic. Southern Bohemia, Šumava Mts, Prachatice, Záblatí: the canyon-like valley of Blanice, mixed forest on the right bank of the rivulet, WNW foothill of Mt. Panský vrch [834], 1.4–1.5 km NNW of the castle-ruin Hus, alt. 668 m, 48.96947N, 13.92164E, on dry wood of a huge stump of a conifer (?*Abies alba*), 12 July 2022, Z. Palice 34118 (PRA, holotype).
Sequences from the holotype: ITS (OQ717379); mtSSU (OQ682933).

Diagnostic characters: Thallus endoxylic or epixylic membranaceous, forming green patches surrounding apothecia, associated with algae of *Coccomyxa*-morphology. Apothecia usually up to 0.5 mm diam., initially immersed in wood, without internal pigmentation. Exciple surface obscured by colourless crystals, glowing white in polarized light and dissolving in KOH and HNO_3.

Morphology-anatomy: Thallus not apparent, endoxylic or partly epixylic, membranaceous, usually observable as a green patch surrounding groups of apothecia. Photobiont (if present) *Coccomyxa*-like, of ellipsoid to obtusely rectangular or cuboid cells, ~2–3 × 2–6 µm. However, other green algae are usually loosely associated. Apothecia round, 0.1–0.5 mm diam., with cream-white urceolate disc and white margin. Apothecial primordia visible as white spots among wood fibres, ~0.10–0.15 mm diam., may appear to be hairy due to outgrowing hyphae. Young apothecia fully immersed in wood, of perithecial character, enclosed in exciple (Fig. 8A). Mature apothecia often half-immersed to almost sessile, with exposed disc and strongly raised margin (Fig. 8B). True exciple cup-shaped (i.e. continuous under subhymenium), up to 0.1 mm thick, colourless throughout (Fig. 8D), differentiated into two parts. (i) The outer part of exciple consists of ~1.0–1.5 µm thick, densely intertwined, thin-walled hyphae with the uppermost cells thicker and shorter, sometimes almost spherical, ~2–3 µm diam., but some of the uppermost hyphae protruding outward as ~5–10 µm long, thin and fragile projections. Upper surface of exciple usually obscured by colourless crystals, glowing white in polarized light and dissolving in KOH and HNO_3. Excipular tissue adjacent to the wood sometimes enclasping small external particles and algal cells. (ii) Inner part of exciple, adjacent to the hymenium, of cellular character. On thin sections, thin-walled cells are visible, which are angular-ellipsoid to rectangular-isodiametric, ~3–5 × 2–4 µm in diam. Hymenium 50–80 µm tall, colourless, without crystals in epihymenium. Subhymenium ~25 µm thick, of ~0.5–1.0 µm thick, contorted, thin-walled hyphae, colourless, without crystals. Paraphyses straight to flexuose, unbranched or sparsely branched and anastomosed, ~1 µm thick, not or only slightly broadened at tips. Periphysoids not developed. Asci cylindrical, ~40–60 × 5–7 µm (Fig. 8E). Ascospores 8 in asci, acicular, straight or sometimes slightly twisted, (12–) 19–30 (–36) × 1.5–2.0 (–2.5) µm, with (1–) 3–7 transverse septa (Fig. 8F–I). Conidiomata not observed.

Chemistry: Hymenium hemiamyloid, I+ pale blue (in a lower concentration of Lugol's solution) to orange (higher concentration; Fig. 8C), KI+ blue. True exciple and subhymenium not amyloid. Colourless crystals present in the outer surface of exciple, POL+, dissolving in KOH and HNO_3. No substances detected by TLC (in sample Palice 34310).

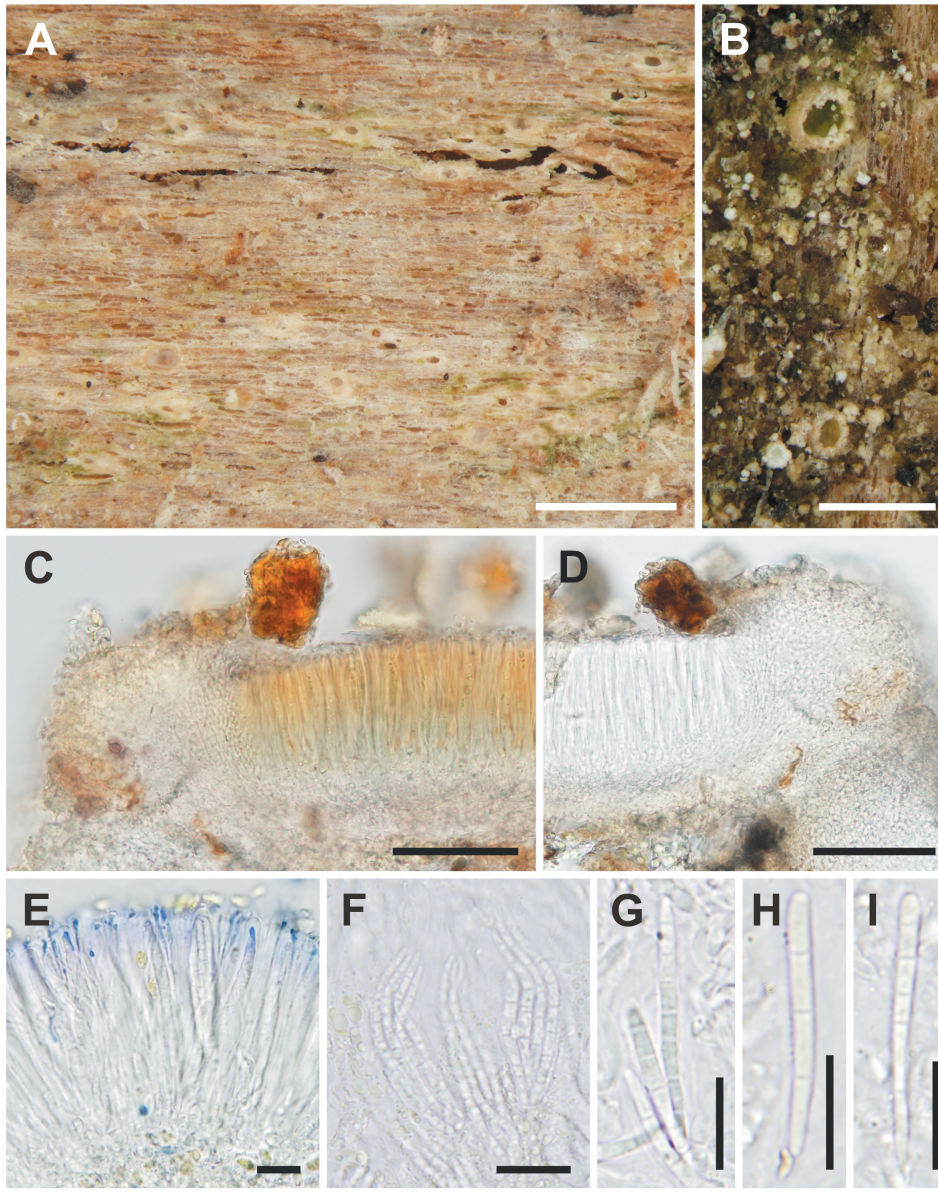

Fig. 8. *Cryptodiscus neglectus* (Palice 34310, PRA; but A, holotype). A, thalli with green spots of photobiont colonies and with young apothecia; B, mature apothecia with algae on the disc; C, hemiamyloid hymenium in vertical section; D, vertical section through apothecium without internal pigmentation; E, detail of hymenium; F, ascospores in asci; G–I, ascospores. C, observed after the treatment with Lugol's solution; D, F–I, in water; E, partly stained by cotton blue. Bars: A, B, 0.5 mm; C, D, 50 µm; E–I, 10 µm.

Ecology and distribution: Specimens are so far available only from four localities in the Czech Republic and Austria, where *C. neglectus* occurs on soft, water-soaked conifer wood (stump, logs) in microsites rather sheltered from rain. This inconspicuous

semilichen is more frequent in the Czech Republic than available specimens imply. According to eDNA, *C. neglectus* has been detected at nine out of 12 sites at altitudes > 500 m, but usually in low numbers of reads. It is likely to be rarer at lower altitudes, as it was found at only three of eight sites at < 500 m, and in low abundances.

Similar species: Shape, septation and dimensions of ascospores are usually species specific in *Cryptodiscus* (Baloch et al. 2009, Fernández-Brime et al. 2018). Comparatively short acicular ascospores similar to or identical with those of *C. neglectus* are produced only by *C. gloeocapsa*, which differs in the association with *Gloeocystis*-like algae, the presence of a yellow to orange pigment in the exciple and subhymenium, and POL+ crystals in the excipular surface which are insoluble in KOH and HNO_3. It mainly occurs in microbial crusts overgrowing acid soil and epigeic bryophytes, but rarely switches to wood. *Cryptodiscus epicladonia* has distinctly longer acicular ascospores and is lichenicolous (Pino-Bodas et al. 2017). Another putative species (Palice 32004, PRA; as *C. gloeocapsa* in Vondrák et al. 2023), which occurred on decaying wood at the foot of a *Picea* snag, is identical with *C. neglectus* in most characters, including the frequent association with *Coccomyxa*-like algae, but differs in the absence of crystals in the exciple. Sequences of this putative species differ considerably from those of *C. neglectus* holotype: ITS is 85% identical and mtSSU 95%.

DNA data: Sequences were obtained from two specimens; 95% identical in ITS and 98% in mtSSU. In the ITS phylogeny, the relationship of *C. neglectus* to other *Cryptodiscus* spp. is unresolved (Supplementary Fig. S6); in mtSSU, the closest relatives to *C. neglectus* are *C. epicladonia* (KY661680) and *Cryptodiscus* sp. (OQ646209) represented by the specimen Palice 32004, PRA; see above (Supplementary Fig. S7).

Paratypes: Austria. Niederösterreich, Ybbstaler Alpen, Wildnisgebiet Dürrenstein, Rothwald-Grosser Urwald, valley of Rothausbach, primeval fir-beech forest, 1.5 km SE of Mt. Kleiner Dürrenstein [1624], alt. 1061 m, 47.78264N, 15.09244E, on decaying wood of a hugh log of *Abies alba* (vertical to overhanging part), 27 August 2024, Z.Palice 37614, J. Vondrák 28482 et F. Berger (PRA). Czech Republic. Southern Bohemia, Šumava Mts, Volary: nature reserve Mrtvý luh - boggy pine-birch forest, S marginal part (lagg) of the peatbog, alt. 735 m, 48.86388N, 13.87103E, on vertical side of decaying wood of *Pinus* log, 27 October 2022, Z. Palice 34310 (PRA; ITS: PP410493, mtSSU: PP407379). Lenora: nature reserve Malá Niva, boggy forest of *Pinus sylvestris, P. uliginosa* and *Picea abies*, alt. 753 m, 48.91017N, 13.81525E, on dry side of trunk lying partly in peat, 6 August 2015, F. Berger et Z. Palice 20964 (PRA).

Gyalidea gabretae Vondrák et Palice, spec. nova

MycoBank: MB#8555539; Fig. 9
Etymology: Specimens are available only from the type locality in the heart of the Šumava Mts (i.a. Silva *gabreta* in Latin).
Type: Czech Republic. Western Bohemia, Šumava Mts, Modrava, Javoří Pila, 500 m south of the top of Mt. Smrkový vrch [1112 m], alt. 1100 m, 49.02712N, 13.42445E, on shaded bark at the foot of veteran *Acer pseudoplatanus*, 17 October 2017, J. Vondrák 20680 (PRA; holotype) et Z. Palice 26050 (PRA; isotype).
Sequences from the holotype: ITS (OQ717661); mtSSU (PP407380).

Diagnostic characters: Apothecia tiny, yolk yellow, translucent when wet, enclosed in a true exciple in initial stages. Ascospores submuriform to muriform, 12–23 × 5–10 µm, with 4–6 transverse septa and 1–7 longitudinal septa. Photobiont chlorococcoid. Epiphytic on bark, not overgrowing bryophytes.

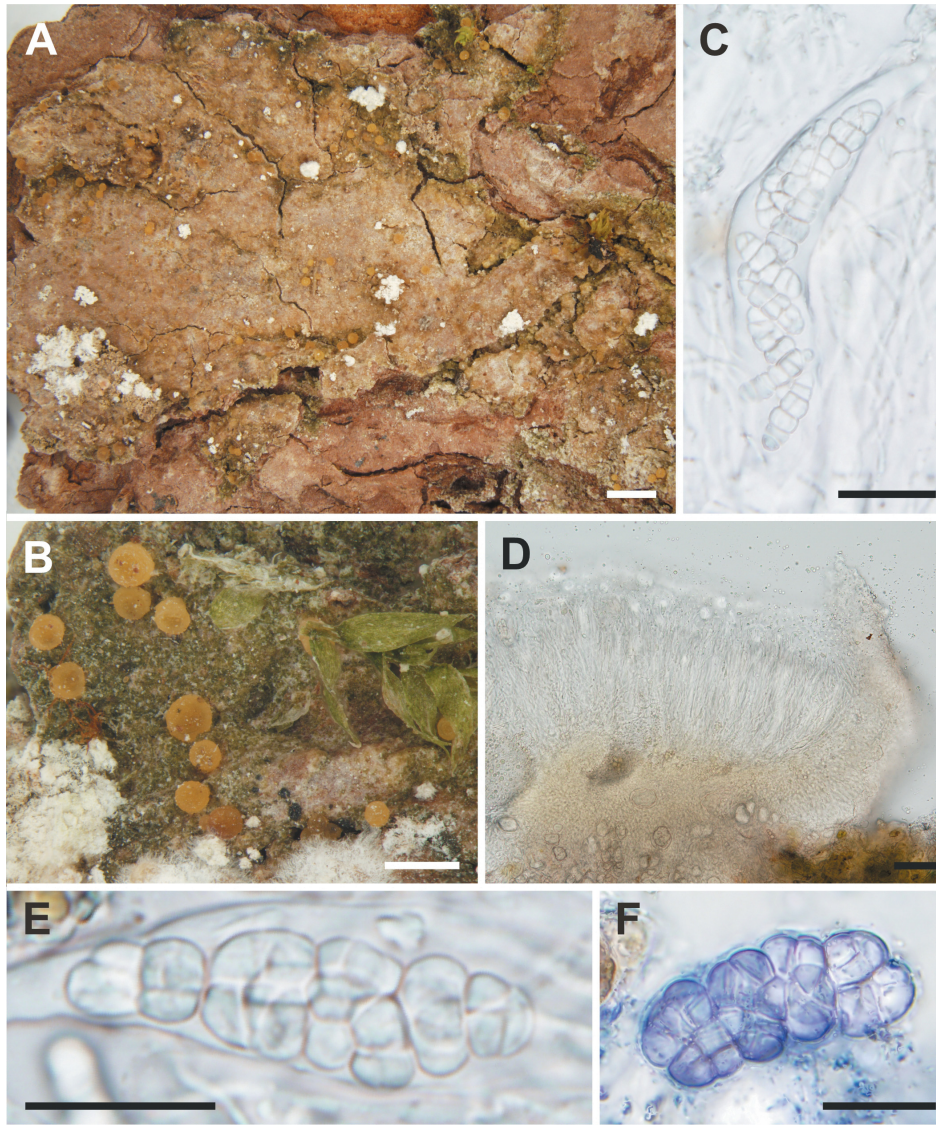

Fig. 9. *Gyalidea gabretae* (holotype). A, B, thallus with apothecia; C, ascus with eight ascospores; D vertical section through apothecium with pale yellowish pigmentation in hypothecium/subhymenium; E, F, well-developed ascospores, some of the largest observed. C–E, observed in water; F, stained by cotton blue. Bars: A, 1 mm; B, 0.5 mm; C, D, 20 µm; E, F, 10 µm.

Morphology-anatomy: Thallus epiphloedal, green, formed of scattered to merged goniocyst-like structures of ~10–35 µm diam. Thallus height hardly reaches 50 µm; cortical and medullar tissues absent. Photobiont chlorococcoid, the mature cells up to 17 µm diam., dividing into numerous, 5–7 µm wide daughter cells. Apothecia round, uniformly yolk-yellow in fresh specimens (but fading in herbarium), translucent when wet, sessile, ~0.1–0.3 mm diam, initially globose with the true exciple almost enclosing disc, later

becoming barrel-shaped, up to 0.2 mm high, with weakly exposed disc (Fig. 9A, B). Mature apothecia biatorine, with broadly exposed flat discs and thin persistent margin, about 0.05 mm wide. True exciple 40–65 µm thick, cup-shaped, i.e. continuing below subhymenium and indistinguishable from the hypothecial tissue; formed of short hyphae of gelatinized walls and lumina ~3–6 × 2.5–3.0 µm, arranged in palisades towards the surface. Superficial cells almost spherical with lumina about 3 µm diam. Hymenium up to ~150 µm tall in the middle, inspersed in the lower part, formed of numerous, ~1–1.5 µm wide paraphyses, with gelatinized walls and unthickened or slightly broadened (up to 2 µm) terminal cells; branching rarely observed. Epihymenium indistinct. Subhymenium forming a distinct thin layer of intricate hyphae, with pale yellowish pigmentation (Fig. 9D). Young asci cylindrical, ~60–70 × 12–13 µm, but mature asci shorter and more broadly clavate, ~40–50 × 17–22 µm. Ascospores eight per ascus (Fig. 9C), irregularly ellipsoid, colourless, submuriform to muriform, 12–29 × 5–14 µm, with thin cell walls, with 4–7 transverse septa and ~1–20 longitudinal septa (some oblique), often slightly constricted at septa; with ~6–30 cells; perispore indistinct (Fig. 9E, F). Conidiomata not observed.

Chemistry: Apothecial sections with no substances glowing in polarized light; the only apothecial pigment, pale yellow in subhymenium, KOH–, HNO_3–. Exciple and hymenium I–, exciple and wall of asci sometimes KI+ pale blue. Content of young asci I+ and KI+ strongly orange, but ascospores I–, KI–. Thallus tissues I–, patchily KI+ pale blue. TLC: no substances in thallus and apothecia (holotype).

Ecology and distribution: Both voucher specimens are from the type locality in the Šumava Mts, Czech Republic, where the species occurred directly on bark (not overgrowing epiphytic bryophytes) at the base of trunk of an old *Acer pseudoplatanus*. Co-occurring lichen species include *Anisomeridium polypori*, *Bacidina modesta*, *Lepraria finkii*, *Phlyctis argena* and the liverwort *Metzgeria furcata*. According to data from eDNA, the species also occurs in the Žofínský prales old-growth forest reserve (plot ZF1) in Novohradské hory Mts (Czech Republic). Its presence in only one of 126 eDNA samples indicates its general rarity. It probably requires a particular microclimate (high humidity).

Similar species: The closely related *Gyalidea cylindrica* is indistinguishable in phenotype. It shares relatively high yolk-yellow apothecia which are translucent when wet, but it overgrows epiphytic bryophytes and differs microscopically in the transversally septate, not submuriform to muriform ascospores (Etayo & Vězda 1994). *Gyalidea minuta* is another European epiphytic species related to *G. gabretae*. It differs in almost colourless apothecia with faintly brownish or ochraceous, not yellow tinge, smaller ascospores with fewer septa and smaller and thinner asci (van den Boom & Vězda 1995, Kubiak & Malíček 2012). A further European corticolous species of *Gyalidea*, *G. fruticola*, was described by Svensson & Thor (2007), but was later synonymized with *Thelenella pertusariella* (Svensson et al. 2017). It differs from *G. gabretae* in having white-grey apothecia that tend to be immersed in thallus/substrate, hymenium KI– (pale blue/yellow in *G. gabretae*), much longer and thinner asci and thinner ascospores (Svensson & Thor 2007). *Gyalidea gabretae* may resemble some species of *Coenogonium*, e.g. *C. tavaresianum*, but these have trentepohlioid photobiont and also differ greatly in apothecial characters.

DNA data: ITS sequence was obtained from the holotype. The NCBI Blast search shows closest relationships to *Gyalidea cylindrica* (OQ717664, 92% identity, 100% cover) and *G. minuta* (OQ717859, 90% identity, 43% cover). Other available sequences of

Gyalidea and *Gyalideopsis* species are far more distant. The mtSSU sequence obtained from the holotype has no close NCBI sequences; members of *Ostropomycetidae* (e.g. *Xylographa*) are up to 79% identical.

Karstenia dryina Vondrák, Palice et Šoun, spec. nova

MycoBank: MB#855540; Fig. 10
Etymology: The epithet refers to the occurrence on oak bark.
Type: Czech Republic. Central Bohemia, Křivoklátsko, protected area Týřov, on south slope, close to bottom of valley of Úpořský potok stream, alt. 360 m, 49.96459N, 13.81690E, on bark of *Quercus petraea*, 6 October 2020, J. Vondrák 24227 (PRA, holotype).
Sequences from the holotype: ITS (PP410494); mtSSU (OK465505).

Diagnostic characters: Apothecia immersed in oak bark, with "perithecial" appearance when young. True exciple strongly developed, formed of angular cells, enclosing the grey or white pruinose discs in young apothecia. Periphysoids forming a compact palisade tissue with obtuse triangular terminal cells. Hymenium 120–150 µm tall, epihymenium with brown-green to olive-green pigment (KOH+ brown, HNO_3+ emerald green). Ascospores 30–80 × 4–8 µm, with 6–20 transverse septa (rarely with 1 longitudinal septum in the largest cells).

Morphology-anatomy: Thallus immersed in bark, inapparent. Groups of algal cells often present around apothecia and in contact with the fungal tissue. Apothecia immersed in bark, at first globose, enclosed in excipular tissue, with an ostiole-like pore at top, later with an exposed, grey to white-pruinose, up to 1 mm wide disc (Fig. 10A). True exciple 40–120 µm thick, formed of angular, 4–6 µm diam. wide cells (Fig. 10B), colourless inside, with a brown to olive-green tinge at surface. Inner excipular surface below the ostiole-like pore formed of a palisade tissue of periphysoids, ~20 µm tall and 1.5–2.0 µm wide, with obtuse-triangular terminal cells forming a "papillose" surface. Hymenium 120–150 µm tall, colourless, but epihymenium with a brownish-green to olive-green pigment (KOH+ brown, HNO_3+ emerald green); subhymenium thin and indistinct. Hypothecium colourless, up to 25 µm thick, formed of compact tissue of short ~2 µm wide hyphae, ~2 µm thick, perpendicular to hymenial hyphae. Paraphyses numerous, straight, ~1 µm thick in lower part, up to 2.5 µm thick in upper part. Asci narrowly cylindrical, ~80–120 × 12–15 µm, thin-walled, without broadened tholus. Ascospores 8 in asci, colourless, with 6–20 transverse septa, some with 1 or few longitudinal septa in the largest cells (Fig. 10 C, D), 30–80 × 4–8 µm (but slightly narrower in KOH), thin-walled, without perispore. Conidiomata not observed.

Chemistry: The olive-green pigment in epihymenium and exciple (KOH+ brown, HNO_3+ emerald green) is characteristic. This pigment matches the Caesiocinerea-green pigment sensu Meyer & Printzen (2000). Hymenium I+ pale blue (low concentration) or red (high concentration); KI+ dark blue (i.e. hemiamyloid, type RB, sensu Baral 2009). Exciple and hypothecium I–, KI–. TLC not performed.

Ecology and distribution: *Karstenia dryina* is known from weathered bark of older *Quercus petraea* in unmanaged oak forests. It occurs in lichen communities with e.g. *Acrocordia gemmata*, *Bacidia rubella*, *Bacidina* sp. div., *Caloplaca lucifuga*, *Hypocenomyce scalaris*, *Hysterium pulicare* and *Karschia cezannei*. The only four voucher specimens came from warm areas of the Czech Republic: three close sites in the Křivoklátsko region in central Bohemia and one site in Podyjí in southern Moravia. Data from eDNA

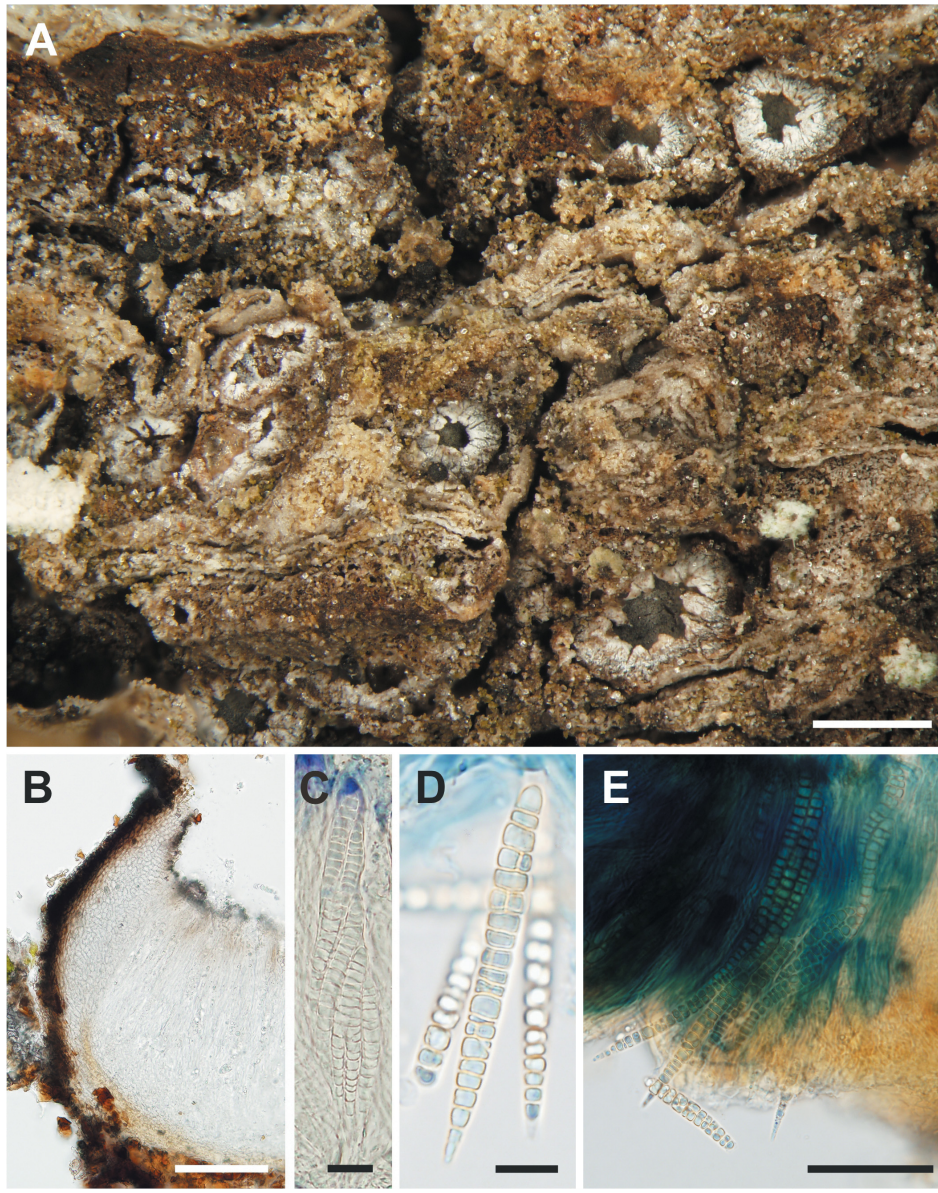

Fig. 10. *Karstenia dryina* (A, Palice 29691, PRA; B, C, Šoun 1711; D, E, holotype). A, apothecia with typically torn margins; B, true exciple with angular cells; C, ascospores in ascus; D, ascospore; E, ascospores releasing from asci. B, C, observed in water; D, E, after KOH and Lugol's treatment. Bars: A, 0.5 mm; B, E, 50 µm; C, D, 10 µm.

confirm the occurrence of *K. dryina* in the Křivoklátsko region (plots TY1 and TY2), but the species was not detected from other sampled sites.

Similar species: In outer appearance, *K. dryina* may resemble also some members of other ostropomycetous genera, e.g. *Cryptodiscus*, *Ramonia*, *Schizoxylon*, *Odontotrema* and *Stictis* (Vězda 1966, Sherwood 1977). However, the combination of long and multiseptate

ascospores, epihymenium pigmentation and apothecial anatomy is very characteristic and not found in any other known species of the *Ostropomycetidae*. Even in *Karstenia*, the known species differ considerably morphologically and ecologically (Sherwood 1977, 1980).

DNA data: Sequences of ITS and mtSSU were obtained from the holotype. The mtSSU phylogeny confirms the placement of *K. dryina* in *Karstenia*, close to *K. idaei* and *K. rhopaloides* (*Odontotremataceae, Ostropomycetidae*). In the mtSSU phylogeny, *K. idaei* OL473426 is the closest species (Supplementary Fig. S8). In ITS, the closest species in NCBI is also *K. idaei*, but only 84% identical.

Paratypes: Czech Republic, central Bohemia, Křivoklátsko, protected area Týřov, on east slope above Prostřední potok stream, alt. 390 m, 49.95729N, 13.79833E, on bark of *Quercus petraea*, 19 October 2020, J. Vondrák 24323 (PRA); ibid., well-lit forest on rocky, steep S-SSW-facing slope of the hill Průhonek [472], alt. 325 m, 49.96500N, 13.81125E, on weathered bark of *Quercus petraea*, 6 October 2020, Z. Palice 29691, S. Svoboda et J. Vondrák (PRA). Southern Moravia, National Park Podyjí, loc. Ledové sluje, alt. 390 m, 48.88419N, 15.84286E, on bark of *Quercus petraea*, 29 October 2021, J. Šoun 1711 (herb. Šoun).

Micarea lobarica Vondrák et Malíček, spec. nova

MycoBank: MB#855541; Fig. 11
Etymology: The epithet refers to the peculiar occurrence of lobaric acid in thallus and apothecia.
Type: Czech Republic. Central Bohemia, Beroun, Broumy, protected area Týřov, rocks above right side of stream Úpořský potok, alt. 320–370 m, 49.96573N, 13.80936E, on wood of log of *Quercus petraea* in forest-steppe, 9 June 2020, J. Vondrák 23708 (PRA, holotype).
Sequence from the holotype: ITS (OQ717941); published under the name *Micarea globulosella* TYPE2 by Vondrák et al. (2023).

Diagnostic characters: Thallus and apothecia containing lobaric acid. Thallus grey, areolate or endoxylic. Apothecia black (superficially similar to *Micarea denigrata* when growing on sun-exposed sites). Epihymenium brown, hypothecium colourless. Mature ascospores 1- to 3-septate, ~ (13–) 17–23 (–27) × (1.5–) 1.8–2.5 (–3) µm. Pycnidia numerous, of two types, producing microconidia 4–5.5 × 1 µm or mesoconidia 2.5–4.0 × 1.2–1.5 µm. Epihymenium and pycnidial wall contain Sedifolia-grey (KOH+ purple-violet) and a brown pigment dissolving in KOH.

Morphology-anatomy: Thallus partly endoxylic, inapparent, partly epixylic, of pale-grey convex, ~0.2–0.3 mm diam wide, up to 200 µm thick areoles, with Sedifolia-grey (KOH+ violet) in low concentration in superficial tissues. Cortex and medulla absent; prothallus indistinct. Photobiont micareoid, of spherical, 5–7 µm wide cells. Apothecia numerous in observed specimens, usually black (but some poorly pigmented in shades of brown or grey), 0.2–0.6 mm diam. (up to 0.8 mm when tuberculate), strongly convex, without margin (Fig. 11A, B). Hymenium 40–60 µm tall, colourless, but epihymenium brown (Fig. 11C; pigment dissolving in KOH; Sedifolia-grey also present in epihymenium, but observable only after dissolving the brown pigment as purple-violet tinge, Fig. 11E). Hypothecium colourless, up to 200 µm tall in central part. True exciple indistinct. Paraphyses numerous, flexuose, branched and anastomosed, of two types: (1) 1.5 µm wide, with tips broadened up to 3 µm and (2) of short and swollen cells, up to 4 µm wide. Both types of paraphyses convoluted together (Fig. 11H). Asci broadly clavate, 27–35 × 12–16 µm (Fig. 11F, G). Ascospores 8 in asci, narrowly bacilliform to fusiform-acicular, (0–) 1–3-septate, (13–) 17–23 (–27) × (1.5–) 1.8–2.5 (–3) µm, straight or often slightly curved (strongly resembling those of *Micarea globulosella*; Fig. 11I–K). Pycnidia

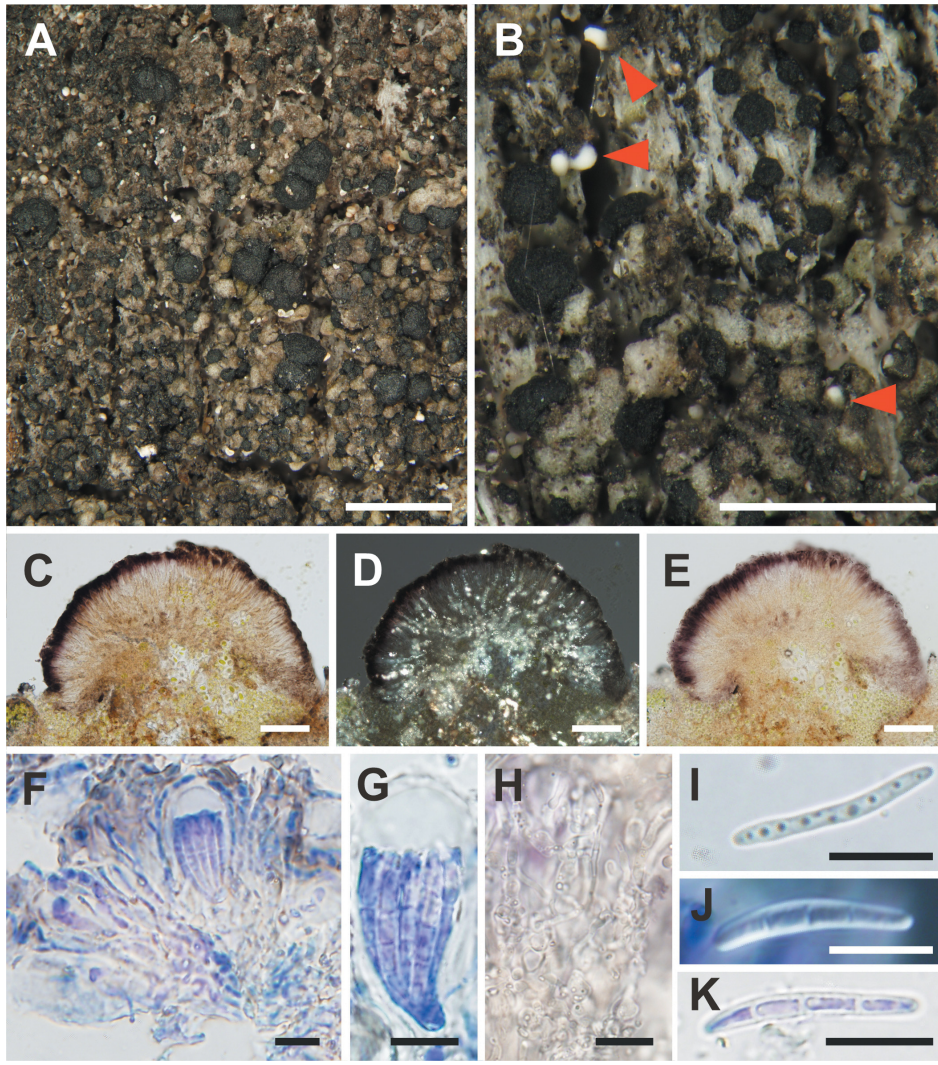

Fig. 11. *Micarea lobarica* (Vondrák 24501, PRA). A, thallus with apothecia and micropycnidia; B, thallus with mesopycnidia recognisable by a white drop of released mesoconidia (arrowheads); C, vertical section through apothecium; D, the same apothecium in polarized light, with lobaric acid glowing; E, the same apothecium after treatment with KOH; F, hamathecium and ascus with young, 1-septate ascospores; G, ascus with mature 3-septate ascospores; H, hamathecium of two types of paraphyses; I, young aseptate ascospore; J, K, mature ascospores. C, D, observed in water, E, after KOH treatment; F-K, in water or stained by cotton blue. Bars: A, B, 1 mm; C-E, 50 µm; F-K, 10 µm.

numerous in observed specimens, of two types: (a) micropycnidia (more frequent), black, 50–100 µm wide, sessile to rarely shortly stalked, with a dark brown wall (pigment dissolving in KOH; Sedifolia-grey also present, but only visible as a purple-violet hue after dissolution of the brown pigment), microconidia narrowly rhomboid, 4–5.5 × 1 µm; (b) mesopycnidia, usually brown to grey (with lower concentrations of the same pigments

as in micropycnidia), partly immersed in thallus, ~50–100 µm wide, readily recognisable from micropycnidia by a white drop of released conidia at the ostiole (Fig. 11B); mesoconidia ellipsoid to shortly cylindrical or dacryoid, 2.5–4.0 × 1.2–1.5 µm.

Chemistry: Lobaric acid detected by TLC in all studied specimens. This substance obscures the inner tissues in thallus and apothecia and glows in polarized light; Fig. 11D (observed only before KOH treatment). Spot tests K–, C–, P–, UV–. Sedifolia-grey (KOH+ violet, HNO_3+ red) in epihymenium, pycnidial wall and, in lower concentrations, in tissues at thallus surface. Additional brown (KOH dissolving) pigment present in epihymenium and pycnidial wall. This brown pigment probably matches the pigment described in *Micarea elachista* (Elachista-brown; Meyer & Printzen 2000).

Ecology and distribution: Voucher specimens are known from wood of pine and oak logs in dry conditions of ecotones between xerothermic forests and steppe-like grasslands. It occurs in communities of common epixylic lichens: e.g. *Hypocenomyce scalaris*, *Lecidea nylanderi*, *Micarea denigrata* and *Trapeliopsis flexuosa*. It is so far known only from three sites in the Czech Republic at altitudes 320–420 m. According to eDNA, the species was detected in four of eight sites at altitudes up to 500 m, in two of eight sites at 500–1000 m and in two of four sites above 1000 m; abundances significantly decreased with increasing altitude.

Similar species: The long and thin, at maturity 3-septate ascospores of the new species are rather characteristic, but ascospores of the same or similar dimensions are also produced in *Micarea globulosella*, *M. neostipitata*, *M. pycnidiophora*, *M. stipitata* and *M. synotheoides*. *Micarea globulosella* differs unambiguously in the presence of gyrophoric acid (and the absence of lobaric acid) in its olive-grey thallus, pycnidia and apothecia. It also has different ecology, occurring on bark in montane (especially spruce-dominated) forests, being almost absent at low altitudes. *Micarea neostipitata* is an eastern North American species, also producing lobaric acid in thallus and apothecia, but in addition it contains fumarprotocetraric acid in thallus and has pale apothecia and pale stipitate pycnidia (Coppins & May 2001). Both *M. pycnidiophora* and *M. stipitata* have whitish apothecia and stipitate pycnidia without internal pigmentation, the former also produces gyrophoric acid. *Micarea synotheoides* in the sense of Coppins (1983) includes at least two species: *M. longispora* with distinctly longer ascospores and with oceanic distribution in Europe (Coppins et al. 2021) and *M. synotheoides* s. str. described from Japan and supposed to occur in central Europe (Czarnota 2007, Coppins et al. 2021). These taxa differ from the new *M. lobarica* in the absence of secondary metabolites in TLC, the olive thallus and the absence of the brown pigment (dissolving in KOH) in epihymenium and pycnidial wall (Czarnota 2007). It also differs ecologically by preferring colder boreal habitats (Czarnota 2007, Coppins et al. 2021). *Micarea denigrata* is similar in outer appearance, but contains gyrophoric acid and has shorter, usually 1-septate ascospores. *Micarea elachista* is also similar, especially in having the brown pigment that dissolves in KOH, but it lacks secondary metabolites and also differs in the shorter, 0–1-septate ascospores.

DNA data: ITS sequences from two specimens are 99% identical and have no close NCBI Blast matches. The closest species, *Micarea denigrata* and *M. nitschkeana* are only 85–87% identical. Other *Micarea* spp. are less than 84% identical. For the ITS phylogeny of *M. lobarica* and related species see Supplementary Fig. S9. Repeated attempts to sequence mtSSU were unsuccessful.

Paratypes: Czech Republic. Central Bohemia, Beroun, Broumy, protected area Týřov, below hill Tok, alt. 420 m, 49.95976N, 13.82327E, on wood of log of *Pinus sylvestris* in forest-steppe, 9 November 2020, J. Vondrák 24501 (PRA; ITS: OK332999). Central Bohemia, Bernartice, Sedlice: Hadce u Želivky National Nature Monument, pine forest with serpentinite outcrops at right bank of Želivka dam, 49.68667N, 15.10139E, alt. 380–390 m, on stump of *Pinus sylvestris*, 17 October 2013, J. Malíček 6164 et al. (herb. Malíček; sub *Micarea globulosella* in Malíček et al. 2014).

Monilibrachium Vondrák, Palice et M. Svenss., gen. nov.

MycoBank: MB#855542

Monilibrachium splendens Vondrák, Palice et M. Svenss., spec. nova

MycoBank: MB#855543; Fig. 12
Etymology: The generic name refers to the shape of conidia which are formed by chains (arms) of moniliform cells. The epithet reflects the aesthetic value of the peculiar conidia.
Type: Russia. Caucasus, Republic of Adygea, ca. 6.5 km S of village Guzeripl', Mt. Abago [2628], montane mixed forest on NW-facing slope of the point 1778, alt. 1720 m, 43.93611N, 40.14722E, on mossy bark of *Fagus orientalis*, 13 June 2016, Z. Palice 21314 (PRA, holotype).
Sequences from the holotype: ITS (PP410497); mtSSU (PP407383).

Diagnostic characters: Thin pale grey thallus with trebouxioid photobiont with white spherical to hemispherical sporodochia, 0.1–0.2 mm diam. Conidiophores of spherical cells, ~3.5–5 µm diam. Conidia of globose cells, 1.5–2.5 µm diam., arranged in straight to flexuose arms (chains of 3–8 cells), often branched into short secondary arms.

Morphology-anatomy: Thallus indistinct to pale grey, membranaceous, lichenized, formed of ~25–50 µm wide goniocysts. Photobiont trebouxioid, of ~10–15 µm wide mature cells., dividing into several ~5–8 µm wide daughter cells. Sexual fruiting bodies not observed, but sparse to numerous sporodochia present (Fig. 12A, B). Sporodochia white, spherical to hemispherical, 0.1–0.2 mm diam. Conidiophores of colourless, thin-walled, short swollen hyphae or more frequently of spherical ~3.5–5.0 µm wide cells (Fig. 12E). Conidia arising mainly from terminal cells of conidiophores, but also from lower cells. One to four multi-armed conidia arising from each conidiogenous cell. Conidia colourless, formed by chains of thin-walled, globose, ~1.5–2.5 µm wide cells, (Fig. 12C, D), the chains straight or flexuose, strongly constricted at septa, consisting of 3 to 8 cells in a row, often branched into secondary and tertiary arms of up to 5 cells in a row.

Chemistry: Sporodochial tissue I+ yellow, with no substances glowing in polarized light. Thallus and sporodochia K–, C–, KC–, P–, UV–; no substances detected by TLC (in specimen Palice 21314).

Ecology and distribution: In the Czech Republic, Russia and Ukraine, numerous specimens of *Monilibrachium splendens* have been collected on various substrata, mostly on bark of *Abies nordmanniana*, *Fagus orientalis* and *F. sylvatica*, and once of *Betula*, but also on wood, and bryophytes overgrowing bark. All records came from montane old-growth, often beech-dominated woodlands. Most collections are from the Caucasus, but a single record is from the Czech Republic and two are from the Ukrainian Carpathians. In Sweden, *M. splendens* has been collected on *Salix caprea*, in three cases within old-growth stands, but in one case in a stand of deciduous trees rather close to an agricultural field. Among associated species, *Biatora* spp. and *Cheiromycina* spp. were recorded several times; *M. splendens* may prefer humid sites with a long-lasting snow cover. In eDNA data, *Monilibrachium* was detected in a single site (RD1) where it was also recorded by the taxonomic survey.

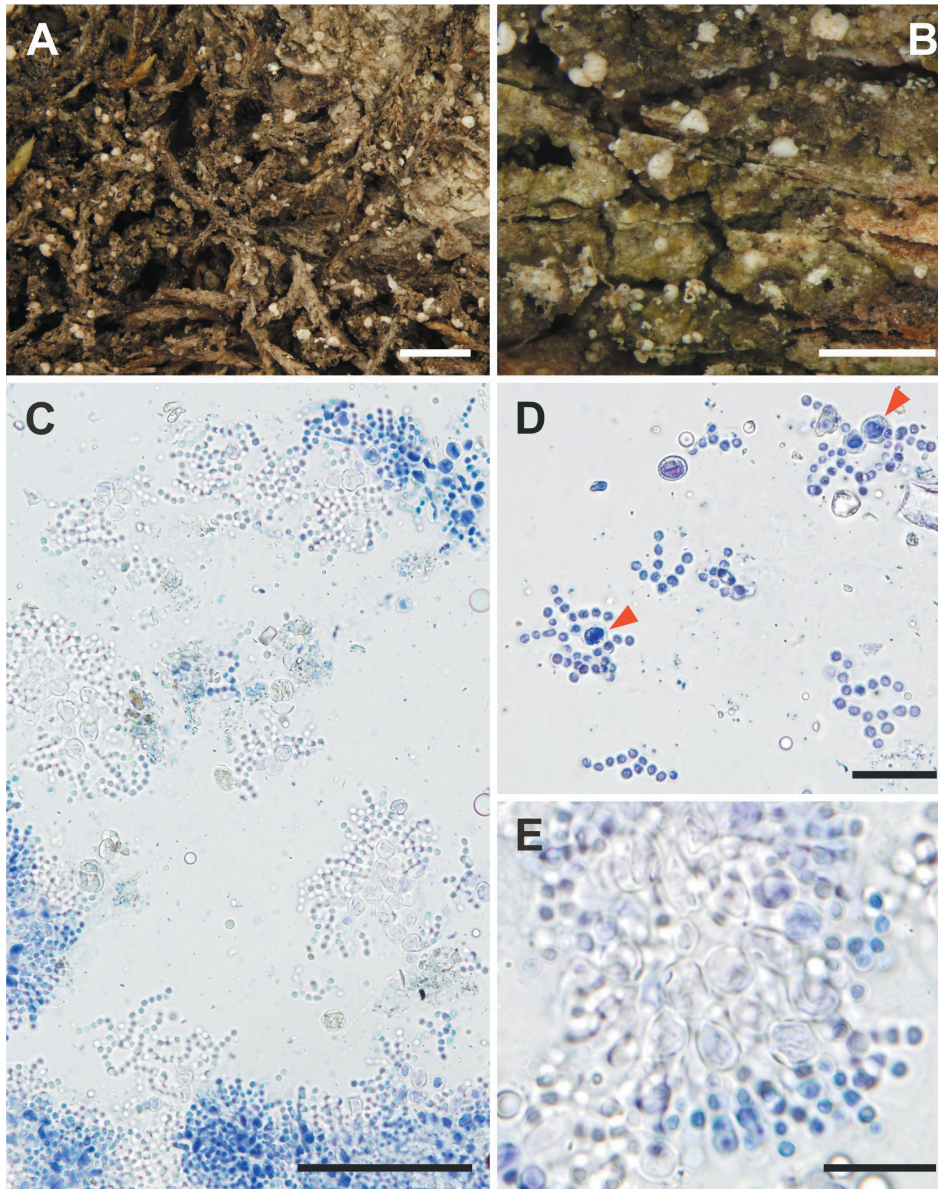

Fig. 12. *Monilibrachium splendens* (holotype, but B, Palice 32012, PRA). A, sporodochia on bryophytes; B, sporodochia on bark; C, mass of conidia and conidiogenous cells in the squash preparation of a sporodochium; D, released conidia, some still attached to conidiogenous cells (arrowheads); E, conidiophore of large spherical cells and attached branched conidia. C–E, partly stained by cotton blue. Bars: A, 1 mm; B, 0.5 mm; C, 50 µm; D, E, 10 µm.

Similar species: The sporodochia of *Monilibrachium* are somewhat similar to those of e.g. *Cheiromycina*, young *Dictyocatenulata*, *Sporodophoron*, *Tylophoron* and *Xyleborus*. However, the conidia formed of branched moniliform chains of globose cells are diagnostic. The new species may be confused with the poorly known anamorphic species

tentatively described in the genus *Cheiromycina* as *C. globosa* (Aptroot & Schiefelbein 2003). This anamorphic fungus with excavate sporodochia and globose conidia has an unknown generic affiliation (Muggia et al. 2017). The conidia in *C. globosa* are released singly but are of similar size to the conidiogenous cells of *Monilibrachium*. *Cheiromycina globosa* has a clearly different ecology from *Monilibrachium*. It was collected in a strongly nitrophilous lichen community in a wound track on bark of a road-side *Acer platanoides* (Aptroot & Schiefelbein 2003).

DNA data: Identical mtSSU sequences from four specimens have no close matches in NCBI, except for 100% match with OP161937, a sequence published as *Austropeltum glareosum* by Svensson & Fryday (2022). This sequence, however, is the result of a lab error, as a Swedish specimen of *M. splendens* was extracted at the same time as the specimen of *A. glareosum*. Barring that sequence, the closest NCBI Blast identities (85–88%) belong to the *Ostropomycetidae*: *Karstenia*, *Phlyctis*, *Sphaeronema truncatum* and *Thrombium*, and we suggest to place *Monilibrachium* into *Ostropomycetidae*, incertae sedis. The three ITS sequences obtained are 97% identical and have no close matches in NCBI and their phylogenetic relationship to other lichens remains unresolved.

Paratypes: Czech Republic. South Bohemia, Šumava Mts, Volary, České Žleby, N part of Radvanovický hřbet, old-growth mixed forest on E-facing slope, just ENE of the point-peak 930 m, alt. 905 m, 48.90650N, 13.80133E, on bark of old *Fagus sylvatica*, associated with *Lecanora argentata*, 20 October 2021, Z. Palice 32012 (PRA; ITS: PP410495; mtSSU: PP407381). Russia. Caucasus, Maykop, Guzeripl, protected area Kavkazskiy zapovednik, alt. 1465 m, 43.96475N, 40.13073E, on bark of *Abies nordmanniana*, 11 June 2016, J. Vondrák 15173 (PRA; mtSSU: PP407382), Caucasus, Republic of Adygea, ~3.5 km S of village Guzeripl', Mt. Abago [2628], mixed forest on crest just E of the point 1431, alt. 1465 m, 43.96500N, 40.13138E, on wood of snag (*Abies nordmanniana*), 14 June 2016, Z. Palice 23402 (PRA); ibid., 43.93611N, 40.14677E, on dry bark of *Fagus orientalis*, Z. Palice 22836 (PRA; mtSSU: PP407384); ibid., on wood of Abies *nordmanniana* snag, Z. Palice 23397 (PRA); Caucasus, Republic of Adygea, ca. 8 km S-SSE of village Guzeripl', Mt. Abago [2628], subalpine forest at N-facing slope of the point 2093, alt. 1900 m, 43.92611N, 40.15166E, on bark of dead *Betula*, 12 June 2016, Z. Palice 22808 (PRA); Adler, Krasnaya Polyana, primeval fir-beech forest below timberline, alt. 1690 m, 43.69721N, 40.35687E, on bark of *Abies nordmanniana*, 27 June 2019, J. Vondrák 23356 (PRA). Sweden. Värmland, Övre Ullerud par., ~7.5 km N of Deje, 200 m W of Upplanda, alt. 57 m, 59.68128N, 13.47513E, on *Salix caprea*, 10 October 2020, M. Westberg s.n. (UPS L-1020373). Ångermanland, Tåsjö par., 4.5 km NW of Tåsjö church, about 50–100 m N of the road fork between Skänknäsberget and Viken, alt. 240 m, 64.24774N, 15.83430E, on bark of *Salix caprea* in old growth deciduous forest regenerated after fire, 24 July 2017, M. Svensson 3059 (UPS L-1091426).; ibid., 2.7 km SE of Tåsjö church, N of Kroknäset, along the first stream E of the stream Hansbäcken, alt. 304 m, 64.20653N, 15.94372E, small stream in old-growth coniferous forest, on fallen *Salix caprea*, 24 July 2017, M. Westberg ULR074 (UPS L-880094). Lule Lappmark, Jokkmokk par., 1 km W of Ålloluokta chapel, N of the northern bay of Oalloluokta, alt. 375 m, 67.12388N, 19.47537E, on bark of *Salix caprea*, 3 August 2019, M. Svensson 3659 (UPS L-1091429). Ukraine. Zakarpattia, eastern Carpathians Mts, Khust, Uglya, Velyka Uhol'ka, beech forest at artificial tree line on S slope of Mt. Manchul, alt. 1200 m, 48.29795N, 23.66658E, on bark of *Fagus sylvatica*, 17 May 2015, J. Vondrák 14197 (PRA). Irshava, valley of Irshavka river, alt. 770 m, 48.45120N, 23.08508E, on bark of *Fagus sylvatica*, 6 September 2023, J. Vondrák 28142 (PRA; ITS: PP410496).

Psoroglaena neglecta Vondrák, Malíček et Palice, spec. nova

MycoBank: MB#855544; Fig. 13

Etymology: According to eDNA, the species is nearly ubiquitous in central-European forests, but overlooked (neglected).

Type: Czech Republic. Šumava Mts, Prášily, Mt. Ždánidla, E slope, alt. 1130 m, 49.10217N, 13.35788E, on bark of *Fagus sylvatica*, 29 September 2021, J. Vondrák 25856 (PRA, holotype).

Sequences from the holotype: ITS (OQ717579); mtSSU (PP407386).

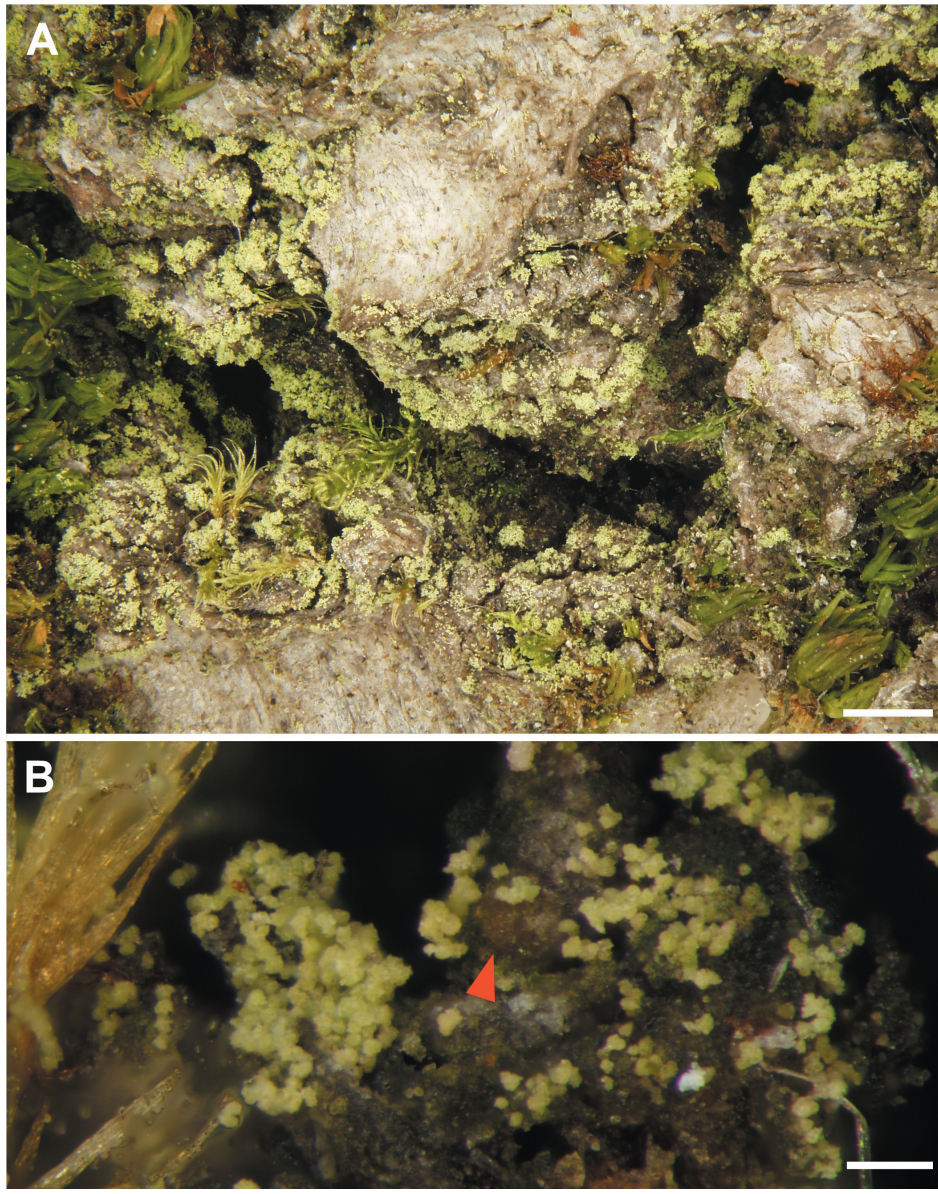

Fig. 13. *Psoroglaena neglecta* (holotype). A, sorediate thallus; B, thallus and a perithecium of co-occurring *Macentina abscondita* (arrowhead). Bars: A, 1 mm; B, 0.1 mm.

Diagnostic characters: The endophloedal thallus with punctiform to confluent pale green to grey green soralia can hardly be distinguished from sorediate *Bacidina* spp., *Micarea* spp., or some other sorediate *Verrucariaceae* and the DNA-based identification is recommended.

Morphology-anatomy: Thallus endophloedal, inapparent, soralia bright pale green, sometimes with somewhat yellowish or greyish tinge, punctiform, convex, 0.1–0.4 mm

diam., sometimes groups of soralia confluent (Fig. 13A). Soredia 12–20 µm diam., containing only few (~2–10) algal cells, without conspicuous papillae (known in goniocysts/filaments of *P. stigonemoides*). Photobiont chlorococcoid, the mature cells ~8–12 µm diam., dividing into several daughter cells, ~3–5 µm diam. Perithecia not observed. (The ochre to orange perithecia (Fig.13B), translucent when wet, occasionally present in close proximity to soralia of *P. neglecta*, apparently belong to the co-occurring *Macentina abscondita*). Conidiomata not observed.

Chemistry: TLC: no substances in thallus (holotype).

Ecology and distribution: On bark, usually at bases of tree trunks, in communities of nitrophilous epiphytes; also recorded on wood and eventually spreading to bryophytes. The five voucher specimens come from very different forest types and substrates: bark of *Fagus sylvatica* in a montane old-growth beech forest at 1130 m (holotype), wood of *Sorbus aucuparia* in spruce forest at 1070 m (Malíček 16698), bark of *Sambucus nigra* in young mixed managed forests at ~500 m (Malíček 16095 et 16410), and an unmanaged deciduous forest at 270 m (published as *Psoroglaena* sp.; Malíček & Konečná 2023). The occurrence on the bark of *Sambucus* (in two out of five specimens) supports the nitrophilic character of *P. neglecta*. According to eDNA, it was detected in 18 of 20 sites of various forest types along the altitudinal gradient in the Czech Republic and was abundant in many samples across altitudes. However, it was absent from the two spruce forest sites, so it probably avoids growing on acidic spruce bark.

Similar species: The sterile crust with green soralia differs from other known members of European *Psoroglaena: P. stigonemoides* forms coralloid goniocysts with papillate surface and *P. dictyospora* is known only in fertile state and forms thallus of tiny goniocysts, not aggregated in soralia. *Psoroglaena neglecta* is however hardly distinguishable from sorediate members of *Bacidina*, *Micarea* and some other sorediate *Verrucariaceae*.

DNA data: ITS sequences obtained from three specimens are 97.5% identical and form a sister group to *Psoroglaena stigonemoides* (Supplementary Fig. S10), which has ITS sequences < 90% identical. The sequence of *P. dictyospora* (OQ718037; Vondrák et al. 2023) is even less close. Two sequences of mtSSU are about 99% identical and they are 98–99% identical with those of *P. dictyospora* (OQ683138; Vondrák et al. 2023) and 91–93% *P. stigonemoides* (OQ683139; Vondrák et al. 2023).

Paratypes: Czech Republic. Eastern Bohemia, Český ráj Protected Landscape Area, Sedmihorky, Bažantník Nature Reserve, mixed deciduous lowland forest with old oaks, alt. 260–270 m, 50.55639N, 15.19000E, on bark of *Sambucus nigra*, 12 October 2022, J. Malíček 15935 et E. Konečná (herb. Malíček; ITS: OR724069). Krkonoše Mts, Velká Úpa, Sargasserovy boudy, margin of spruce forest, alt. 1070 m, 50.70420N, 15.77017E, on exposed wood of living *Sorbus aucuparia*, 28 January 2024, J. Malíček 16698 (herb. J. Malíček). Southern Bohemia, Blanský les Protected Landscape Area, Holubov, Bořinka Nature Reserve, serpentinite rocks above Křemžský potok, alt. 490–500 m, 48.89520N, 14.31010E, on bark of *Sambucus nigra*, 12 October 2023, J. Malíček 16410 (herb. Malíček; ITS: PP410498; mtSSU: PP407385); ibid., Holubovské hadce Nature Reserve, N-facing slopes on serpentinite above Křemžský potok brook, alt. 460–490 m, 48.89139N, 14.34139E, on bark of *Fraxinus excelsior*, 9 November 2022, J. Malíček 16095 et H. Ghlimová (herb. Malíček).

Toniniopsis pruinosa Vondrák, Svoboda et Palice, spec. nova

MycoBank: MB#855545; Fig. 14
Etymology: The white pruina on margins of young apothecia is very characteristic.
Type: Ukraine. Zakarpattia, Eastern Carpathians Mts, Shyrokyi Luh, Pryhid, protected atea Shyrokyi Luh, valley of river Luzhanka, alt. 540 m, 48.30739N, 23.73130E, on mossy bark at base of *Fagus sylvatica* trunk, 24 May 2019, J. Vondrák 21364 (PRA, holotype).
Sequences from the holotype: ITS (PP410501); mtSSU (PP407387).

Diagnostic characters: The almost stipitate young apothecia with white pruinose margin are diagnostic. The apothecia have specific pigments: green in epihymenium (KOH–, HNO_3+ purple-violet; sometimes in low concentration or absent), brown-red in hypothecium (KOH+ deep purple, HNO_3+ red intensifying) and usually an additional olive-green pigment in upper hypothecium and outer exciple (KOH+ dark brown, HNO_3+ purple). Ascospores 18–38 × 1.5–2 µm, with up to 7 transverse septa. Pycnidia with brown-red wall (KOH+ deep purple, HNO_3+ red intensifying), conidia bacilliform, 4–6 (–8) × 1–1.5 µm.

Morphology-anatomy: Thallus green to pale grey-green, formed of tiny granules (goniocysts) or in part gradually confluenting into ±continuous crust with finely roughened surface, up to 50 µm thick. Granules 20–35 µm diam., without pigmentation. Cortex and medulla absent; prothallus inapparent. Photobiont chlorococcoid, the mature cells up to 13 µm diam., dividing into several ~4–6 µm wide daughter cells. Apothecia black, or rarely with disc in shades of grey or reddish/rosish brown. Young apothecia 0.2–0.4 mm diam., sessile, frequently with white pruina on margin (Fig. 14A), with constricted base or almost shortly stipitate (Fig. 14B), and with concave disc. Mature apothecia up to 0.7 mm diam. (tuberculate apothecia up to 1 mm), with strongly convex discs and diminishing margin (Fig. 14C). Hymenium colourless, 45–80 µm tall, with epihymenium green (KOH–, HNO_3+ purple-violet) or not distinctly coloured in less pigmented apothecia. Paraphyses hardly visible, ~1–1.5 µm wide, straight to flexuose, with terminal cells widened up to 4 µm. Asci cylindrical, 40–70 × 7–9 µm, with amyloid, KI+ blue tholus, with a deeper-staining internal "beak" (Fig. 14F). Ascospores 8 in asci, acicular, straight or twisted in asci, with obtuse ends, 18–38 × 1.5–2 µm, colourless, with up to 7 transverse septa (Fig. 14G); sometimes slightly thicker in the upper half (Fig. 14H). True exciple well developed, but hidden below convex discs in mature apothecia, formed of palisades of short-celled hyphae with thick and gelatinized walls. Outer cells spherical, the lumina ~3–4 µm. Gelatinous matrix around cell lumina strongly brown-red pigmented (KOH+ deep purple, HNO_3+ red intensifying), sometimes outer part with additional dark olive-green pigment (KOH+ dark brown, HNO_3+ purple). In young apothecia, the exciple is strongly inspersed with colourless crystals of irregular shapes (POL+, insoluble in KOH, soluble in HNO_3; Fig. 14E). Hypothecium dark brown-red throughout, up to 300 µm thick in central part, of irregularly shaped cells in gelatinous matrix with the brown-red pigment (KOH+ deep purple, HNO_3+ red intensifying). An additional dark olive-green pigment (KOH+ dark brown, HNO_3+ purple) is sometimes present in the upper part of hypothecium. Pycnidia sparse, sometimes absent, sessile, globose to barrel-shaped, ~0.08–0.12 mm wide and 0.1–0.2 mm tall, black or reddish to dark brown. Pycnidial wall brown-red (KOH+ deep purple, HNO_3+ red intensifying). Conidiogenous cells obtuse-triangular, ~4–5 µm tall and ~2 µm wide at base. Conidia colourless, simple, bacilliform, 4–6 (–8) × 1–1.5 µm.

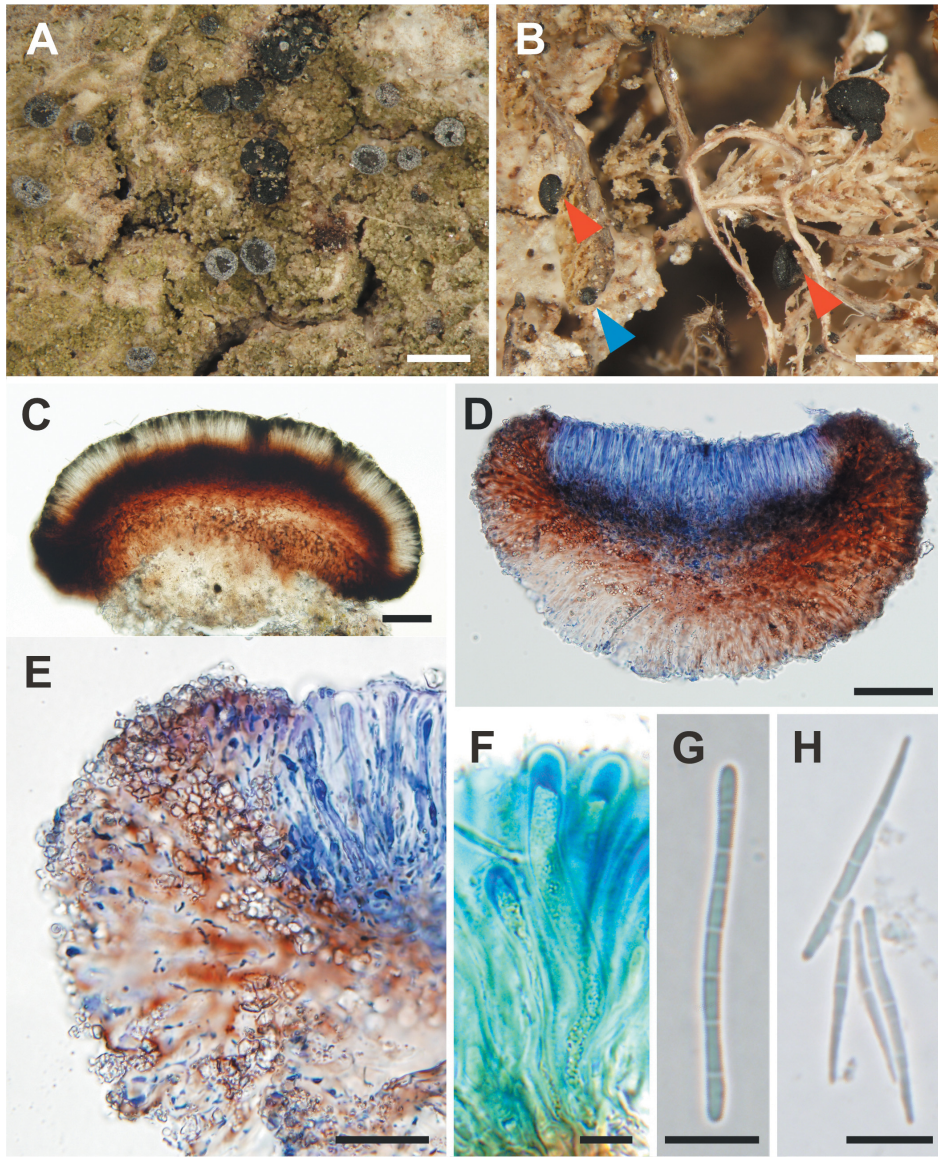

Fig. 14. *Toniniopsis pruinosa* (holotype; but B, Palice 19884, PRA). A, thallus and apothecia on bark; B, thallus with substipitate apothecia (red arrowheads) and pycnidia (blue arrowhead) on bryophytes; C, vertical section through a large, slightly tuberculate apothecium; D, vertical section through a young apothecium with a distinct margin; E, true exciple with colourless crystals inside and on the surface; F, asci with a dark blue "beak" in tholus; G, well-developed, 7-septate ascospore; H, 1–3-septate ascospores. C, G, H, observed in water; D, E, stained by cotton blue; F, after KOH and Lugol's treatment. Bars: A, B, 0.5 mm; C, 100 µm; D, 50 µm; E, 20 µm; F–H, 10 µm.

Chemistry: TLC: no substances in thallus and apothecia. Apothecial pigments are of three types: (1) green pigment in epihymenium (KOH–, HNO_3+ purple-violet) corresponding to Bagliettoana-green sensu Meyer & Printzen (2000), sometimes in low

concentration or absent; (2) brown-red pigment in hypothecium and inner exciple and also in pycnidial wall (KOH+ deep purple, HNO_3+ red intensifying); (3) olive-green pigment in upper hypothecium and outer exciple (KOH+ dark brown, HNO_3+ purple), not always present. Apothecial crystalline pruina of colourless crystals of irregular shapes (POL+, insoluble in KOH, soluble in HNO_3).

Ecology and distribution: On shaded, rain-sheltered parts of trunks, on slightly acidic bark where it usually overgrows epiphytic bryophytes in the humid microclimate of old-growth forests. Usually recorded on tree bases or exposed roots of *Fagus sylvatica* together with *Alyxoria varia*, *Anisomeridium polypori*, *Lepraria finkii*, *Opegrapha trochodes* and *Pyrenula nitida*. Most of the specimens come from the southern and eastern Carpathians and one from the Czech Republic. According to eDNA, it is a rare species, detected only in the site ZF1 (beech-dominated old-growth forest where the species was not recorded during the taxonomic survey).

Similar species: *Toniniopsis pruinosa* is most similar to four non-epiphytic species of calcareous substrates, usually occurring in high-montane areas. The most similar species is probably the arctic-alpine muscicolous/terricolous *T. illudens* (the later synonym, *T. obscura* is also the generic type; Ekman 1996, Kistenich et al. 2018), which also may have apothecia with white-pruinose margins (Nylander 1870, as *Lecidea illudens*; Vainio 1922, as *Bacidia muscorum* var. *irrorata*). The ascospores of *T. illudens* are broader than those of *T. pruinosa* (2.5–3.0 µm for the former species; Nimis 2024). Further, available ITS and mtSSU sequences of *T. illudens* (MG926037, MG925943; Kistenich et al. 2018) are clearly different from those of *T. pruinosa*.

According to Vězda (1961), white-pruinose apothecia are also characteristic of *Bacidia caesiomarginata* and *Toniniopsis coelestina*. The former is a poorly known taxon distinguished by short and broad 3-septate ascospores (14–20 × 2.5–3.0 µm; Vězda 1961), whereas the latter differs from *T. pruinosa* in having a distinct granulose-subsquamulose thallus, by often being associated with cyanobacteria or cyanolichens, and by having longer (–40 µm) and broader (–4 µm) ascospores (Timdal 1992, Halıcı et al. 2021, Cannon et al. 2023, Nimis 2024). *Toniniopsis bagliettoana* is also similar, but differs in apothecial characters, such as longer (–45 µm) and somewhat broader (–3 µm) ascospores and by the pale brown to colourless lower hypothecium (Cannon et al. 2023). Also, the white pruina on margins of young apothecia has not been reported for this species. The two hitherto known epiphytic species of *Toniniopsis*, *T. dissimilis* and *T. separabilis*, have similar apothecial pigmentation to *T. pruinosa*, but usually weaker, with almost colourless outer exciple and lower hypothecium. They also differ in the absence of white pruina on margins of young apothecia and have broader (–4 µm) ascospores (Gerasimova et al. 2021). *Toniniopsis separabilis* is also distinct by the conidia, which are allegedly filiform, 10–20 × 0.8 µm (Cannon et al. 2023); however, we have never seen pycnidia in *T. separabilis*. Both *T. dissimilis* and *T. separabilis* are also clearly distinct from *T. pruinosa* in ITS and mtSSU sequences.

DNA data: ITS sequences obtained from four specimens are more than 99.5% identical and they have the closest NCBI Blast hits to numerous sequences of *Toniniopsis separabilis* (identities 85–86%), as well as species of *Bibbya*, *Kiliasia*, *Toniniopsis* and *Waynea*, which are about 85% identical. Two mtSSU sequences of *T. pruinosa* are more than 99.5% identical and the closest NCBI sequences are from *T. coelestina* (MG925933; 93.5% identical) and *T. bagliettoana* (MG925847; 93.5% identical). Single-locus phylo-

genetic trees of ITS and mtSSU (Supplementary Figs S11 et S12) do not support the monophyly of *Toniniopsis* and therefore the current generic placement of *T. pruinosa* may have to be changed.

Paratypes: Czech Republic. Southern Bohemia, Šumava Mts, Volary, České Žleby: Mt. Spáleniště [960], old-growth mixed forest on the top crest, alt. 940 m, 48.87722N, 13.79389E, on shaded foot of old *Fagus*, 20 October 2016, Z. Palice 22161 (PRA; ITS: OQ717710). Romania. Transylvania, Southern Carpathians, Făgăraş Mts, Sibiu County: valley of Arpaşu Mare, silver-fir-beech forest on W-facing slope, 10 km S-SSW of Victoria, alt. 1171 m, 45.64131N, 24.66981E, on mossy bark of *Fagus sylvatica*, 13 August 2024, J. Halda et Z. Palice 37610 (PRA); ibid., alt. 1132 m, 45.63722N, 24.66630E, 14 August 2024, Z. Palice 37484 (PRA); ibid., alt. 1178 m, 45.63697N, 24.66847E, on bryophytes on bark of snag of *Fagus sylvatica*, 14 August 2024, J. Halda et Z. Palice 37783 (PRA). Ukraine. Eastern Carpathians Mts, Shyrokyi Luh, Pryhid, protected area Shyrokyi Luh, valley of river Luzhanka, alt. 540 m, 48.30739N, 23.73130E, on exposed roots of *Fagus sylvatica*, 24 May 2019, J. Vondrák 21510 et 21670 (PRA; ITS: PP410499, PP410500; mtSSU: PP407388). Khust, Velyka Uhoľka, valley of V. Uhoľka, old-growth hornbeam-beech forest, alt. 500 m, 48.25083N, 23.69639E, on bryophytes on shaded bark of old *Fagus*, with *Thelopsis rubella* associated on bark, 13 May 2015, F. Berger s.n., specimen ID: Palice 19884 (PRA).

Xylopsora diffissa Vondrák, Šoun, Svoboda, Malíček, Palice et Timdal, spec. nova

MycoBank: MB#855546; Fig. 15
Etymology: The epithet "diffissa" (= divided) refers to the thallus squamules, which in the early stages completely break up into piles of soredia.
Type: Czech Republic. Southern Bohemia, Šumava Mts, Prachatice, Včelná pod Boubínem, protected area Čertova stráň, alt. 720 m, 49.00768N, 13.88561E, on rotten wood of stump (*Abies alba*), 22 September 2021, J. Vondrák 25810 (PRA, holotype).
Sequences from the holotype: ITS (OQ718153); mtSSU (OQ646511).
Sequence from the photobiont in the holotype: *rbc*L (PP438761).

Diagnostic characters: Thallus consisting of up to 0.3 mm high piles of pale-grey to brown soredia which originate from squamules in early stages. Soredia containing friesiic acid and an orange-red pigment (KOH+ brown, HNO_3+ brown-red).

Morphology-anatomy: Thallus primarily of pale brown to pale grey squamules, adjacent to the substrate to erect, up to 0.6 mm diam. and up to 200 µm thick. Squamules composed of a thick algal layer below an alveolate cortex (sensu Vondrák et al. 2009) up to 40 µm thick and an up to 20 µm thick epinecral layer. Medulla present in places, thin and composed of loose proso-plectenchymatous tissue. Lower cortex absent. At an early stage, each squamule fully dissolves into a pale-grey to brown, up to 0.3 mm high pile of soredia and consoredia (Fig. 15A, C). Soredia ~20–30 µm diam., consoredia up to 100 µm diam. Cell structure in soredia (Fig. 15F) obscured by high concentration of friesiic acid (glowing in polarized light, Fig. 15D, and dissolving in KOH). Exposed soredia at thallus surface containing an orange-red pigment (Fig. 15E; KOH+ brown, HNO_3+ brown-red). Photobiont trebouxioid, the mature cells up to 13 µm diam. dividing into several, ~4–6 µm wide daughter cells. The *rbc*L sequence of the photobiont from the type specimen matches *Trebouxia jamesii*. Apothecia and conidiomata not observed.

Chemistry: Friesiic acid detected by TLC (glowing in polarized light, dissolving in KOH). Spot tests: K–, C+ fleeting rose, KC+ fleeting rose, P–, UV+ white (but shaded by an orange-red pigment). Orange-red pigment in soredia (KOH+ brown, HNO_3+ brown-red).

Ecology and distribution: Specimens of *Xylopsora diffissa* are so far available from less than a dozen localities in the Czech Republic and one in Austria. It occurs in old-growth and old managed, mainly pine dominated forests (but also fir-beech forests),

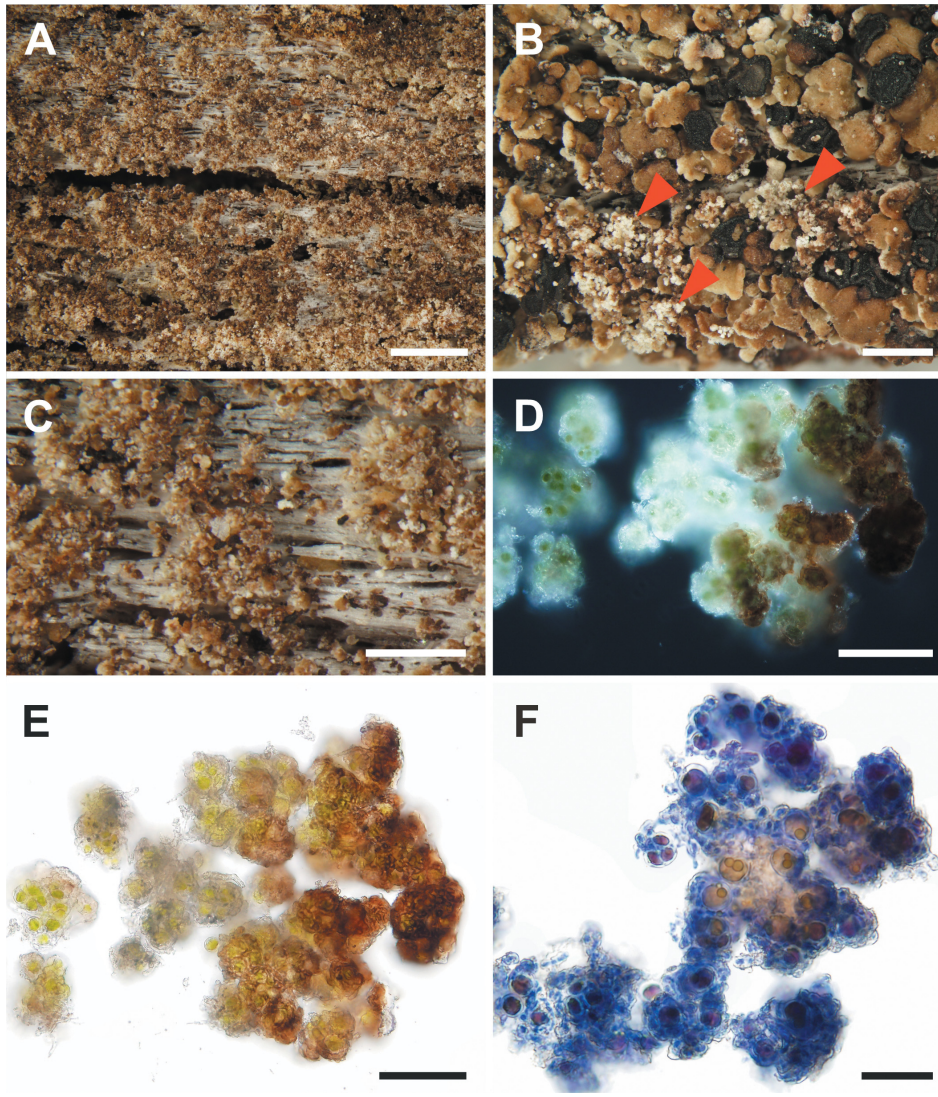

Fig. 15. *Xylopsora diffissa* (A, C, holotype; B, Šoun 732; D-F, Šoun 1717). A, sorediate thallus; B, mechanical hybrid of *X. diffissa* (arrowheads) with predominant *X. friesii* s. lat.; C, detail of diffused soralia; D, soredia/consoredia in polarized light; E, orange-brown pigment in exposed soredia, inner soredia unpigmented; F, structure of soredia; D, E, observed in water; F, stained by cotton blue. Bars: A, 1 mm; B, C, 0.5 mm; D, E, 50 µm; F, 20 µm.

either on steep, often rocky slopes or on peat-bogs. The species occurs on hard, slowly decaying wood in microhabitats sheltered from rain in communities with e.g. *Chaenotheca ferruginea*, *C. xyloxena*, *Micarea melaena*, *Pycnora sorophora*, *Hypocenomyce scalaris* and *Xylopsora friesii* s. lat. It frequently forms "mechanical hybrids" with *X. friesii*, giving the impression that *X. diffissa* represents soralia of *X. friesii* (Fig. 15B). The species was detected in eDNA samples from 14 sites at altitudes between 340 and

1270 m, but mostly in low abundances (probably detections of diaspores, i.e. soredia). Markedly high abundances were detected only in sites CS1 and CS2, where the species was confirmed also by the taxonomic survey.

Similar species: The thick brown to grey sorediate crust and the soredia containing friesiic acid and an orange-red pigment are diagnostic compared to sorediate crusts of other genera. *Xylopsora caradocensis* and *X. friesii* s. lat. may occasionally form sorediate morphotypes too (Palice, Peksa, Šoun, unpublished data), but these are distinctly squamulose, not dissolved into a sorediate crust. *Xylopsora canopeorum* is somewhat similar, but forms squamules dissolving into coralloid isidia that are larger than the soredia of *X. diffissa* (cf. Bendiksby et al. 2018).

DNA data: Six ITS sequences, more than 99.5% identical, were generated for *X. diffissa*, and they form a distinct clade within the genus (Fig. 16). There are actually seven clades and one singleton in our ITS phylogeny, which we regard as putative species hypotheses. Three species names are available: *X. caradocensis* is the clade forming a basal lineage, and *X. canopeorum* is the sister of a clade that might be named *X. friesii* s. str. We refrain from formal epitypification at this point, however, because we think this complex consists, at least partly, of apparently cryptic species which need closer study by more markers and a deeper understanding of their ecology and distributions. Here we merely recognize *X. diffissa* as a taxonomic species due to its deviating morphology and dispersal strategy. We confirmed that the sequences of *X. diffissa* (i.e. the morphotype fully dissolved into a sorediate crust) are different from co-occurring populations of *X. friesii* s. lat. (i.e. fertile, non-sorediate morphotype). A sequence of mtSSU was obtained only from the type specimen; the mtSSU is, however, almost identical among lineages of *X. friesii* s. lat. (and *X. canopeorum* and *X. diffissa*).

Paratypes: Austria, Lower Austria, Thayatal National Park, Hardegg, Fugnitztal, S-facing rocks 0.7 km SW of Hardegg castle, alt. 340–400 m, 48.84889N, 15.84972E, on stump of *Pinus sylvestris*, 9 August 2022, J. Malíček 15591, F. Bouda, E. Konečná, M. Schultz et J. Vondrák (herb. Malíček; ITS: PP410503). Czech Republic, central Bohemia, Křivoklátsko, Rakovník, Skryje: Jezírka nature reserve, steep WSW-facing slope with mixed forest on the right bank of the Zbirožský potok brook, alt. 325 m, 49.94232N, 13.75103E, on wood of stump of *Pinus sylvestris*, 26 November 2019, J. Šoun 1718 (herb. Šoun; ITS: PP410504). Beroun, Nižbor: well-lit oak-pine forest on S facing slope of the hill Koš [383], 0.9 km W of the settlement Krkavčí hora, alt. 328 m, 50.00775N, 14.01883E, on hard wood of *Pinus* stump, 22 May 2024, Z. Palice 37215 et J. Vondrák (PRA). Southern Bohemia, Blanský les Mts, Český Krumlov, Třísov: steep SE-facing rocky slope Uhlířská stráň with a fragment of relic pine forest above the left bank of the Vltava river, alt. 470 m, 48.89495N, 14.35673E, on wood of standing decorticated *Pinus sylvestris*, 20 May 2010, J. Šoun 732 et V. Pouska (herb. Šoun). Šumava Mts, Prachatice, Včelná pod Boubínem: nature reserve Čertova stráň, old-growth fir-beech forest at steep S-facing slope above the valley of Boubínský potok, alt. 740 m, 49.00775N, 13.88508E, on hard wood of *Pinus*/*Abies* stump, 22 September 2021, Z. Palice 32382 (PRA; type locality); ibid., 10 October 2021, J. Šoun 1720 (herb. Šoun; ITS: PP410505). Šumava Mts, Prachatice, Krejčovice: the valley of Blanice, 0.5 km E of the chapel of St. Joseph, steep, SSE-facing rocky slope with a fragment of relic pine forest above the left bank of the rivulet, alt. 652 m, 48.97914N, 13.91314E, on burnt wood of *Pinus sylvestris*, 10 August 2021, Z. Palice 31929 (PRA). Šumava Mts, Prachatice, Záblatí: the valley of Blanice, a bouldery scree covered by a relic pine forest on S-facing slope, 0.4–0.5 km SW of the point 776, 1.5 km WSW of the settlement Hlásná Lhota, on wood of *Pinus sylvestris* snag, alt. 709 m, 48.97714N, 13.91717E, 11 July 2022, Z. Palice 34449 (PRA; ITS: PP410502). Třeboň area, Suchdol nad Lužnicí, Třebnouze: nature reserve Žofinka, a boggy pine forest, alt. 473 m, 48.82153N, 14.88439E, on wood of *Pinus sylvestris* snag, 23 September 2021, Z. Palice 32315 (PRA); ibid.: alt. 474 m, 48.81758N, 14.88439E, 21 June 2022, Z. Palice 33785 (PRA). Western Bohemia, Křivoklátsko, Rokycany, Ostrovec-Lhotka: Lípa nature reserve, edge of E-facing dacite rock outcrops on the left bank of the Zbirožský potok brook, alt. 325 m, 49.93860N, 13.74637E, on wood of stump, 16 June 2020, J. Šoun 1717 (herb. Šoun; ITS: PP410506).

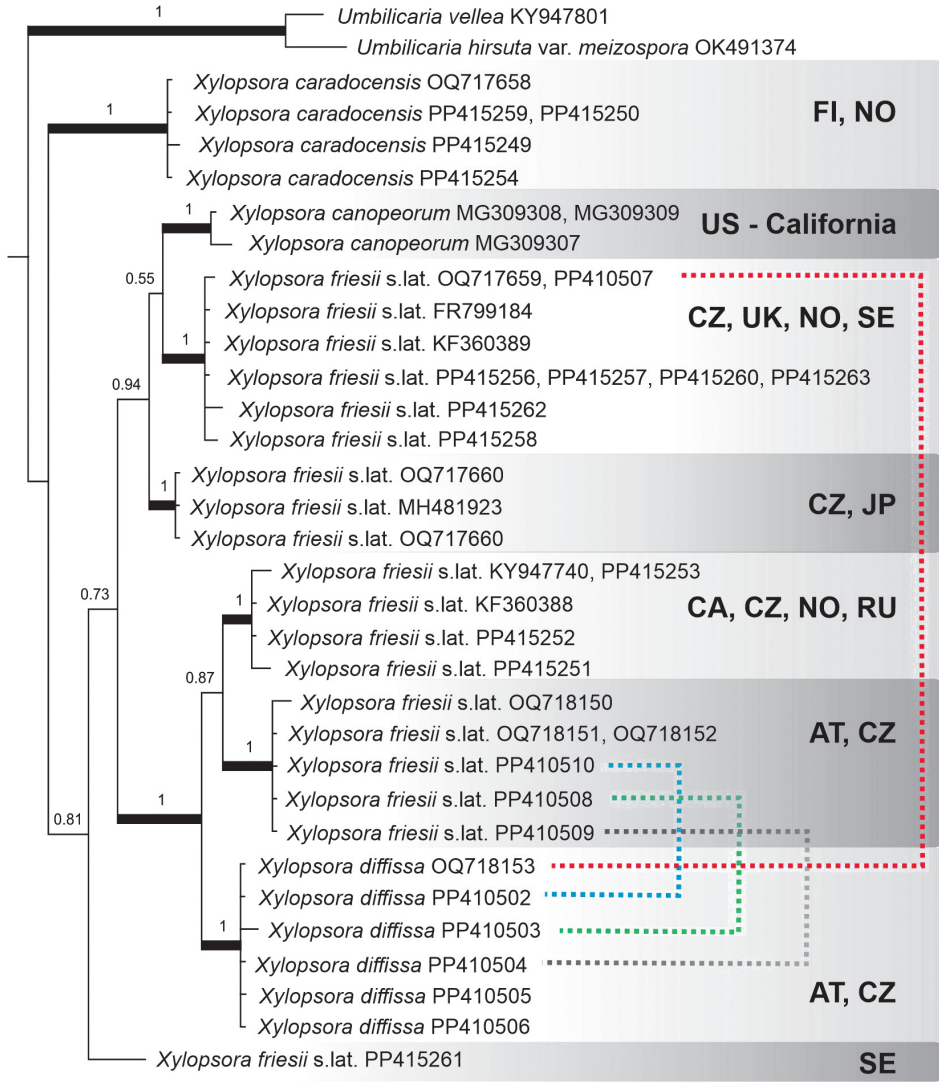

Fig. 16. Phylogenetic tree of *Xylopsora* based on ITS sequence data showing positions of *Xylopsora diffissa* and several lineages of *X. friesii* s. lat. GTR model with gamma distribution and proportion of invariable sites was used as a model of sequence evolution. The tree was constructed using Bayesian inference run for 144000 generations, and was rooted with *Umbilicaria*. Dotted lines connect the sequences of *X. diffissa* and *X. friesii* s. lat. occurring together in individual specimens and forming "mechanical hybrids". Country codes for sequenced specimens are given for each *Xylopsora* clade.

Species new to the Czech Republic or rediscovered after more than 25 years

Absconditella amabilis T. Sprib.

This species was previously known from a single locality in British Columbia on the bark of *Tsuga heterophylla* from a waterfall spray zone (Spribille et al. 2009). In the description it was compared with *A. lignicola*, from which it was distinguished by its smaller apothecia and spindle-shaped ascospores with acute ends. DNA sequences for *A. amabilis* are, however, not available in NCBI yet. A specimen from the Czech Republic, originally called *Absconditella* sp. (Palice 29411, PRA), was sequenced for purposes of the DNA barcode database (Vondrák et al. 2023; ITS: OQ717674, mtSSU: OQ646064) and examinations of the phenotype revealed ascospores fully corresponding with *A. amabilis*. Other species with similar ascospores are *A. celata* and *A. fossarum*, but these have higher urn-shaped apothecia containing a distinct orange-red pigment. Apothecia in the dried Czech specimens of *A. amabilis* are pale, sordid or creamy white, with a faint yellowish-ochre hue (not almost purely white like in *A. lignicola* or *Absconditonia sublignicola*), translucent when wet, microscopically either with a slight yellow-orange tint in the subhymenium (observed on thicker sections in freshly collected material) or fully colourless (as reported for *A. amabilis*). The sequenced Czech specimen comes from wood subjected to water spray and inundation in a brook bedrock (not bark as the type of *A. amabilis*) and the apothecia are slightly larger, i.e. 0.1–0.2 mm in diam. vs. 0.06–0.13 mm. The second Czech specimen produces smaller apothecia up to 0.15 mm, and was growing on a living *Picea* twig in a humid spruce plantation on a boggy place in a slope of a deep brook valley. For the time being, we prefer to use the name *A. amabilis* for Czech specimens (and the corresponding European population) unless it turns out that the American population is significantly different in DNA. According to eDNA, *A. amabilis* is distinctly rarer than both *Absconditella lignicola* and *Absconditonia sublignicola*, but is still present in seven of 20 surveyed sites: one of eight at altitudes below 500 m, three of eight at 500–1000 m, three of four at altitudes above 1000 m. It is likely a narrow-niche, hygrophilic lichen preferring stable, humid microsites.

Voucher specimens from the Czech Republic: northern Bohemia, Jizerské hory Mts, Josefův Důl: valley of Jedlový potok, nature reserve Jedlový důl, bedrock of the brook, alt. 753 m, 50.79572N, 15.24536E, on soaked hard wood of a conifer log, 14 July 2020, Z. Palice 29411 (PRA). Krkonoše Mts, Harrachov: SW foothill of Mt. Jakšín [1115], a managed boggy spruce forest on WSW facing slope above the brook Kamenice, alt. 790 m, 50.78250N, 15.43653E, on living twig of *Picea*, 24 June 2015, Z. Palice 20873 et P. Uhlík (PRA).

Absconditonia sublignicola Suija et van den Boom

A common species that used to be identified as *Absconditella lignicola* until Suija and van den Boom (2023) recognized it on the basis of substantial differences in three-loci DNA sequences and minute differences in morphology (smaller apothecia and ascospores, different exciple structure). Both species have similar ecology, but *Absconditonia sublignicola* is notably more frequent in eDNA data (present in 17 of 20 forest sites), whereas *Absconditella lignicola* was present in only nine sites and always in lower abundances.

Andreiomyces obtusaticus (Tønsberg) B. P. Hodk. et Lendemer

Morphotypes with abundant anthraquinones are easily spotted in the field as a finely sorediate greenish "*Lepraria*-like" crust with rust red to orange spots. Such specimens were frequently recorded in the spruce forest site BO1. However, this species also occurs

with low amounts of anthraquinones, and possibly also without them (Vondrák et al. 2023), and such morphotypes are hardly distinguishable from *Lepraria* sp. div. and some frequently sterile sorediate *Chaenotheca* species (e.g. *Chaenotheca stemonea*). According to eDNA, *Andreiomyces obtusaticus* occurs in five of 20 forest sites. It is very rare in sites below 500 m, where a low abundance was recorded from a single site PO1. Most records come from altitudes between 500 and 1000 m, where high abundances were detected in sites CS1 and CS2; probably a morphotype with low amounts of anthraquinones, which was not revealed by the taxonomic survey. At altitudes above 1000 m, it was only recorded in BO1. During recent field inventories the species was recognized as locally quite frequent in constantly humid forested areas, namely in deep brook valleys, usually as a bark dwelling species, but occurring also on wood, epilithic bryophytes, or directly on rock. The majority of the specimens listed below contained a comparatively high amounts of anthraquinones which were already noticed in the field. Attention was paid to the presence of stunted orange thalli of *Chaenotheca ferruginea*, which can cause confusion when overgrown by, or associated with, finely sorediate thalli of other *Chaenotheca* or *Lepraria* species.

Selected voucher specimens from the Czech Republic: southern Bohemia, Šumava Mts, Volary, Zátoň, Mt. Boubín, east slope, below Mt. Basumský hřeben (site BO1), alt. 1260 m, 48.97413N, 13.80077E, on bark of *Picea abies*, 25 May 2020, 28 July 2020 and 9 September 2022, J. Vondrák 23664, 24049, 26191 (PRA; ITS: OQ717300; mtSSU: OQ682850). Šumava Mts, Prachatice, Záblatí: forest on the right bank of the rivulet, 0.7 km N-NNE of the castle ruin Hus, alt. 735 m, 48.96328N, 13.93158E, on shaded foot of old *Picea* near the rivulet, 31 July 2020, Z. Palice 33868 (PRA). Šumava Mts, Prachatice, Záblatí: the valley of Blanice, pine-dominated forest on steep W-facing slope above the right bank of the rivulet, 1.3–1.4 km W of the settlement Hlásná Lhota, alt. 726 m, 48.97961N, 13.91822E, on bark at foot of *Abies alba*, 20 June 2022, T. Hauser et Z. Palice 33868 (PRA; ITS: OQ717301, mtSSU: OQ682851); ibid., small rock outcrops on the right bank of the rivulet, N-NNW foothill of Mt. Panský vrch [834], 1.3 km SW-SSW of the settlement Hlásná Lhota, alt. 665 m, 48.97211N, 13.92617E, on shaded gneiss stone below overhang, 12 July 2022, Z. Palice 34786 (PRA). Šumava Mts, Volary: valley of a right-hand-side tributary of the Hučina brook, humid old-growth spruce-dominated forest, just NW of the settlement Jelení Vrchy, alt. 881 m, 48.81931N, 13.87075E, on bark of young *Picea* snag, 6 July 2023, Z. Palice 35956 (PRA); ibid., NW foothill of Mt. Spálený [818], a managed spruce forest with dispersed pines and birches on NNW-facing slope near the forest road Spálená cesta, 0.8–0.9 km SE of the railway-station Černý Kříž, alt. 744 m, 48.85539N, 13.86964E, on bark of old *Pinus sylvestris*, 16 June 2024, Z. Palice 37226 (PRA). Novohradské hory Mts: nature reserve Hojná voda, old-growth beech-dominated forest on E-facing slope, alt. 860 m, 48.70592N, 14.75242E, on bark of shaded dry foot of *Fagus*, 21 May 2020, Z. Palice 29017 et J. Malíček (PRA; mtSSU: OQ646080). Western Bohemia, Šumava Mts, Hamry: nature reserve Bílá strž, forest in the valley of the brook Bílý potok, alt. 798 m, 49.19530N, 13.15669E, on bark of *Picea* near brook, 15 July 2019, Z. Palice 28214 et P Uhlík (PRA); ibid., alt. 810 m, 49.19444N, 13.15758E, on bark of young *Picea*, 16 July 2019, Z. Palice 28222 et P. Uhlík (PRA); ibid., alt. 745 m, 49.19873N, 13.15502E, on dry bark of old *Abies alba*, 19 October 2020, Z. Palice 30093 (PRA); ibid., alt. 767 m, 49.19667N, 13.15517E, on wood of thin snag of *Picea abies*, 19 October 2020, Z. Palice 30097 (PRA); ibid., just W-WNW of the waterfall Suťový vodopád, alt. 895–900 m, 49.19203N, 13.15764E, on polypore on a snag of *Abies alba*, 23 June 2020, Z. Palice 30442 et P. Uhlík (PRA); ibid., alt. 803 m, 49.19478N, 13.15719E, on dry wood of stump (*Abies*/*Picea*), 14 July 2022, Z. Palice 33961 (PRA). Šumava Mts, Srní, Povydří: old bridge over Popelný potok brook near Turnerova chata cottage, alt. 800 m, 49.08067N, 13.51533E, on bryophytes on vertical siliceous rock, 18 January 2020, J. Malíček 13444 (herb. Malíček). Šumava Mts, Horská Kvilda: forested bouldery scree on the right bank of the valley of Hamerský potok brook, alt. 1018 m, 49.05667N, 13.53889E, on wood of a small *Picea* snag, 24 May 2022, Z. Palice 33578 (PRA). Northern Bohemia, Jizerské hory Mts, Josefův Důl: valley of Jedlový potok, nature reserve Jedlový důl, old-growth forest on the left bank, alt. 735 m, 50.79419N, 15.24536E, on bark of *Acer pseudoplatanus*, 15 July 2020, Z. Palice 29391 (PRA); ibid., 50.79431N, 15.24547E, Z. Palice 29471 (PRA).

Arthonia apatetica (A. Massal.) Th. Fr.

Only two ITS2 reads obtained, 99.7% identical with the barcode OQ717303 from a specimen from Ukrainian Carpathians (Vondrák et al. 2023). Both reads came from the lowland floodplain forest, site RN2. Not yet found in the Czech Republic by taxonomic surveys.

Arthonia bueriana (J. Lahm ex Arnold) Zahlbr.

A little-known species, reported from oak bark in Germany (Redinger 1937, Wirth et al. 2013). Our barcode specimen (Vondrák 24332, PRA) comes from oak bark in central Bohemia, Czech Republic and is so far the single documented record from the Czech Republic. However, the eDNA revealed this species from seven of eight sites at altitudes below 500 m, being abundant in sites PO1, PO2, TY1. The species was not detected in any site above 500 m.

Voucher specimen from the Czech Republic: central Bohemia, Rakovník, Skryje, on east slope above Prostřední potok, alt. 360 m, 49.96160N, 13.80138E, on bark of *Quercus petraea*, 19 October 2020, J. Vondrák 24332 (PRA; ITS: OQ717692).

Arthopyrenia subcerasi (Vain.) Zahlbr.

Only two ITS2 reads obtained, 99.5–100% identical with the barcode OQ717630 (specimen Vondrák 23176, PRA) from Russian Caucasus. Both reads came from the upland site CS1. Not yet found in the Czech Republic by taxonomic surveys.

Bacidia absistens (Nyl.) Arnold

This species with a rather oceanic distribution in Europe was surprisingly recorded in eDNA from sites RD1 (in four samples) and ZD1 (three samples). It was detected only in the mtSSU dataset and the abundances were low in all samples; the total number of obtained sequences was 29. The sequences were 97–98.5% identical with MG925845 from Norway (Kistenich et al. 2018). Failure to detect the species in the ITS likely means that the sequences in the eDNA were less than 97% identical to the barcode sequences of AF282085 (Norway) and MW523506 (Caucasus). The two ITS barcodes are only 92.5% identical, suggesting that multiple species may exist within the current understanding of *B. absistens*. Not yet found in the Czech Republic by taxonomic surveys.

Bacidina violacea van den Boom et Magain

A recently-described sorediate *Bacidina* from Macaronesia, where it was recorded fertile with a typical apothecial pigmentation (van den Boom & Magain 2020). According to ITS sequences from herbarium specimens, the same species also occurs in Europe, but often in the sterile state. Our barcode sequence, OQ717328 (Vondrák et al. 2023), is from the Czech Republic (Palice 31173, PRA), and is > 98% identical with the sequence of the type specimen. The Czech specimen was collected on a leaning trunk of *Salix euxina* in a well-lit riparian forest along a rivulet, overgrowing both epiphytic bryophytes and bark. It is mostly sterile, formed by extensive yellowish-green sorediate coverings, initially with delimited soralia, later merging into irregular sorediate patches. Only a few apothecia are present. The specimen basically matches the description of *B. violacea*, but the

apothecial margin is pale, not with a violaceous tinge, as noted in the original material. The red-brown pigment in hypothecium fits the Arnoldiana-brown (KOH+ dark brown to greenish, HNO_3–; Meyer & Printzen 2000, Ekman 2023). van den Boom and Magain (2020) reported Superba-brown (described from *Porpidia superba*) in the hypothecium of *B. violacea*, but this was not confirmed by Ekman (2023) who reported Arnoldiana-brown pigment for this species. This is in concordance with our observations in the Czech specimen which was originally assigned to *Bacidina flavoleprosa*, a taxon described from a siliceous rock in the Czech Republic (Czarnota & Guzow-Krzemińska 2012), although all later reported occurrences are epiphytic (Ekman 2023). While the apothecia of the saxicolous specimen of *B. flavoleprosa* have a dark hypothecium containing Arnoldiana-brown pigment, most of the epiphytic specimens are pallid, almost pigment-deficient (Ekman 2023). van den Boom and Magain (2020) did not compare *B. violacea* and *B. flavoleprosa*, although these two taxa seem to be very similar. According to Ekman (2023) *B. violacea* has more yellowish thallus than *B. flavoleprosa* (in spite of the epithet of the latter species), dissolving completely into vegetative propagules, and forming also darker apothecia. Due to the limited material of *B. violacea* studied so far the diagnostic features of the species should be refined as more specimens become available.

ITS sequences obtained from a single specimen from Luzern, Switzerland, called *Bacidina arnoldiana* agg. (KX098347, KX098348, KX132958; Mark et al. 2016) apparently belong to *B. violacea* too, as they are 95–98% identical to the sequence from the type specimen (NR_184913). In our study, it was only detected by ITS2 barcode (mtSSU barcode is not obtained yet) from sites TY1, RD1, ZF1, ZF2, with low abundances in all cases.

Voucher specimen from the Czech Republic: southern Bohemia, Třeboň area, nature reserve Stará a Nová řeka, margin of a small wood on the right bank of the river Lužnice, 0.55–0.60 km NNE of the church 'kostel sv. Máří Magdaleny', alt. 435 m, 48.97028N, 14.86728E, on mossy bark (both over bryophytes and bark) of *Salix euxina* on the bank of the river, 6 June 2020, Z. Palice 31173 (PRA; ITS: OQ717328).

Biatora albidula Willey [= *Myrionora albidula* (Willey) R. C. Harris]

A rare species with a circumpolar distribution in the temperate zone of the northern hemisphere. Only a single historical record exists from central Europe: Schwarzwald, Germany (Palice et al. 2013). In the eDNA data, it was detected only by ITS1 as a single read (97.5% identical to OQ717973 from Caucasus; Vondrák et al. 2023) from the site CS1. The second closest known species is *B. ligni-mollis*, which is ~93.5% identical in ITS1. Unlike the latter species, *B. albidula* has not yet been found in the Czech Republic by taxonomic surveys.

Biatora hemipolia (Nyl.) S. Ekman et Printzen

This species was first validly described from Finland by Nylander in 1873 as *Lecidea arceutina * hemipolia*, four years later after its first mention in literature (cf. Printzen 2014), but it has only a few later records, mainly from Fennoscandia and North America. It is certainly one of the rarest *Biatora* species in central Europe (e.g. Nimis et al. 2018). In the Czech Republic, we collected voucher specimens from a single tree in RD1, where the species was also abundant in eDNA. Low abundances in eDNA were also detected in ZF1, ZD2. *Biatora hemipolia* probably prefers montane ravine and beech dominated old-growth forests.

Voucher specimens from the Czech Republic: southern Bohemia, Šumava Mts, Volary, Soumarský most, scree forest on Radvanovický hřbet, alt. 910 m, 48.90667N, 13.80091E (site RD1), on bark of *Acer platanoides*, 9 August 2021, J. Vondrák 25080 (PRA; ITS: OQ717332, mtSSU: OQ682885); ibid., 20 October 2021, Z. Palice 31937 (PRA).

Calicium episcalare Tibell et Knutsson

Described by Tibell and Knutsson (2016; as *C. episcalaris*) from a single locality in Sweden. Recent taxonomic surveys, however, revealed its occurrence in other European countries including the Czech Republic (see below), and in North America (Selva et al. 2023). It was also detected in our eDNA data by the barcode loci ITS1 and ITS2 from the sites PO2, TY1 and TY2.

The lichen has already been reported from the Czech Republic from Týřov, Křivoklát region, as *C. montanum* (Vondrák et al. 2022). The confusion was caused by the then unknown fact that *C. episcalare* may form its own autonomous thallus in maturity and is overall similar to *C. montanum*.

Voucher specimens from the Czech Republic: central Bohemia, Rakovník, Skryje, protected area Týřov, open scree on west slope above Prostřední potok stream, alt. 380 m, 49.95901N, 13.80149E, on bark of *Pinus sylvestris*, 7 August 2020, J. Vondrák 24110 (PRA; ITS: OQ717763, mtSSU: OQ646151, sub *C. montanum*); ibid., alt. 360 m, 49.95894N, 13.80122E, 6 October 2020, Z. Palice 29699, S. Svoboda et J. Vondrák (PRA). Kublov, protected area Jouglovka, alt. 560 m, 49.93180N, 13.83913E, on wood of oak log, lichenicolous on *Hypocenomyce scalaris*, 12 March 2023, J. Vondrák 27600 (PRA). Velká Buková (distr. Rakovník), PR Nezabudické skály, oak-pine forest on steep slope, alt. 332 m, 50.02178N, 13.84833E, on *Hypocenomyce scalaris* on snag of *Pinus sylvestris*, 30 December 2021, J. Šoun 1013 (herb. Šoun; ITS: OQ717343). Western Bohemia, Křivoklátsko Protected Landscape Area, Zbiroh, Třebnuška: SW-facing mixed forest on steep slope above Zbirožský potok brook, 0.1 km E of Sýkorův mlýn, alt. 370 m, 49.89483N, 13.73478E, on stump of *Pinus sylvestris*, parasitic on squamules of *Cladonia* sp. and *Hypocenomyce scalaris*, 4 July 2024, J. Malíček 16875 et Z. Palice (herb. J. Malíček).

Candelariella rubrisoli Dong Liu et Hur

The species was recently described from China on the basis of sterile thalli consisting of tiny yellow squamules and marginal soralia (Liu et al. 2019). The Chinese specimens morphologically match European lichens previously called *Candelariella xanthostigmoides* and the ITS from one Chinese specimen is more than 98% identical with the European sequences. DNA sequences from the North American *C. xanthostigmoides* are still not available and the relationship between European and North American populations is not resolved yet. It is a common central-European species frequently occurring from lowlands to upper mountains. It was recorded in eDNA from 19 of 20 studied sites.

Chaenotheca nitidula Tibell

Described from eastern North America (Tibell & Koffman 2002), *Chaenotheca nitidula* is hardly distinguishable from the common European species *C. xyloxena*, which is, however, not closely related. In our eDNA specimens, it was detected only by ITS2, but very abundantly in site OS1. The reads were 97–98% identical to the AF492386 barcode from an eastern Canadian specimen. The closest related species is *C. gracilenta*, but its sequences are only about 90% identical to our reads. Although the ITS2 reads from central Europe are slightly distinct from American sequences, we still consider the European population conspecific with *C. nitidula*, unless closer study can show that the populations are phenotypically different. Not yet found in Europe by taxonomic surveys.

Chaenothecopsis consociata (Nádv.) A. F. W. Schmidt

The species is obligately lichenicolous on thalli of *Chaenotheca chrysocephala*, probably rare and/or overlooked in Europe. According to our eDNA data, it is a rare species in the Czech Republic that was detected only as seven ITS2 reads from the sites ZF1 and BO1. The reads were 97.2% identical with the AY795851 barcode generated from a Swedish specimen (Tibell & Vinuesa 2005). The rather low identity of the Czech reads to the barcode sequence can be explained by the geographic distance. The only previously published Czech record is from 1993, from the Šumava Mts (Kocourková 2000).

Recent specimen from the Czech Republic: southern Bohemia, Šumava Mts, Volary: nature reserve Mrtvý luh - boggy pine-birch forest, S marginal part (lagg) of the peatbog, alt. 735 m, 48.86298N, 13.87246E, associated with thallus of *Chaenotheca chrysocephala* on wood of *Betula* snag, 27 October 2022, Z. Palice 34322 (PRA).

Chaenothecopsis haematopus Tibell

Described from New Zealand (Tibell 1987), but with a world-wide distribution, including Europe (Tibell 1999). It is considered to be rare in Europe and the eDNA detection by ITS2 from 12 of 20 sites from lowland to mountains is surprising. The high detection rate is probably due to the presence of stages without fruiting bodies. Remarkably, a distinctive anamorphic stage of *C. haematopus* was described as a new hyphomycetous genus and species, *Catenomycopsis rosea* Constant. (Tibell & Constantinescu 1991). This anamorph, allegedly easily obtained in culture (Tibell 1997), is possibly widespread in nature, but not yet observed in the field by experts. Not yet found in the Czech Republic by taxonomic surveys.

Chaenothecopsis orientalis Tibell

Described from India and the Russian Far East (Tibell & Vinuesa 2005) as a species similar to *C. savonica*, but distinct by the stalk pigmentation and other minute anatomical characters. Our barcode sequences were generated from a Czech specimen (Vondrák et al. 2023) which has the ITS which is 96% identical with the Russian specimen (AY795863), 91% with the Indian one (AY795864) and 100% with a specimen from Germany (MW325682; Dornes & Beck, unpublished). Other known *Mycocaliciaceae* sequences in NCBI are up to 85% identical. In eDNA, *Chaenothecopsis orientalis* was detected by all barcode loci in sites MK1, OS1 and BO1, but abundant only in OS1 where it was also recorded by the taxonomic survey.

Voucher specimens from the Czech Republic: western Bohemia, Český les Mts, Tachov, Lesná, protected forest area Ostrůvek, alt. 740 m, 49.76429N, 12.45725E (site OS1), on bark of *Picea abies*, 21 July 2020, J. Vondrák 23994 (PRA; ITS: OQ717805, mtSSU: OQ646193); ibid., J. Vondrák 23995 (PRA).

Chaenothecopsis cf. *tasmanica* Tibell

Described from Tasmania, but with a world-wide distribution, including a few records from central Europe (e.g. Palice 1999). Our barcode sequence is from a Scottish specimen (Vondrák et al. 2023). The specimen matches the concept of *C. tasmanica* by Groner (2006), but its true identity with the original specimen is questionable. In eDNA, the species was detected by ITS1 and ITS2, but only in low abundance in a single site, OS1.

Furthermore, it was detected by mtSSU from 10 of 20 sites throughout the altitudinal gradient. However, sequences 99–100% identical to the barcode (Vondrák 19728, PRA, OQ646192, from Scotland; Vondrák et al. 2023) were only present in samples from OS1. Sequences only 97–99% identical to the barcode were detected in samples from other sites. We suppose, that the "sensu stricto" taxon represented by the barcode was only present in OS1, but another related taxon/taxa was/were present in other sites. *Chaenothecopsis tasmanica* was preciously recorded from the Czech Republic only by Palice (1999) and both records from the Šumava Mts are more than 20 years old.

Cladonia bacilliformis (Nyl.) Sarnth.

A boreal lichen characteristic by yellowish-green podetia containing usnic acid, with subulate tips and pale ochraceous apothecia. We detected only five ITS2 reads in samples from upper montane sites BO1, BO2, and ZD2, 97.5–99.5% identical to the MK179529 barcode from the Caucasian specimen of *C. bacilliformis* (Stenroos et al. 2019). We are not entirely convinced that all reads must belong to *C. bacilliformis*, at least those with lower identities to the barcode, because some species are also close in ITS2: e.g. *Cladonia norvegica*. *Cladonia bacilliformis* has not yet been found in the Czech Republic by taxonomic surveys, but its presence in the montane spruce forests is rather probable.

Cliostomum corrugatum (Ach.) Fr.

Our barcode sequences come from the Czech specimen listed below, which was collected from bark of old oak trees in a parkland landscape, where it was common, but with numerous pycnidia only (i.e. without apothecia). In eDNA, *C. corrugatum* was detected only by mtSSU in sites PO1 and CS1, and the taxonomic identity of the only two available mtSSU reads is uncertain, as they are 97–98% identical with the barcode sequence. The mtSSU reads may belong to another related species, as also suggested by the absence of *C. corrugatum* in eDNA data from ITS1 and ITS2.

Voucher specimens from the Czech Republic: southern Bohemia, Třeboň, protected area Stará a Nová řeka, Novořecká Bašta, alt. 430 m, 49.00183N, 14.84798E, on bark of *Quercus robur*, 22 October 2021, J. Vondrák 25744 (PRA; ITS: OQ646207, mtSSU: OQ717824); ibid., 48.99501N, 14.85103E, J. Vondrák 25745 (PRA).

Cliostomum haematommatis (Keissl.) D. Hawksw., Earl.-Benn. et Coppins

Detected in the eDNA data from a single site, CS1, by ITS1 (two reads 100% identical to the MK446224 barcode) and mtSSU (two reads 99.5% identical with MK446223). The barcode sequences are from a Swiss specimen (Dietrich & Malíček 2019). Not yet found in the Czech Republic by taxonomic surveys.

Cryptodiscus muriformis Fern.-Brime, Olariaga, Baral, Friebes, Jaklitsch, Senn-Irlet et Wedin

In the Czech Republic, the species was only detected in a single eDNA sample from the site CS2 (three reads of mtSSU; identities with MG281972 barcode 98–99.5%). The barcode sequence is from a Swedish specimen (Fernández-Brime et al. 2018). Not yet found in the Czech Republic by taxonomic surveys.

Cryptodiscus pini (Romell) Baloch, Gilenstam et Wedin

Detected in the eDNA from the sites PO2 and CS2 by the barcode loci: ITS1 (single read 100% identical to HM244762) and ITS2 (reads 97–99.5% identical to HM244762). The barcode sequence is from a Swedish specimen (Baloch et al. 2010). Only recently documented in the Czech Republic from a native forest during an accidental field research.

Voucher specimen from the Czech Republic: southern Bohemia, Šumava Mts, Volary: nature reserve Mrtvý luh - boggy pine-birch forest, SW marginal part (lagg) of the peatbog, 0.7–0.8 km NW-WNW of the railway-station Černý Kříž, alt. 735 m, 48.86412N, 13.87033E, on dry, slowly decaying wood of high *Pinus* stump (sheltered side), 4 February 2024, Z. Palice 36733 (PRA).

Cryptodiscus tabularum Kirschst.

Detected in the eDNA from the sites PO2, CS2 by all barcode loci with identities 98–100% to the barcodes OQ717827 and OQ646211 obtained from a Czech specimen (Vondrák et al. 2023).

Voucher specimens from the Czech Republic: southern Moravia, Vranov nad Dyjí, locality Ledové sluje, alt. 390 m, 48.88372N, 15.84286E (site PO1), on wood of oak log, 30 June 2021, S. Svoboda s.n., specimen ID: Vondrák 25892 (PRA; ITS: OQ717827, mtSSU: OQ646211); ibid., alt. 375 m, 48.88372N, 15.84225E, on wood of *Pinus* snag, 27 August 2021, Z. Palice 32843 (PRA). Vranov nad Dyjí, Feliciino údolí, alt. 330 m, 48.89310N, 15.81480E, on wood of *Pinus* log, 14 July 2022, J. Vondrák 27359 (PRA). Southern Bohemia, Šumava Mts, Prachatice, Záblatí: the wooded canyon-like valley of Blanice, foot of a low rock-outcrop above the right bank of the rivulet, 1.1 km SW of Hlásná Lhota, 1.5–1.6 km ESE of the settlement Krejčovice, alt. 630 m, 48.97405N, 13.92575E, on wood of *Pinus* snag, 13 July 2022, Z. Palice 35247 (PRA).

Graphis betulina (Pers.) Ach.

A species from the difficult *Graphis scripta* complex that was recently studied on a morphological basis (Neuwirth & Aptroot 2011) and with the use of DNA data (Kraichak et al. 2015), however the phylogeny does not match the morphological concept. Due to an unresolved taxonomy, we generally call our specimens *G. scripta* s. lat., but this case is exceptional. Here we adopted the concept by Singh et al. (2019) who accept the name *G. betulina* within the complex. Our barcode specimen from the Czech Republic (Vondrák et al. 2023; as *Graphis scripta* TYPE2) had a distinctly whitish thallus and was 99.8% identical with the specimen from Poland called *G. betulina* (Singh et al. 2019). We sequenced other specimens of *G. scripta* s. lat., but with greyish thalli, and they vere distinct in ITS and belong to other lineages. We suggest that the notably whitish thallus is one of the diagnostic characters of *G. betulina* (sensu Singh et al. 2019). Whereas *Graphis scripta* s. lat. occurred in most of the surveyed sites, *G. betulina* was detected in eDNA by the barcode loci ITS1 and ITS2 from only two sites, MK1 and RD1.

Voucher specimen from the Czech Republic: southern Bohemia, Novohradské hory Mts, Benešov nad Černou, Žofín, beech forest, alt. 840 m, 48.65947N, 14.69396E (site ZF2), bark of *Fagus sylvatica*, 11 November 2020, J. Vondrák 28320 [originally 25932a] (PRA; ITS: OQ717395).

Lecania falcata van den Boom, M. Brand, Coppins, Magain et Sérus.

Described from the Canary Islands, the Azores and western Europe (Sérusiaux et al. 2012), but also known from the Caucasus (Vondrák et al. 2023). We surprisingly detected it in eDNA by ITS2 and mtSSU, but only from a single site, MK1. The detection by ITS2

revealed numerous reads 97–98% identical with JQ796857 (specimen from the Canary Islands; Sérusiaux et al. 2012), but only three reads from a single sample were detected by mtSSU (99–100% identical with OQ682972 from Caucasus; Vondrák et al. 2023). Not yet found in the Czech Republic by taxonomic surveys.

Loxospora cismonica (Beltr.) Hafellner

In eDNA, this rare species was detected by all barcode loci from a single site, CS1. In the same site, the species was also frequently recorded by the taxonomic survey. Apart from this site, *L. cismonica* is known from only one historical record from the Czech Republic (Hilitzer 1924).

Voucher specimens from the Czech Republic: Záblatí, Řepešín, protected area Čertova stráň, alt. 760 m, 49.00791N, 13.88405E (site CS1), on bark of *Abies alba*, 9 October 2020, J. Vondrák 24437 (PRA; ITS: OQ717930, mtSSU: OQ646299); ibid., alt. 730 m, 49.00764N, 13.88553E, on bark of *Abies alba*, 22 September 2021, Z. Palice 32358 (PRA).

Megalaria pulverea (Borrer) Hafellner et E. Schreiner

This species is well known from central Europe (e.g. Nimis et al. 2018), but considered to be very rare in this region. In eDNA, *M. pulverea* was detected by ITS1 and ITS2 barcode loci from the sites CS2, RD2 (abundant), TY2 and ZD1. In RD2 and ZD1, it was also confirmed by the taxonomic survey.

Voucher specimens from the Czech Republic: southern Bohemia, Šumava Mts, Volary, Soumarský most, Radvanovický hřbet, alt. 820 m, 48.90055N, 13.80778E (site RD2), on bark of *Fagus sylvatica*, 20 October 2021, J. Vondrák 25429 (PRA; ITS: OQ717934); ibid., 21 October 2021, Z. Palice 32047 (PRA). Záblatí, Řepešín, protected area Čertova stráň, alt. 700 m, 49.00705N, 13.88232E, bark of *Ulmus glabra* by stream, 1 May 2024, J. Vondrák 28462 (PRA). Western Bohemia, Šumava Mts, Prášily: SW-SSW-facing slope of Mt. Ždanidla, remnant of montane mixed forest, alt. 1210 m, 49.10073N, 13.34562E (site ZD1), on weathered bark of *Fagus* snag, 12 August 2021, Z. Palice 32876 (PRA); ibid., alt. 1195 m, 49.10050N, 13.34503E, on bark of *Acer pseudoplatanus*, Z. Palice 31815 (PRA).

Melaspilea bagliettoana Zahlbr.

A rare and probably under-recorded species, known from bark of deciduous trees in warm-temperate Europe (Sanderson et al. 2009, Nimis 2024). The barcode sequences come from three Czech specimens (Vondrák et al. 2023). In our eDNA data, *M. bagliettoana* was detected by ITS1 and ITS2 in lowland floodplain forest sites RN1 and RN2. It was detected from 11 of 20 sites by the mtSSU, with high abundances in sites located below 500 m (RN1, RN2, PO1, PO2, TY1). The detection of the latter barcode is possibly overrated by a "false" detection of other closely related species.

Voucher specimens from the Czech Republic: southern Moravia, Lanžhot, floodplain forest in protected area Ranšpurk, alt. 180 m, 48.67958N, 16.94568E (site RN1), on twig of *Acer campestre*, 17 September 2020, J. Vondrák 24810 (PRA; ITS: OQ717935, mtSSU: OQ646303); ibid., 48.67961N, 16.94586E, on dry twigs of *Alnus glutinosa*, 17 September 2020, Z. Palice 30599 (PRA; ITS: OQ717936). Vranov nad Dyjí, Feliciino údolí, alt. 330 m, 48.89157N, 15.81277E, on bark of *Corylus avellana*, 14 July 2022, J. Vondrák 27338 (PRA).

Micarea eximia Hedl.

Records of this rare species are almost absent from central Europe (e.g. Nimis et al. 2018). In eDNA, it was detected in the upper montane site ZD1, but only by two ITS2 reads, which are 99% identical with MT981599 and 97.2% with MT981600. Both barcode sequences are from Finnish specimens (Kantelinen et al. 2021). Other known species of the genus *Micarea* are more divergent (< 90% identity). Not yet found in the Czech Republic by taxonomic surveys.

Micarea hypoviolascens Czarnota et Coppins

A very rare species, described from Scotland (Czarnota & Coppins 2005) and so far not known from most of Europe. In our eDNA dataset, it is only represented by a single mtSSU read from the upper montane site BO2, which is 98% identical to the barcode OQ646313 from a Scottish specimen (Vondrák et al. 2023). The taxonomic identity of the single read is uncertain, as it is only 98% identical with the barcode, i.e. it may belong to an unknown, closely related species; however, other *Micarea* species available for comparison in NCBI are < 92% identical. The absence of *Micarea hypoviolascens* in eDNA data from ITS1 and ITS2 also supports the possibility of detection an unknown closely related species by mtSSU. Not yet found in the Czech Republic by taxonomic surveys.

Micarea isidioprasina van den Boom, Guzow-Krzem., Sérus. et Kukwa

Recently described from Europe (Guzow-Krzemińska et al. 2019) and we used the barcode specimen from the Czech Republic (Vondrák et al. 2023). In eDNA, *M. isidioprasina* was detected by the barcode loci ITS1 (98–100% identical to the barcode) and ITS2 (99–100%) from sites RD1, RD2, ZD1, ZF1 and ZF2. In ZF1, it was also confirmed by the taxonomic survey.

Voucher specimen from the Czech Republic: southern Bohemia, Novohradské hory Mts, virgin forest Žofínský prales, NE-facing slope above the brook Tisový potok, alt. 785 m, 48.66983N, 14.70950E (site ZF1), on strongly weathered, soft wood of a big conifer log (vertical part), 30 July 2020, Z. Palice 29526 et J. Vondrák (PRA; ITS: OQ717943).

Micarea melanobola (Nyl.) Coppins

Recently reported from Finland and Switzerland, and shown to be distinct from the closely related *Micarea prasina* s. str. (Launis et al. 2019b). We used barcode specimens from Finland. In eDNA, *M. melanobola* was detected by ITS1 (98–100% identical with MK454950) and ITS2 (99–100% identical with MK454949) from sites BO1, BO2, CS1, OS1 and ZF1. In ZD1, it was recorded by the taxonomic survey.

Voucher specimens from the Czech Republic: western Bohemia, Šumava Mts, Prášily: SW-SSW-facing slope of Mt. Ždanidla, remnant of montane mixed forest, alt. 1210 m, 49.10089N, 13.34533E (site ZD1), on strongly decayed wood of *Picea/Abies* stump, 12 August 2021, Z. Palice 32115 (PRA). Western Bohemia, Šumava Mts, Hamry: nature reserve Bílá strž, forest on the left bank of the brook Bílý potok above the waterfall, alt. 940 m, 49.18953N, 13.15611E, on wood of dry *Picea* snag near the brook, 16 September 2019, Z. Palice 27947 (PRA).

Micarea pseudomicrococca Launis et Myllys

Recently described from Finland and Scotland (Launis et al. 2019a); we used the barcode specimen from Scotland (Launis171141; H). In eDNA, *M. pseudomicrococca* was detected by all barcode loci from 13 of 20 sites. It is apparently more abundant in the mountains. Although it is a common species, it has not yet been confirmed from the Czech Republic by taxonomic surveys because it is difficult to distinguish from *M. micrococca* s. str.

Mycocalicium victoriae (C. Knight ex F. Wilson) Nádv.

Described from Australia, but with a world-wide distribution including southern Europe (Tibell 2007). Characterized by a reddish pigment in outer tissues of the stalk and exciple (KOH+ intensifying), but pale internal tissues (Tibell 1987). Our barcode specimens are from central Europe (Vondrák et al. 2023; as *Mycocalicium* sp.) and their identity with *M. victoriae* is based on morphological similarities. In our eDNA data, *M. victoriae* was detected by all barcode loci from eight of 20 forest sites: abundant in sites at altitudes below 500 m, especially MK1, PO1, PO2 and TY2, very rare in sites with altitudes between 500 and 1000 m (only in RD1 and ZF2) and absent in sites higher than 1000 m. The species has also been recorded by recent taxonomic surveys in the Czech Republic (e.g. sub *Chaenothecopsis rubescens* in Vondrák et al. 2022). It occurs on bark and wood of old oak trees and snags. The ecology is distinct from that of *M. subtile*, which occurs predominantly on the wood of conifers and its eDNA abundances increase from lowlands to montane forests.

Voucher specimens from the Czech Republic: central Bohemia, Rakovník, Skryje, protected area Týřov, rocks above right side of stream Úpořský potok, alt. 320–370 m, 49.96573N, 13.80936E, on bark of *Quercus petraea*, 9 June 2020, J. Vondrák 23725 (PRA, sub *Chaenothecopsis rubescens*). Rakovník, Skryje, on east slope above Prostřední potok, alt. 410 m, 49.95904N, 13.79847E, on wood in hollow of *Quercus petraea* trunk, 19 October 2020, J. Vondrák 24321 (PRA, sub *Chaenothecopsis rubescens*). Southern Moravia. Vranov nad Dyjí, locality Ledové sluje, alt. 390 m, 48.88372N, 15.84286E, on bark of *Quercus petraea*, 27 August 2021, J. Vondrák 25443 (PRA, sub *Mycocalicium* sp.; ITS: OQ717966, mtSSU: OQ646342); ibid., on bark of *Quercus petraea*, 15 June 2022, J. Vondrák 26031 (PRA, sub *Mycocalicium* sp.). Znojmo, Šobes, alt. 330 m, 48.82178N, 15.97238E, on wood of snag of *Quercus petraea*, 22 June 2022, J. Vondrák 26633 (PRA, sub *Mycocalicium* sp.). Čížov, rocks above Dyje river against Einsiedler, alt. 380 m, 48.86149N, 15.87094E, on wood of *Quercus* log, 17 September 2022, J. Vondrák 26653 (PRA, sub *Mycocalicium* sp.). Jevišovická pahorkatina, distr. Znojmo, Štítary: hornbeam-oak forest on a crest above the meander of the brook Štítarský potok, NW of the tourist crossroads Švýcarské údolí, alt. 395 m, 48.92231N, 15.83314E, on bark of *Quercus petraea*, 26 August 2021, Z. Palice 32636 (PRA).

Parmelia encryptata A. Crespo, Divakar et M. C. Molina

Described as a morphologically and chemically identical to *P. sulcata*, but genetically different (Molina et al. 2011). However, Ossowska et al. (2021) reported on diagnostic characters in rhizines: the rhizines are predominantly simple, and squarrose ones appear only in the central parts of the thalli of *P. encryptata*, whereas squarrose rhizines occur over the whole lower surface of the thallus of *P. sulcata*. Our eDNA data support the suggestion by Ossowska et al. (2021) that *P. encryptata* is rare in central Europe. It was detected only by ITS1 (7 reads 98–100% identical to OQ717535) from sites at low altitudes, PO1 and TY1. In contrast, the only confirmed taxonomic record comes from the mountains (see below).

Voucher specimen from the Czech Republic: southern Bohemia, Šumava Mts, Nová Pec, Protected area Smrčina, spruce-beech forest at forest road Saitzova cesta, alt. 1190 m, 48.75005N, 13.92728E, on bark of *Acer pseudoplatanus*, 19 May 2020, J. Vondrák 23821 (PRA; ITS: OQ717535, mtSSU: OQ683106, sub *Parmelia sulcata*).

Pertusaria alpina Hepp ex Ahles

We are uncertain how to distinguish *P. alpina* from *P. constricta*. The latter allegedly differs in perithecial blisters with constricted bases, but sequences of both morphotypes were identical and we decided to view them as synonymous, using the older name, *P. alpina* (Vondrák et al. 2023). In our eDNA data, *Pertusaria alpina* was detected by the barcode loci ITS1 and ITS2 from sites RD1, ZD1, ZF2. All eDNA records were identical with the barcode from the Norwegian specimen (ITS: MK811905, Marthinsen et al. 2019). Not yet found in the Czech Republic by taxonomic surveys, but *P. constricta* was several times recorded historically (Kuťák 1952, Hanko 1983) and also recently (Malíček & Palice 2013). At least some of these data probably refer to *P. alpina* as we understand it.

Phlyctis agelaea (Ach.) Flot.

In eDNA, *P. agelaea* was detected by the ITS1 and ITS2 barcode loci from sites PO1 and RN1. It was also detected by the taxonomic survey from sites MK1 and RN1 (but without documentary specimens). *Phlyctis agelaea* is very rare in central Europe and apparently restricted to thermophilous forest localities below 500 m.

Voucher specimen from the Czech Republic: southern Moravia, National Park Podyjí, locality Ledové sluje, alt. 400 m, 48.88349N, 15.84325E, bark of *Quercus petraea*, 4 May 2024, T. Hauser 637 et J. Vondrák (herb. T. Hauser).

Porina multipuncta (Coppins et P. James) Ertz, Coppins et Frisch (= *Opegrapha multipuncta* Coppins et P. James)

Overlooked and probably rare in continental Europe, with the vast majority of records in Atlantic and Mediterranean areas (e.g. Pentecost & James 2009). In our eDNA dataset, detected only by a single mtSSU read from the site CS1, which is 99.5% identical with MN687915 barcode from a Welsh specimen (Orange et al. 2020). Not yet found in the Czech Republic by taxonomic surveys.

Rinodina sheardii Tønsberg

A sorediate and often sterile species described from northwestern Europe and northwestern North America (Tønsberg 1992). In central Europe it has not been so far recorded outside the Alps, where it is rare in the montane belt (Nimis et al. 2018). Detected in the eDNA data from a single site, TY1, by all three barcodes. ITS2 detection was most powerful, with 200 reads 98–99.5% identical to the MK778640 barcode from a Caucasian specimen (Urbanavichus et al. 2020). Not yet found in the Czech Republic by taxonomic surveys.

Sclerophora farinacea (Chevall.) Chevall.

Probably the rarest central-European *Sclerophora*, known in the Czech Republic only from two historical records (Suza 1921) and recently, a hundred years later, rediscovered. In eDNA data, it was detected by all barcode loci from the lowland floodplain oldgrowth forest Ranšpurk (RN1). In this site, the species was also recorded by the taxonomic survey, being abundant on two trunks of old *Acer campestre*.

Voucher specimens from the Czech Republic: southern Moravia, Lanžhot, protected area Ranšpurk, alt. 160 m, 48.67944N, 16.94653E (site RN1), on rain-sheltered bark of *Acer campestre*, 17 September 2020, J. Vondrák 24055 et Z. Palice 29597 (PRA); ibid., J. Vondrák 24825 (PRA; ITS: OQ718085, mtSSU: OQ646450).

Scutula cf. *igniarii* (Nyl.) S. Ekman

Possibly an undescribed species related to lichens currently placed in the genus *Scutula,* morphologically most similar to the boreal species *S. igniarii* (for which no data exist in Genbank). It is represented by the barcode specimen Palice 33295, PRA, from the Czech Republic (Vondrák et al. 2023, as *Bacidia* cf. *igniarii*). It has ascospores similar to those of *S. igniarii*, but slightly wider, i.e. 10–16 × 3.0–3.5 µm, and 1–3-septate. However, it is distinct in a dark red-brown hypothecium (not very pale brown or pale as reported for *S. igniarii*, cf. Ekman 1996 and Cannon et al. 2023) containing large amount of Laurocerasi-brown pigment (sensu Meyer & Printzen 2000) and in the broadly sessile apothecia (not barrel-shaped or inversely cone-shaped as in *S. igniarii*). The species is definitely rare in the Czech Republic and probably restricted to thermophilous forests at low altitudes. It was detected only in two eDNA samples from a single forest site, PO1. The eDNA dataset includes seventeen ITS2 reads 97.3% identical with OQ717312 barcode and eight mtSSU reads 98.5–100% identical with OQ682868.

Voucher specimen from the Czech Republic: central Bohemia, Bohemian Karst, Karlštejn – Budňany, nature reserve Karlštejn, Mt. Prostřední hora [383], well-lit oak dominated forest (*Quercus pubescens*) on SSW-SW-facing slope, alt. 343 m, 49.93750N, 14.16839E, on bark of *Quercus pubescens*, 7 October 2021, Z. Palice 33295 (PRA; ITS: OQ717312, mtSSU: OQ682868).

Sphaeronema truncatum Fr. (= *Sphaeronaema truncatum*)

A recently resurrected old name for a semi-lichenized coelomycete dwelling on moist wood of conifers (Vondrák et al. 2023) but rarely also of broadleaved trees like *Betula* or *Populus tremula* (Svensson et al. 2024). In eDNA data, detected by all three barcode loci, but only in two sites: RD1 and RD2. In both sites, the species was also recorded by the taxonomic survey. In the Czech Republic, it is rather common in humid montane forests and raised-bogs with rich occurrence of lying dead wood of conifers.

Selected voucher specimens from the Czech Republic: southern Bohemia, Šumava Mts, Volary, Soumarský most, scree forest on Radvanovický hřbet, alt. 910 m, 48.90637N, 13.80106E (RD1), on wood of spruce log, 10 September 2021, J. Vondrák 25159 (PRA); ibid., 48.90049N, 13.80787E (RD2), 20 October 2021, J. Vondrák 25841 (PRA). Volary, Černý Kříž: valley of Studená Vltava, 0.9 km E of the railway-station Černý Kříž, wet meadow by road-side, alt. 740 m, 48.86067N, 13.87386E, on decaying wood of drifted *Picea* trunk, 3 March 2019, Z. Palice 26498 (PRA). Nová Pec, Ovesná: Mt. Perník [1049] - E-facing slope, along yellow marked tourist trail Medvědí stezka, managed forest with dispersed older fir-trees, alt. 840 m, 48.81028N, 13.92972E, on wood of log of a conifer, 11 July 2019, Z. Palice 26903, Jul. Palicová et K. Palicová (PRA). Volary: Mrtvý luh – boggy pine forest, lagg - W part of the peatbog, 739 m, 48.86731N, 13.86808E, on slowly decaying, soaked wood of *Pinus* snag, 7 November 2021, Z. Palice 32322 (PRA). Novohradské hory Mts, Pohorská ves,

Žofín: 1.8 km ESE of settlement, alt. 800–810 m, 48.67250N, 14.71806E, beech forest, on tree stump, 27 October 2010, J. Malíček 8949 et L. Syrovátková (herb. Malíček). Novohradské hory Mts, Benešov nad Černou, protected forest area Žofínský prales, on wood of spruce log, alt. 750 m, 48.66933N, 14.70426E, 5 July 2021, J. Vondrák 25158, 25168, 25169 (PRA; ITS: OQ717610, OQ717611, OQ717612); ibid., NE-facing slope above the brook Tisový potok, alt. 780–790 m, 48.66983N, 14.70950E, on decaying wood of a large spruce log, 30 July 2020, Z. Palice 31078 et J. Vondrák (PRA). Třeboňsko, Suchdol nad Lužnicí, Františkov, protected area Široké blato, alt. 500 m, 48.90784N, 14.98592E, on log of *Pinus sylvestris*, 12 June 2020, J. Vondrák 23773 (PRA; ITS: OQ718100; mtSSU: OQ683177); ibid., Třebnouze: nature reserve Žofinka, a boggy pine forest, alt. 474 m, 48.82119N, 14.89025E, on lying soaked wood of *Pinus*, 21 June 2022, Z. Palice 33775 (PRA).

Toniniopsis dissimilis Gerasimova et A. Beck

A recently recognized species, similar and closely related to *T. separabilis* (Gerasimova et al. 2021). In the eDNA data, the barcode loci ITS1 and ITS2 detected *T. dissimilis* in upper montane sites ZD1 and ZD2 (numerous reads 99–100% identical with the MT169983 barcode). The barcode locus mtSSU detected *T. dissimilis* from nine sites of twenty, but the reads 97–99% identical to the barcode sequences (see Gerasimova et al. 2021) are unreliable and probably belong to the closely related and common *T. separabilis*. Most of the mtSSU reads with identities over 99% are from ZD1, ZD2, which matches the data from ITS. *Toniniopsis dissimilis* is probably a rare montane species with a single specimen-based record from the Czech Republic.

Voucher specimen from the Czech Republic: southern Bohemia, Šumava National Park, Nová Pec, beech forest on NE-exposed slope of Hraničník Mt., 2 km SW of Klápa settlement, 48.75278N, 13.91389E, alt. 1145 m, on bark of *Sorbus aucuparia*, 25 September 2012, J. Malíček 724 (herb. Malíček).

Availability of data and materials. The eDNA datasets generated and analysed during the current study are available in Dryad (doi:10.5061/dryad.fqz612k0g).

Supplementary materials

Data S1. Species diversity data obtained with eDNA and from taxonomic surveys.
Figs S1–S12. Phylogenetic trees with the new species.
Table S1. Square hectare sites under study, and their abbreviations, altitudes, slopes and positions.
Table S2. Information on DNA barcodes.
Table S3. Groups of indistinguishable taxa in barcode databases.
Table S4. Species detected in a single dataset only (i.e. solely in one of ITS, ITS2, mtSSU and taxonomy).
Table S5. Five-hundred-ninety-five species detected by eDNA in at least one of the 20 sites, their abundances in eDNA and taxonomic surveys, and their known occurrences in the Czech Republic.

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Environmentální DNA data v kombinaci s taxonomickým průzkumem poskytují nebývalé porozumění rozmanitosti lišejníků a usnadňují objevování nových druhů

Sekvenování environmentální DNA (eDNA) se stále častěji používá k odhalování biologické rozmanitosti na různých taxonomických a prostorových úrovních. Většina takových studií však obvykle pracuje s abstraktními čísly, která nejsou propojena s názvy druhů, což ztěžuje hodnocení a následné využití výsledků. V našem průzkumu epifytických lišejníků v České republice se nám podařilo propojit sekvence eDNA s druhovými jmény s využitím existující referenční databáze čárových kódů DNA. Na plochách o rozloze 1 ha v různých typech středoevropských lesů se nám podařilo porovnat DNA data z environmentálních vzorků s výsledky paralelního taxonomického průzkumu a údaji o početnosti druhů v celostátním měřítku. V eDNA jsme zjistili velké množství druhů silně podhodnocených v taxonomických průzkumech a v předchozích údajích o rozšíření v České republice. Většina těchto druhů jsou buď velmi malé nebo málo známé mikrolišejníky, často notoricky přehlížené taxonomy. Některé z nich jsou vzácné a mají specifické ekologické nároky, mnohé jsou však poměrně hojné. Byla zjištěna řada pro vědu nových druhů. Dvanáct druhů a dva rody jsou zde nově popsány: *Allarthothelium endochlorum*, *Atrodiscus fagicola* (nový rod), *Bacidina omnicola*, *Biatorella ligni-putridi*, *Cryptodiscus neglectus*, *Gyalidea gabretae*, *Karstenia dryina*, *Micarea lobarica*, *Monilibrachium splendens* (nový rod), *Psoroglaena neglecta*, *Toniniopsis pruinosa* a *Xylopsora diffissa*. V popisech jsou poprvé v lichenologii využita data z eDNA pro charakteristiku ekologie a rozšíření nových druhů. Pomocí eDNA jsme zjistili 43 druhů nových pro Českou republiku a 23 z nich bylo potvrzeno také paralelním taxonomickým průzkumem. *Absconditella amabilis* a *Chaenotheca nitidula* jsou nové pro Evropu.

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