

Methods for obtaining more complete species lists in surveys of lichen biodiversity

Jan Vondrák, Jiří Malíček, Zdeněk Palice, Brian Coppins, Martin Kukwa, Paweł Czarnota, Neil Sanderson and Andy Acton

J. Vondrák (j.vondrak@seznam.cz), Inst. of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ-252 43 Průhonice, Czech Republic. JV also at: Dept of Botany, Faculty of Biological Sciences, Univ. of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic. – J. Malíček, Charles Univ. in Prague, Faculty of Sciences, Dept of Botany, Benátská 2, CZ-128 01 Praha 2, Czech Republic. – Z. Palice, Inst. of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ-252 43 Průhonice, Czech Republic. – B. Coppins, 37 High Street, East Linton, Scotland, United Kingdom. – M. Kukwa, Dept of Plant Taxonomy and Nature Conservation, Univ. of Gdańsk, Wita Stwosza 59, PL-80-308 Gdańsk, Poland. – P. Czarnota, Dept of Agroecology, Faculty of Biology and Agriculture, Univ. of Rzeszów, Ćwiklińskiej 1a, PL-35-601 Rzeszów, Poland. – N. Sanderson, 3 Green Close, Woodlands, Southampton, SO40 7HU, United Kingdom. – A. Acton, Dailnamac, Taynuilt, Argyll, Scotland, United Kingdom.

We tested two methods to obtain more complete species inventories in surveys of lichen biodiversity. The first was to employ eight lichenologists (all experienced, some specialists) acting as individuals in parallel in a competitive survey. The second was to organize those lichenologists into two competing teams. We show that overall recorded biodiversity is distinctly higher than the part of lichen biodiversity recorded by each single lichenologist (45–66%) or team (79–83%). Use of these methods in a survey of epiphytic and epixylic lichens resulted in a list containing 112 species in 1 ha, 192 species in 12.5 ha and 212 species for 30 km² of lowland floodplain old-growth forest in southeastern Czech Republic. Eleven recorded species are new to the country; four are rediscovered after more than 50 years. In comparison, few previous surveys of mixed montane forests in the same region have yielded more than 200 species, even though it is certain that those forests have greater lichen diversity than our lowland forest.

Biodiversity inventories are undoubtedly an indispensable part of basic research, but it is very difficult, perhaps impossible, to obtain complete lists of species present in a large area. The problem applies to many groups of organisms (Chiarucci and Palmer 2009, Chiarucci et al. 2011) including lichens (Hunter and Webb 2002, Will-Wolf et al. 2004). The difficulty decreases as the investigated area becomes smaller, and for sufficiently small plots a complete list is achievable, e.g. as concluded by Klimeš et al. (2001) for vascular plants and by McCune and Lesica (1992) for lichens and bryophytes. Modern work on lichen biodiversity usually uses surveys of small plots, from which the number of species in a larger region is extrapolated (McCune et al. 1997, Nascimbene et al. 2010, Dymytrova et al. 2013, 2014, Ravera and Brunialti 2013). Only a few studies primarily focused on cryptogams simultaneously used different methodological approaches for getting relevant data, i.e. the combination of random (probabilistic) approach (sampling plots/quadrats or transects) and a non-random ‘floristic’ research focusing on specific microhabitats (Edwards et al. 2004, Newmaster et al. 2005, Ravera and Brunialti 2013). Newmaster et al. (2005) found that plot sampling of bryophytes is much less efficient for detecting rare species.

McCune and Lesica (1992) investigated which size of plot is best suited for making bryophyte and lichen inventories in various habitats. They concluded that the use of numerous small plots gives reproducible results, but fails to capture many of the species present in the habitat. Use of fewer but larger plots captures more species, but many records have a ‘random’ character: they represent rare species not found in most plots. In addition, any survey faces the practical problem that different recorders have different levels of skill, and many researchers have ‘blind spots’ for some groups of taxa (Ketchledge and Leonard 1984, McCune and Menges 1986, McCune et al. 1997, Klimeš et al. 2001).

Here we present and test methods for obtaining α and γ biodiversity data. We applied them to epiphytic and lignicolous lichens in a large old-growth floodplain woodland in the Czech Republic, where they appeared to give good results for both completeness and reproducibility. The underlying drawback is that they will be costly because they require the participation of several skilled lichenologists.

The methods tested include simple floristic surveys at three levels and with different intensities of study, as follows: 1) detailed survey of a 1 ha plot, 2) detailed survey in a 12.5 ha area of a well-preserved woodland, and 3) surveys in the

whole floodplain woodland of 30 km², in seven spots with a total area of 25 ha. Levels 1) and 2) used several well-trained field lichenologists working as individuals or in teams, and with an element of competition among individual researchers or between teams. We expected that a competitive element would increase motivation of involved researchers, both during the field work and in subsequent identification. Differences in results of individual recorders involved in inventories have been studied previously by botanists (Petřík and Boublík 2003) and can also be traced in lichenological literature with multiple-expert comparisons (McCune et al. 1997, 2009, Löhmus et al. 2012), but the importance of competition has not been evaluated.

Material and methods

Surveyed territory and field work

We selected a large flood-plain forest between the rivers Dyje and Morava in southern Moravia (Fig. 1A–C, Table 1) covering approximately 30 km². It was selected because it consists of fairly homogeneous lowland forest formations of native tree species (Table 1), it was presumed to have high lichen species richness (numerous tree species of variable age, bark texture and acidity), and is partly comprised of preserved old growth forests, i.e. the protected areas Cahnov (locations 1 and 2 in Table 1), Ranšpurk (location 3) and Soutok (location 7). Eight researchers (the authors) were involved in the main experiments (Table 1). All of them were experienced in

collecting and identifying European epiphytic lichens. The experiment was conducted over the period 30 March – 4 April 2014, the dates being chosen to provide good conditions for field work (good light conditions as leaves were absent, mild temperatures, and no mosquitoes). We examined the territory at three different spatial scales employing different methods (below).

One ha plot experiment

A single 1 ha square plot was randomly marked out by people not involved in the experimental surveys (location 1 in Table 1, Fig. 1C). The plot was intensively surveyed for 3 h by two independent teams, each team containing four co-operating specialists (details in Table 1). The teams operated mostly on separate half hectare areas, though there was some slight overlap. Records were listed for each half hour period, i.e. in six periods. Data from both teams were used to create species accumulation curves.

Floristic 12 h experiment

This took place in the territory circumscribed by the fence within the protected area ‘Cahnov’ (location 2 in Table 1, Fig. 1B–C) but excluding the 1 ha area used for the 1 ha plot experiment. Each of the eight investigators, working independently this time, recorded for 12 h (2 days; 6 h day⁻¹). Records were listed for each 1 h period. Data from all recorders were used to create species accumulation curves.

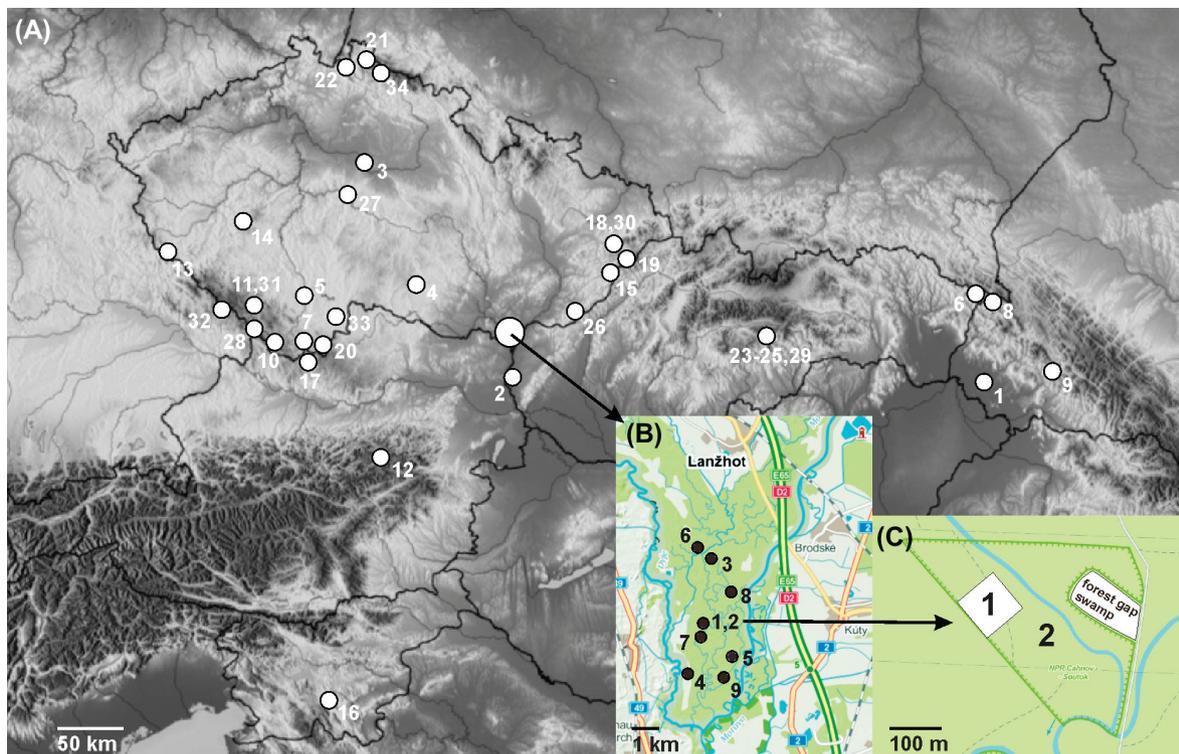


Figure 1. (A) location of the investigated woodland (large circle) and localities of various old-growth forest types used for biodiversity comparison, no. 1–34 correspond with Table 2, (B) visited sites inside the investigated woodland, no. 1–9 correspond with Table 1, (C) localities of experiments (sites 1 and 2) within the protected area Cahnov (circumscribed by green line).

Table 1. Sites observed in the investigated woodland (see also Fig. 1B–C). Person-hours reflect intensity of research. Detailed information to the sites 1–3 in Vrška et al. (2006).

site no	coordinates	Tree species (dominants/less frequent)	forest quality	area	person/hours	recorded species	visiting researchers/date
1	48°39'21"N, 16°56'21"E	AC, CB, FA/TC, UL, QR (QR - old, huge trees, often dead)	natural, old-growth	1 ha	27	112	AA, BC, PC, MK, JM, ZP, NS, JV/3 Apr 2014
2	48°39'22"N, 16°56'27"E	QR (huge trunks, often dead trees, highest age 400–450 years), AC, CB, FA/TC, UC, UL (presence of numerous decorticated snags and fallen trees)	natural, old-growth	12.5 ha	104	194	AA, BC, PC, MK, JM, ZP, NS, JV/1–2 Apr 2014
3	48°40'41"N, 16°56'48"E	As in the previous locality (2), but the forest floor is more shaded because of massive undergrowth of young trees	natural, old-growth	19.2 ha	24	101	JM, JV/11–12 Oct. 2013
4	48°38'19"N, 16°55'57"E	SF, POP, QR, FA (dominants)	managed forest margin	<1 ha	10	56	AA, BC, PC, MK, JM, ZP, NS, JV, F. Bouda, O. Peksa/31 Mar 2014
5	48°38'45"N, 16°57'28"E	CB (dominant)	managed, ca 80 years	<1 ha	20	83	AA, BC, PC, MK, JM, ZP, NS, JV, F. Bouda, O. Peksa/31 Mar 2014
6	48°40'59"N, 16°56'15"E	AC, CB, FA, QR (dominants)	managed, 80–130 years	<1 ha	30	93	AA, BC, PC, MK, JM, ZP, NS, JV, F. Bouda, O. Peksa/31 Mar 2014
7	48°39'8"N, 16°56'22"E	AC, CB, FA, QR/TC, UC, UL	natural, old-growth	1.2 ha	27	100	AA, BC, PC, MK, JM, ZP, NS, JV, J. Kocourková/3 Apr. 2014
8	48°40'6"N, 16°57'38"E	CB, FA (dominants)	managed, 140 years	ca 1 ha	14	88	JV, J. Šoun / 29 Mar 2014
9	48°38'23"N, 16°57'21"E	FA (dominant)	managed, 110 years	<<1 ha	5	24	AA, BC, PC, MK, JM, ZP, NS, JV, F. Bouda, O. Peksa/31 Mar 2014

Additional floristic research

The aim was to show differences between α diversity in the experimental site 'Cahnov' (locations 1 and 2 in Table 1, Fig. 1B–C) and γ diversity of the whole 30 km² area. Floristic research was performed in 7 sites scattered over the whole floodplain woodland of ca 30 km² (locations 3–9 in Table 1, Fig. 1B) were also investigated for lichen biodiversity. They were selected to cover the habitat variability within the floodplain forest and their total area is about 25 ha. Because this stage of the work involved both a larger area and greater habitat diversity, comparisons of the results with those from the earlier stages must be made with caution. This work used a total of 130 person-hours, with person-hours per site varying from a minimum of 5 to a maximum of 30. As in the 12 h experiment, recorders worked independently. Table 1 and Supplementary material Appendix 1, Table A1 have further details.

Material and data analyses

Epiphytic and lignicolous lichens, lichenicolous fungi and non-lichenized micro-fungi were recorded (Supplementary material Appendix 1, Table A1), but only lichens and facultatively lichenized fungi were included in analyses. By the latter we mean the genera *Chaenothecopsis*, *Leptorhaphis* and non-lichenized, non-lichenicolous species of the genera *Anisomeridium*, *Arthonia*, *Arthopyrenia*, *Lithothelium*, *Ramonia*, and the species *Melaspilea proximella*. To minimize errors in identification of lichens in the field, most species were collected, often repeatedly, and their vouchers are available in the herbaria of the authors (Supplementary material Appendix 1, Table A1). Only about twenty easily identifiable species were recorded without herbarium vouchers. TLC was used to identify some lichens (notes in Supplementary material Appendix 1, Table A1).

Data from recorders were collated by the first three authors, who also revised the suspicious records (possibly incorrectly identified or ambiguously identified specimens). Unidentified specimens (usually fragments of sterile thalli or some crusts with pycnidia only) were ignored. Several records do not match any species known to us. These are included in the analyses, marked either by 'cf.', or by the suffix 'nom. ined.' if the taxon will be formally described elsewhere (Supplementary material Appendix 1, Table A1).

Comparison with other inventories

We extracted presence/absence data for epiphytic lichens from 34 central European old-growth forest inventories to compare the number of species reached in our experiments with existing inventories of various forest types. We extracted data from Kondratyuk et al. (1997), Guttová and Palice (1999, 2002, 2004), Kondratyuk and Coppins (1999), Hafellner and Komposch (2007), Bilovitz et al. (2011), Guttová et al. (2012), Dymytrova et al. (2013), Malíček and Palice (2013), Malíček et al. (2013), Malíček and Vondrák (2014), Vondrák et al. (2015), and from eighteen unpublished inventories (Supplementary material Appendix 1, Table A3). Data extraction and work with our own dataset used the same taxonomic concepts.

Results

Overall, the 1 ha plot yielded 112 lichen species (Supplementary material Appendix 1, Table A1), but each research team recorded only 89 and 93 species (79% and 83% of this total). The species accumulation curves have a broadly similar shape for each team, though one team appears to have been a little more productive in the first half of the recording period and less productive in the second half (Fig. 2). Neither the species accumulation curves of each team or nor the total accumulation curve had flattened at the end of the 3 h recording period.

The 12 h experiment yielded a total of 194 species (Fig. 3, Supplementary material Appendix 1, Table A2) from the 12.5 ha area. The eight individual researchers recorded from 87 to 128 species (only 45% to 66% of the accumulated total). The individual species accumulation curves differ, but not dramatically. The three lowermost curves, which clearly cluster separately from the other six, belong to investigators without previous field experience in central Europe. The five upper curves have less scatter, with 114 to 128 species recorded at the end of the experiment (Fig. 3). None of the individual curves had completely flattened at the end of the recording period, though some appear to have been approaching saturation. The positive effect of an increasing number of researchers is evident; only 46 species (mostly common macro-lichens) were recorded by all researchers but 40 species were uniquely recorded by only one researcher. The number of recorded species is positively correlated with the number of researchers contributing to the investigation (Fig. 4).

The survey of another 7 sites (location 3–9 in Table 1) within the whole floodplain woodland (involving a further 130 person-hours of recording) increased only slightly the total number of species recorded (γ diversity of the whole 30 km² floodplain forest area), to 212 (112.5% of the 12.5 ha α diversity). The increase of the number of captured species from the 1 ha plot experiment to the whole 30 km² area is

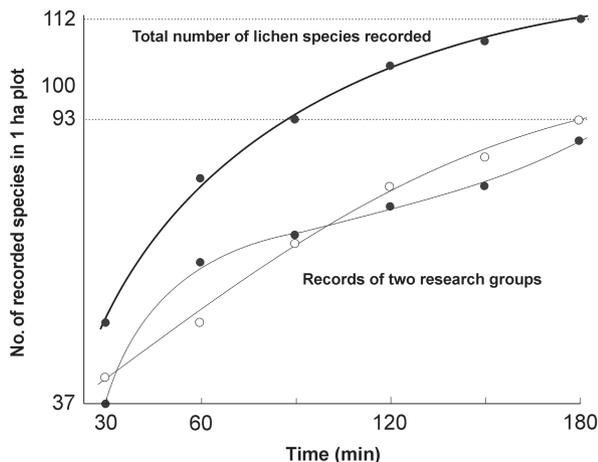


Figure 2. Number of lichen species recorded during the 1 ha plot experiment. Cumulative numbers are shown for six 30 min periods; results of two groups of researchers as well as total results are plotted. Results are approximated by 'species accumulation curves'; total curve is thicker. Curves drawn by hand.

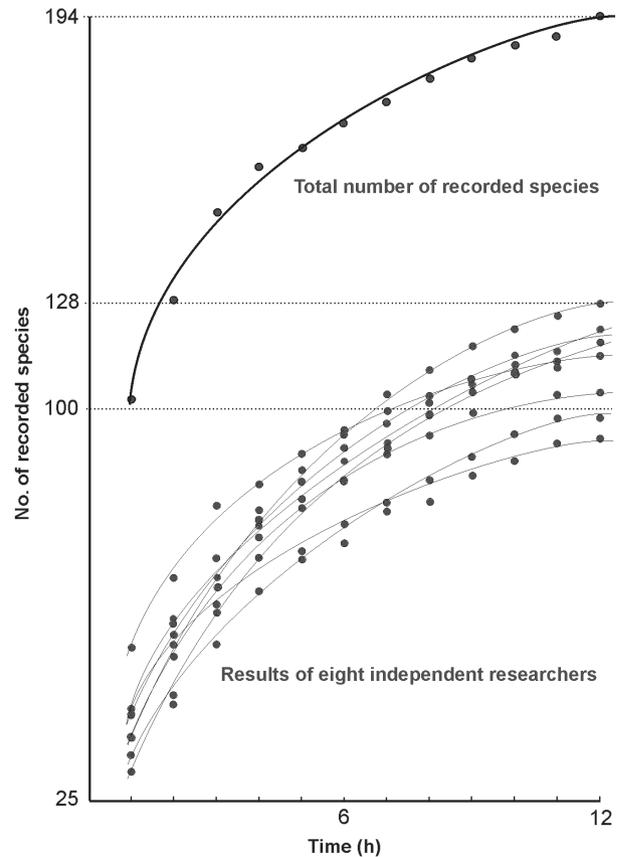


Figure 3. Number of lichen species recorded during the 12 h experiment (12.5 ha). Cumulative numbers are shown for twelve 1 h periods; results are shown for individual researchers as well as the total. Results are approximated by 'species accumulation curves'; total curve is thicker. Curves drawn by hand.

demonstrated by the species–area curve, shown in Fig. 5A. The total number of recorded species increased much more between 1 ha and 12.5 ha than between 13.5 ha and 30 km², but because the sampling effort per area decreased (for reasons of practicality) in larger territories, this observation must be interpreted with caution (see Discussion). Selected characters of the lichen biodiversity (γ diversity) captured within the project are summarized in Supplementary material Appendix 1, Table A2.

During our research, several unexpected species were recorded. *Agonimia borysthena*, *Anisomeridium macrocarpum*, *Biatora pontica*, *Chaenothecopsis rubescens*, *Lecanora quercicola*, *L. subcarpineae*, *Lithothelium hyalosporum*, *L. phaeosporum*, *Phaeophyscia rubropulchra*, *Strigula affinis* and *Verrucaria* cf. *viridigrana* were new for the Czech Republic. *Bacidia auerswaldii*, *Cresporhaphis wienkampii*, *Melaspilea proximella*, *Diplotomma pharcidium* and *Phaeophyscia pusilloides* were rediscovered in the Czech Republic after more than 50 years (cf. Liška et al. 2008). Some noteworthy species recorded during our research, e.g. *Arthonia pruinata*, *Arthothelium spectabile* and *Bactrospora dryina*, have already been published in a separate paper (Malíček et al. 2014). Three probably undescribed species were recorded during the lichen inventory (Supplementary material Appendix 1, Table A1: *Bacidia 'albogranulosa'*, *Micarea 'substipitata'* and *M. 'inconspicua'*).

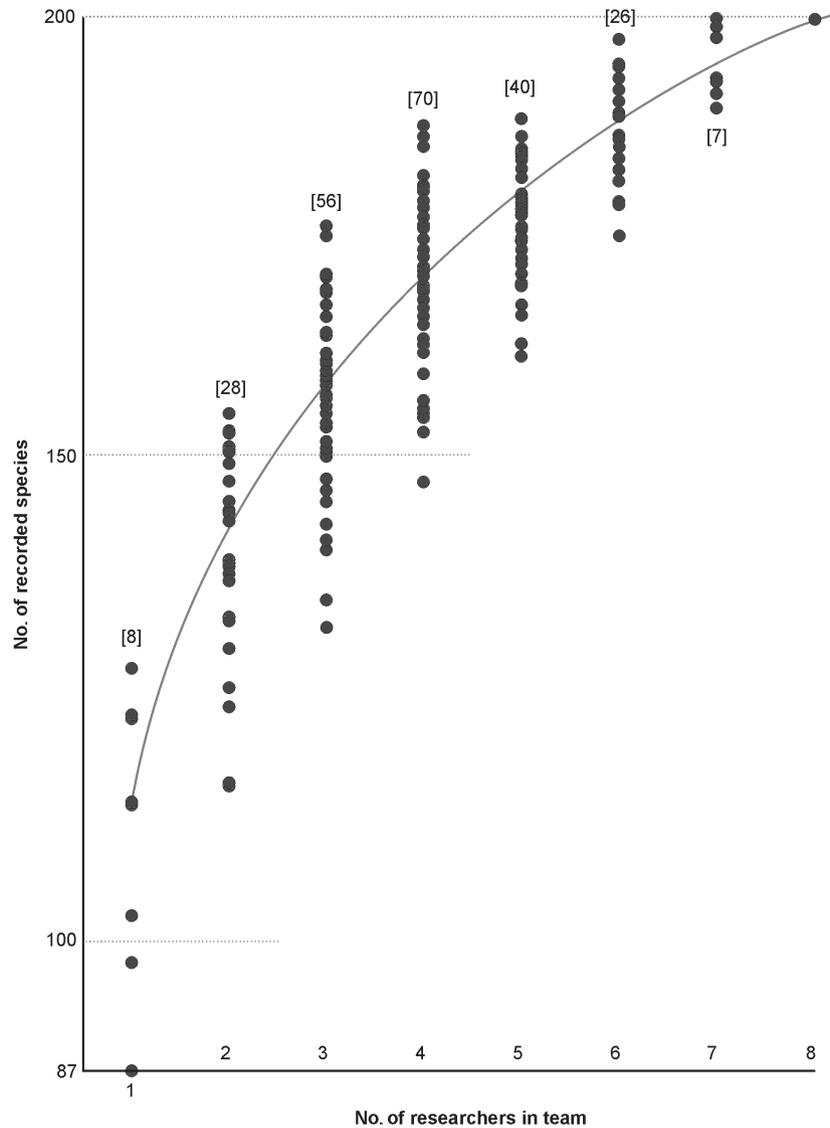


Figure 4. Relation between the number of included researchers and the number of recorded species (based on our datasets from eight researchers for the 12 h experiment). Possible combinations are in square brackets. Data approximated by a logarithmic curve with formula $114.1025 + 89.1625 \times \log_{10}(x)$.

Discussion

Raising number of researchers and competitive effect

In this paper, we evaluate the additional effect of raising the number of competing researchers/teams in a lichen inventory. Results from the 12 h experiment (Fig. 3) demonstrate that no one of the eight lichenologists managed to record more than 70% of the total species list obtained by collecting and correcting data from all researchers, even though the recorders are experienced and skilled workers. Similar results were obtained from the 1 ha plot experiment when the two four-member teams recorded about 80% of all recorded species (Fig. 2). However, these results are inevitably affected by dividing the area into two subareas surveyed separately by one of the teams (Methods). Clearly, raising the number of contributing lichenologists involved improves completeness

of lichen inventories (Fig. 4). Of course, there must come a point when further addition of researchers has negligible benefit, though we find it difficult to estimate just where that point would occur, even when including up to eight researchers in a team. Any estimate from our data might not work with different recorders or in other field conditions.

Employing numerous lichenologists and taking advantage of competition does not guarantee a complete lichen inventory, but the species list should be close to complete if individual species accumulation curves (Fig. 3) reach plateaus. The approach employing a group of researchers with an element of competition probably work best for small territories, up to tens of hectares, because individual accumulation species curves would not plateau in a reasonable time span in larger territories. However, even in larger territories, this approach will probably work better than inventories performed by a single researcher, even if his accumulation curve reached plateau.

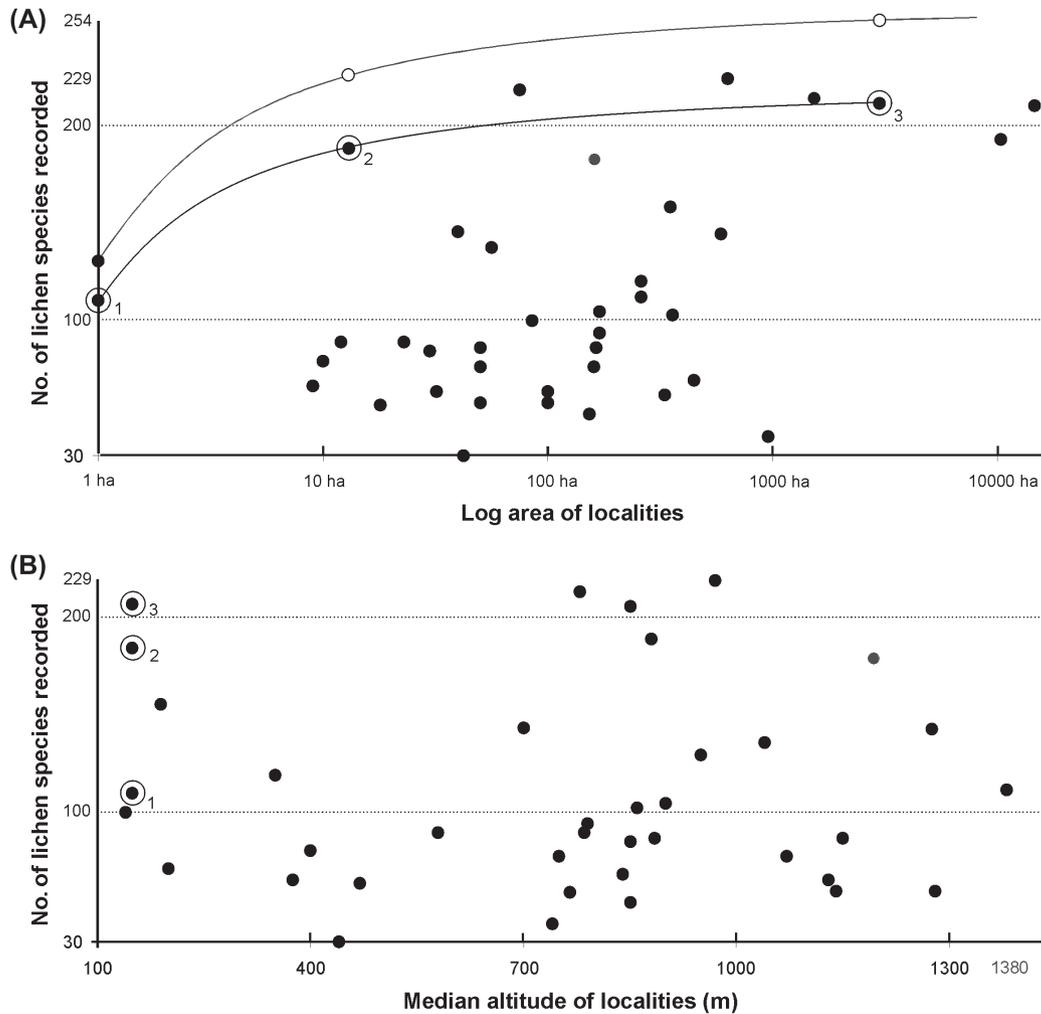


Figure 5. Our data (shown as dots in rings; 1 = 1 ha plot experiment, 2 = 12 h experiment, 3 = γ diversity in 30 km²) and results from 34 inventories of central European old-growth forests (Supplementary material Appendix 1, Table A3). (A) species–area relation (sampling effort per area has not been standardized due to missing data for the extracted inventories). Lower curve: species–area curve based on our three datasets; upper curve: hypothetical species–area ‘minimal’ curve for mixed mountain forests (explained in text). (B) species–altitude relation.

The effect of competition among lichenologists cannot easily be quantified and tested, but that there is such an effect is an obvious consequence of human nature (Kilduff et al. 2010). It will obviously tend to improve the completeness of species lists. In lichen survey work the ‘stakes’ are probably far too low for any undesirable effects of competition (such as identifying additional taxa on dubious grounds) to be a concern.

Our data in the context of central European surveys

The quality of inventory data obtained by our methods is demonstrated by comparison with 34 central European old-growth forest inventories (Fig. 1A, Supplementary material Appendix 1, Table A3). These diversity data produced by different methods for contrasting spatial scales are inevitably somewhat inconsistent, and we are aware of limitations in comparisons among the three data sets. Presumably these 34 inventories vary in quality according to who did the work, and how thoroughly. Methods of inventories, especially

intensity of research, must also differ among various spatial scales. This methodological drawback strongly influenced also our experiments in the three spatial scales: the number of person-hours ha⁻¹ decreased from 27 in the 1 ha experiment, to 8.3 in the 12.5 ha plot, and to 0.0014 in the 30 km² area.

The numbers of species recorded by these inventories are compared with ours in Fig. 5A–B. We recorded fewer species in the 1 ha plot than Hafellner and Komposch (2007) who precisely studied a 1 ha plot in a beech-dominated virgin montane forest remnant. This is consistent with our experience that montane forests generally have higher lichen biodiversity than lowland ones. The reason is that montane forests have a mix of both deciduous and coniferous phorophytes that support both lowland and montane species. Lower air pollution and higher humidity are also factors that may contribute to a higher diversity in montane regions. The higher species richness in montane mixed forests should be apparent in Fig. 5B, where maximum biodiversity would be expected at altitudes between 500–1200 m a.s.l. Despite the

large scatter, this is apparent in the chart (although the high number of species captured in our own detailed lowland inventories disturbs the pattern). We suggest that the relation between species numbers and altitude would show much less scatter and would have a unimodal distribution if all sites had been surveyed by a detailed standardized procedure, such as by using our methods.

Assuming similar species–area relations for lowland floodplain forests and for montane mixed forests, our datasets and the data by Hafellner and Komposch (2007) yield a hypothetical species–area ‘minimal’ curve for mixed forests (upper curve in Fig. 5A). Although Hafellner and Komposch (2007) made their 1 ha inventory carefully, they used only two recorders; our eight recorders captured noticeably more species. Our method, if employed in well-preserved montane mixed forests would probably get numbers of species above this species–area ‘minimal’ curve.

Problems in lichen survey methods

A serious difficulty in surveying epiphytic lichens is their uneven vertical distribution. Some species do not usually occur on the lowermost 2 m of the trunk, the part of the tree that is most accessible (Eversman et al. 1987, Fritz 2009, Ellis 2012, Marmor et al. 2013). The overlooked richness of lichen biodiversity in tree canopies was noted by Jarman and Kantvilas (1995) and Boch et al. (2013). The latter authors found that information on more than 50% of the lichen diversity may be lost if canopy lichens are not considered. Some otherwise detailed recent forest lichen inventories that unfortunately suffer from this problem (Dymytrova et al. 2013, Malíček and Palice 2013). Their species lists lack some canopy lichens and some common lichens restricted to twigs. To avoid this problem, we specifically searched for lichens on twigs and in the upper parts of trunks by observing fallen twigs (which is occasionally performed in lichen inventories, McCune et al. 1997) and by climbing trees. We also made the experiments in a locality containing a natural forest gap (Fig. 1C), where lichens on lower branches and sun-exposed young trees could easily be observed. The canopy makes a significant contribution to epiphytic lichen biodiversity (Supplementary material Appendix 1, Table A2); 24 of our species were observed only on twigs, and even this probably underestimates the diversity of canopy lichens, which were sampled mainly from fallen twigs.

The forests have many kinds of heterogeneity (Fritz and Heilmann-Clausen 2010, Löhmus et al. 2012, Blasy and Ellis 2014). Supplementary material Appendix 1, Table A1, A2 show many niche specialists (e.g. *Arthonia pruinata*, *Biatora veteranorum*, *Chaenotheca hispidula*, *Chaenothecopsis rubescens*, *Schismatomma pericleum* and *Verrucaria* cf. *viridigrana*) restricted to one substrate type. Many micro-lichens have been recorded from only one site (and usually recorded only once during the project); that is partly explained by the overlooking of some micro-habitats. More than half of the recorded taxa have been found at one, two or three sites only, but some of them have probably been overlooked in other sites.

Involving more lichenologists with different field experience of lichens in specialized niches is undoubtedly beneficial

for obtaining more complete species lists. Our study could be practical for assessment of the thresholds for an acceptable minimum number of contributing researchers (Fig. 4). In our 12 h experiment, species lists from single recorders varied between 50 and 65% of the accumulated total, while a combination of two recorders increase species capture to 60–75%, etc. If we suggest that our accumulated total list from 12.5 ha forest (12 h survey) is approaching 100% of species present, then the combination of five researchers is a reasonable minimum for reaching a threshold 75% of recorded species, which is a rather discouraging conclusion. We suggest that smaller plots should be selected for detailed inventories when completeness of species lists is the aim.

Acknowledgements – Linda in Arcadia and Christopher Ellis kindly revised the manuscript. František Bouda, Eva Budějcká-Jungwirthová, Jana Kocourková, Ondřej Peksa, Jaroslav Šoun, and David Svoboda generously provided their unpublished floristic data and/or helped in preparing field experiments. Petr Macek helped us with data analysing. We are supported by the long-term research development project no. RVO 67985939, by the program NAKI of the Ministry of Culture of the Czech Republic (DF12P01OVV025) and the Grant Agency of Faculty of Environmental Sciences, CULS, Prague, 42900/1312/3114. Andy Acton received a grant covering travel expenses from the British Lichen Society.

References

- Bilovitz, P. O. et al. 2011. Epiphytic lichen mycota of the virgin forest reserve Rajhenavski Rog (Slovenia). – *Herzogia* 24: 315–324.
- Blasy, V. and Ellis, C. J. 2014. Life on deadwood: cut stumps as a model system for the succession and management of lichen diversity. – *Lichenologist* 46: 455–469.
- Boch, S. et al. 2013. Up in the tree. The overlooked richness of bryophytes and lichens in tree crowns. – *PLoS One* 8: e84913.
- Chiarucci, A. and Palmer, M. W. 2009. The inventory and estimation of plant species richness. *Environmetrics. Encyclopedia of life support systems*. – <www.eolss.net/ebooklib/ebookcontents/E4-26-ThemeContents.pdf>.
- Chiarucci, A. et al. 2011. Old and new challenges in using species diversity for assessing biodiversity. – *Phil. Trans. R. Soc. B* 366: 2426–2437.
- Dymytrova, L. et al. 2013. Primeval beech forests of Ukrainian Carpathians are sanctuaries for rare and endangered epiphytic lichens. – *Herzogia* 26: 73–78.
- Dymytrova, L. et al. 2014. Topographic and forest-stand variables determining epiphytic lichen diversity in the primeval beech forest in the Ukrainian Carpathians. – *Biodiv. Conserv.* 23: 1367–1394.
- Edwards, T. C. Jr et al. 2004. Assessing rarity of species with low detectability: lichens in Pacific northwest forests. – *Ecol. Appl.* 14: 414–424.
- Ellis, C. J. 2012. Lichen epiphyte diversity: a species, community and trait-based review. – *Persp. Plant Ecol. Evol. Syst.* 14: 131–152.
- Eversman, S. et al. 1987. Vertical distribution of epiphytic lichens on three tree species in Yellowstone National Park. – *Bryologist* 90: 212–216.

- Fritz, Ö. 2009. Vertical distribution of epiphytic bryophytes and lichens emphasizes the importance of old beeches in conservation. – *Biodiv. Conserv.* 18: 289–304.
- Fritz, Ö. and Heilmann-Clausen, J. 2010. Rot holes create key microhabitats for epiphytic lichens and bryophytes on beech (*Fagus sylvatica*). – *Biol. Conserv.* 143: 1008–1016.
- Guttová, A. and Palice, Z. 1999. Lišajníky Národného parku Muránska planina I - Hrdzavá dolina [Lichens of National Park Muránska planina I - the Hrdzavá dolina Valley]. – In: Uhrin, M. (ed.), *Výskum a Ochrana Prírody Muránskej Planiny 2*. Správa Národného parku Muránska planina, Revúca (Slovakia), pp. 35–47.
- Guttová, A. and Palice, Z. 2002. Lišajníky Národného parku Muránska planina II - Javorníková dolina. [Lichens of National Park Muránska planina II - the Javorníková dolina Valley]. – In: Uhrin, M. (ed.), *Výskum a Ochrana Prírody Muránskej Planiny 3*. Správa Národného parku Muránska planina, Revúca (Slovakia), pp. 53–68.
- Guttová, A. and Palice, Z. 2004. Lišajníky Národného parku Muránska planina III - Cigánka. [Lichens of the Muránska Planina National Park III - Cigánka]. – *Reussia, Suppl.* 1: 11–47.
- Guttová, A. et al. 2012. Lišajníky Národného parku Muránska planina IV - Fábova hoľa. [Lichens of the Muránska planina National Park IV - Fábova hoľa]. – *Acta Rerum Nat. Mus. Nationalis Slovaci* 58: 51–75.
- Hafellner, J. and Komposch, H. 2007. Diversität epiphytischer Flechten und lichenicoler Pilze in einem mitteleuropäischen Urwaldrest und einem angrenzenden Forst. – *Herzogia* 20: 87–113.
- Hunter, M. L. and Webb, S. L. 2002. Enlisting taxonomists to survey poorly known taxa for biodiversity conservation: a lichen case study. – *Conserv. Biol.* 16: 660–665.
- Jarman, S. J. and Kantvilas, G. 1995. Epiphytes on an old Huon pine tree (*Lagarostrobos franklinii*) in Tasmanian rainforest. – *N. Z. J. Bot.* 33: 65–78.
- Ketchledge, E. H. and Leonard, R. E. 1984. A 24-year comparison of the vegetation of an Adirondack mountain summit. – *Rhodora* 86: 439–444.
- Kilduff, G. J. et al. 2010. The psychology of rivalry: a relationally dependent analysis of competition. – *Acad. Manage. J.* 53: 943–969.
- Klimeš, L. et al. 2001. Scale-dependent biases in species counts in a grassland. – *J. Veg. Sci.* 12: 699–704.
- Kondratyuk, S. et al. 1997. Lobarion lichens as indicators of primeval forests in the Ukrainian part of the international biosphere reserve 'eastern Carpathians': distribution, ecology, long-term monitoring and recommendations for conservation. – *Roczniki Bieszczadzkie* 6: 65–87.
- Kondratyuk, S. and Coppins, B. 1999. Basement for the lichen monitoring in Uzhansky national nature park, Ukrainian part of the biosphere reserve 'eastern Carpathians'. – *Roczniki Bieszczadzkie* 6: 149–192.
- Liška, J. et al. 2008. Checklist and red list of lichens of the Czech Republic. – *Preslia* 80: 151–182.
- Löhmus, P. et al. 2012. Old selectively cut forests can host rich lichen communities. – *Nova Hedw.* 95: 493–515.
- Maliček, J. and Palice, Z. 2013. Lichens of the virgin forest reserve Žofínský prales (Czech Republic) and surrounding woodlands. – *Herzogia* 26: 253–292.
- Maliček, J. and Vondrák, J. 2014. Příspěvek k poznání lichenoflóry Rašeliniště Jizery a Rašeliniště Jizerky. – *Bryonora* 53: 16–26.
- Maliček, J. et al. 2013. Lišajníky zaznamenané během podzimního bryologicko–lichenologického setkání v Novohradských horách. – *Bryonora* 51: 22–35.
- Maliček, J. et al. 2014. New lichen records and rediscoveries from the Czech Republic and Slovakia. – *Herzogia* 27: 257–284.
- Marmor, L. et al. 2013. Lichens on *Picea abies* and *Pinus sylvestris*. – *Lichenologist* 45: 51–63.
- McCune, B. and Lesica, P. 1992. The trade-off between species capture and quantitative accuracy in ecological inventory of lichens and bryophytes in forests in Montana. – *Bryologist* 95: 296–304.
- McCune, B. and Menges, E. S. 1986. Quality of historical data on midwestern old-growth forests. – *Am. Midland Nat.* 116: 163–172.
- McCune, B. et al. 1997. Vertical profile of epiphytes in a Pacific northwest old-growth forest. – *Northwest Sci.* 71: 145–152.
- McCune, B. et al. 2009. Macrolichen diversity in Noatak National Preserve, Alaska. – *North Am. Fungi* 4: 1–22.
- Nascimbene, J. et al. 2010. Effect of reduction in sampling effort for monitoring epiphytic lichen diversity in forests. – *Community Ecol.* 11: 250–256.
- Newmaster, S. G. et al. 2005. The ones we left behind: comparing plot sampling and floristic habitat sampling for estimating biodiversity. – *Divers. Distrib.* 11: 57–72.
- Petrík, P. and Boublík, K. 2003. Sources of variation in botanical grid mapping. – *Novitates Bot. Univ. Carolinae* 17: 17–23.
- Ravera, S. and Brunialti, G. 2013. Epiphytic lichens of a poorly explored National Park: is the probabilistic sampling effective to assess the occurrence of species of conservation concern? – *Plant Biosyst.* 147: 115–124.
- Vondrák, J. et al. 2015. Epiphytic lichens of Stužica (east Slovakia) in the context of central European old-growth forests. – *Herzogia* 28: 108–130.
- Vrška, T. et al. 2006. Dynamika vývoje pralesovitých rezervací v ČR II - Lužní lesy (Cahnov–Soutok, Ranšpurk, Jiřina) [Developmental dynamics of virgin forest reserves in the Czech Republic II - The lowland floodplain forests (Cahnov–Soutok, Ranšpurk, Jiřina)]. – *Academia, Praha*.
- Will-Wolf, S. et al. 2004. Lichenized fungi. – In: Mueller, G. M. et al. (eds), *Biodiversity of fungi: inventory and monitoring methods*. Academic Press, pp. 73–195.

Supplementary material (Appendix NJB-01053 at <www.nordicjbotany.org/appendix/njb-01053>). Appendix 1.

Supplementary material

Supplementary material Appendix 1, Table A1. Species recorded in the surveys. Bold font denotes species used in the analysis (lichens or similar); other (lichenicolous fungi or epiphytic micro-fungi) are listed but were not analyzed. Substrate abbreviations: AC = *Acer campestre*, AG = *Alnus glutinosa*, CB = *Carpinus betulus*, FA = *Fraxinus angustifolia*, POP = *Populus*, QU = *Quercus robur*, SAL = *Salix alba / fragilis*, TIL = *Tilia*, ULM = *Ulmus minor / laevis*. Collector abbreviations: AA, BC, JM, JV, MK, NS, PC, ZP are acronyms of the authors; FB, František Bouda; JŠ, Jaroslav Šoun. Abundance: 1 = rare, recorded from only one visited site; 2 = occasional, recorded from 2–3 sites; 3 = common, recorded from 4 and more sites.

Supplementary material Appendix 1, Table A2. Selected characteristics of epiphytic lichen diversity in whole studied territory (γ -diversity). Percent from all lichen species in the list are in brackets.

Supplementary material Appendix 1, Table A3. Basic data, including number of recorded lichen species, from 34 central European old-growth woodland inventories.

Vondrák et al. 2016. Methods for obtaining more complete species lists in surveys of lichen biodiversity. – Nordic Journal of Botany 34: 619–626.

Supplementary material Appendix 1, Table A1. Species recorded in the surveys. Bold font denotes species used in the analysis (lichens or similar); other (lichenicolous fungi or epiphytic microfungi) are listed but were not analysed. Substrate abbreviations: AC Acer campestre, AG Alnus glutinosa, CB Carpinus betulus, FA Fraxinus angustifolia, POP Populus, QU Quercus robur, SAL Salix alba / fragilis, TIL Tilia, ULM Ulmus minor / laevis. Collector abbreviations: AA, BC, JM, JV, MK, NS, PC, ZP are acronyms of the authors; FB, František Bouda; JŠ, Jaroslav Šoun. Abundance: 1, rare, recorded from only one visited site; 2, occasional, recorded from 2-3 sites; 3, common, recorded from 4 and more sites.

species	substrate	sites (according to Table 1 & Fig. 1B)									vouchers	abundance	note to identification
		1	2	3	4	5	6	7	8	9			
Absconditella lignicola	wood	x	x								JV, MK, PC(2), ZP(2)	2	Sample ZP17555 approaching <i>Absconditella amabilis</i> T.Sprib. Usually with perithecia, but anamorphic crusts seen (JV11974). BC specimen with smaller spores (14.5-16.5 µm long) somewhat resembling <i>A. cavata</i> .
Acrocordia gemmata	AC, CB, FA, QU, TIL, ULM, Crataegus	x	x	x		x	x	x	x		BC, JM(2), JŠ, JV(5), MK(2), PC(2), ZP(3)	3	
Agonimia allobata	AC, FA, QU, TIL, ULM, wood	x	x				x		x		JM, JV(2), ZP	3	
Agonimia borysthenea	CB, TIL, ULM	x	x						x		JV, PC, ZP(2)	2	It matches the description by Dymytrova et al. (2011) except the thallus - distinct hyaline hairs up to 10 µm long observed in our material - are not mentioned in the protologue. Our examination of the isotype from W showed hairs only on several juvenile areoles; otherwise the isotype fits our specimens: overall habit, almost isodiametric globose areoles, black non-furrowed perithecia with 8 ascospores in asci.
Agonimia repleta	TIL		x								PC, ZP	1	
Agonimia tristicula	CB, QU, wood (bryophytes)		x								BC, PC ZP (cum Placynthiella icmalea)	1	Only sterile specimens recorded; but their subsquamulous thalli are distinct from other lichens.
Ahlesia lichenicola	wood		x										
Amandinea punctata	AC, CB, FA, QU, SAL (also twigs), TIL, ULM, Crataegus, Euonymus, wood	x	x	x	x	x	x	x	x	x	FB, JM, MK(3), PC(3), ZP	3	
Amphisphaeria fallax	AC							x			JV		
Anaptychia ciliaris	FA (twigs)		x						x			2	

Ascodichaena sp.	FA (twig)		x		x					JV		
Bacidia albogranulosa	nom. ined.	AC	x	x					x	JM(3), JŠ, JV(4), MK, ZP	2	Grey-white soresiate crust; apothecia and pycnidia absent; TLC: atranorin; ITS nrDNA sequence data for two (JV) samples obtained Hypothecium colourless, epihymenium brown, ascospores 25-32 x 4.5-6.5 µm; thallus of tiny granules (smaller than in B. subincompta), sometimes with blackish pigmentation
Bacidia auerswaldii		AC	x	x					x	JM, JŠ, JV(2), PC(2)	2	
Bacidia circumspecta		AC, CB	x	x					x	JV, PC(3), ZP	2	
Bacidia fraxinea		AC, CB, FA, QU	x	x	x			x	x	FB, JM, JV, MK, PC(2), ZP	3	
Bacidia incompta		AC, FA, ULM	x	x	x				x	AA, JM(2), JV, NS, ZP(2)	3	
Bacidia pycnidiata		AC, QU, wood	x	x						JM, ZP(2), PC	2	
Bacidia rubella		AC, CB, FA, POP, TIL	x	x	x			x	x	JV(2), MK, PC	3	
Bacidia subincompta		AC, TIL	x		x					NS	2	
Bacidia trachona		AG, CB (trunk base)		x	x					BC, JV	2	Apothecia absent; pycnidia large black; wall K+ purple; conidia 3-4 x 1.5 µm; thallus K+ yellow; TLC: no substances.
Bacidina brandii		QU	x							PC	1	Thallus not soresiate/blastidiate; apothecia with brownish discs and paler margins; hypothecium with Arnoldiana-brown pigment.
Bacidina chlorotricula		AC, FA, ULM (twigs), CB		x					x	JV, ZP(2)	2	Minute white apothecia found together with immersed pycnidia with crescent/narrowly-sickle shaped macroconidia (ca 15 x 1.5 µm) that are not mentioned in Ekman (1996) for this taxon.
Bacidina cf. neosquamulosa		FA, wood, fallen branch	x	x	x					MK, PC	2	Thallus areolate, hypothecium very pale/colourless, epihymenium olive, K-, excipulum at the top orange brown, K+ darkening, ascospores acicular, c. 40 x 1.5 µm.
Bacidina sulphurella		AC, CB, FA, QU, TIL, wood	x	x	x				x	JM, JV, MK, PC, ZP(3)	3	Specimen ZP 17707 is richly fertile but its pycnidia contain non-hooked conidia. Hooked conidia found in some other specimens (e.g. JV11934).
Bactrospora dryina		FA, QU, TIL	x	x	x				x	JM(3), JŠ, JV(2), PC, MK	3	Ascospores filiform, breaking down within asci into cylindrical part-spores, up to 8 µm long c. 3 µm wide
Biatora albohyalina		CB		x						BC, JM, JV	1	All specimens in anamorphic stage.
Biatora globulosa		QU							x	JM	1	

Biatora pontica	CB	x	x							JM, MK(2), NS(2), ZP	2	TLC (ZP, MK samples): thiophanic acid, asemone, pontica unknown (in 366 UV++ white after reaction with sulphuric acid)
Biatora veteranorum	QU (bark, wood), (rarely TIL)	x	x	x						JM, JV(2), PC, MK, ZP(2)	2	Mostly in anamorphic stage.
Biatoridium monasteriense	AC, ULM		x	x		x	x			JM(2), MK(2), ZP(2)	3	
Bryoria cf. fuscescens	dry wood, fallen twig		x						x	JV	2	
Buellia griseovirens	CB, FA (also twigs), Crataegus, Euonymus, wood	x	x	x		x		x	x	JV(2), PC	3	
Calicium adpersum	CB, QU		x				x	x		BC, JM, JV, ZP	3	In anamorphic stage. Thallus with Norstictic acid (K reaction is distinct; confirmed by TLC)
Calicium glaucellum	wood	x	x						x	JM(2), MK, PC(4), ZP	2	
Calicium salicinum	QU		x				x			PC(2)	2	
Caloplaca obscurella	AC, CB, FA, POP, PYRUS, QU, SAL, TIL, ULM, wood	x	x	x	x	x	x	x	x	JM(4), JV, PC(2), ZP	3	
Caloplaca pyracea	AC, FA, SAL, POP (often twigs)		x		x			x	x	ZP	3	
Caloplaca substerilis	AC, (rarely CB)	x	x						x	JM(2), JV(2), MK, ZP(2)	2	Sterile, rarely with yellow pycnidia.
Candelaria concolor s.str.	FA, QU, ULM	x	x		x					JM	2	
Candelariella efflorescens s.lat.	AC, CB, FA, QU, SAL, TIL, ULM (often twigs), wood	x	x	x	x		x	x	x	MK, PC	3	Sterile thalli with marginal soralia on squamules (not <i>C. reflexa</i>)
Candelariella vitellina	FA, wood		x		x					MK, PC	2	
Candelariella xanthostigma	AC, CB, FA, POP, QU, SAL (often twigs), wood	x	x	x	x	x		x	x	PC, ZP	3	
Catillaria fungoides	AC, FA, QU (twigs), POP		x	x	x	x			x	JM, JV, PC, ZP	3	
Catillaria nigroclavata	AC, CB, FA, QU, POP, SAL, TIL, ULM (often twigs), Crataegus	x	x	x	x	x	x	x	x	FB, JM(2), JV, MK(3), PC(2), ZP	3	

Catinaria atropurpurea	FA, QU	x						JM, JV(2), PC(3), ZP	1	Pycnidia present (unknown in literature) and some crusts only with pycnidia: pycnidia mostly immersed in thallus; pycnidial wall pale (yellow-orange), rarely blackened around ostiole, C-, K-, N-, conidia 4-5 x 1.5-3 µm
Chaenotheca brachypoda	AC, FA, QU	x	x				x	JM, JV	2	
Chaenotheca brunneola	wood of snag	x						MK	1	
Chaenotheca chrysocephala	FA, CB, QU, TIL, wood of snag	x	x	x			x	AA, ZP	3	AA sample; sterile yellow crust, partly with leprose appearance
Chaenotheca ferruginea	FA, POP, QU, TIL	x	x	x			x	ZP	3	
Chaenotheca hispidula	AC	x						PC	1	Two apothecia present only; thallus endophloedal, with <i>Trentepohlia</i> .
Chaenotheca phaeocephala	FA (rarely CB, TIL, QU, POP)	x	x	x			x	FB, JM(4), JŠ, JV(4), MK(2), NS(2), PC, ZP(2)	3	Some specimens with colourless stalks (albinomorphs)
Chaenotheca stemonea	CB, FA, QU, TIL	x	x				x	JM, MK, PC, ZP	3	Usually sterile; TLC (ZP17665): barbatic and cf. obtusatic acids. PC7696 is fertile.
Chaenotheca trichialis	AC, CB, FA, QU, TIL, ULM, wood	x	x	x			x	JV	3	
Chaenotheca xyloxena	wood	x					x	JM, JV, NS, PC	2	
Chaenothecopsis debilis	wood	x						AA, FB, JV, NS, PC, ZP	1	Stalk with prevailing reddish pigment, N+ purple; hypothecium ±black-green, N-; ascospores 1-septate, with dark septa, 5-7(9) x 2-3 µm; on hard wood; photobiont absent. (concept of Groner 2006)
Chaenothecopsis cf. nigra	CB (wood in hollow trunk), QU	x	x				x	AA, BC(2), JM, MK, ZP(2)	2	Hypothecium brown or olive green, stalk orange-brown, K-, N± intensively orange, ascospores 1 septate, pale, with darker septa, 5-7 x 2-2.5 µm; ± associated with <i>Stichococcus</i> . (orange-brown pigment in stalk does not fit <i>C. nigra</i> sensu Groner 2006); ZP17739 associated with <i>Stichococcus</i> algae on bark of <i>Quercus</i> matches <i>Ch. nigra</i> well
Chaenothecopsis pusilla	wood	x					x	AA, JM, JV, MK, NS, PC(2)	2	Green pigment in hypothecium and stalk, N- or N+ green intens., K+/- brown; ascospores 1-septate, 5-6 x 2 µm, with pale septum; some samples on <i>Stichococcus</i> crust. (concept of Groner 2006)
Chaenothecopsis rubescens	QU	x						JV	1	Distinct K+ red reaction of hypothecium; 0-septate ascospores; on dead white <i>Trentepohlia</i> crust in old QU bark fissures
Chrysothrix candelaris	QU	x	x					JM	2	
Cladonia cenotea	wood	x	x				x	JM, JV, ZP	2	

Cladonia chlorophaea	wood		x		x					MK, PC(2)		2	
Cladonia coniocraea (incl. <i>Cl. ochrochlora</i>)	CB, SAL, dry wood, mossy wood	x	x	x	x	x	x	x	x	JM		3	
Cladonia digitata	wood	x	x				x	x				3	
Cladonia fimbriata	AC, FA, QU, SAL, Crataegus, wood	x	x	x	x	x	x	x	x	JM, JV, ZP		3	TLC (ZP17684): fumarprotocetraric and cf. physodic acids
Cladonia glauca	wood		x							MK		1	
Cladonia incrassata	wood	x	x							JM, MK(2), PC, ZP		2	TLC (ZP, MK12509): didymic and squamatic acids, ±usnic acid; (MK12489): barbatic and thamnolic acids
Cladonia macilenta	dry wood	x	x	x	x		x	x		ZP		3	TLC (ZP17674): thamnolic, barbatic and didymic acids
Cladonia parasitica	dry wood		x					x		JM(2), JV(2), MK(3), PC		2	Squamules K+ yellow and Pd+ intensely yellow
Cladonia pyxidata	wood		x									1	
Cladonia squamosa	wood		x									1	
<i>Clypeococcum hypocenomyces</i>	Hypocenomyce scalaris		x					x					
Coenogonium pineti	CB, QU, TIL, wood	x	x	x			x	x	x	PC, ZP(2)		3	Sometimes only with pycnidia.
Cresporhaphis wienkampii	SAL QU, on Verrucaria						x			BC		1	
<i>Dactylospora</i> sp.	viridigrana		x					x		JV			
Diplotomma pharcidium	CB		x							BC		1	Apothecia little developed but with distinct true exciple; ascospores 3-septate, 18-20 x 6-7 µm, dark; conidia straight, 6-8 x 1 µm; norstictic acid absent.
Eopyrenula leucoplaca	AC, CB, FA, QU AC, CB, FA, QU, SAL, TIL (often twigs), Crataegus,	x	x			x	x	x	x	JV(6), MK, NS, PC(3), ZP(5)		3	Perithecia rare, (eg. JV12009); anamorphic crusts common, recognizable by broadly ellipsoid, slightly melanized (blue-grey), 1-septate conidia, 7.0-8.5×3.5-4.0 µm.
Evernia prunastri	Euonymus, wood	x	x	x	x	x	x	x	x	MK, PC		3	
<i>Exarmidium inclusum</i>	wood		x							x ZP(2)			

Flavoparmelia caperata	AC, CB, FA, QU, SAL, TIL (also twigs), Crataegus, Euonymus, wood	x	x	x		x	x	x	x	FB, JM	3	
Graphis scripta s.lat.	CB, (rarely ULM)	x	x	x		x		x	x	JM, JV(3), MK(2), ZP	3	Samples of MK identified as <i>Graphis betulina</i>
Halecania viridescens	AC, CB, FA, QU, TIL (twigs)	x	x	x		x	x	x	x	JM, JV(2), MK, PC, ZP(2)	3	
Hyperphyscia adglutinata	AC, CB, FA, QU, POP (usually twigs)		x		x	x				JM, JV(2), PC, ZP(2)	2	Only young thalli observed
Hypocenomyce scalaris	QU, wood		x	x		x	x	x	x		3	
Hypogymnia physodes	AC, CB, FA, QU, SAL (mostly twigs), Crataegus, Euonymus, dry wood	x	x	x	x	x	x	x	x	FB	3	
Hypogymnia tubulosa	FA, QU, TIL, SAL (twigs), Euonymus	x	x	x	x	x	x	x	x		3	
Hysterium angustatum	POP, ULM		x							JV, ZP		
Hysterium pulicare	FA, QU		x							BC, MK		
Hysterobrevium sp.	wood		x							ZP		
Hysterographium fraxini	FA (twig), decorticated branch on dead tree		x							BC, AA		Brown muriform spores 32x15 µm
Illosporopsis christiansenii	Physcia adscendens, P. tenella		x				x			MK		
Imshaugia aleurites	dry wood		x								1	Excipulum present also in lower side of perithecium; ascospores 22-24-28 x (7)8-11 µm, finely dotted. Sample JV11976 with ascospores 26-30 x 7-9 µm, brown, with smooth wall, hamathecium with dense paraphysoids.
Kirschsteiniothelia aethiops	AC, FA, TIL		x	x						BC, JV, ZP		
Kirschsteiniothelia recessa	CB, ULM		x							JV(2)		
Lecania croatica	AC, CB, FA, QU, TIL, ULM	x	x	x		x	x	x	x	FB, JM(4), JV, MK, PC(2), ZP(3)	3	Only sterile specimens recorded.

Lecania cyrtella	AC, CB, FA, POP, QU, SAL, TIL, ULM (twigs), Sambucus	x	x	x	x		x	x	x	JM, JV, MK, PC, ZP	3	
Lecania cyrtellina	AC, CB		x				x			JM, JV(2), PC(2)	2	Ascospores usually simple, very thin (2-3 µm), conidia curved of two types (thin, non-septate and thicker, 1-septate); different ecology than in <i>L. cyrtella</i> - on shaded trunks of old-growth <i>Acer campestre</i> .
Lecania naegelii	AC, CB, FA, POP, QU, SAL, TIL (twigs), Crataegus, Sambucus	x	x	x	x	x	x	x	x	JM, JV(2), MK, PC(3), ZP(2)	3	
Lecanidion atratum	wood		x							ZP		Det. M.Šandová
Lecanora argentata	CB	x	x	x		x		x		JV, NS, ZP	3	
Lecanora chlarotera	CB, FA	x	x		x					JV(2), MK(3), PC	2	
Lecanora compallens	QU		x							MK	1	TLC: usnic acid, zeorin, +1 terpenoid (trace)
Lecanora conizaeoides	QU, dry wood	x	x		x		x	x		NS, PC	3	
Lecanora dispersa s.lat.	wood				x					MK(2)	1	
Lecanora expallens	AC, CB, FA, POP, PYRUS, QU, SAL, TIL, ULM, wood	x	x	x	x	x	x	x	x	JM(2), JV(3), MK(8), PC(2), ZP(3)	3	TLC (MK, all specimens): usnic acid, thiophanic acid, zeorin, cf. arthothelin, expallens unknown, ice blue terpenoid just above thiophanic acid
Lecanora glabrata	CB	x	x	x						JM(2), MK, NS, ZP	2	
Lecanora leptyroides	CB	x	x	x		x		x	x	JM(3), MK(2), PC(2)	3	Some specimens may be identified as <i>L. carpineae</i> , but they fall into <i>L. leptyroides</i> sensu Lumbsch et al. 1997
Lecanora persimilis	wood		x			x				JV	2	
Lecanora pullicaris	CB		x							ZP	1	
Lecanora quercicola	wood				x					MK	1	Distinguished from <i>L. saligna</i> on basis of conidial size (van den Boom & Brand 2008)
Lecanora saligna	AC twigs, wood	x	x	x	x	x	x	x		JV, PC(2), ZP	3	
Lecanora saxicola	wood				x					MK, PC	1	

Marchandiomyces corallinus	Hypogymnia, Parmelia, Physcia, Xanthoria		x				x				MK(2)		
Melanelixia glabratula	AC, CB, FA, QU, TIL, Crataegus	x	x	x	x	x	x	x	x	x	JM, ZP	3	
Melanelixia subargentifera	FA (twigs), FA, QU (twigs), Crataegus,		x							x	ZP	2	
Melanelixia subaurifera	Euonymus, Juglans		x	x		x	x	x	x		NS	3	
Melanohalea elegantula	AC, FA, QU (twigs), Euonymus	x	x		x	x				x	JM(3), JV(2), MK, ZP(2)	3	TLC (JV12018): no compound detected
Melanohalea exasperatula	AC, FA, POP, QU, SAL, ULM, Juglans (twigs), Crataegus	x	x	x	x	x	x	x	x		ZP(2)	3	Apothecia and pycnidia with purplish, K+ dark green pigment like in <i>Micarea nigella</i> , Redinger (1937) reports tiny pycnidia with small bacilliform conidia while the present material contains large pycnidia (0.15-0.35mm, only apically pigmented) with large simple conidia 7.5-9.5 x (4-)4.5-5 µm
Melaspilea gibberulosa	AC, (rarely CB, FA, QU)	x	x	x					x		FB, JM(3), JŠ, JV(6), MK(2), PC(6), ZP	3	
Melaspilea proximella	QU				x						JV	1	
<i>Melaspilea</i> sp.	wood		x					x			JV, NS		Non-lichenized taxon with ciliate ends of spores
Micarea botryoides	wood		x					x			PC(2), ZP	2	Anamorphic state.
Micarea byssacea	wood		x								JM, MK	1	TLC (both samples): methoxymicareic acid.
Micarea substipitata nom. ined.	wood		x								NS, ZP	1	Undescribed species close to <i>Micarea myriospora</i> . Habitually similar to <i>Biatora veteranorum</i> .
Micarea denigrata	wood		x			x	x				JM, JV, NS, PC(3), ZP	2	Anamorphic state.
Micarea inconspicua nom. ined.	wood	x	x								PC(2), MK, ZP	2	Undescribed species, member of <i>Micarea prasina</i> group. Apothecia very small, hyaline, spores ovoid, 1-septate, thallus inconspicuous.
Micarea melaena	wood		x								BC	1	
Micarea micrococca	wood		x	x		x			x		JM, JV(4), MK, PC, ZP JM, JV(3), MK, NS, PC(3), ZP	3	TLC (MK12387, ZP17695): methoxymicareic acid; apothecia white
Micarea misella	wood	x	x			x	x				ZP	3	

Micarea prasina	QU, rotten wood	x	x								JM(3), JV, MK(2), PC(2), ZP(2)	3	TLC (JM, MK12441, ZP17697): micareic acid; dark form, epihymenium C+ violet, K+ violet, section not red in C
Muellerella hospitans	on Bacidia fraxinea (ap)										PC		
Mycocalicium subtile	dry wood		x								MK		Eurotiomycetes
Ochrolechia turneri	FA, QU		x								JŠ, MK, NS, PC	2	K-, C+ yellowish, KC+ yellow-orange. TLC (MK12399): variolaric acid, two unknowns (traces)
Opegrapha niveoatra	AC, CB, FA, QU, TIL	x	x	x	x	x	x	x	x		JŠ, JV(4), MK(3), PC(8), ZP(3)	3	Three types of conidia found within samples: (1) straight or slightly curved, 2.5-4 x 1.5 µm; (2) curved, 5-6 x 1-1.5 µm; (3) curved, 7-9 x 1-1.5 µm. Sometimes only with pycnidia.
Opegrapha rufescens	AC, CB, FA, PYRUS, QU (often young trees)	x	x	x		x	x	x	x	x	JM(3), JV(6), PC, ZP JM(4), JV(5), MK(3), PC(2), ZP(5)	3	
Opegrapha varia	AC, CB, FA, QU, ULM	x	x	x		x	x	x	x		PC(2), ZP(5)	3	
Opegrapha vermicellifera	AC, CB, FA, QU, TIL, ULM, wood	x	x	x			x	x	x		FB, JM, MK(3), PC(2), ZP	3	
Opegrapha viridis	CB (rarely FA, TIL)	x	x	x							JM(4), JV(3), PC(2), ZP	2	
Pachyphiale fagicola	fallen branch		x								MK	1	
Parmelia sulcata	AC, CB, FA, QU, SAL, TIL, ULM (twigs), Crataegus, Euonymus, wood	x	x	x	x	x	x	x	x	x	MK(2), ZP	3	
Parmeliina tiliacea	AC, CB, FA, POP, QU, SAL, TIL, ULM (twigs), wood	x	x	x	x	x	x	x	x		PC, ZP(2)	3	
Parmeliopsis ambigua	dry wood		x									1	
Peridiothelia fuliguncta	CB, ULM		x								JV, ZP(2)		
Pertusaria albescens	AC, CB, FA, QU, SAL	x	x	x							JM, MK, PC, ZP	3	TLC (MK12400): allopertusaric acid, dihydropertusaric acid, unknown fatty acid
Pertusaria amara	CB, FA, QU, wood		x	x		x						3	
Pertusaria coccodes	CB, TIL		x			x					FB, JM, JV	2	With high amount of norstictic acid (crystals).
Pertusaria coronata	CB		x								PC	1	K+ yellow, UV+ orange

Pertusaria leioplaca	CB (rarely TIL)	x	x	x		x		x	x	JM, JV, MK, ZP(2)	3	
Pertusaria pertusa	CB			x		x				JV	2	
Phaeophyscia endophoenicea	AC, CB, FA, QU, TIL, Crataegus, Sambucus	x	x	x		x	x	x	x	JM(3), MK, PC(3), ZP(2)	3	This species occurs in the area, but <i>P. rubropulchra</i> is common and may be misidentified as this species.
Phaeophyscia nigricans	AC, FA, SAL (twigs)		x		x			x			2	
Phaeophyscia orbicularis	AC, CB, FA, POP, QU, SAL, ULM (twigs), Juglans, Sambucus	x	x	x	x	x		x	x	MK	3	
Phaeophyscia pusilloides	AC (twig)		x							ZP	1	Rather young thallus, but distinguished from other similar taxa.
Phaeophyscia rubropulchra	AC, CB, QU AC, CB, FA, QU, TIL, ULM, Crataegus,						x			ZP(3)	1	More delicate lobes and smaller thallus than in <i>P. endophoenicea</i> ; more rough (almost blastidiate) soredia than in <i>P. endophoenicea</i> ; anthraquinones in higher concentration - continuous red layer well visible in section; soralia usually without anthraquinones.
Phlyctis argena	Euonymus	x	x	x	x	x	x	x	x		3	
Physcia adscendens	AC, CB, FA, QU, SAL, TIL, Juglans (usually twigs), Crataegus, Euonymus, Sambucus, wood	x	x	x	x	x	x	x	x		3	
Physcia aipolia	FA, ULM (twigs)	x	x							JV, MK(2), ZP(2)	2	
Physcia aipolioides	QU, POP		x		x					PC	2	
Physcia dubia	fallen branch		x							MK	1	
Physcia stellaris	AC, FA, QU, SAL (twigs)	x	x	x	x	x	x	x	x	BC, MK	3	
Physcia tenella	AC, CB, FA, QU, SAL, TIL (usually twigs), Euonymus, Sambucus, ULM, wood	x	x		x	x	x	x	x		3	

Physciella chloantha	AC, CB, FA, Sambucus	x	x		x	x	x		JM(2), JV(3), PC, ZP	3	
Physconia distorta	FA (twigs)	x						x	PC	2	
Physconia enteroxantha	AC, FA, QU, SAL, TIL, ULM (usually twigs)	x	x	x	x	x	x	x	JM, JV(4), ZP	3	Some specimens keyed out as " <i>Ph. detersa</i> " - medulla K-, TLC: no substances. ITS fingerprint showed placement of one such specimen into <i>Ph. enteroxantha</i> . <i>Ph. detersa</i> is a boreal taxon, perhaps absent in C Europe.
Physconia grisea	AC, CB, FA, QU, POP, PYRUS, SAL, ULM (often twigs), Crataegus	x	x	x	x	x		x	JM(2), JV(2), ZP	3	
Physconia perisidiosa	AC, FA	x	x					x	JM(2), JV(2)	2	
Piccolia ochrophora	AC, CB, FA (twigs), Sambucus	x	x			x			JM, JV, ZP(2)	2	
Placynthiella dasaea	rotten wood	x	x		x	x	x	x	PC, NS	3	
Placynthiella icmalea	wood	x	x	x		x	x	x	MK, PC(3), ZP(3)	3	
Platismatia glauca	dry wood	x								1	
Polycoccum sp.	on <i>Xanthoria parietina</i>				x				PC		Perithecia simple in small galls 4/5 to 3/5 immersed in host thallus and apothecia; spores 12-14 x 6-8 µm, brown; asci cylindrical, 8-spored, paraphyses present.
Porina aenea	AC, CB, FA, TIL, ULM, Crataegus	x	x	x		x		x	BC(2), JV(2), PC, ZP	3	
Protoparmelia hypotremella	CB	x							JM, MK, PC, ZP	1	TLC (ZP17693): lobaric acid, aliphatic unknowns (?contamination); thallus Pd-, UV+ glaucous white.
Psamma cf. inflata	on bark (QU) and/or unidentified sterile thallus (? <i>Bactrospora dryina</i>)							x	ZP		
Pseudevernia furfuracea	TIL, QU (twigs), Euonymus	x	x	x		x		x		3	
Punctelia jeckeri	AC, CB, FA, QU, SAL, TIL, ULM, Juglans (usually twigs), Crataegus, Euonymus, wood	x	x	x	x	x	x	x	JV, MK, NS, PC	3	

Punctelia subrudecta	AC, CB, FA, QU, SAL, Juglans (usually twigs), Euonymus	x	x	x		x	x		x	ZP	3			
Pycnora sorophora	Wood (QU)			x										
Pyrenula nitida	CB	x	x	x				x	x	FB, JM, ZP	3			
Pyrenula nitidella	CB, (rarely FA)	x	x	x		x			x	JM, JV, MK, NS	3	Real <i>Pyrenula nitidella</i> (with narrower spores) is rare, but young specimens of <i>P. nitida</i> (resembling the former taxon) are common.		
Ramalina farinacea	AC, FA, SAL (often twigs)			x	x			x			3			
Ramalina fastigiata	FA			x						x	2			
Ramalina pollinaria	FA, POP			x	x			x	x	x	3	MK, NS		
Ramonia chrysophaea	CB (wood in hollow trunk)	x									1	ZP		
Rebentischia massalongii	AC, CB, POP			x		x	x					ZP(3)		
Rhagadostoma sp.	AC	x	x					x		x		BC, JV(3), NS	Perithecia vertically compressed with wall cracked into polygons, carbonized, thick, developed also below hymenium; ascospores 35-50 x 10-15 µm, 1-septate, colourless; often grows together with <i>Lecania croatica</i> (lichenicolous?).	
Rinodina degeliana	AC, FA, TIL			x	x							JM, JV, ZP	2	
Rinodina exigua	CB							x				JV	1	
Rinodina freyi	POP, QU twigs			x		x						MK, ZP	2	Ascospore sizes in MK specimen fit better <i>R. septentrionalis</i> , but distinguishing between these taxa is not clear to us.
Rinodina pyrina	FA (twigs), wood			x		x						BC, PC	2	
Schismatomma decolorans	CB, FA, QU, TIL	x	x	x		x	x	x	x			FB, JŠ, JM(3), JV11347, MK(2), PC(3), ZP(2)	3	
Schismatomma pericleum	FA			x	x							JV(2)	2	
Scoliciosporum chlorococcum	wood	x	x									PC(2), ZP	2	
Scoliciosporum sarothamni	AC, AG, CB, FA, POP, QU, TIL (twigs), wood	x	x	x	x			x	x	x		JM, MK(2), ZP(2)	3	Rarely fertile; usually as C+ red sorediate crust

Steinia geophana	wood	x	x	x				JV, ZP(4)	2	In two samples of ZP, apothecia are accompanied with tiny synnematous anamorphic stage (cf. <i>Graphium aphotosae</i>); perhaps not previously reported for the species.
Strangospora pinicola / moriformis	wood		x	x				NS, PC	2	We suggest <i>S. pinicola</i> and <i>S. moriformis</i> being synonyms; transitional forms are commonly collected.
Strigula affinis	AC							x ZP(2)	1	
Strigula sp.	AC, ULM		x				x	BC(2), ZP	2	Thallus pale green-brown, inconspicuous, with Trentepohlia; perithecia partly immersed, < 0.2 mm diam; wall brown-olive, K-; asci c. 70-80 x 8-12 µm; paraphysoids 2 µm wide, non-branched; ascospores 3-septate, 16-26 x 5-7 µm, slightly constricted at septa; pycnidia numerous, <0.1 mm diam; conidia (0-)1-septate, 13-20 x 1.5-3 µm, straight to slightly curved, without distinct gelatinose appendages. Perhaps undescribed taxon. ZP specimen contains pycnidia only.
Taeniolella punctata	Graphis scripta		x				x	JV, MK		
Taeniolella sp.	Pertusaria leioplaca		x					MK, NS		
Thelenella vezdae	AC, FA, TIL	x		x			x	JM(2), JV, ZP	2	
Thelocarpon intermediellum	wood		x					JM, ZP	1	Usually with perithecia but occasionally in anamorphic stage.
Trapeliopsis flexuosa	wood	x	x	x		x	x	PC	3	
Trapeliopsis glaucolepidea	wood	x	x	x				JM, JŠ	2	Including " <i>Trapeliopsis percrenata</i> " morphotype
Trapeliopsis granulosa	wood	x	x		x	x	x	PC	3	
Tremella christiansenii	Physcia stellaris (thallus)		x					MK		Galls and probasidia as on Fig. 25 (galls) and Fig. 26 (probasidia) in Diederich (1996)
Trichonectria hirta	Placynthiella icmalea		x					MK		Perithecia pink-orange, with hairs, ascospores transversely septate, with obtuse ends, 105×6, 92×6, 85×7.5, 71×6.5 µm
Usnea hirta	FA (twigs), Euonymus, dry wood		x			x	x	JV	3	TLC: usnic acid, murolic acid
Usnea substerilis	QU		x					JM	1	
Verrucaria cf. viridigrana	AC, QU, ULM		x			x	x	JM, JV(4), PC(3), ZP(2)	2	The thallus quite variably developed. Internal structure of perithecia approaching both <i>V. viridigrana</i> and <i>V. bryoctona</i> .
Vezdaea cf. retigera	QU, SAL (wood and bryophytes)		x			x		ZP(3)	2	Only goniocysts present with blunt spines ca 2µm long.

Vondrák et al. 2016. Methods for obtaining more complete species lists in surveys of lichen biodiversity. – Nordic Journal of Botany 34: 619–626.

Supplementary material Appendix 2, Table A2.

Selected characteristics of epiphytic lichen diversity in whole studied territory (γ -diversity). % from all lichen species in the list are in brackets.

Substrate

substrate	nr of species	nr of specialists
Acer campestre	84 (39%)	6 (2.8%)
Carpinus betulus	91 (42%)	17 (7.9%)
Fraxinus angustifolia	93 (43%)	7 (3.2%)
Quercus robur	92 (43%)	10 (4.6%)
Salix alba / fragilis	35 (16%)	1 (0.5%)
Tilia	57 (26%)	1 (0.5%)
Ulmus minor / laevis	39 (18%)	1 (0.5%)
wet wood	24 (11%)	15 (6.9%)
dry wood	54 (25%)	24 (11.1%)
twigs (canopy)	52 (24%)	24 (11.1%)
tree bases	not calc.	6 (2.8%)

Growth forms

fruticose	foliose	microlichens (lichen crusts)
17 (8%)	35 (17%)	159 (75%)

Abundance in the studied territory

recorded on one site only	recorded on 2-3 sites	recorded on more than 3 sites
48 (22%)	70 (33%)	94 (45%)

Supplementary material Appendix 3, Table A3. Basic data, including number of recorded lichen species, from 34 central European old-growth woodland inventories.

forest type (country abbreviation)	locality (nr on fig. 1A)	species nr	median altitude (m)	area (ha)	source
floodplain (UKR)	Otok, Mukachevo (1)	161	190	350	our unpublished data
floodplain (SK)	Horný les (2)	101	140	85	our unpublished data
floodplain (CZ)	Libický luh (3)	70	200	446	our unpublished data
oak-horn beam (CZ)	Údolí Oslavy a Chvojnice (4)	130	350	261	J. Šoun (unpublished)
oak-horn beam (CZ)	Hluboká n Vltavou (5)	81	400	10	our unpublished data
beech-fir (SK)	Stužica (6)	230	970	630	Vondrák et al. 2015
beech-fir (CZ)	žofín (7)	223	780	98	Malíček & Palice 2013 Kondratyuk et al. 1998,
beech-fir (UKR)	Stuzhitsia (8)	218	850	2492	Kondratyuk & Coppins 2000
beech-fir (UKR)	Ugolka (9)	197	880	10380	Dymytrova et al. 2013
beech-spruce-fir (CZ)	Hraničník (10)	188	1150	165	our unpublished data
beech-fir (CZ)	Boubín (11)	140	1040	56	E. Budějcká (unpublished)
beech-fir (A)	Neuwald (12)	133	950	1	Hafellner & Komposch 2007
beech (CZ)	Čerchov (13)	106	900	170	O. Peksa (unpublished) O.
beech (CZ)	Chejlava (14)	90	580	12	Peksa (unpublished)
beech-fir (CZ)	Razula (15)	90	785	23	our unpublished data
beech-fir (SLO)	Rajhenavski Rog (16)	87	885	50	Bilovitz et al. 2011
beech-fir (A)	Luxensteinwand (17)	85	850	30	Malíček et al. 2013
beech (CZ)	Čertův mlýn (18)	77	1070	50	our unpublished data
beech-fir (CZ)	Salajka (19)	57	765	18	our unpublished data
beech-fir (CZ)	Hojna voda (20)	67	840	9	Malíček et al. 2013
beech (CZ)	Jizerskohorske bučiny (21)	40	740	952	our unpublished data
beech (CZ)	Karlovské bučiny (22)	30	440	42	our unpublished data
mixed on scree (SK)	Cigánka (23)	148	700	40	Guttová & Palice 2004
mixed on scree (SK)	Hrdzava (24)	104	860	357	Guttová & Palice 1999
mixed on scree (SK)	Javornikova dolina (25)	95	790	170	Guttová & Palice 2002
mixed on scree (CZ)	Javořina (26)	77	750	160	our unpublished data
mixed on scree (CZ)	Ve studeném (27)	64	375	32	our unpublished data
spruce (CZ)	Trojmezná (28)	147	1275	588	our unpublished data
spruce (SK)	Fábova hola (29)	114	1380	260	Guttová et al. 2012
spruce (CZ)	Kněhyně (30)	64	1130	100	our unpublished data
spruce (CZ)	Boubín - top (31)	58	1280	100	our unpublished data
spruce (DE)	Reschbach Klause (32)	58	1140	50	our unpublished data
peat-bog pine (CZ)	Červené blato (33)	62	470	330	our unpublished data
peat-bog spruce, pine (CZ)	Rašeliniště Jizery (34)	52	850	153	Malíček & Vondrák 2014