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From Cinderella to Princess: an exceptional hotspot of lichen diversity in a long-inhabited central-European landscape

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Abstract: Biodiversity is a key criterion in nature protection and often indicates habitats and localities rich in endangered species. Our research, using 48 one-man one-day field trips, located an exceptional lichen diversity hotspot and refugium for rare species, the Týřov National Nature Reserve (Czech Republic, central Bohemia). Within its 410 hectares, we detected 787 species of lichens and related taxa (675 lichens, 35 semilichens, 58 lichenicolous fungi and 19 bark microfungi). This is more species of these organisms than has ever been recorded from such a small area, up to 10 km^2, anywhere in Europe (and probably anywhere in the world). The species richness is positively correlated with the habitat heterogeneity within Týřov, which is very far from uniform. In most of the reserve, the species richness is fairly typical for the broader region, and only three sites, with an overall area of a mere 80 hectares, have distinctly higher species richnesses. The most species-rich site, with 502 species, is only about 25 hectares and is distinctly more diverse in habitats than other sites. The enormous importance of Týřov for biodiversity protection is emphasized by the nine species described as new to science: *Acarospora fissa*, *Bacidia hyalina*, *Buellia microcarpa*, *Micarea substipitata*, *Microcalicium minutum*, *Rufoplaca griseomarginata*, *Verrucaria substerilis*, *V. tenuispora* and *V. teyrzowensis*. Three species are new to Europe, 55 to the Czech Republic and 191 species are included in the national Red-list.

Keywords: biodiversity hotspot, DNA barcoding, lichenized fungi, *Verrucaria*

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Introduction

Biodiversity inventories contain unique data on a diversity of various types of organisms and consequently can be used to identify and so help in the conservation of biodiversity hotspots (Zechmeister et al. 2003) and protection of rare species (Jetz et al. 2019). In the Czech Republic, hundreds of small nature reserves are being surveyed for various organisms including bryophytes, fungi and lichens, with the aim to record in detail the diversity of particular groups of organisms. A common practice is to visit a reserve a few times within a year or two, but this superficial approach easily overlooks any areas rich in small organisms with a sparse distribution and peculiar niches, such as lichens (Lõhmus et al. 2018, Vondrák et al. 2018).

In most central-European landscapes north of the Alps, superficial surveys are unlikely to distinguish between areas rich in species of lichens and impoverished ones, because in rich localities the additional species are often scarce. Only a small proportion of these additional species will be detected by a perfunctory survey. One may ask why localities in central Europe tend to have a higher ratio of sparsely occurring species than some other European areas. The main reason is that specific niches preferred by lichens, e.g. various types of rock or tree-related microhabitats, are rare and sparsely distributed in ancient central European landscapes that have been transformed to agricultural land, woodland plantations or urbanized sites. As a result, most habitats rich in lichens have only a limited number of microsites suitable for a great diversity of lichens (Fritz & Heilmann-Clausen 2010, Boch et al. 2016, Hofmeister et al. 2016). Another reason is that high air pollution in the past greatly reduced the populations of many species, especially epiphytic lichens, and any survivors became restricted to especially favourable microsites (Hauck et al. 2013, Pescott et al. 2015, Malíček et al. 2019). Also, small-sized localities of epilithic lichens became, due to an absence of traditional extensive farming, overgrown by shrub and forest vegetation (Nascimbene et al. 2013, Kubiak & Osyczka 2020). These processes resulted in lichen communities in these ancient landscapes being impoverished. Only a few sites, largely untouched by humans and mostly in the mountains, are known to have distinctly more species (Malíček & Palice 2013, Malíček et al. 2019). However, it is now apparent that hotspots of lichen diversity can still be found, even at low altitudes, within landscapes with a long tradition of human exploitation, but only if they are thoroughly searched. In this study, we report the lichen diversity in one such unexpectedly species-rich area and its linkage with habitat heterogeneity.

Materials and methods

Study area

We studied the Týřov National Nature Reserve (Czech Republic, about 40 km south-west of Prague), a 420-hectare spot of natural habitats, protected since 1933 to conserve rich biodiversity occurring there in streams, forests and on rocks. It is located in the Protected Landscape Area Křivokátsko (Fig. 1), which consists mainly of agricultural land and woodland plantations. Natural forests and rocky habitats are limited to the surroundings of solitary peaks and to steep slopes above deeply incised rivers and streams. Křivoklátsko is one of the warmer and drier parts in the Czech Republic, with annual precipitation about 530 mm and mean annual temperature 7–8 °C.

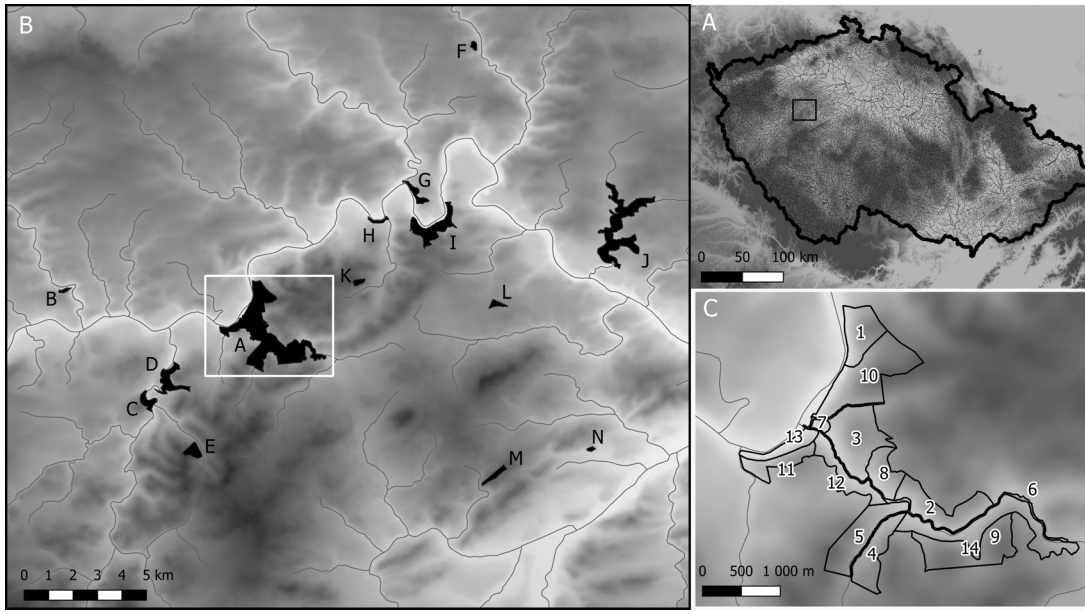


Fig. 1. Study area, the Týřov National Nature Reserve. (A) Location in the Czech Republic. (B) Locations of the study area (in white frame) and reference localities B–N in the region. (C) Division of the study area into 14 sampling units.

Predominant habitats in the study area are forests (~410 hectares), with patches of rocks and steppes (~10 hectares) in valleys of the streams Úpořský potok and Prostřední potok, and in an adjacent valley of the river Berounka, altitudes range from 250–520 m. Woodland vegetation is predominantly oak-hornbeam forests (of *Acer campestre*, *Carpinus betulus* and *Quercus petraea*, with less frequent *Sorbus torminalis*) but there are also scree forests rich in tree species (dominated by *Tilia cordata* / *T. platyphyllos* and *Fraxinus excelsior*, and with less frequent *Acer platanoides*, *A. pseudoplatanus*, *Fagus sylvatica,* and *Ulmus glabra*; *Taxus baccata* is locally frequent). Forest-steppes, frequently coverring upper parts of sun-exposed slopes, consist of sparse *Pinus sylvestris*, *Quercus petraea* or *Fraxinus excelsior* with an admixture of various shrub and tree species (e.g. *Crataegus* spp., *Prunus spinosa* and *Sorbus collina*). *Alnus glutinosa* stands are frequent in the bottoms of valleys. The bedrock is volcanic, formed of andesites, rhyolites and dacites (Mašek et al. 1997). Andesites, which form the major part of the local bedrock, are generally base-rich, occasionally with calcareous inclusions, but exposed surfaces are often base-poor. They weather easily and crumble into small pebbles. Rhyolites, restricted to the southernmost part, are always base-poor, without calcareous inclusions and weather slowly into large boulders. Dacites, forming rocks in the eastern part, have an intermediate character between andesites and rhyolites.

The landscape of Týřov is currently almost entirely forested, but was not so in the past. According to forest maps, the present age of most stands in the area ranges between 100 and 150 years, though some trees are undoubtedly much older. In some places, current stands are a first generation of forest after a period of deforestation, but not a tree-less landscape used for extensive grazing. The picture from 1822 shows only solitary trees and

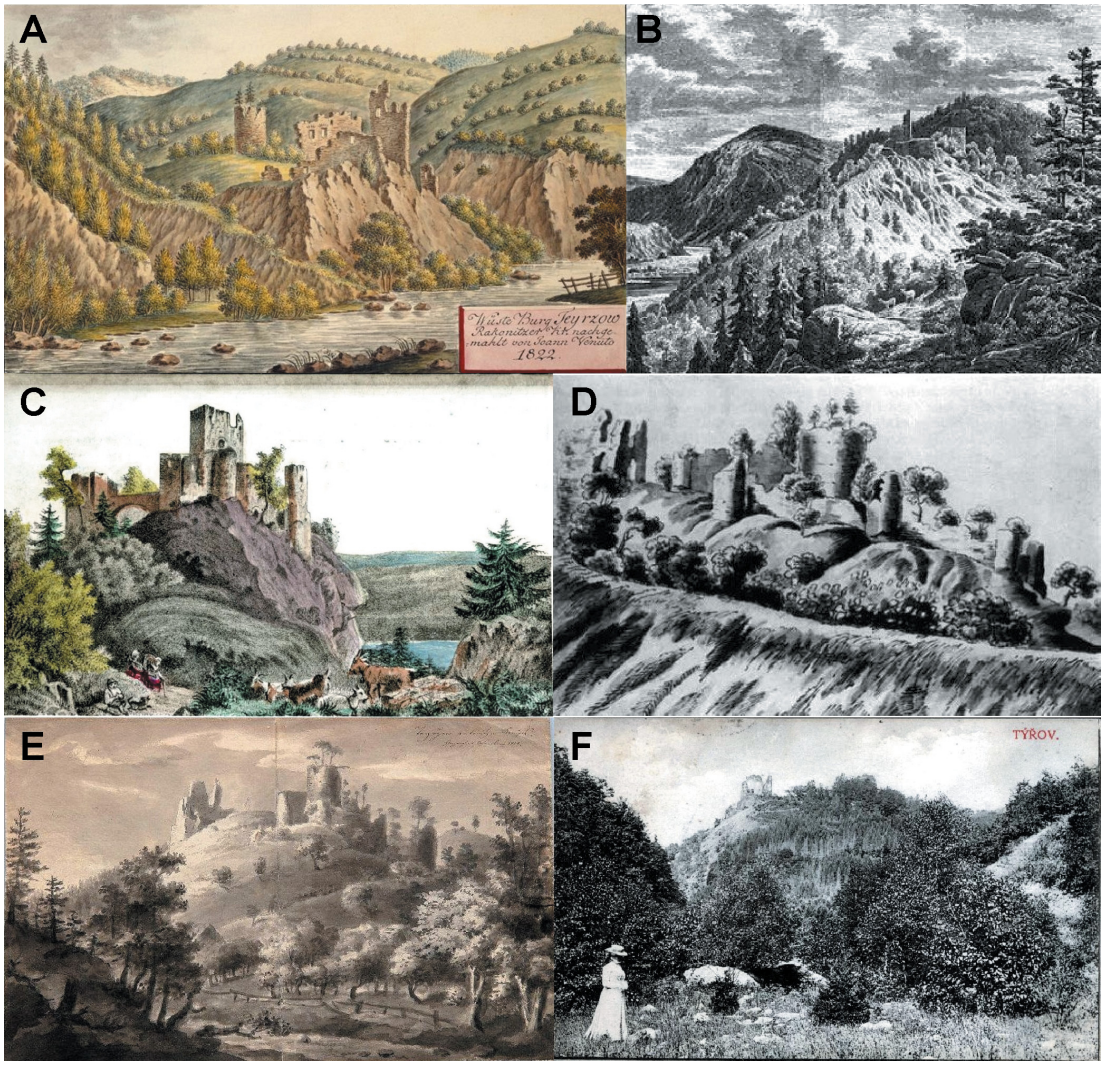


Fig. 2. Forestless landscapes at the ruin of Týřov in 18th, 19th and early 20th century. Most of these landscapes are covered by forest nowadays. (A) Forestless hills from the left: Na Budkách, Vápenný vrch, Vosník, painted by Johann Venuto, 1822. (B) The ruin viewed from the hill Vosník, anonymous author, 18th century. (C) Goats and herdsman on a pasture at ruin, postcard, 1901. (D) Almost treeless surroundings of the ruin, anonymous author, early 19th century. (E) Valley of the Úpořský potok stream, Josef Šembera, 1824. (F) An early stage of forest expansion in the valley of the Úpořský potok stream; postcard, 1914.

shrubs on the hills Vosník and Vápenný vrch, which are now covered by forest (Fig. 2A). The valley of Úpořský potok is similar; in the 19th century it was pasture with groups of shrubs and trees (Fig. 2E). The hill with the ruin of the castle of Týřov was still largely uncovered by forest at the beginning of the 20th century and served as goat pasture (Figs 2B–D). Initial phases of forest invasion in the valley and a young conifer plantation on the south-east slope of the castle hill are depicted on a postcard dated 1914 (Fig. 2F).

Division into sampling sites

Týřov was divided into fourteen sampling sites distributed fairly uniformly over the whole area (Fig. 1). The time spent surveying each locality was 20 hours on average, with some variation from site to site based on their lichenological interest. The sites and notes on their habitats are listed below. The areas cited are all approximate. The number of well-developed habitats (as well as the total number of habitats) is given for each site.

(1) Týřovické skály rocks (area: 25 ha; altitudes 250–450 m; available habitats: 14 (22); survey: 79 man-hours; species detected: 502). It has the best developed rocky habitats uncovered by forest within Týřov (Supplementary Fig. S1A). Rocky slopes, mainly facing west, formed of andesites with variable attributes. Some exposed rocks, obviously unweathered, are hard and acidic, without traces of base enrichment. In other places, andesites (or their tuffs) are soft, weathered and crumbling into gravel; such rocks are usually base-rich. Calcareous inclusions are frequent in lower parts of rocky outcrops and seepage water from these inclusions has sometimes calcified large surfaces of the volcanic rocks (Supplementary Fig. S1B). Less exposed rocky surfaces are covered by forest-steppe with predominant oak and ash trees. Decorticated logs of oaks are rather frequent in forest-steppes and their wood resists rotting and persists in a hard state for a long time. Oak-hornbeam and scree forests only occur at the margins of this locality.

(2) Southern rocky slopes above the stream Úpořský potok, below the hill Průhonek (area: 25 ha; altitudes 300–400 m; available habitats: 12 (21); survey: 40 man-hours; species detected: 337). The area is mainly covered by oak-hornbeam and scree forests along a strong gradient of humidity, from damp forests at the base of the slope to dry stands of shrubs or forest-steppes with mainly oak, ash and pine on the upper parts of the slopes and in rocky stands. Sun-exposed rocks and tree-less steppes are present in a few places. Rocks are formed of andesite, which is partly base-rich, with a few calcareous inclusions.

(3) South-western rocky slopes above the stream Úpořský potok, below the hill Vápenný vrch (area: 30 ha; altitudes 270–420 m; available habitats: 13 (18); survey: 31 man-hours; species detected: 340). Largely covered by forest-steppe with oak, ash and pine. Damp scree forests occur on the lower parts of the slopes, and drier oak-hornbeam forests occur localy, mostly at the margins. Rocky outcrops are frequent and well developed, formed of andesite with numerous base-rich microhabitats and some calcareous inclusions.

(4) Western slope above Prostřední potok (area: 20 ha; altitudes 280–430 m; available habitats: 8 (11); survey: 18 man-hours; species detected: 202). The area has a colder and more humid mesoclimate than other sites and harbours a distinct lichen flora, not observed elsewhere in Týřov. Rhyolite screes and rocky outcrops are partly covered by sparse pine forest or by solitary pine trees. Fir used to be a common tree on this slope, but currently only rather young trees remain, with a lot of decaying fir wood (stumps and logs) with a locally unique lichen biota.

(5) Eastern slope above Prostřední potok (area: 30 ha; altitudes 280–500 m; available habitats: 4 (8); survey: 10 man-hours; species detected: 188). Mostly covered by oak-hornbeam and scree forests, partly by forest-steppes and tree-less rocky habitats; screes occur in a few places. Bedrock is an acid rhyolite.

(6) Valley bottoms of the streams Úpořský potok and Prostřední potok (area: 25 ha; altitudes 250–370 m; available habitats: 4 (9); survey: 23 man-hours; species detected:

198). Damp stones in and around streams are a characteristic habitat, together with damp forests dominated by *Alnus glutinosa* and locally by *Carpinus betulus*, *Acer* spp. and *Fagus sylvatica*.

(7) The hill with the ruin of the castle of Týřov (area: 2 ha; altitudes 260–320 m; available habitats: 2 (10); survey: 12 man-hours; species detected: 211). A single area with an anthropogenic habitat represented by the ruined walls of the castle. Tree-less andesite rocky slopes on the south slope of the hill are the most xerothermic site in the whole area and have some thermophilous lichens. The andesite rocks are locally enriched by lime from concrete in the ruined walls. The northern and eastern slopes are covered by scree forest.

(8) South-western rocky slopes above Úpořský potok, below Průhonek (area: 17 ha; altitudes 280–400 m; available habitats: 7 (16); survey: 10 man-hours; species detected: 207). Covered by oak-hornbeam forest, or by shrubs or forest-steppe in more sun-exposed rocky sites. Rocky steppes occur in places. Andesite outcrops are locally base-rich, but calcareous inclusions are very rare.

(9) The rocky hills U Tří skalek and Tok (area: 40 ha; altitudes 380–490 m; available habitats: 3 (8); survey: 14 man-hours; species detected: 161). Mostly covered by woodlands including conifer plantations, oak-hornbeam and beech forests. Tree-less sites with rhyolite outcrops are well developed in two places. The north slope of Tok has a few open rhyolite screes with unique communities of montane lichens.

(10) Rocky ridge and slopes of the hill Vysoký vrch (area: 60 ha; altitudes 250–520 m; available habitats: 2 (10); survey: 7 man-hours; species detected: 219). The area includes slopes on both sides of the SW–NE oriented ridge. The north-west slope is covered mainly by scree forests, but forest plantations predominate in the south-east. Forest steppes with oak, pine and ash trees are present in places on the ridge and the south-east slopes. Tree-less rocky steppes occur on a few small andesite outcrops, which are locally base-rich to calcareous.

(11) Upper part of the north-western slope of the hill Vosník (area: 14 ha; altitudes 300–420 m; available habitats: 2 (9); survey: 7 man-hours; species detected: 147). Covered by oak-hornbeam and scree forests with frequent *Taxus baccata*. Andesitic, partly lime-enriched rocky outcrops are mostly shaded by trees. Only a few acidic outcrops at the top of the hill are more directly exposed to sun.

(12) North-eastern slope above Úpořský potok (area: 15 ha; altitudes 270–350 m; available habitats: 4 (8); survey: 9 man-hours; species detected: 129). Mostly covered by damp scree forest. Andesitic outcrops are largely in shade, below trees, but two rocks rise above the tree canopy. Base-rich and lime enriched rocks are restricted to a few spots.

(13) Lower part of the north-western slope of Vosník (area: 7 ha; altitudes 250–300 m; available habitats: 2 (6); survey: 10 man-hours; species detected: 135). Currently covered by scree forest with yew more than 100 years old (Supplementary Fig. S1C), but in the past largely deforested and much eroded (Fig. 2A). The frequent andesite outcrops are usually base-rich and locally calcareous. They are mostly shaded, but some sites by the river Berounka are partly directly exposed to sun light and have distinct lichen communities.

(14) Northern slope above Úpořský potok (area: 47 ha; altitudes 270–350 m; available habitats: 3 (7); survey: 10 man-hours; species detected: 122). Mostly covered by damp scree forest. Only small rocky outcrops, shaded by trees, are present.

Lichen biodiversity survey

Most of the fieldwork, which consisted of 48 one-man one-day visits, was done in the years 2018–2021 (Vondrák 33 visits, Malíček 8 and Palice 7). A total of 318 hours was spent in the field. Supplementary Table S1 lists the details. Important additional floristic data, especially of lichenicolous fungi, was provided by J. Kocourková based on visits in the years 1996–2005. Further additional data come from visits by Palice and Vondrák in 2005, Šoun in 2020 and Thüs in 2021.

We aimed to cover all microhabitats and substrates, to maximize detection of species. We recorded species that are clearly lichenized, and also fungi that appear to have a loose association with algae, though they lack a typical algal layer (we call these fungi "semilichens"). Genera with semilichens include: *Anisomeridium*, *Arthonia*, *Arthopyrenia*, *Chaenothecopsis*, *Cyrtidula*, *Eopyrenula*, *Epigloea*, *Julella*, *Leptorhaphis*, *Lichenothelia*, *Melaspilea*, *Microcalicium*, *Mycocalicium*, *Stenocybe*, *Strigula* and *Thelocarpon*. We also recorded bark microfungi that are clearly not lichenized, such as *Amphisphaeria*, *Kirschsteiniothelia*, *Peridiothelia*, *Rebentischia*, *Requienella* and *Strickeria*, and lichenicolous fungi. The catalogue of all the species recorded is attached in Supplementary Data S1 and data for all 6526 records are in Supplementary Table S2.

Some species were identified based only on their morphology. Numerous others were identified, or their identification was confirmed using data from thin layer chromatography (henceforth abbreviated to TLC; done for 81 species) or DNA sequences. Voucher specimens for records of Palice and Vondrák are deposited in PRA (herbarium of the Institute of Botany of the The Czech Academy of Sciences). Specimens recorded by Kocourková, Malíček and Šoun are in their personal herbaria; a few specimens are in the public herbaria PRM and STU. Detailed data for all records of lichens and semilichens are deposited in the national database of lichens and bryophytes (dalibor.ibot.cas.cz) and are publicly available online in the Atlas of Czech lichens (https://dalib.cz; Malíček et al. 2021). Lichen nomenclature used here follows the current version of that Atlas. (The records in the Atlas are indexed by today's names, but in the future, they may also be indexed under other names.)

Sequencing for barcoding purposes

DNA was extracted using a cetyltrimethylammonium bromide (CTAB)-based protocol (Aras & Cansaran 2006). Two DNA loci were amplified: mitochondrial small subunit ribosomal DNA (mtSSU in further text), and internal transcribed spacer (ITS) region of nuclear ribosomal DNA (ITS in further text). For DNA barcoding purposes, we generated the 297 ITS and 262 mtSSU sequences listed in Supplementary Table S3.

Polymerase chain reactions were performed in a reaction mixture containing 2.5 mmol/l MgCl2, 0.2 mmol/l of each dNTP, 0.3 µmol/l of each primer, 0.5 U Taq polymerase (Top-Bio, Praha, Czech Republic) in the manufacturer's reaction buffer, and sterile water to make up a final volume of 10 µl. The primers and the cycling conditions are summarized in Supplementary Table S4. Successful amplifications were sent for Sanger sequencing (GATC Biotech, Konstanz, Germany). Sequences were edited using BioEdit v.7.0.9.0 (Hall 1999).

ITS trees in Verrucariaceae

ITS sequences of two poorly known groups of *Verrucariaceae* were aligned by MAFFT v.7 (Katoh & Standley 2013; available online at http://mafft.cbrc.jp/alignment/server) using the Q-INS-i algorithm with the gap opening penalty set to 1. Ambiguously aligned positions at the beginning of ITS1 region were excluded and gaps coded as missing data. The best-fit model of sequence evolution was selected using the Akaike information criterion calculated in jModelTest v.0.1.1 (Posada 2008). Phylogenetic relationships were assessed using Bayesian inference as implemented in MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). Two runs starting with a random tree and employing four simultaneous chains each (one hot, three cold) were executed. The temperature of a hot chain was set empirically to 0.05, and every 100th tree was saved. The analysis was considered to be completed when the average standard deviation of split frequencies dropped below 0.01. The first 25% of trees were discarded as the burn-in phase and the remaining trees were used for construction of a 50% majority consensus tree.

Catalogue of habitats and habitat heterogeneity

We put together a catalogue of regionally available habitats for lichens (Supplementary Data S2). It consists of four sections reflecting substrate preferences: habitats for (i) saxicolous, (ii) terricolous, (iii) epiphytic and (iv) lignicolous lichens.

The classification of habitats for saxicolous lichens is based on the availability of carbonates and other minerals (acidic siliceous, base-rich siliceous, calcareous), availability of light (exposed to sun light, shaded), availability of water (e.g. seapage rocks, stones in streams, river side rocks, vs. overhanging rocks or air-dried rocks), extrinsic nutrient enrichment (bird perches) and size (rocks, stones, pebbles).

Habitats for terricolous lichens are classified according to the predominant lichen growth form (*Cladonia* carpets vs. other types) and according to presumed availability of carbonates and light. Habitats for epiphytic and lignicolous lichens are classified according to the light and humidity conditions and predominant tree species. Habitats with a negligible contribution to lichen biodiversity in the area studied, such as conifer plantations, are not included.

Based on the catalogue of regionally available habitats, we calculated a value of habitat heterogeneity for the sites sampled in order to compare them with reference localities (Fig. 2B). Our measure of habitat heterogeneity is based on pooling the numbers of well developed habitats and poorly developed (or poorly inhabited) habitats divided by two. The spatial aspect and grain size of habitat heterogeneity are not included in this measure.

Regional context of lichen species richness

The relationship between numbers of species present and the area and heterogeneity of the habitats at Týřov were compared with that at 13 other reserves within 20 kilometers of Týřov (Fig. 2B). For this, we used lichen biodiversity inventories that were compiled in 2010–2020 using similar methods to our study. The results of these other inventories are either published or held, as manuscripts, by the Nature Conservation Agency of the Czech Republic.

Results

Lichen biodiversity survey

We detected a total of 787 species in the 48 one-day, one-man field trips (Supplementary Data S1 – Catalogue of all recognized taxa). The maximum number of species recorded in a single day was 235; on another three days we recorded more than 200 species. About one third of the total richness (280 species) was detected in the first three visits. Visits 4–25 produced an average increase of 15.4 species per field trip and visits 26–48 produced an average of seven species (Fig. 3).

The species list includes 675 lichens (88% of total), 35 semilichens (see above definition), 58 non-lichenized lichenicolous fungi and 19 non-lichenized bark microfungi occurring in lichen microhabitats. Nine species are described as new to science: *Acarospora fissa*, *Bacidia hyalina*, *Buellia microcarpa*, *Micarea substipitata*, *Microcalicium minutum*, *Rufoplaca griseomarginata*, *Verrucaria substerilis*, *V. tenuispora* and *V. teyrzowensis*.

Three species are new to Europe: *Lecidea fuscoatrina*, *Leprocaulon nicholsiae* and *Lichenothelia papilliformis*. Fifty-five are new to the Czech Republic: *Arthonia thoriana*,

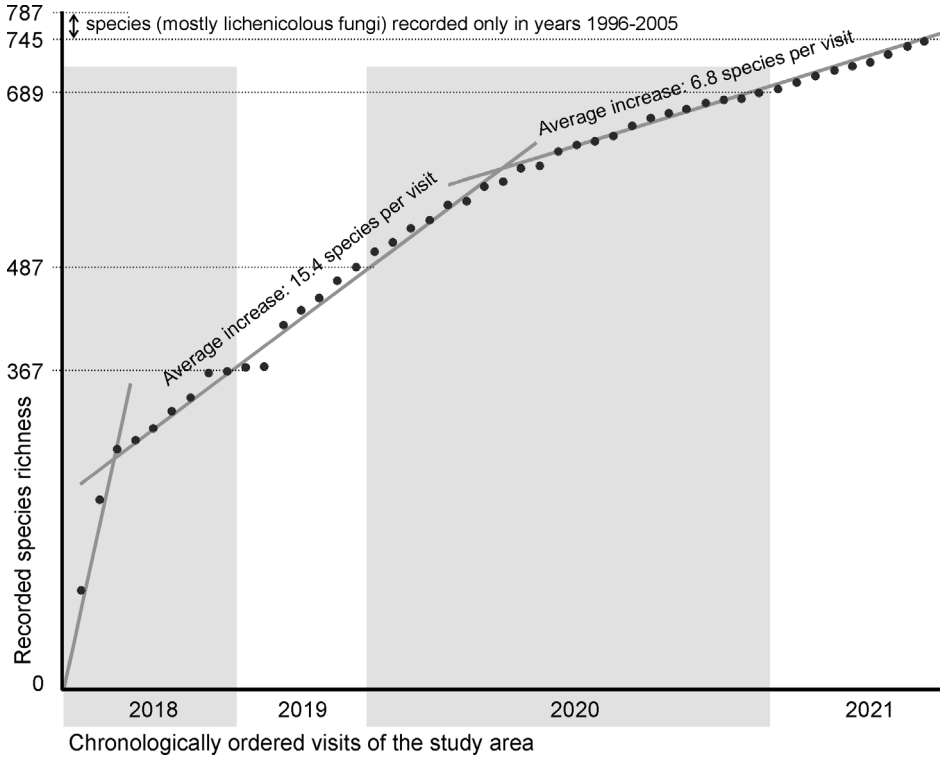


Fig. 3. Cumulative counts of detected species during the 48 visits of the study area.

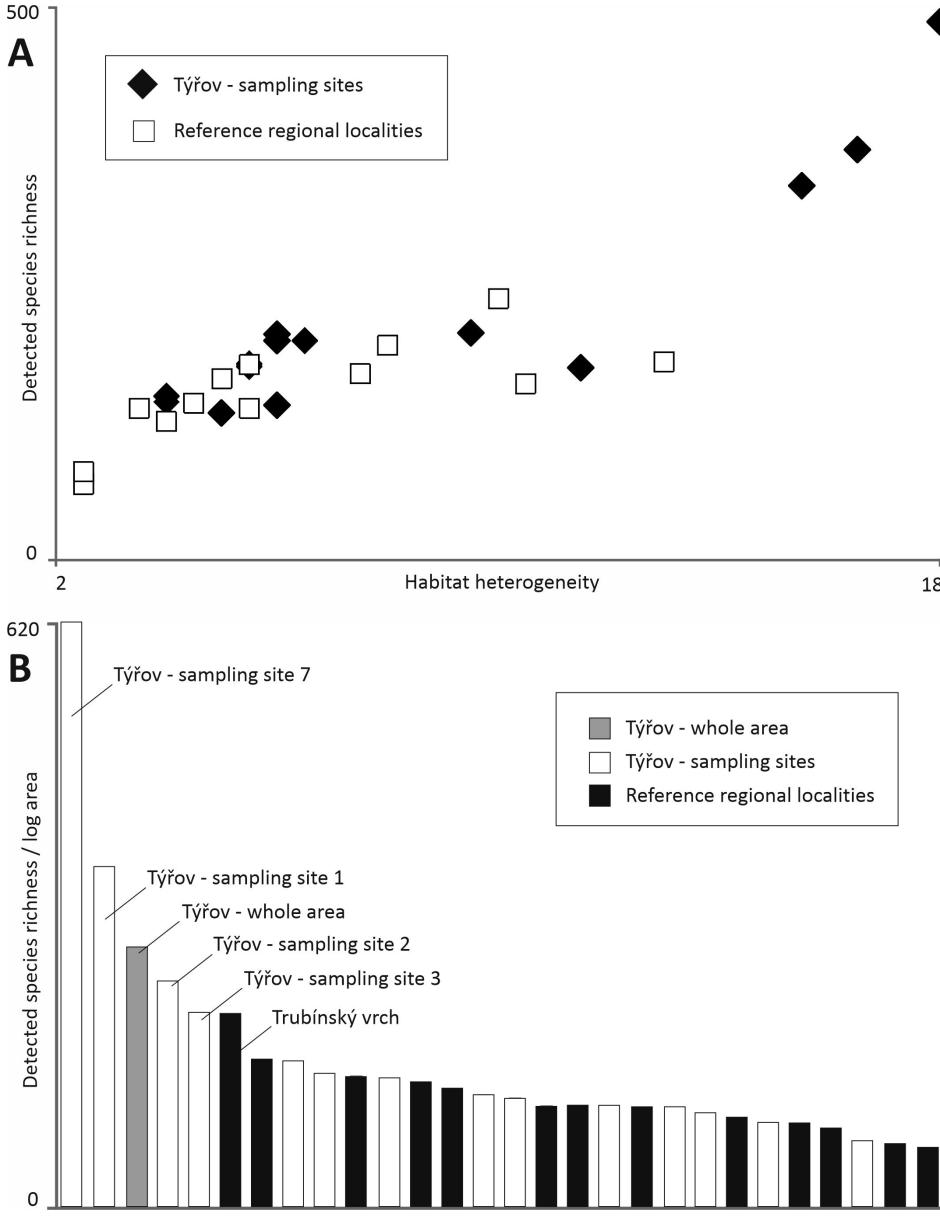


Fig. 4. Species richness in the study area and its sampling sites in context of regional reference localities (see Fig. 1B and Tables 1, 2 for details). (A) Relationship between species richness and habitat heterogeneity, calculated as a count of well developed habitats, plus a count of poorly developed habitats divided by two (half-weighted). (B) Species richness per area (log) in the study area and its sites and in reference localities.

Table 1. Habitats detected in sampling units of the study area and in reference localities. * well developed; (*) poorly developed habitats. Habitats are described in the Supplementary Data S2.

Habitats	Sampling units of the study area (A on Fig. 1B)															Reference localities (B–N on Fig. 1B)												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		B	C	D	E	F	G	H	I	J	K	L	M	N
For epilithic lichens:																												
1. Pebbles and stones in forest	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)	(*)		(*)		(*)	(*)	(*)	(*)		(*)	(*)	(*)			
2. Acidic rock outcrops in shade	*	*	*	*	*	*	*	(*)	(*)	(*)	(*)	*	(*)				*	*			*	*	*	*	(*)		*	
3. Sun-exposed acidic rocks	*	*	*	*	(*)		(*)	*	*	*	(*)	*					*	*			*	*	*	*	*		*	*
4. Base-rich outcrops in shade	*	(*)	(*)			(*)		(*)		(*)	(*)	(*)	(*)								*	*	(*)	(*)				(*)
5. Sun-lit base-rich rocks	*	(*)	*				*			(*)											*			(*)				*
6. Pebbles and stones in rocky steppes and dry screes	*	*	*				(*)	(*)									*	*			*	(*)	*	(*)				*
7. Calcareous inclusions in andesite outcrops	*	(*)	*					(*)			(*)										(*)							
8. Lime enriched seapage rocks with cyanolichens	*	(*)	(*)																									
9. Boulders in damp screes	(*)			*	(*)				*									*			(*)							
10. Extremely exposed hard rocks	*		(*)																				(*)				(*)	
11. Nutrient-rich outcrops at river bank	*												*															
12. Stones and concrete in ruin wall								*																(*)				
13. Stones in streams						*												(*)						*				
For terricolous lichens:																												
1. Bryophytes and plant debris in lime enriched sites	*	(*)	*				(*)	(*)		(*)											(*)							
2. Soil and bryophytes on rocky steppes	*	*	*					(*)									*	*			*		*	(*)				*
3. *Cladonia* carpets on acid soils	(*)	*		*					(*)									(*)			(*)	(*)		(*)	(*)		(*)	
4. Soil patches in damp sites	(*)			(*)					(*)										(*)	(*)	(*)							
For epiphytic lichens:																												
1. Hornbeam stands	(*)	*	*		*	*	(*)	*				(*)		(*)		(*)	(*)	(*)		*	(*)		(*)	*	(*)	(*)		
2. Damp scree forests	(*)	*	*			(*)	(*)			(*)	(*)	(*)	(*)	*		(*)	*	(*)	*	*	(*)	(*)	(*)	(*)				
3. Sparse oak forests and forest-steppes	*	*	*	(*)	*			*		(*)	(*)					(*)	(*)	*	(*)				(*)	*	*	*	(*)	
4. Haw and blackthorn shrubs		*					(*)	*				*		*			(*)	(*)			*		(*)					*
5. Hazel stands		(*)	*			(*)		(*)						(*)				(*)			(*)							
6. Pine stands	(*)	(*)		*						(*)	(*)										(*)							
7. Sparse ash stands on nutrient-rich sites on rocky slopes	*	*	*					*																				
8. Beech stands		(*)				(*)								(*)					*									
For lignicolous lichens:																												
1. Rapidly decaying dead wood	(*)	*	(*)	*	(*)	*	(*)	(*)	*			*	*	*		(*)			*	(*)						(*)		
2. Wood resisting decay in dry and lit sites	*	*	*	*	*			*	(*)	*	*						*	*	*	(*)	*		(*)	*	*	(*)		
3. Dead wood in remnants of fir-pine stands				*																								
Number of well developed habitatas	8	9	5	3	4	5	8	9	5	8	8	4	4	4		5	3	7	3	4	10	3	8	8	4	3	3	1
Number of poorly developed habitatas	14	12	13	8	4	4	2	7	3	2	1	4	2	3		0	6	7	4	2	8	3	4	6	3	1	2	5
Total number of available habitats	22	21	18	11	8	9	10	16	8	10	9	8	6	7		5	9	14	7	6	18	6	12	14	7	4	5	6

Arthopyrenia fallaciosa, *Arthopyrenia inconspicua* auct., *Aspicilia brucei*, *Bertia gigantospora*, *Buellia ocellata*, *B. sandstedei*, *Byssoloma diederichii*, *Cheiromycella microscopica*, *Cyrtidula quercus*, *Endococcus brachysporus*, *E. karlstadtensis*, *Eopyrenula avellanae*, *Gonohymenia schleicheri*, *Hyalotrochophora lignatilis*, *Immersaria cupreoatra*, *Inoderma sorediatum*, *Ionaspis obtecta*, *Lecanora subravida*, *Lecidea plebeja*, *Lepraria humida*, *Lichenostigma chlaroterae*, *Lichinella myriospora*, *Lithothelium septem-septatum*, *Micarea coppinsii*, *M. herbarum*, *M. microsorediata*, *Muellerella polyspora*, *Nectriopsis micareae*, *Paranectria oropensis*, *Peltigera neocanina* ined., *Pertusaria stalactiza*, *Placopyrenium cinereoatratum*, *Porocyphus rehmicus*, *Porpidia contraponenda*, *Protoparmelia oleagina*, *Reichlingia zwackhii*, *Requienella fraxini*, *Rhizocarpon fratricida*, *R. geminatum* var. *citrinum*, *R. rubescens*, *Rinodina obnascens*, *R. poeltiana*, *Sarea coeloplata*, *Spirographa ciliata*, *Stigmidium lichenum*, *Strigula taylori*, *Tremella candelariellae*, *T. diploschistina*, *T. wirthii*, *Verrucaria devensis*, *V. elaeina*, *V. tabacina*, *Waynea giraltiae* and *Xanthoparmelia plittii*. Five species are rediscovered in the Czech Republic after more than 80 years: *Arthopyrenia fraxini*, *Callome multipartita*, *Dermatocarpon meiophyllizum*, *Ramalina obtusata* and *Rinodina archaea*. 44 tentative taxa are classified as "known unknowns", i.e. taxa that are well defined but did not match any species known to us. Three of the "known unknowns" belong in *Bacidina* and 22 in *Verrucaria*.

Other remarkable records are e.g. *Caloplaca ulcerosa*, *Calicium abietinum*, *C. montanum*, *Gyalecta* (five species), *Gyalideopsis helvetica*, *Hertelidea botryosa*, *Lecania suavis*, *Lecanora impudens*, *Micarea tomentosa*, *Miriquidica intrudens*, *Pleopsidium flavum*, *Pterygiopsis neglecta*, *P. umbilicata*, *Rinodina moziana*, *R. fimbriata*, *Rostania occultata*, *Stigmidium rivulorum* and *Xanthoria sorediata*.

An astonishing number of red-list lichen species (cf. Liška et al. 2008) was recorded: six regionally extinct, 25 critically endangered, 56 endangered and 104 vulnerable. Species classified as data deficient are also numerous (185 species).

Epiphytic (including lignicolous) and saxicolous species were about equally frequent; 362 and 386 species, respectively. Fewer species were recorded on soil and humus (72). We obtained the high total number of epiphytic lichens even though most trees are poor in epiphytes. It is a result of (i) the affinity of rare species for some exceptional trees, with specific microhabitats and microclimate, sparsely scattered in the area studied and (ii) the high species turnover among these trees.

Macrolichens, with foliose (91 species) and fruticose (54) thalli, represent ~20% of the local species richness; microlichens (i.e. crustose lichens and semilichens) predominate.

Species richness at the sites sampled

The numbers of species detected at each of the above 14 sites varied considerably, which indicates that lichen diversity is not spatially uniform at Týřov. The richest site (Týřovické skály rocks, with 502 species in 25 hectares) has more than four times as many species as the poorest, site 14 with 122 species. Note, however, that even the richest site includes fewer than two thirds of all the species detected.

All the sites sampled, even the least species rich, contributed to the total biodiversity with at least one species that was not detected elsewhere in the reserve. About one third of species (250) were recorded at only a single site, and each site sampled had from 1 to 104 species recorded only at that site. At each site the number of species not recorded else-

where is correlated with the total number of species at that site. The richest site, with 104 species found only at that site, stands out. None of the others had more than 27 such species.

Species richness correlates with habitat heterogeneity

Lichen species richness increased with habitat heterogeneity at the sites sampled (Fig. 4A). The three most species rich sites also have distinctly more habitats than the others. Moreover, some habitats, e.g. lime enriched seepage rocks, only occur at these sites (Table 1). The three most species-rich sites sampled, covering 20% of the reserve and with 337–502 species, are outstanding hotspots in a regional context and on average have distinctly more habitats. At the other 11 sites, both species richness (122–219 species) and habitat heterogeneity are comparable with those recorded in thirteen nearby reserves (with 62–213 species) (Fig. 4A).

Above-average species richness per area in a regional context

Four of the sites sampled have distinctly higher species richness per area than the protected areas surveyed in the same region (Fig. 4B). Site 7, the ruin of castle Týřov and surrounding slopes, with its 211 species on a mere 2 hectares, is the most species rich site per area, but its position in first place may be an artefact arising from its very small area. It is followed by the three most species-rich but larger sites and by the whole area of Týřov (Fig. 4B). The remaining sites with fewer species have species richness per area comparable with protected areas surveyed in the same region (reference localities B–N in Fig. 1B and Table 2).

Record frequencies in the area studied

The 787 species detected were from a total of 6526 records. More than a quarter of the species, i.e. 225, have only a single record. About half, i.e. 384 species, have up to three records and most of them are considered exceptionally rare in the area, though in a few cases this may be an artefact of under-recording (e.g. the most inconspicuous lichens and lichenicolous fungi). 233 species have between four and 10 records and only 170 have more than 10 records.

Epiphytic cyanolichens

Cyanolichens are uncommon in most Czech landscapes, and Týřov, with 42 species, is undoubtedly the richest locality known in the Czech Republic. The richness of epiphytic cyanolichens is even more outstanding because, with the exception of *Peltigera praetextata*, they have not been detected in the vast majority of the localities surveyed within Czechia. In Týřov, we recorded four predominantly epiphytic cyanolichens (*Scytinium lichenoides*, *S. subtile*, *S. teretiusculum* and *Rostania occultata*) and a few generally saxicolous or terricolous species on bark (e.g. *Collema flaccidum*, *Scytinium magnussonii*, *S. pulvinatum* and *Peltigera* spp.). They form communities especially on trunk bases and exposed roots of ash trees, where several cyanolichens occur together.

Table 2. List of reference localities, ordered according to decreased detected species richness. Some data are not published, but deposited in the archive of the Nature Conservation Agency of the Czech Republic (AOPK).

Locality	Number of species	Area (km^2)	Reference
Regional level (indexed according to Fig. 1B)			
(G) Stříbrný luh	213	1.50	Vondrák 2020 (AOPK)
(I) Na Babě	175	0.24	Malíček & Kocourková (2014)
(J) Vůznice	162	2.31	Halda 2012 (AOPK)
(E) Kohoutov	159	0.29	Malíček (2020)
(C) Lípa	152	0.26	Šoun 2020 (AOPK)
(K) Vysoký tok	148	0.09	Kocourková 2018 (AOPK)
(D) Jezírka	144	0.59	Šoun 2019 (AOPK)
(H) U Eremita	128	0.08	Kocourková 2018 (AOPK)
(M) Vraní skála	124	0.21	Malíček & Kocourková (2014)
(N) Trubínský vrch	124	0.04	Lenzová & Svoboda (2015)
(F) Svatá Alžběta	113	0.07	Kocourková 2018 (AOPK)
(L) Červený kříž	72	0.13	Kocourková 2018 (AOPK)
(B) Dubensko	62	0.05	Šoun 2018 (AOPK)
Country-wide level (inventories including >200 species)			
Černé a Čertovo jezero	450	2.80	Palice (unpublished estimation)
Kar Plešného jezera	400	0.78	Palice (unpublished estimation)
Boubín	356	6.86	Vondrák et al. 2020 (AOPK)
Cigánka (Slovakia)	338	44.00	Guttová & Palice (2005)
Ralsko & Vranovské skály	298	0.36	Malíček & Vondrák (2018b)
Mohelenská hadcová step	272	1.90	Malíček et al. (2017)
Čertova stráň	250	0.47	Vondrák et al. 2020 (AOPK)
Bílá strž	250	0.76	Palice 2021 (AOPK)
Karlštejn	246	15.47	Svoboda 2013 (AOPK)
Děvín-Kotel-Soutěska & Tabulová	245	4.90	Malíček & Vondrák (2018a)
Vývěry Punkvy	242	5.50	Kocourková 2006 (AOPK)
Velká kotlina	236	28.00	Halda (2017)
Vyšenské kopce	207	0.55	Vondrák (2006)
Kleť	204	0.65	Vondrák et al. 2019 (AOPK)
European level (inventories including >500 species)			
Mercantour - Roya-Bévéra (France)	1018	>100	Roux C. et al. (2012)
Bödmeren Forest-Silberen (Switzerland)	1000	24	Groner (2016, 2020)
Koralpe (Austria)	910	>100	Hafellner (2008)
Mercantour - Haut-Var (France)	815	312	Roux C. et al. (2013)
Mercantour - Haute-Ubaye (France)	805	>100	Roux C. et al. (2011a)
Stubalpe (Austria)	739	445	Hafellner & Obermayer (2007)
Mercantour - Haute-Vésubie (France)	720	>100	Roux C. et al. (2015)
Mercantour - Haute- et de Moyenne-Tinée (France)	709	>100	Roux C. et al. (2014)
Mercantour - Haut-Verdon (France)	661	>100	Roux C. et al. (2011b)
Hochschwab-Massiv (Austria)	640	750	Hafellner et al. (2005)
Cévennes (France)	540	937	Roux C. et al. (2007)
Venezia Giulia (Italy)	527	212	Nimis & Martellos (2020)
Muniellos Preserve (Spain)	502	59.7	Pérez-Ortega (2004)

Atypical substrates

Numerous species were recorded on atypical substrates. Some usually saxicolous species were occasionally recorded on tree bark, e.g. on roots and trunk bases of old trees, e.g. *Aquacidia trachona*, *Chrysothrix chlorina*, *Lecanora orosthea*, *Lepraria borealis*, *L. caesioalba*, *Leprocaulon nicholsiae*, *Physcia dimidiata*, *Rinodina oxydata* and *Xanthoparmelia* spp. Some usually epiphytic species were occasionally recorded on rock, e.g. *Anisomeridium polypori*, *Bacidia rubella*, *Catillaria nigroclavata*, *Fellhanera bouteillei*, *F. subtilis*, *F. viridisorediata*, *Fuscidea pusilla*, *Halecania viridescens*, *Hyperphyscia adglutinata*, *Hypotrachyna revoluta*, *Imshaugia aleurites*, *Lecania cyrtella*, *L. naegelii*, *Micarea coppinsii*, *Opegrapha niveoatra*, *Phlyctis argena*, *Physconia perisidiosa*, *Strigula jamesii* and *S. taylori*. The rich, but strictly saxicolous *Caloplaca ulcerosa* is perhaps the most remarkable; this species is locally a common epiphytic lichen in coastal areas of Europe, with previously only a single known saxicolous occurrence inland in Europe (Vondrák et al. 2009).

Beneficial effect of DNA-barcoding (particularly for Verrucaria species)

A substantial part of the species richness would have remained undiscovered if DNA-barcoding had not been used. For example, the sterile sorediate crust, *Leprocaulon nicholsiae*, previously known only from North America, would have been identified as one of the European crusts with the same secondary metabolites, e.g. *Lecanora orosthea*. The same is true for numerous sterile crusts, some of them new to the Czech Republic (*Aspicilia brucei* and *Lepraria humida*).

We found DNA-barcoding especially beneficial in taxon identification within *Verrucaria*, a species-rich genus, which contains many species with poorly understood delimitations. ITS (and partly mtSSU) sequences supported delimitation of three *Verrucaria* species new to science and 22 taxa for which no published name could be found (known unknowns) in addition to 24 named species. These numbers are remarkably high for a central European site not in the Alps, which indicates that numbers of known species in *Verrucaria* are likely to continue to rise substantially, even in lichenologically well surveyed areas.

Discussion

Common and rare species: distinct components of biodiversity

Most lichen inventories include all the common species, but rather few of the rare ones (Lõhmus et al. 2018, Vondrák et al. 2018). However, detection of regionally common species has little significance, because they are expected, with a probability close to 100%, to be present at a site even before the survey starts. For example, the same 100 epiphytic species known to be common in the Šumava mountains, Czech Republic (Vondrák & Kubásek 2019) would certainly be recorded in most sites in the region. Similarly, 160 species are present on the species lists of all the sites sampled with suitable habitats at Týřov. Moreover, these species will probably be present and easily recorded at numerous suitable sites in a broad surrounding area (Křivoklátsko in this case).

Rare species carry much more information, and differences in their number determine which sites are valuable biodiversity hotspots and refuges for endangered and specialized lichens. Unfortunately, most inventories reported in the Czech Republic, and perhaps elsewhere, have been compiled too quickly and not thorough enough for a reasonable assessment. They usually involve few visits and are terminated long before species saturation. The previous survey at Týřov is an example (Bouda 2012). The species list, based on three one-day visits, contained 150 species, mostly common ones. On the basis of this result, the assessment of Týřov as a lichenological locality would be "species-poor". Our survey increased the number of species by more than a factor of five, which makes Týřov exceptionally species-rich. Such large and rather alarming differences in the assessments of a single locality warn us against drawing conclusions from hasty surveys and their often very incomplete species lists.

Recognition of species-rich localities

The number of species detected is thus a misleading indicator for the recognition of biodiversity hotspots, owing to the incomplete detection of rare species. Complete species lists of lichens are achievable for only very small sites, such as a part of a tree branch or plots of 0.01–0.1 m^2 on tree bark (McCune & Lesica 1992). Even lists for such small areas may not be complete, as diaspores and initial stages of thalli will be overlooked (Keepers et al. 2019). The problem increases with increasing area: it would be very difficult to obtain a complete species list even from a single tree or a single rock outcrop and almost impossible at larger scales (Vondrák et al. 2016).

When comparing species lists from two areas, we must be aware that both lists are incomplete and that the extent of imperfect detection probably differs, perhaps greatly. The quality of a species list is strongly influenced by the abilities of the surveyors and lists prepared by several experts working in parallel are distinctly longer than those made by a single researcher (Vondrák et al. 2016, 2018). Species lists are important and are often all that is available for a site, but we conclude that they may be a misleading tool for appraising local species richness unless supported by further information. The most important additional information is a cumulative species curve, and habitat heterogeneity assessment. This additional information, together with a good survey, will usually permit the reliable identification of biodiversity hotspots.

(i) Cumulative species curves: The idea is that common species are recorded during the first few visits, but rare species randomly during initial and later visits. The number of species recorded during the first visit to a site will be high and will gradually decrease with subsequent visits. Eventually each visit reveals few or no new species, i.e. the cumulative species curve reaches a plateau. The number of visits required to reach the plateau depends on the number of rare species present, which in turn depends on whether the locality is exceptionally species rich (i.e. a biodiversity hotspot) or not. The curve for Týřov has not reached a plateau after 48 one-man one-day visits, or come even close to doing so (Fig. 3). An exhaustive survey of such a locality might take years and then the final results might be influenced by temporal species turnover. We presume that accumulation curves from less diverse central-European areas of comparable size (1–10 km^2) would reach a plateau at much lower numbers of species and after far fewer visits.

(ii) Habitat heterogeneity: (See the Methods for our measure of habitat heterogeneity) Lichen biodiversity increases with habitat (microhabitat) heterogeneity (Lesica et al. 1991, Gignac & Dale 2005, Vondrák et al. 2019). Although this correlation is challenged by some researchers (e.g. Bässler et al. 2016), it is generally supported by the unpublished experience of most lichenologists working in Europe. For that reason, we encourage specialists involved in lichen biodiversity surveys to supplement their species lists with a catalogue of local habitats plus notes on their size, frequency and the estimated value for local biodiversity (i.e. saturation by species). This information is useful when the expected general pattern is not recorded (i.e. species richness does not increase with habitat heterogeneity), because potentially suitable habitats are not saturated by species (e.g. impoverished by air pollution). Field research aimed at revealing all (or almost all) of the local habitats is time consuming, especially in large and diverse areas, but it requires far less time than an attempt to obtain a complete list of species.

As an example, the catalogue of habitats for Týřov is attached below (Supplementary Data S2). It was prepared from data gathered during just a few visits, although some of the listed habitats have very limited distributions in the area studied (Table 1) and might take longer to find. At the sites sampled, species richness is positively correlated with the number of available habitats, which demonstrates the value of a catalogue of habitats. This relationship works well at the regional level too (Fig. 4A).

Is this Czech locality outstanding?

Is 787 species recorded in an area of 410 hectares an exceptional count? We suggest that it is, on the basis of the high heterogeneity of habitats and the very high numbers of: (i) red-list species, (ii) species new to the Czech Republic and Europe, (iii) species new to science, (iv) known unknowns, and (v) other remarkable records.

However, we cannot conclude that Týřov is an outstanding locality merely on the basis of the high numbers of habitats and species recorded. To claim Týřov is a biodiversity hotspot we need reliable species lists for areas of comparable size. For this reason, we summarize below the lichen biodiversity data at three levels: (i) regional, (ii) country-wide and (iii) European (Table 2). We are aware that the efforts employed in the reference surveys are likely to have differed and as a consequence our conclusions may be biased to some extent.

Týřov is exceptionally species-rich at the regional scale. We were able to compare our data with thirteen inventories of lichen biodiversity recently compiled by various authors in nearby natural reserves from 5 to 231 hectares in area. Most of the sites sampled at Týřov had species richness comparable to these reference inventories, but species richness at three sites was distinctly higher and it occurred together with higher habitat heterogeneity (Fig. 4A, Table 1). However, a considerable part of the species list for Týřov comes from lichen communities at sites with lower habitat heterogeneity, which indicates that the above-average size of this nature reserve contributes quite significantly to the exceptional species richness (Johansson & Ehrlén 2003, Johansson et al. 2012).

Týřov is exceptional at a national level. Numerous lichen surveys have been carried out in the Czech Republic at various nature reserves from a few hectares to a few square kilometres in area. Most resulted in lists of fewer than 200 species. The few that reported

more species are listed in Table 2, but the maximum is 450 species, which is still about 300 fewer than at Týřov.

Týřov match the best in Europe. We have searched for lichenological surveys in Europe that report more than 500 species. Our search of the literature was supplemented by obtaining the views of experienced European lichenologists. We were unable to find any species list with more than 500 species from an area of less than 10 km^2. This makes Týřov by far the most species rich locality in Europe at a scale up to 10 km^2. We doubt that it would retain that position if some other areas were surveyed with equal thoroughness. Localities with far higher habitat heterogeneity and longer climatic gradients than at Týřov occur elsewhere and are probably at least as rich. Perhaps the most comparable work is from the Swiss Alps and reports on 1000 species recorded from an area of 24 km^2 over a period of several decades (Groner 2016, 2020). A few other studies from Europe also report more than 500 species, especially those from the French Alps (Roux et al. 2011a, 2012, 2013), but these refer to distinctly larger areas, all exceeding 100 km^2 (Table 2).

Conclusions

Lichen diversity hotspots probably occur on all continents, and a detailed survey of each of them would result in very large numbers of species. Our seemingly "ordinary" central-European locality in a long-inhabited landscape is an example. On the basis of existing data, our locality is by far the most species rich in Europe, at a scale up to 10 km^2. Three factors led to this result: (i) exceptional habitat heterogeneity, (ii) variable but favourable natural conditions on steep slopes of several valleys, and (iii) the substantial effort put into the survey and species identification. We hope that our conclusion will motivate other lichenologists to seek hotspots comparable with or even richer than that at Týřov.

Descriptions of new species

Acarospora fissa K. Knudsen et Vondrák, spec. nova

MycoBank: MB#841124; Fig. 5A–C

Etymology: The epithet refers to the process of it splitting apart to replicate by division.

Type: Czech Republic. Central Bohemia: district Rakovník, protected area Týřov, Týřovické skály rocks, alt. 370 m, 49.98412N, 13.79375E. On sun-exposed andesite rock, 28 April 2020, coll. J. Vondrák (PRA-JV23503, holotype).

Type sequences: ITS (MW989377), mtSSU (MW989378), nLSU (MW989379).

Diagnosis: Similar to the areolate *A. scrobiculata* but differing in having squamules, which are wider (1.0–3.5 mm vs. 1.0–2.0 mm) and taller (1.0–2.0 mm vs. 0.5–0.8 mm).

Morphology and anatomy: Thallus variable in size, consisting of few squamules, covering tens of cm^2. Squamules up to 3.5 mm wide, convex, up to 2 mm tall, developing from areoles 0.5–1.0 mm wide, flat, angular. Upper surface light brown, crosshatched with fissures, replicates by division. Lower surface brown or becoming blackened. Epicortex uneven, 10 µm or less thick. Cortex ~50 µm thick, upper layer light brown, thickness variable, lower layer colourless, cells 2–5 µm wide, round to irregular. Algal layer 100–150 µm thick, dense, uninterrupted, algal cells 7–12 µm wide. Medulla obscure,

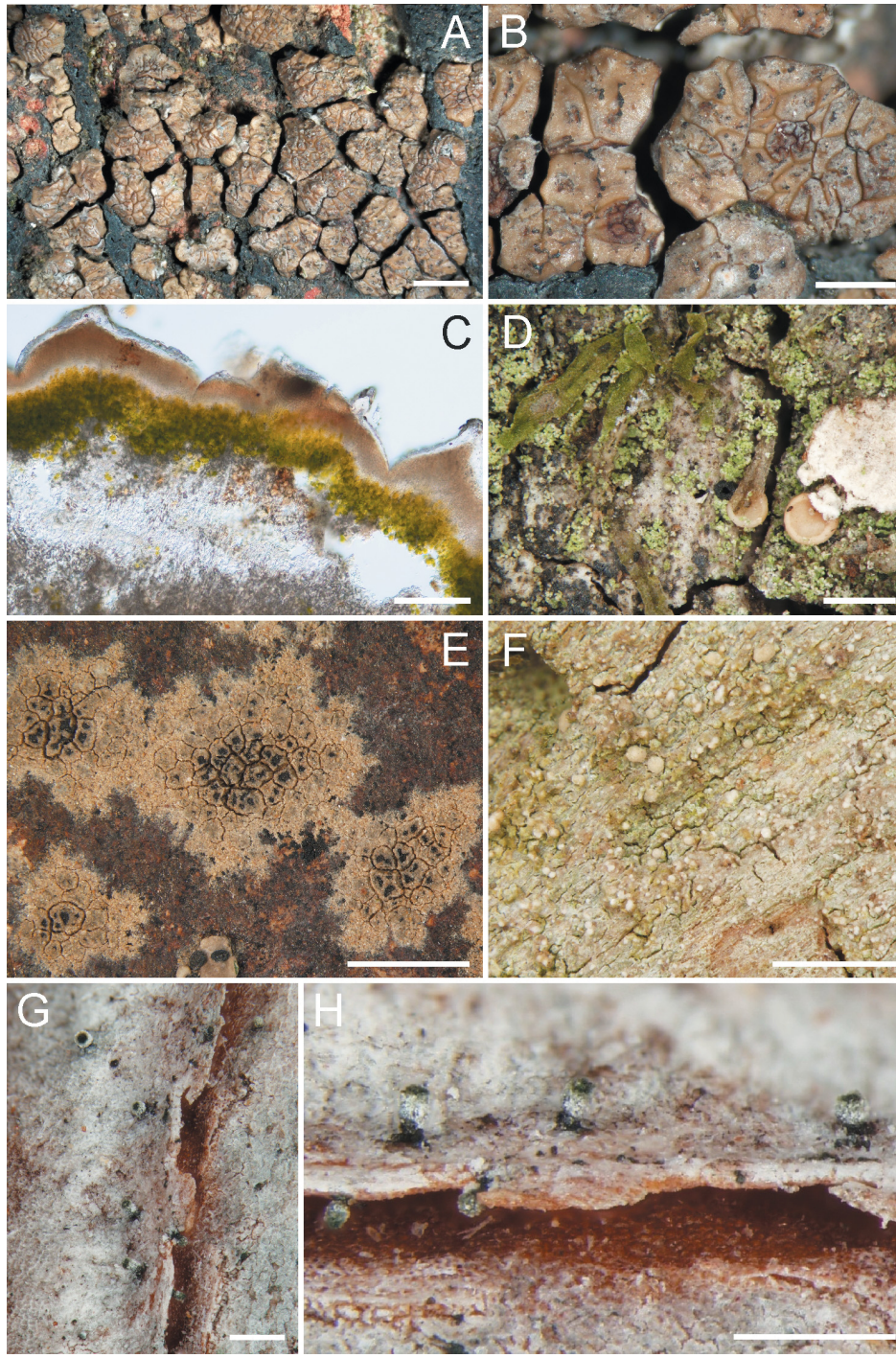


Fig. 5. New species: (A-C) *Acarospora fissa*, holotype; A, B, outer appearance; C, vertical section in thallus. (D) *Bacidia hyalina*, holotype. (E) *Buellia microcarpa*, holotype. (F) *Micarea substipitata*, PRA-ZP27411. (G, H) *Microcalicium minutum*, holotype. Scales: A, B, E, F – 1 mm, D, G, H – 0.5 mm, C – 100 µm.

200 µm thick in young areoles, but up to 850 µm tall in biggest squamules. Stipe less than half the width of squamule, up to 1 mm tall. Most squamules and areoles sterile, apothecia usually on about half or fewer areoles, 1 or 2 each per areole. Apothecia punctiform, immersed, disc reddish, epruinose. 0.1–0.2 mm wide, best seen when wet. Parathecium IKI-, expanding around disc, 10–20 µm wide. Hymenium 110–120 µm tall, epihymenium narrow and light brown, paraphyses mostly 2 µm wide, hymenial gel IKI+ blue (not dark blue), asci 50–70 × 17–20 µm, ascospores 3–4 × 2 µm. Subhymenium 20–30 µm thick, IKI+ blue. Hypothecium ~10 µm thick, golden in water, IKI-. Pycnidia rare, conidia 0.5 × 0.5–1.0 µm.

Chemistry: Gyrophoric acid. Spot tests: cortex C+, KC+ red.

Similar species: *Acarospora scrobiculata* H. Magn., known from Greenland and Norway, has a thallus surface similarly cross-hatched with fissures but has areoles instead of squamules (Magnusson 1935). Both replicate by division as their primary mode of reproducing. *Acarospora applanata* H. Magn., common in the Organ Mountains in southern New Mexico, is similar in being rarely fertile, having fissures and reproducing primarily by division. It differs from *A. fissa* in being a facultative lichenicolous lichen and in having smaller areoles or squamules, not exceeding 1.5 mm in width and 0.5 mm in height (Knudsen, unpublished).

Ecology and distribution: Known from andesitic rocks completely exposed to sunlight at its single locality.

DNA data: This species belongs to the *Acarospora* s.str. clade, but is distinct from all other species for which there is sequence data. Details are published by Knudsen et al. (2021).

Paratypes: Two additional specimens sampled at the type locality: PRA-JV21129 (19 September 2019), PRA-JV21144 (13 October 2019).

***Bacidia hyalina* Vondrák, spec. nova**

MycoBank: MB#841125; Fig. 5D

Etymology: Named after its colourless (hyaline) apothecia.

Type: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, southern slope above valley of 'Úpořský potok' stream, alt. 300–340 m, 49.96482N, 13.81124E, on bark of *Acer platanoides*, 21 August 2020, coll. J. Vondrák (PRA-JV24084-holotype).

Type sequences: ITS (MZ968995), mtSSU (OK019727).

Diagnostic characters: Thallus formed of dispersed green soralia. Soredia fine, 20–30 µm in diam., consoredia up to 50 µm in diam. Apothecia rare, without pigmentation, with distinct margin. Ascospores 35–80 × 2.5–3.5 µm.

Morphology-anatomy: Green sorediate crust, hardly distinguishable from thalli of sorediate *Bacidina* spp. or *Lecania croatica*. Thallus formed of an inconspicuous, thin green crust, densely covered by pale green soralia. Soralia usually discrete, punctiform, ~0.2–0.5 mm diam., sometimes confluent into more extensive sorediate spots. Soredia fine, 20–30 µm in diam., consoredia up to 50 µm in diam. Photobiont *Trebouxia*-like. Apothecia usually absent; when present, then only few detected; colourless or pale beige, without internal and superficial pigmentation or with a yellowish tinge in outer excipular tissue. Discs flat; margin distinct, ~50–80 µm wide, slightly raised above disc. Exciple of

radially arranged anastomosing hyphae with thick, conglutinated walls; lumina ~1–2 µm thick in KOH. Hymenium ~50–80 µm tall. Paraphyses sparsely branched, ~1–2 µm thick, with slightly thickened uppermost cell, ~2.5 µm thick. Asci clavate, ~50–70 µm tall. Ascospores 4–10-septate, 35–80 × 2.5–3.5 µm; the length varied among specimens: 40–70 µm (holotype), 40–70 µm (JV24092), 35–50 µm (JV24158).

Chemistry: No secondary substances detected by TLC of the type specimen.

Ecology and distribution: Known from a few sites in the Týřov National Nature Reserve (Czech Republic). Occurring on nutrient-rich bark of *Acer platanoides*, *A. pseudoplatanus* and *Quercus petraea* in scree forests, often together with *Bacidia rubella*.

Similar species: *Bacidia hyalina* presumably occurs predominantly in a sterile state, without apothecia. Unfortunately, these occurrences are hardly distinguishable from other sorediate crusts with discrete green soralia, e.g. *Bacidina* spp. and *Lecania croatica*. Apothecia are similar to those of various species of *Bacidia* s. lat., especially the pale morphs of *B. rubella* or pallidomorphs of a common *B. subincompta* s. lat. The combination of the green sorediate thallus and the colourless apothecia producing long ascospores is diagnostic.

DNA data: Sequence data: ITS (24084, 24092, 24210), mtSSU (24084, 24158, 24274). Closest NCBI Blast records in ITS include various *Ramalinaceae* (*Bacidina* spp. and *Toniniopsis* spp.; idnetities ~85%), in mtSSU *Toniniopsis coelestina* and *T. subincompta* (94%). On the basis of our data, *Bacidia hyalina* belongs to *Ramalinaceae*, but its generic position is tentative, as it is probably outside the genera currently known for *Bacidia* s.lat.

Paratypes: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, south-western slope with scree forest adjacent to the nature reserve above stream 'Úpořský potok', alt. 340 m, 49.96985N, 13.82595E, on bark of *Quercus petraea*, 5 Sept. 2020, coll. J. Vondrák (PRA-JV24092, 24210); Ibid.: in valley of the Úpořský potok stream, alt. 320 m, 49.96794N, 13.82400E, on bark of *Acer platanoides*, 5 Sept. 2020, coll. J. Vondrák (PRA-JV24158); Ibid.: on bark of *Acer platanoides*, 6 Oct. 2020, coll. J. Vondrák 49.96399N, 13.80945E (PRA-JV24274); Ibid.: rocks Týřovické skály, alt. 300–400 m, 49.98412N, 13.79375E, on bark of *Acer pseudoplatanus*, 28 Apr. 2020, coll. J. Vondrák (PRA-JV23489).

Buellia microcarpa **Vondrák et Malíček, spec. nova**

MycoBank: MB#841126; Fig. 5E

Etymology: Named after its minute apothecia, which are exceptionally small for *Buellia*.

Type: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, Týřovické skály rocks, alt. 350 m, 49.98387N, 13.79361E. On andesitic stones in rocky steppes, 13 Oct. 2019, coll. J. Vondrák (PRA-JV21161, holotype).

Type sequence: ITS (MZ968996).

Diagnostic characters: Tiny ochre-brown to grey thalli, rimose in centre, diffuse at margins; minute, immersed apothecia; no secondary substances.

Morphology-anatomy: Thallus orbicular, a few mm diam., rimose-areolate in inner part, with diffuse margin. Thallus pale ochre-brown to grey, approximately 100 µm thick

(but up to 200 µm around apothecia), gradually thinning to thallus margin. Cortex and medulla absent. Epinecral layer present, up to 30 µm thick. Areoles flat, angular, 0.2–0.6 mm diam. Apothecia immersed in thallus (*Buellia aethalea*-type), 0.05–0.2 mm in diam., round to angular. Ascospores melanized, 11–13 × 6–7.5 µm; superficial ornamentation absent (light microscopy).

Chemistry: No secondary substances detected by TLC of the type specimen.

Ecology and distribution: A pioneer species on stones and pebbles at xerothermic sites (e.g. on rocky steppes) accompanying nitrophilous and acidophilous lichen species (e.g. *Acarospora* spp., *Amandinea punctata*, *Rinodina aspersa*). So far known from five localities in central Bohemia where the species is abundant at suitable sites.

Similar species: Several *Buellia* species have apothecia immersed in the thallus, but these taxa are distinct and have different ecologies: (i) *B. aethalea* differs e.g. in grey thallus surface, larger apothecia (0.2–0.4 mm diam.) and the presence (usually) of stictic/norstictic acid. (ii) *B. jugorum* and *B. ocellata* have yellow thalli and contain xanthones. (iii) *B. miriquidica* and *B. uberior* are lichenicolous on *Schaereria fuscocinerea* and contain gyrophoric acid and miriquidic acid, respectively. (iv) *B. spuria* has large apothecia and contains atranorin and norstictic acid. (v) *B. stellulata* contains atranorin, confluentic acid and 2'-O-methylperlatolic acid.

DNA data: We sequenced ITS (PRA JV20925, 21161, JM13917; sequences identical) and mtSSU (JM13917). Available data indicate that *Buellia microcarpa* is related to *B. aethalea*. Closest NCBI Blast relative is *B. aethalea* with 98.5% identity in mtSSU and 92.5% in ITS.

Paratypes: Czech Republic. Central Bohemia: district Rakovník, nature reserve Stříbrný luh, alt. 375 m, 50.02108N, 13.89817E, on andesitic stone, 19 Aug. 2020, coll. J. Vondrák (PRA-JV24599); Ibid., Týřov National Nature Reserve, SW slope of hill Vápenný vrch, alt. 300–400 m, 49.97114N, 13.79466E, on andesitic stone, 12 Sept. 2019, coll. J. Vondrák (PRA-JV20923); Ibid. (PRA-JV20925, 20965); district Příbram, Dubenec, uranium heap 11A W of Bytíz settlement, ~49°40'57"N, 14°04'11"E, alt. 520–540 m, on siliceous stone, 18 Sept. 2020, coll. J. Malíček et al. (JM13917); Ibid. Lešetice, S side of uranium heap no. 15 between Lešetice and Brod, 49°39'07"N, 14°00'55"E, alt. 530 m, on siliceous stone, 19 Oct. 2020, coll. J. Malíček (JM13963).

***Micarea substipitata* Palice et Vondrák, spec. nova**

MycoBank: MB#841127; Fig. 5F

Etymology: Named after its sessile pycnidia that are similar to those of *Micarea stipitata*, but shorter.

Type: Russia. Krasnodar Territory, Sochi, Estosadok, Mt. Tabunnaya [2351], fir-beech primeval forest on WSW descending crest of 'Psekhano' along a tourist trail E of the Olympic-games ski-complex 'Pichtovaya polyana', 43°41'50"N, 40°21'25"E, on dry decaying wood of a moribund snag of *Abies nordmanniana*, alt. 1670–1700 m, 27 June 2019, coll. Z. Palice (PRA-ZP27411, holotype).

Type sequences: ITS (MZ968997), mtSSU (OK019728).

Diagnostic characters: Apothecia infrequent, small, 0.15–0.3 mm diam., pallid to whitish, occasionally with beige to pale ochre tint, translucent when wet, initially ± flat, later becoming distinctly convex to subglobose, matt, finely roughened on the upper surface,

internally without psammoid granules and pigmentation; pycnidia numerous, sessile to shortly stipitate, white, translucent when wet, becoming confluent with age, covered by a gelatinous mass (blobs) with released conidia; conidia 2.5–3.5 × 1.0–1.5 µm; thallus immersed to semiimmersed, effuse, pale green-grey, not forming distinct continuous crust, photobiont cells small, globose to (more-usually) broadly ellipsoid, 3–6 (7) µm in diam., tightly arranged in colonies, *Stichococcus*-like. No substances detectable by TLC.

Morphology-anatomy: Thallus immersed in substrate or rarely exposed as a thin roughened undifferentiated greenish crust, partly farinose. Sometimes forming patches of a few mm in diam., but more often recorded as an extensive crust covering larger areas. Thallus less than 50 µm thick, cortex indistinct. Photobiont *Stichococcus*-like. Apothecia pallid, with pale rose, beige to ochre tint in herbarium, translucent when wet, almost flat to distinctly convex, emarginate, not tuberculate, 0.15–0.3 mm diam., infrequent but present in approximately half of examined specimens. Hymenium not pigmented, 30–40 µm high, without differentiated epihymenium but older apothecia often with amorphous, uneven epinecral layer up to 5 µm with embedded extraneous material. Asci ±clavate, about 25 × 8–12 µm, *Micarea*-type. Excipulum reduced, eventually limited to a narrow zone consisting of narrow paraphyses-like hyphae. Hamathecium of narrow, branched, and in part anastomosing paraphyses, 0.9–1.3 µm wide; rarely less branched broader paraphyses present (1.5–2.0 µm wide). Ascospores (0-)1-septate, cylindrical, narrowly ellipsoid, ovoid, or almost pyriform, apically rounded, often tapering to one end, (6–) 7–10 (–11) × (2.0–) 2.2–3.5 (–3.8) µm. Pycnidia abundant, sessile to shortly stipitate, up to 0.2 mm wide and 0.25 mm tall (but initial conical pycnidia only 15–35 µm in diam); with broad ostiole when mature; ocassionally merged into "conpycnidia"; white, translucent when wet, smooth; often with a shiny drop of released conidia on the top. Conidia shortly cylindrical, (2.8–) 3.0–3.5 (–4.0) × 1–1.6 µm.

Chemistry: No secondary substances detected by TLC (holotype and two paratypes tested).

Ecology and distribution: On wood that is externally hard and dry but internally rotten and wet, in sites sheltered from rain, but with higher air humidity. Usually recorded in overhanging sides and shallow cavities on vertical surfaces of stumps and snags in old-growth forests. Recorded in many forest habitats in central and eastern Europe from low altitudes to upper mountains (e.g. beech forests at timber line in Carpathians). It inhabits wood of various trees, e.g. beech and fir. Only a few records are from outside old-growth forests, such as in old parklands or on decaying veteran trees left in managed forests. So far known from the Czech Republic (Šumava Mts, Český les Mts, Krkonoše Mts, Českomoravská vrchovina Mts, Beskydy Mts, Jeseníky Mts, Křivoklátsko), Slovakia (W Carpathians), Russia (Caucasus) and Ukraine (Eastern Carpathians).

Similar species: Most likely to be confused with the unrelated *Biatora veteranorum*, which may occur in similar micro-habitats, and which also usually form extensive coverings, and produce numerous and distinctive pale pycnidia. *Biatora veteranorum* also has quite similar asci, similarly sized ascospores and conidia. It differs from *M. substipitata* by its pruinose, cylindrical to barel-shaped (usually not conical) pycnidia and pruinose apothecia. The pruina of *Biatora veteranorum* may diminish with age or due to environmental conditions, but is still detectable microscopically as psammoid granules soluble in KOH in apothecial or pycnidial sections. Epruinose elderly apothecia of *B. veteranorum* are more convex, basally constricted and tend to become tuberculate apothecia that are

not recorded in *Micarea substipitata*. The lignicolous populations of *B. veteranorum* seem to prefer more decayed, softer and drier wood than *M. substipitata*.

Micarea stipitata and *M. pycnidiophora* are similar but usually corticolous species of more oceanic woodlands (the former often overgrowing bryophytes), not occurring on rotten wood sheltered from rain. Both mentioned taxa have distinctly longer conidia. Moreover *M. stipitata* usually has taller pycnidia and *M. pycnidiophora* contains gyrophoric acid (Coppins 2009). The above species also differ from *M. substipitata* in their photobiont, which is never *Stichococcus*-like. *Micarea myriocarpa* also occupies sheltered niches and contains *Stichococcus*-like photobiont (Czarnota 2007). However, pycnidia of *M. myriocarpa* are usually smaller and pigmented. In addition, *M. myriocarpa* has a pigmented hypothecium and usually forms distinctly episubstratal thalli.

DNA data: DNA sequences were obtained from the holotype (Caucasus), one Ukrainian (Eastern Carpathians) and three Czech paratypes (from Šumava Mts, Český les Mts and Týřov). The four ITS sequences are variable only in a single nucleotide position. The five mtSSU sequences are identical. The closest Blast record is *Micarea myriocarpa* in ITS (92% identity) and *Micarea contexta* and *M. doliiformis* in mtSSU (93–94% identity).

Paratypes: Czech Republic. Central Bohemia: Týřov, 49.95624N, 13.80047E, on rotten wood of *Abies alba* stump, alt. 400 m, 24 Apr. 2021, coll. J. Vondrák (PRA-JV24847; ITS-MZ968998; mtSSU-OK019729); Western Bohemia: Český les Mts, Domažlice, Vranov, old ash-maple forest on hill with ruin of Starý Herštýn, SE-S-SW slope, alt. 830–870 m, 49°28'17"N, 12°42'50"E, on wodd of stump of tree, 13 Apr. 2016, coll. J. Vondrák (PRA-JV14631); Přimda, nature reserve Diana, old-growth mixed forest with predominant beech, 49°37'55"N, 12°34'46"E, on dry decaying wood of *Picea* stump, alt. 515 m, 12 Apr. 2016, coll. Z. Palice (PRA-ZP21040); Šumava Mts, Lenora: Mt Zátoňská hora – old-growth mixed forest on S facing slope, 48°56'25.2"N, 13°49'47.4"E, on wood of snag of *Abies alba*, alt. 892 m, 20 Sept. 2018, coll. J. Malíček & Z. Palice (PRA-ZP26038); Volary, Zátoň, Mt Boubín, southern slope between ways "Knížecí" and "Lukenská", alt. 1070 m, 48.98021N, 13.81854E, on wood of snag in old-growth beech forest, 6 May 2020, coll. J. Vondrák (PRA-JV23523; ITS-MZ968999; mtSSU-OK019730); Volary, Stožec: Mt. Stožec, locality 'Medvědice', old-growth scree forest on NE-facing slope, 48°52'48.9"N, 13°50'18.9"E, on decaying wood of a conifer, alt. 900 m, 17 Oct. 2016, coll. Z. Palice (PRA-ZP24611); Nová Pec, Mt. Hraničník – NNE slope, a fragment of climax spruce forest surrounded by large clearings, 130m NNE from the top, N48°45'03", E013°54'19", on wood of *Picea* snag, alt. 1233 m, 28 Oct. 2014, coll. Z. Palice (PRA-ZP18480); Ibid.: northern slope of Mt Hraničník, old-growth beech-dominated forest, alt. 1170 m, 48.75364N, 13.90472E, on wood of snag in old-growth beech forest, 15 June 2017, coll. J. Vondrák (PRA-JV18769); Ibid.: alt. 1165 m, N48°45'14", E013°54'16.5", on wood of snag of *Picea abies*, 12 Aug. 2017, coll. Z. Palice (PRA-ZP24423); Novohradské hory Mts, Pohorská Ves: nature reserve Pivonické skály, old managed beech forest on N facing slope of Mt Stříbrný vrch [936], 48°39'35"N, 14°41'50"E, on dry bark/wood of *Fagus* stump, alt. 835 m, 17 Aug. 2016, coll. Z. Palice (PRA-ZP22240). N Bohemia, Krkonoše Mts, distr. Semily, E-facing slope above Jizerka brook, 1.2 km N of Vítkovice settlement (church), 50°41'42.5"N, 15°31'36.6"E, on slowly decaying wood of stump of conifer along road-side in a chalet area, on forest margin, alt. 660 m, 1 May 2015, coll. Z. Palice (PRA-ZP19035); S Moravia, distr. Jihlava, Třešť: close-to-primeval forest (*Fagus sylvatica*, *Abies alba*,

Picea abies, *Acer platanoides*) on W-facing slope of Mt. Velký Špičák [734], just 0.2 km WNW of the top, 2.6 km NE of Třešť, 49°18'43.58"N 15°30'31.51"E, on wood of *Abies* stump, alt. 690–700 m, 8 Sept. 2010, coll. I. Černajová, J. Malíček & Z. Palice (PRA-ZP13993); N Moravia: Jeseníky Mts, NR Praděd, Mt. Vysoká hole, old-growth spruce forest on E-facing slope above the chalet 'Eustaška', 50°03'35.1"N, 17°15'12.7"E, on dry wood of big *Picea* stump, alt. 1220 m, 21 Aug. 2015, coll. Z. Palice (PRA-ZP20247); Moravsko-Slezské Beskydy Mts, distr. Frýdek-Místek: Mt. Lysá hora, old-growth climatic spruce forest, WSW facing slope, 49°32'42"N, 18°26'45"E, on dry hard wood of *Picea* snag, alt. 1230 m, 30 Aug. 2016, coll. J. Malíček & Z. Palice (PRA-ZP21707). Mt. Kněhyně, fragment of old-growth spruce forest with some beech and sycamore, E facing slope, 49°29'47.8"N, 18°18'56.1"E, on dry hard wood of *Picea* snag, alt. 1190 m, 31 Aug. 2016, coll. J. Malíček & Z. Palice (PRA-ZP21707, 21773); Vysočina Region: Černovice, park of town, alt. 580 m, 49.3668153N, 14.9585858E, on wood of *Tilia* stump, 9 February 2018, coll. J. Vondrák (PRA-JV19311). **Russia**. Republic of Adigea: Maykop, Guzeripl, protected area Kavkazskiy zapovednik, on wood of snag in virgin forest, alt. 1465 m, 43.96475N, 40.13073E, 11 June 2016, coll. J. Vondrák (PRA-JV15383); Ibid.: alt. 1724 m, 43.93632N, 40.14730E, 10 June 2016, coll. J. Vondrák (PRA-JV16149); Ibid.: alt. 1911 m, 43.92619N, 40.15161E, 9 June 2016, on log, coll. J. Vondrák (PRA-JV15403). Lago-Naki plateau, just SW of the pass Azishiskiy pereval, a hill ~300 m E of the Hotel Oshten, a montane mixed forest dominated by *Abies nordmanniana*, 44°04'40"N, 40°00'50.5"E, on dry decaying wood of snag of *Abies nordmanniana*, alt. 1830 m, 18 June 2016, coll. Z. Palice (PRA-ZP23223, 23429). **Slovakia**. W-Carpathians, distr. Revúca, Stolické vrchy Mts, Mt Stolica [1476], W-facing slope, a mountain spruce forest, margin of a clearing, N48°46'20.9" E020°11'57.1", on hard wood of *Picea* stump, alt. 1285 m, 30 May 2017, coll. D. Blanár, A. Guttová & Z. Palice (PRA-ZP23853). **Ukraine**: Zakarpatska Oblast Region, Eastern Carpathians, Khust, Velyka Uhol'ka, old-growth beech forest on a ridge 2 km SSW-SW of Mt Menchul [1501], 6.5 km E of Zabrid', 48°17'52"N 23°39'59"E, on dry wood of a very large *Fagus* log in a glade, alt. 1210 m, 17 May 2015, coll. Z. Palice (PRA-ZP19376; ITS-MG773659, mtSSU-MG773688).

***Microcalicium minutum* Vondrák et Svoboda, spec. nova**

MycoBank: MB#841128; Fig. 5G, H

Etymology: Named after the small size of the apothecia.

Type: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, on west slope above 'Prostřední potok' stream, in open rhyolite scree with solitary pines, alt. 380 m, 49.95901N, 13.80149E, on sun-exposed bark of *Pinus sylvestris*, 7 Aug. 2020, coll. J. Vondrák (PRA-JV24173, holotype).

Type sequences: ITS (MZ969000), mtSSU (OK019731).

Diagnostic characters: Apothecia 0.1–0.15 mm diam., sessile to shortly stipitate; ratio of height to width less than two. Ascospores simple (rarely 1–2-septate), 7–18 × 3–4.5 µm. Pycnidia frequent. Pycnidia and apothecia with olive green pigment.

Morphology-anatomy: Thallus inconspicuous, apparently not lichenized, forming bleached to white spots on sun-lit squamules of conifer bark. (It is plausible that the bark bleaching is caused by the *Microcalicium.*) Apothecia sessile to shortly stipitate,

0.10–0.15 mm wide and 0.1–0.2 mm tall. Stipe, if present, about 0.1 mm wide, with smooth surface, without sclerotized hyphae. Mazaedium dark green, stipe and exciple black, but exciple sometimes white pruinose (frequently pruinose in the type specimen). Pigment in stipe, exciple and ascospores olive green, KOH+ orange-brown, HNO^{3+} bright green. (Lower content of pigment in ascospores causes a less obvious colour change after KOH into yellow-brown.) Mazaedium not higher than the width of the capitulum. Paraphyses dissolving at an early stage, absent from upper mazaedium. Ascospores usually remaining non-septate, even in the latest stages of development; 1 or 2 septa observed in only a few ascospores. Ascospore size usually 7.5–12 × 3.0–3.5 µm; a few ascospores up to 18 × 4.5 µm. The secondary ascospore wall ornamentated by spirally arranged ridges. Pycnidia numerous, sessile, black, 0.05–0.10 mm diam. Pycnidial wall olive green, with same pigment as apothecia. Conidia colourless, broadly ellipsoid, non-septate, 2.0–3.5 × 1.5–2.0 µm.

Ecology and distribution: Known from two localities of old and sparse conifer forest on steep sun-lit slopes. *Microcalicium minutum* was detected on several trees in both localities. It occurs on insolated bark of *Picea abies* and *Pinus sylvestris*, on bare bark, too dry for lichens; the only observed co-occuring species was *Chaenothecopsis pusilla*. *Microcalicium disseminatum*, co-occurring in these localities, avoids such extreme microsites.

Similar species: Only four species were previously known in *Microcalicium* (Tibell 1978) and they are distinct from *M. minutum*: (i) *M. disseminatum* is similar in the frequent pycnidia, the sizes of conidia and the sessile to shortly stipitate apothecia, but differs in its larger apothecia and typically 1–3-septate ascospores; (ii) *M. ahlneri* has similarly small apothecia, but usually with longer stipes. It has no pycnidia, thinner 1-septate ascospores and is predominantly lignicolous. (iii) *M. arenarium* has tall, stipitate apothecia, and is lichenicolous on *Psilolechia*. (iv) *M. conversum*, not known from Europe, has a different, reddish-brown pigment in pycnidia and apothecia.

DNA data: ITS and mtSSU sequenced from the holotype and from PRA-JV24396. Both obtained mtSSU sequences are variable in six nucleotide positions, ITS in thirty positions. The closest relative in mtSSU is *Microcalicium ahlneri,* which is distinct in eight nucleotide positions. *Microcalicium disseminatum* is distinctly less related and *M. arenarium* is most distant. ITS sequences of *M. disseminatum* and *M. arenarium* are very distinct from *M. minutum* (with differences in more than 60 nucleotide positions).

Paratype: **Czech Republic**. Southern Bohemia: district Prachatice, nature reserve Čertova stráň, south slope of 'Boubínský potok' stream with old and sparse fir-pine-spruce forest, alt. 760 m, 49.00791N, 13.88405E, on sun-lit bark of *Picea abies*, 9 Oct. 2020, coll. J. Vondrák (PRA-JV24396; ITS and mtSSU sequenced); type locality, 7 Aug. 2020, coll. J. Vondrák (PRA-JV24127).

Rufoplaca griseomarginata Vondrák et Svoboda, spec. nova

MycoBank: MB#841129; Fig. 6A

Etymology: Named after the diagnostic grey margin of apothecia, uncommon in *Rufoplaca*.

Type: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, Berounka river bank below Týřovické skály rocks, alt. 250 m, 49.98227N, 13.79289E. On base-rich siliceous rock at riverside, 28 April 2020 coll. J. Vondrák (PRA-JV23413, holotype).

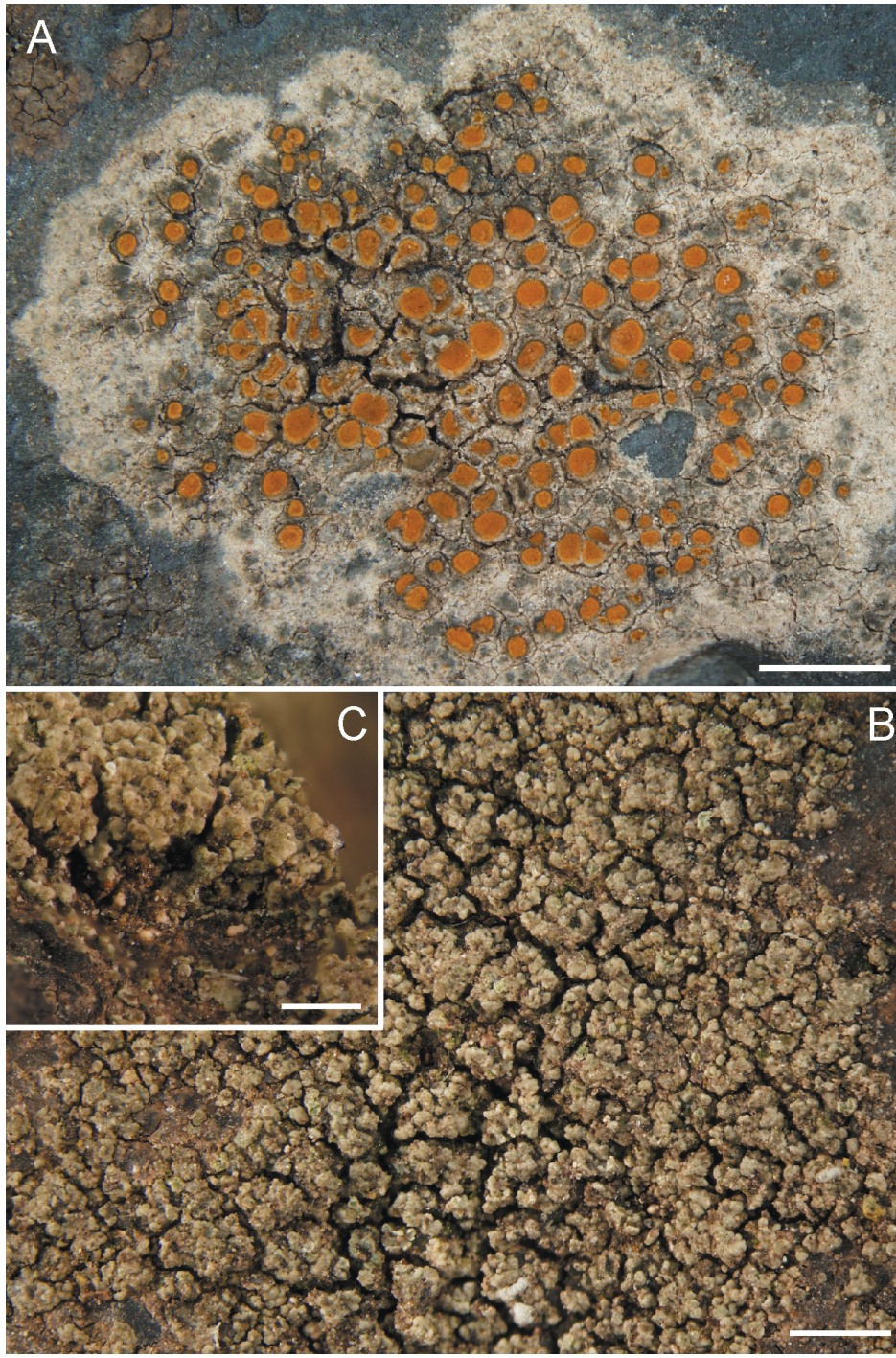


Fig. 6. New species: (A) *Rufoplaca griseomarginata*, holotype. (B, C) *Verrucaria substerilis*, holotype; C, Vertical structure of thallus. Scales: A, B – 1 mm, C – 0.5 mm.

Type sequences: ITS (MZ969001), mtSSU (OK019732).

Diagnostic characters: Apothecia typically adnate, only slightly raised above thallus surface, 0.1–0.3 mm in diam., with outer exciple visible as a grey ring.

Morphology-anatomy: Thallus rimose-areolate, up to 100 µm thick, pale to dark grey (with *Sedifolia*-grey pigment). Initially lichenicolous on *Rinodina oxydata*, later forming independent patches of up to ~1 cm diam. Apothecia 0.1–0.3 mm diam., orange, usually immersed in thallus or adnate and only slightly raised above thallus surface; rarely sessile with constricted base. True exciple orange in the part adjacent to disc, but grey, with *Sedifolia*-grey in the outer part. Thalline exciple present but thin or indistinct. Ascospores 11.0–14.0 × 4.5–6.5 µm; septum 2.5–3.0 µm wide.

Chemistry: Non-chlorinated anthraquinones in apothecia. TLC (apothecia): Parietin (major), 2 unidentified anthraquinones (Rf5, 6 in solvent C). *Sedifolia*-grey in outer exciple and in the thallus.

Ecology and distribution: Occurring on base-rich siliceous outcrops and stones accompanied by e.g. *Caloplaca atroflava*, *C. chlorina* and *C. subpallida*. So far known from central and south-eastern Europe, Iran and Turkey.

Similar species: Morphotypes of *Caloplaca conversa* with orange apothecial discs are probably the most similar lichens in their outward appearance, though not closely related. They differ in their broader ascospores (~6–9 µm) with thicker septa (4.0–8.5 µm). The known species of *Rufoplaca* generally lack the distinct grey ring surrounding the orange true exciple. Mature apothecia of other *Rufoplaca* species differ in their larger size, commonly exceeding 0.5 mm in diam. Apothecia of most *Rufoplaca* species are typically sessile, not immersed to adnate.

DNA data: ITS sequenced from four specimens, mtSSU sequenced from the type. The ITS sequences form a distinct clade close to *Caloplaca subpallida*, *Rufoplaca oxfordensis* and *R. tristiuscula*. mtSSU sequence supports the placement in *Rufoplaca*.

Paratypes: Czech Republic. Sedlčany, Nalžovické Podhájí, nature reserve Drbákov-Albertovy skály in valley of river Vltava, alt. ~300–400 m, 49°43'33"N, 14°22'5"E, on schist stones, 18 April 2008, coll. J. Malíček (JM1309); Ibid.: coll. J. Vondrák (PRA-JV6318); Greece. Methana, Agioi Theodori, volcanic outcrops on E-coast of Methana peninsula, alt. 0–30 m, 37°36'47"N, 23°24'53"E, on volcanic stone, 29 Oct. 2010, coll. J. Vondrák (PRA-JV8748); Iran. Hashtpar (Talesh), stones above road near Khotbeh Sara, alt. 30 m, 38°02'43.61"N, 48°53'33.81"E, on coastal base-rich siliceous outcrops, 5 May 2007, coll. J. Vondrák (PRA-JV5848); Turkey. Ordu, coastal rocks near Mersin, 41°07'08.59"N, 37°45'27.06"E, on siliceous coastal rocks, 22 April 2007, coll. J. Vondrák (PRA-JV5624).

Verrucaria substerilis Vondrák et Thüs, spec. nova

MycoBank: MB#841130; Figs 6B, C, 7A

Etymology: Named after its predominantly sterile occurrences. Perithecia, if present, inconspicuous, immersed in thallus.

Type: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, Berounka river bank below Týřovické skály rocks, alt. 250 m, 49.98227N, 13.79289E. On base-rich andesitic stone, 25 October 2019, coll. J. Vondrák (PRA-JV21184, holotype; STU, isotype).

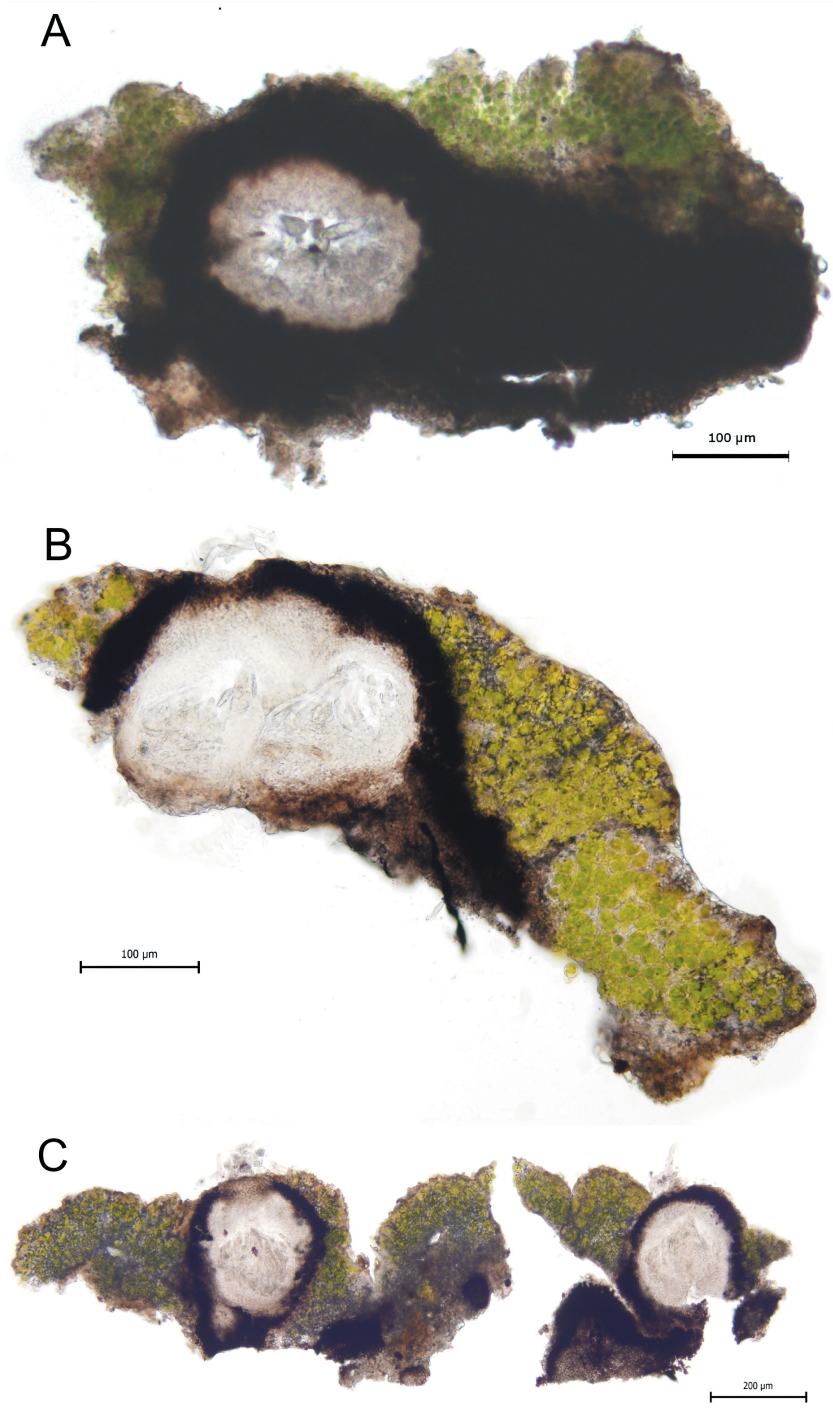


Fig. 7. Anatomical features of new species: (A) *Verrucaria substerilis*, isotype. (B, C) *Verrucaria tenuispora*, holotype. Scales: A, B – 0.1 mm; C – 0.2 mm.

Type sequences: ITS (MZ969002; holotype); mtSSU (OK019734; PRA-JV23705, paratype).

Diagnostic characters: Thallus pale grey-green, granular to squamulose. The squamules typically divided into secondary granules. Perithecia rare, immersed in thallus. Involucrellum laterally spreading in basal part, merging between neighbouring perithecia and forming a local carbonized basal layer. Ascospores ellipsoid (length/width ratio 1.8–2.7), 18.0–26.0 × 7.5–10.5 µm.

Morphology-anatomy: Thallus pale grey-green, forming a granular crust or a multilayer complex of squamules and secondary granules. Thalli start as scattered granules, 50–200 µm in diam., later merging and forming squamules, 0.2–1.0 mm in diam. Secondary granules are formed on the squamules, first along margins and later on the entire surface. Mature thalli are formed by a mixture of primary granules and squamules covered by few to numerous secondary granules with a total thallus height of up to 245 µm. Thin pseudocortex formed by colourless to weakly yellowish brown pigmented, more or less isodiametric fungal cells. Algal cells dispersed throughout the thallus from top to bottom except for areas with basal layer. Algal cells in tightly packed clusters, not in vertical stacks; mature cells in the direct vicinity of the perithecia, mostly 7–12 µm, in thallus squamules also 15–20 µm in diam, the largest dividing into several (3–6 in optical view) daughter cells of 5–10 µm in diam. Perithecia immersed in the thallus, involucrellum 0.2–0.3 mm in diam., apical part of exciple and involucrellum poorly separated and 30–50 µm thick, exciple transparent to partly dark brown, 15–25 µm thick, in young perithecia the pigmented parts of the exciple are surrounded by a thin (10–15 µm), weakly pigmented inner layer of involucrellum followed by its fully carbonized outer parts. In the basal part, the involucrellum is laterally spreading and can form a local black basal layer between neighbouring perithecia. Periphyses ~24–30 µm long, cytoplasm filled lumen ~1,6–2 µm wide. Only a few asci with well developed ascospores were observed; ascospores 18–26 × 7.5–10.5 µm, ellipsoid with a length/width ratio 1.8–2.7 (n=20).

Chemistry: No acetone-soluble secondary substances detected by TLC in the thallus of the type specimen. Internal pigments (other than melanines) not detected in the thallus.

Ecology and distribution: Known from several sites in the Týřov National Nature Reserve (Czech Republic). Occurring on andesitic rocks and stones along banks of river/stream, occasionally inundated. Co-occurring species: *Caloplaca atroflava*, *C. chlorina*, *Circinaria hoffmanniana*, *Rinodina moziana*, *R. oxydata*, *Protoparmeliopsis muralis*, *Rufoplaca griseomarginata*, *Staurothele fissa*, *Verrucaria tenuispora* and *V. teyrzowensis*.

Similar species: The conspicuous granular-squamulose thallus and the perithecia can be similar to some forms of *V. nodosa* and *V. rosula* (Orange 2013), but in these two species, the photobiont cells are smaller (only up to 10 µm) than those in the sequenced thalli of *V. substerilis*. In *V. rosula* the thallus margins are typically more continuous, although much variation is known to occur in this species. Sterile specimens may be very difficult to separate if the variation in the size of the photobiont cells overlap more regularly with those found in *V. rosula* and *V. nodos*a when more collections of *V. substerilis* become available. Young thalli of *V. hunsrueckensis* (Thüs et al. 2018) can look similar, but this species never develops a truly squamulose thallus, fruiting bodies are formed from an early stage on and the spores have a much higher length/width ratio compared to those of *V. substerilis*. Another granular to squamulose species, *Verrucaria glaucovirens*, could be similar when young, but it has a black prothallus and the thallus becomes much thicker

(up to 400 µm) with a distinct medulla, and differs in the perithecia developing in different levels of the thallus simultaneously. Squamulose forms are also known from the *V. macrostoma* complex, but these do not start their development as scattered granules and form much thicker thalli.

DNA data: ITS sequences (PRA JV21184, 23418, 23501, 23563, 23705, 23952, 23988, 25147) are variable in only ~2% of nucleotide positions and form a distinct clade in the ITS tree with unresolved relationships to related *Verrucariaceae*: *Verrucaria maculiformis*, *V. tenuispora*, *V. teyrzowensis* and *Verrucaria* spp. 7–10 (Fig. 8). mtSSU sequences obtained only for specimens JV23705 and JV25147. Both sequences are identical and have 98.5% identity with the sequence of *V. tenuispora*.

Paratypes: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, Berounka river bank below Týřovické skály rocks, alt. 250 m, 49.98227N, 13.79289E. On base-rich andesitic rock at riverside, 22 October 2019, coll. J. Vondrák (PRA-JV23563, 24693), Ibid.: 23 Apr. 2020 (PRA-JV23418); Ibid.: base-rich seepage rock, alt. 340 m, 49.98355N, 13.79380E, 16 Aug. 2021, coll. S. Svoboda & J. Vondrák (PRA-JV25147); Ibid.: andesitic rocks above right side of stream Úpořský potok, alt. 320 m, 49.96573N, 13.80936E, 9 June 2020, coll. J. Vondrák (PRA-JV23705); Ibid.: rock at Berounka riverside, SW of ruin Týřov, alt. 250 m, 49.9697467N, 13.7828344E, 14 July 2020, coll. J. Vondrák (PRA-JV23986).

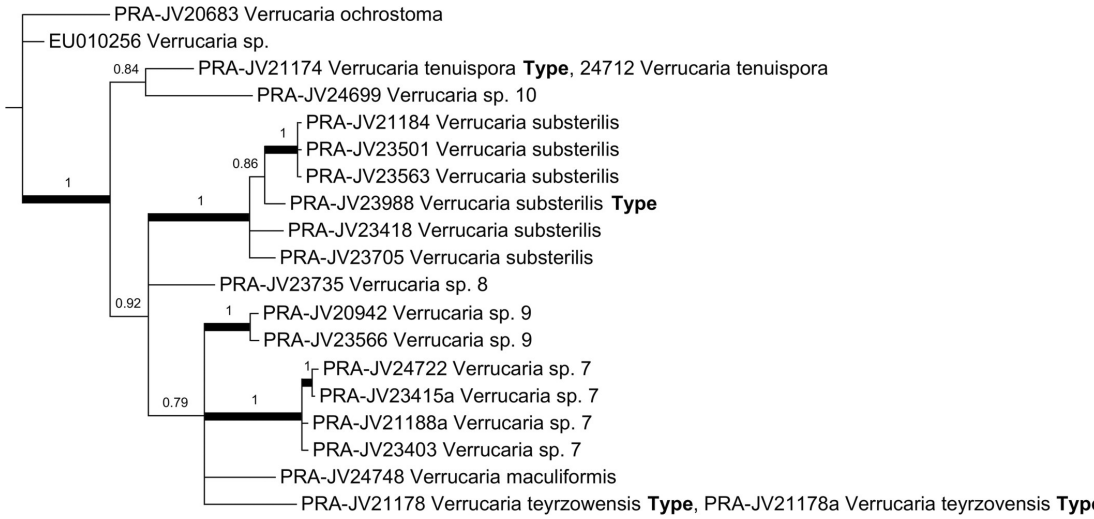


Fig. 8. Phylogenetic tree of the *Verrucaria tenuispora* / *V. substerilis* complex and related taxa based on ITS sequence data. SYM model with gamma distribution and proportion of invariable sites was used as a model of sequence evolution. The tree was constructed using Bayesian inference run for 151000 generations, and was rooted with *Verrucaria ochrostoma* (PRA-JV20683) and *Verrucaria* sp. (EU010256). Numbers on branches indicate posterior probabilities. Bold lines indicate branches with posterior probabilities > 0.95.

***Verrucaria tenuispora* Vondrák et Thüs, spec. nova**

MycoBank: MB#841131; Figs 7B, C, 9A

Etymology: Named after its slender ascospores, unusually elongated compared to most other crustose *Verrucariaceae*.

Type: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, Berounka river bank below Týřovické skály rocks, alt. 250 m, 49.98227N, 13.79289E. On base-rich siliceous rock at riverside, 22 Oct. 2019, coll. J. Vondrák (PRA-JV21174, holotype).

Type sequences: ITS (MZ969004), mtSSU (OK019733; PRA-JV24712, paratype).

Diagnostic characters: Thallus brown (or olive-brown), of thick areoles to squamules. Perithecia largely immersed in thallus, 0.3–0.4 mm in diam. Involucrellum present and often laterally spreading. Ascospores long and slender (length/width ratio mostly between 4.1–4.9), 16–28 × 4–8 µm.

Morphology-anatomy: Thallus brown with olive-green tinge, epilithic, areolate to squamulose. Areoles/squamules ~100–240 µm thick, 0.2–1.2 mm in diam., of variable irregular shape. Margin of older squamules divided into large granular structures. Pseudocortex weakly developed, with dull greyish brown pigment, patchy or entirely absent in some parts. Algal cells dispersed throughout most of the thallus, spherical to slightly elongated (length/width ratio up to 1.6), arranged in clusters, not in vertical stacks; mature cells 12–21 µm in diam, each dividing into several (3–6 in optical view) daughter cells of 6–12 µm in diam. Perithecia from 3/4 to fully immersed in thallus, ~200–300 µm in diam. Melanized perithecial wall in upper third of the fruiting body 30–55 µm wide. Involcurellum indistinguishable from exciple in the upper half, but sometimes distinctly separated in the basal part and spreading laterally to a diameter of up to 450 µm. Exciple brown-orange from top to bottom, turning olive-brown after application of ~10% KOH. Ascospores uniformly slender, (16.0–) 23.6–26.6 (–28.0) × (4.0–) 5.8–6.6 (–8.0) µm in water, only a single spore with an extreme length of 40 µm was seen; length/width ratio (2.9–) 4.1–4.4 (– 5.6); n=40.

Chemistry: No acetone-soluble secondary substances detected by TLC in the thallus of the type specimen. Internal pigments (other than melanins) not detected in thallus.

Ecology and distribution: Known from the type locality only. Occurring on andesitic rocks and stones at river/stream bank, occasionally inundated by water. Co-occurring species: same as for *V. substerilis*.

Similar species: The thallus morphology and the perithecia are very similar to *V. rosula* and *V. nodosa* (Orange 2013), but the spores in these species are wider (7.5–11.5 µm) and of a far less elongated shape (length/width ratio 2.0–3.1). A colour change of the brown exciple pigmentation upon application of KOH is not known from these species. Sterile thalli may be distinguished by the size of mature photobiont cells (just before division) which in *V. rosula* and *V. nodosa* have never been reported larger than 10 µm, only half the maximum size of the algal cells predominantly found in some of the thalli of *V. tenuispora*.

DNA data: ITS sequences of the holotype and PRA JV24712 are identical and form a lineage with unresolved relationships to related species: *Verrucaria maculiformis*, *V. substerilis*, *V. teyrzowensis*, and *Verrucaria* spp. 7–10 (Fig. 8). The mtSSU sequences of both above mentioned specimens are identical and do not have any closer NCBI relatives (identities < 97%), but they are close to the sequence of *V. substerilis* (98.5% identity).

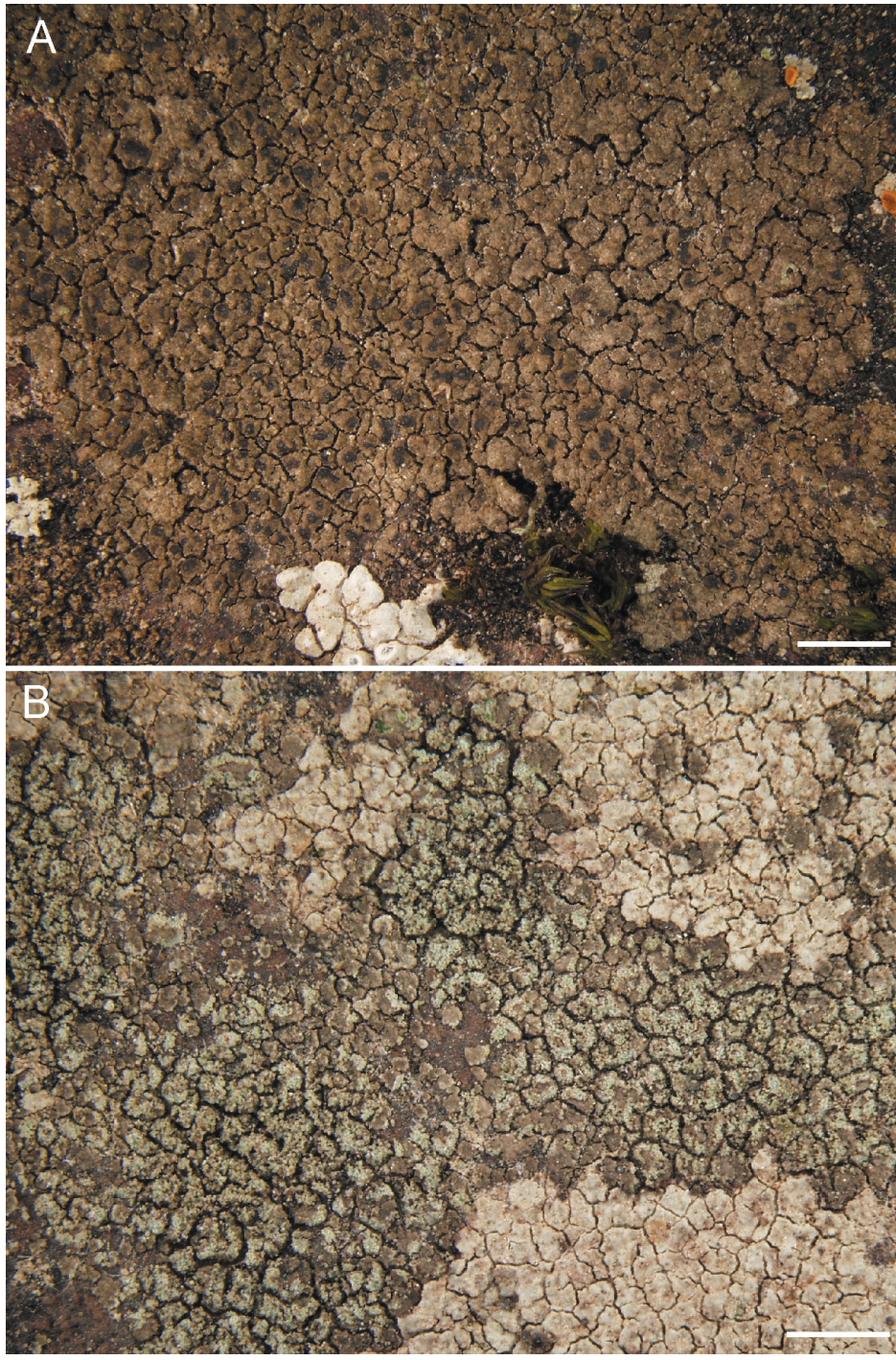


Fig. 9. New species: (A) *Verrucaria tenuispora*, holotype. (B) *Verrucaria teyrzowensis*, holotype, in mosaic with *Rinodina* cf. *oxydata* (pale thallus). Scales: A, B – 1 mm.

Paratypes: Type locality, on periodically inundated andesitic rock, 22 Oct. 2019, coll. J. Vondrák (PRA JV24692); Ibid.: 25 Febr. 2021, coll. J. Vondrák (PRA JV24712).

***Verrucaria teyrzowensis* Vondrák et Thüs, spec. nova**

MycoBank: MB#841132; Fig. 9B

Etymology: Named after the important type locality of this species and the others described here. Teyrzow is the transcription of the locality name (Týřov, Tejřov) used in the 18–19th century.

Type: Czech Republic. Central Bohemia: district Rakovník, Týřov National Nature Reserve, Berounka river bank below Týřovické skály rocks, alt. 250 m, 49.98227N, 13.79289E. On base-rich siliceous rock at riverside, 22 Oct. 2019, coll. J. Vondrák (PRA-JV21178, holotype).

Type sequences: ITS (MZ969005).

Diagnostic characters: Thallus areolate to squamulose, sorediate. Pale grey-green soredia formed in soralia at the margins of squamules. Soredia finely granular, 15–25 µm diam. No other *Verrucariaceae* with vegetative propagules share the above combination of characters.

Morphology-anatomy: Thallus forming extensive crusts of pale grey areoles and squamules. Squamules 0.2–1.0 mm diam., 80–150 µm thick, dispersed (at thallus margin), or densely aggregated in the thallus centre. Soralia pale grey-green, on areole/squamule margins, in some cases extending over the entire upper surface. Soredia fine, 15–25 µm diam., occasionally merged into consoredia, up to 50 µm diam. True cortex or pseudocortex locally developed, up to 20 µm thick, of few layers of isodiametric cells, ~5–7 µm diam. Algal layer of densely arranged clusters of algal cells (not arranged in vertical stacks). Mature algal cells 8–12 (–18) µm diam, each dividing into several (3–6 in optical view) daughter cells of 5–10 µm diam. Epinecral layer and medulla not developed, but black carbonized basal layer present in spots. Perithecia absent in the type material.

Chemistry: No acetone-soluble secondary substances detected by TLC. Internal pigments not detected in thallus.

Ecology and distribution: Known from the type locality only. Occurring on andesitic rocks and stones at river/stream bank, occasionally inundated by water. Co-occurring species: same as for *V. substerilis*.

Similar species: Three *Verrucaria* species with vegetative diaspores are present in the type locality of *V. teyrzowensis* (Týřov), which allows a direct comparison of these taxa under near identical environmental conditions. Based on data from sequenced specimens they show clear morphological differences to the new species: (i) *V. furfuracea* is perhaps the most similar, but differs in the brown colour of aroles and blastidia/soredia, the better developed cortex, containing a brown pigment, and the algal layer arranged into vertical stacks of small algal cells (< 10 µm diam.). (ii) *V. procopii* has a thicker thallus (200–600 µm), consistently well-developed cortex, ~25 µm thick, and an epinecral layer, ~10 µm thick. Its algal layer is formed of small algal cells (< 10 µm diam.) arranged in vertical stacks. Its medulla is well developed, more than 100 µm thick, of loose hyphae. (iii) *V. tectorum* has a thallus of dark brown, smaller areoles (not squamules), ~0.2–0.4 mm diam. and produces brown-black blastidia. In addition to these three formally described species, occasional occurrences of sorediate/blastidiate thalli have also been reported

from lichens which otherwise resemble *V. macrostoma* and *V. nigrescens.* Although the formal status of such forms requires further study, none of them has similarly fine and grey coloured soredia as *V. teyrzowensis*.

Another crustose-sorediate taxon in *Verrucariaceae*, which is known to occur occasionally in a sterile state only, is *Thelidium rimosulum* (Ceynowa-Giełdon 2007). This species occurs on calcareous substrata and differs by more or less rounded, punctiform (not coalescing) cream-coloured soralia developing from the centre of the areoles and a thinner and strictly crustose thallus, which never develops squamules.

Mechanical hybrids of crustose *Verrucariaceae* with sorediate and blastidiate species of *Bacidina* are known and can look like a sorediate *Verrucaria*, but in these forms, the soredia are of a much brighter green colour.

Morphologically the new species resembles sorediate/blastidiate members of *Caloplaca* s.str. (e.g. *C. chlorina*), but these taxa are recognizable by the absence of the black basal layer (melanized medulla) and usualy by the presence of *Sedifolia*-grey pigment (KOH+ sordid violet in section) in the tissues at the thallus surface and in soredia/blastidia.

DNA data: ITS sequences (two isolates of PRA JV21178) are identical in nucleotide positions and are unresolved within related *Verrucariaceae*: *Verrucaria maculiformis*, *V. substerilis*, *V. tenuispora*, and *Verrucaria* spp. 7–10 (Fig. 8). Sequencing of mtSSU failed.

Supplementary materials

Fig. S1. – Recent views of the area studied.
Table S1. – Dates of visits and man-hours spent sampling the fourteen sites.
Table S2. – Data for all the records for the area studied.
Table S3. – NCBI accession numbers for sequences of specimens included in this study.
Table S4. – Details of sequenced loci.
Data S1. – Catalogue of all the recognized taxa with comments on noteworthy records.
Data S2. – Catalogue of habitats.

Supplementary materials are available at www.preslia.cz

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Popelka princeznou – lokalita výjimečné druhové bohatosti lišejníků v dlouhodobě osídlené středoevropské krajině

Lokality s velmi vysokou druhovou bohatostí lišejníků jsou rozptýlené v mnoha oblastech světa, avšak publikované seznamy druhů z takových míst jsou zpravidla jen povrchní. Toto tvrzení podporuje náš detailní průzkum národní přírodní rezervace Týřov na Křivoklátsku. Na zhruba čtyřech čtverečních kilometrech jsme nalezli více druhů, než kdy bylo nalezeno na jiných lokalitách o velikostech do 10 km^2, a to v celosvětovém měřítku. Naše data prokazují, že Týřov je skutečně jedinečným hotspotem biodiverzity lišejníků v regionálním měřítku, především díky mimořádně bohaté škále vhodných substrátů, stanovišť a mikrostanovišť. Zároveň předpokládáme, že ve světě existuje celá řada druhově srovnatelných či dokonce mnohem bohatších lokalit, což patrně ukáže budoucí výzkum. Celkem 43 jednodenních návštěv studovaného území vyústilo v seznam 787 druhů (675 lišejníků, 35 pololišejníků, 58 lichenikolních hub, and 19 lišejníkům podobných hub). Území bylo rozčleněno na 14 dílčích lokalit s odlišnou stanovištní rozrůzněností. Druhová bohatost prokazatelně vzrůstala s množstvím dostupných stanovišť. Počet druhů na většině lokalit nijak výrazně nepřevyšoval regionální průměr, který ovšem výrazně převyšovaly tři stanovištně mimořádně bohaté lokality (s celkovou rozlohou kolem 80 hektarů). Zdaleka nejbohatší lokalitou jsou Týřovické skály, kde bylo nalezeno 502 druhů na rozloze pouhých 25 hektarů. Významnost Týřova je podtržena výskytem devíti druhů nových pro vědu (*Acarospora fissa*, *Bacidia hyalina*, *Buellia microcarpa*, *Micarea substipitata*, *Microcalicium minutum*, *Rufoplaca griseomarginata*, *Verrucaria substerilis*, *V. tenuispora* a *V. teyrzowensis*). Tři druhy jsou nové pro Evropu a 55 pro Českou republiku. Další čtyři byly v ČR znovu objeveny po více než osmdesáti letech. Zaznamenali jsme také velké množství druhů červeného seznamu: RE-6, CR-25, EN-56, VU-104. K řadě nalezených lišejníků jsme nenalezli existující jméno a mnohé z těchto takzvaných "known-unknowns" patrně představují nepopsané druhy.

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