



Japewia aliphatica (Lecanoraceae, lichenized Ascomycota), a new acidophilous, sorediate-blastidiate lichen from Europe

JIŘÍ MALÍČEK^{1,4*}, ZDENĚK PALICE^{1,5}, JAN VONDRÁK^{1,2,6} & TOR TØNSBERG^{3,7}

¹ Institute of Botany of the Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic;

² Faculty of Biological Sciences, University of South Bohemia, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic

³ Department of Natural History, University Museum, University of Bergen, Allégaten 41, P.O. Box 7800, NO-5020 Bergen, Norway;

⁴ ✉ jmalicek@seznam.cz; <https://orcid.org/0000-0002-3119-8967>

⁵ ✉ zdenek.palice@ibot.cas.cz; <https://orcid.org/0000-0003-4984-8654>

⁶ ✉ j.vondrak@seznam.cz; <https://orcid.org/0000-0001-7568-6711>

⁷ ✉ tor.tonsberg@uib.no; <https://orcid.org/0000-0001-9081-0274>

*Corresponding author: ✉ jmalicek@seznam.cz

Abstract

Japewia aliphatica is described as a new species. It is characterized by the usually brown, often areolate, blastidiate to sorediate-blastidiate, rarely fertile thallus containing unknown fatty acid(s). It grows on acidic bark of broad-leaved trees in mainly in montane forests, and is known from Austria, the Czech Republic, Germany, Norway, Russia (European part of the Caucasus), Slovakia and Ukraine. The new species is well characterized by its morphological, chemical and molecular (nrITS, mtSSU) traits. Systematic placement of *Japewia* is briefly discussed and its position within Lecanoraceae confirmed.

Keywords: Aliphatic acids, montane forests, sterile lichens

Introduction

Japewia Tønsberg (1990: 205; Lecanoraceae, lichenized Ascomycota) is a genus of lichenized fungi with crustose thalli, a chlorococcoid photobiont, biatorine apothecia, reduced and weakly gelatinized exciple, frequently branched and anastomosing paraphyses, asci with a distinct axial body, and simple, thick-walled, multilayered ascospores (Tønsberg 1990, Printzen 1999). According to the newest classification of Lecanoromycetes (Lücking *et al.* 2016), the genus has been assigned to the family Ramalinaceae, but molecular data suggests that it rather belongs to Lecanoraceae (Schmull *et al.* 2011, Miadlikowska *et al.* 2014). Two species have so far been described in this genus: *Japewia subaurifera* Muhr & Tønsberg (Tønsberg 1990: 206) and *Japewia tornoensis* (Nyl.) Tønsberg. The third name, *Japewia carrollii* (Coppins & P. James) Tønsberg, became the type species for the genus *Japewiella* (Printzen 1999) and is currently known as *Japewiella tavaresiana* (H. Magn.) Printzen.

Japewia tornoensis, the type species of the genus (Tønsberg 1990), is characterized by its esorediate, dark brown thallus with subglobose, red-brown apothecia. It is widely distributed in temperate and boreal regions of the Northern Hemisphere as an epiphyte (see Czarnota 2009). In Antarctica it occurs on soil, bryophytes and rocks (Øvstedal & Lewis Smith 2001). The second species, *J. subaurifera*, is rarely fertile and further characterized by the golden-brown color of the soralia due to the production of the pigments secalonic acid X2 and Z, and eumitrin L (Elix & Tønsberg 1999) and by the accessory lobaric acid (Tønsberg 1990). That species is a widespread epiphytic lichen mainly in boreal forests in the Northern Hemisphere (Czarnota 2009). The latter species has recently been reported from Tasmania (Kantvilas 2011). However, as that material lacks lichens substances, it may prove to represent an undescribed taxon.

Since the nineties of the 20th century, one of us (TT) was aware of an undescribed taxon similar to *Placynthiella dasaea* (Stirt.) Tønsberg. During recent field work in Central and Eastern European woodlands by JM, ZP, and JV, the same lichen was repeatedly collected. Based on morphological, chemical and molecular characters, we describe this distinctive taxon as a species new to science.

Material and methods

Sampling, morphology and chemistry

Collected specimens are deposited in BG, PRA, and the personal herbarium of J. Malíček. Microscopic descriptions are based on hand-cut sections mounted in water. Lichen secondary metabolites were identified using thin layer chromatography (TLC) in solvents A, B' and C (Orange *et al.* 2010). The pictures were acquired by the (1) stereomicroscope Olympus SZX 12 with the cooled color digital camera Olympus DP 70 (resolution 12.5 Mpx) in the software QuickPHOTO MICRO 3.0 (Promicra), using an extended depth of field module Deep Focus and (2) microscope Olympus BX 43 with color digital camera Promicra 3–5CP (resolution 5 Mpx) in the same software.

DNA extraction, PCR amplification and sequencing

The Invisorb Spin Plant Mini Kit (Invitek) and Chelex protocol (Ferencová *et al.* 2017) were used for DNA extractions. The fungal ITS rDNA (henceforth ITS) and mitochondrial SSU (mtSSU) were amplified with the following primers: ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), mrSSU1, mrSSU2R and mrSSU3R (Zoller *et al.* 1999). The algal ITS rDNA was amplified using the specific primer zeleny_F2 (Moya *et al.* 2018) and the universal primer ITS4 (White *et al.* 1990), following methods by Moya *et al.* (2018). PCR reactions of nrITS and mtSSU were prepared for a 20 µl final volume, containing 14 µl double-distilled water, 4 µl MyTaq polymerase reaction buffer, 0.2 µl MyTaq DNA polymerase, 0.4 µl of each of the 25 mM primers, and 1 µl of the sample. Amplifications of both loci consisted of an initial 1 min denaturation at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 56 °C, 1 min at 72 °C, and a final extension of 7 min at 72 °C. The PCR products were visualized on a 0.8% agarose gel and cleaned with ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific), according to the manufacturer's protocols. In total, 6 new ITS and 6 mtSSU sequences were generated (Table 1).

Sequence alignment and phylogenetic analysis

The newly produced sequences were edited in BioEdit 7.2.5 (Hall 1999). The final analyses included the newly generated sequences, available sequences of the genus *Japewia* and selected closely related taxa according to Schmull *et al.* (2011) and Miadlikowska *et al.* (2014). They all belong to Lecanoraceae, represent most of main lineages in this family and both ITS and mtSSU sequences are available in the Genbank database. Additionally, *Scoliciosporum umbrinum* was included based on its very close phylogenetic position to *Japewia tornuensis* in Zhao *et al.* (2016). *Lecidea nylanderii* (Anzi 1860: 75) Th. Fr. (1874: 462) and *Lecidea roseotincta* Coppins & Tønsberg (1988: 415) were selected as an outgroup because they form a sister clade to all other included species (see Schmull *et al.* 2011, Miadlikowska *et al.* 2014). The ITS and mtSSU regions were aligned separately using MAFFT 7 (Katoh & Standley 2013) with L-INS-i method (Katoh *et al.* 2005). Ambiguous positions were excluded from the analysis using Gblocks 0.91b (Castresana 2000), with a less stringent selection, on the Phylogeny.fr server (Dereeper *et al.* 2008). Gaps were coded in SeqState by simple coding (Simmons & Ochoterena 2000). The final ITS alignment contained 477 positions and 24 sequences; the mtSSU alignment had 717 positions and 22 sequences.

Support values on nodes were checked for single-gene trees and no conflict among well-supported branches (maximum likelihood bootstrap percentages >0.7) was detected. We concatenated the alignments and inferred a phylogeny using MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist *et al.* 2012). Results of jModelTest 2.1.10 (Darriba 2012) suggested the general time reversible model including gamma-distributed rates across sites modelled with four discrete categories and a proportion of invariant sites (GTR+G+I) as the best substitution model for both regions based on the AIC. Each analysis was performed with two runs, each with four MCMC chains (temperature 0.05). Trees were sampled every 500th generation. Analyses were stopped when the average standard deviation of the split frequencies between the simultaneous runs was 0.00997. To eliminate trees sampled before reaching apparent stationarity, the first 25% of entries were discarded as burn-in and the rest were used to compute a majority-rule consensus tree with Bayesian posterior probabilities for the branches.

A maximum likelihood analysis was performed using RAxML-HPC v. 8.2.12 (Stamatakis 2014) using the GTR+G+I model on the CIPRES Science Gateway (Miller *et al.* 2010). Non-parametric bootstrap analysis was performed with 1000 bootstrap replicates. The maximum likelihood consensus tree is not shown, but bootstrap values are indicated at branches in the Bayesian tree (Fig. 2). The alignment used in this study is publicly available in Zenodo as doi: 10.5281/zenodo.3610084.

TABLE 1. GenBank accession numbers and voucher information of specimens used in this study. New sequences are indicated in bold.

Taxon	Source – Specimen	ITS	mtSSU
<i>Frutidella caesioatra</i>	Norway, R. Haugan 10634 (O)	MG925971	MG925872
<i>Japewia aliphatica</i> 1	Czech Republic, Šumava Mts, J. Vondrák 18554 (PRA)	MN547348	MN547347
<i>Japewia aliphatica</i> 2	Czech Republic, Šumava Mts, J. Vondrák 18753 (PRA)	MN547349	–
<i>Japewia aliphatica</i> 3	Czech Republic, Šumava Mts, J. Vondrák 18728 (PRA)	MN547350	MN547346
<i>Japewia aliphatica</i> 4	Russia, Caucasus Mts, J. Vondrák 15548 (PRA)	MN547351	MN547344
<i>Japewia aliphatica</i> 5	Czech Republic, Beskydy Mts, J. Malíček 9764 (PRA; holotype)	MN547352	MN547343
<i>Japewia aliphatica</i> 6	Ukraine, Carpathians, J. Malíček 8238 (hb. J. Malíček)	MN547353	MG773680
<i>Japewia aliphatica</i> 7	Czech Republic, Hrubý Jeseník Mts, Z. Palice 19222 (PRA)	–	MN547345
<i>Japewia subaurifera</i> 1	U.S.A., T. Spribille s.n.	JN009716	–
<i>Japewia subaurifera</i> 2	Japan, R. Haugan (O)	MH481917	–
<i>Japewia tornoensis</i> 1	Canada, C. Printzen (BG)	HQ650656	HQ660559
<i>Japewia tornoensis</i> 2	Canada, C. Printzen 5638 (BG)	EF495163	–
<i>Japewia tornoensis</i> 3	Sweden, M. Wedin (UPS)	–	DQ899301
<i>Japewia tornoensis</i> 4	Norway, J. T. Klepsland (O)	–	MG925874
<i>Lecanora allophana</i>	Finland, Kimito, J. Malíček 9491 (hb. J. Malíček)	KY548051	KY502416
<i>Lecanora argentata</i>	Russia, Caucasus Mts, J. Malíček 9620 (hb. J. Malíček)	MK778604	MK778529
<i>Lecanora caesiorubella</i>	Australia, H. T. Lumbsch 19974	JN943728	JQ782667
<i>Lecanora cinereofusca</i>	U.S.A., North Carolina, J. Lendemer 35007 (NY)	KP224471	KP224464
<i>Lecanora intumescens</i>	Ukraine, Carpathians, J. Malíček 8203 (hb. J. Malíček)	KY548039	KY502443
<i>Lecanora polytropa</i>	U.S.A., Alaska, F. Lutzoni <i>et al.</i> (DUKE)	HQ650643	DQ986807
<i>Lecidea nylanderii</i>	U.S.A., T. Spribille 10020 (hb. Spribille)	HQ650667	HQ660572
<i>Lecidea polytrichina</i>	Sweden, Z. Palice s.n. (hb. Printzen)	HQ650668	HQ660573
<i>Lecidea roseotincta</i>	Norway, T. Tønsberg 34577 (hb. Schmull)	HQ650670	HQ660575
<i>Lecidella elaeochroma</i>	China, Xinjiang	KT453749	KT453835
<i>Lecidella stigmataea</i>	China, Xinjiang	KT453758	KT453852
<i>Protoparmeliopsis muralis</i>	Romania, J. S. Hur	KP059048	KP059054
<i>Scoliciosporum umbrinum</i>	Switzerland	KX133008	–

Taxonomy

Japewia aliphatica Malíček, Palice, Tønsberg & Vondrák *sp. nov.* MycoBank no 832832 (Figs 1 & 2)

Diagnosis: The species is characterized by its brown, often areolate thallus composed of tiny blastidia that frequently develop into pale soredia. Soralia usually irregular, rarely delimited and rounded, sometimes covering most of the thallus. Apothecia very rare, convex, reddish-brown. Ascospores ellipsoid to subglobose, with a thick, multilayered wall. Pycnidia unknown. One or several fatty acids are produced as lichen secondary metabolites.

Type: CZECH REPUBLIC. Beskydy Protected Landscape Area: Frenštát pod Radhoštěm, Kněhyně-Čertův mlýn National Nature Reserve, old-growth spruce forest on E-facing slope of Mt Kněhyně (1257 m), 1190 m elev., 49°29'48"N, 18°18'56"E, on bark of *Fagus sylvatica*, 31 August 2016, J. Malíček 9764 & Z. Palice (holotype: PRA; isotype: herb. Malíček).

Description: Thallus of sorediate aggregations of blastidia, covering patches up to 3(–5) cm or even larger areas when intermixed with other lichens, when well-developed, often organized in areoles about 0.5 mm (up to 1 mm) in diam. and up to 0.3(–0.6) mm thick; in shady places and also in specimens with delimited soralia occasionally with a brownish to green-grey, thin (up to 100 µm high), unevenly verrucose to areolate-squamulose basal thallus. Prothallus absent. Blastidia medium brown, chocolate brown at sun-exposed sites and brown-olive to pale grey-green at shady sites;

(20–)25–35(–45) μm in diam.; wall formed by brown (K+ dark brown) or rarely grey hyphae, composed of ellipsoid to rounded cells, often with flexuous walls, up to 12×4 (–7.5) μm . Soralia often missing, variable, mostly uneven in shape, developing by disintegration of blastidia; becoming confluent and continuously covering the thallus or rarely delimited and rounded, 0.1–0.2 mm in diam. Soredia farinose, whitish or less frequently pale brown to pale green-grey, contrasting the brown blastidia, simple, 20–35 μm in diam., or in consoredia up to 80 μm in diam., in a mount delimited by a colourless or pale brown to grey, more or less compact fungal sheath without projecting hyphae.

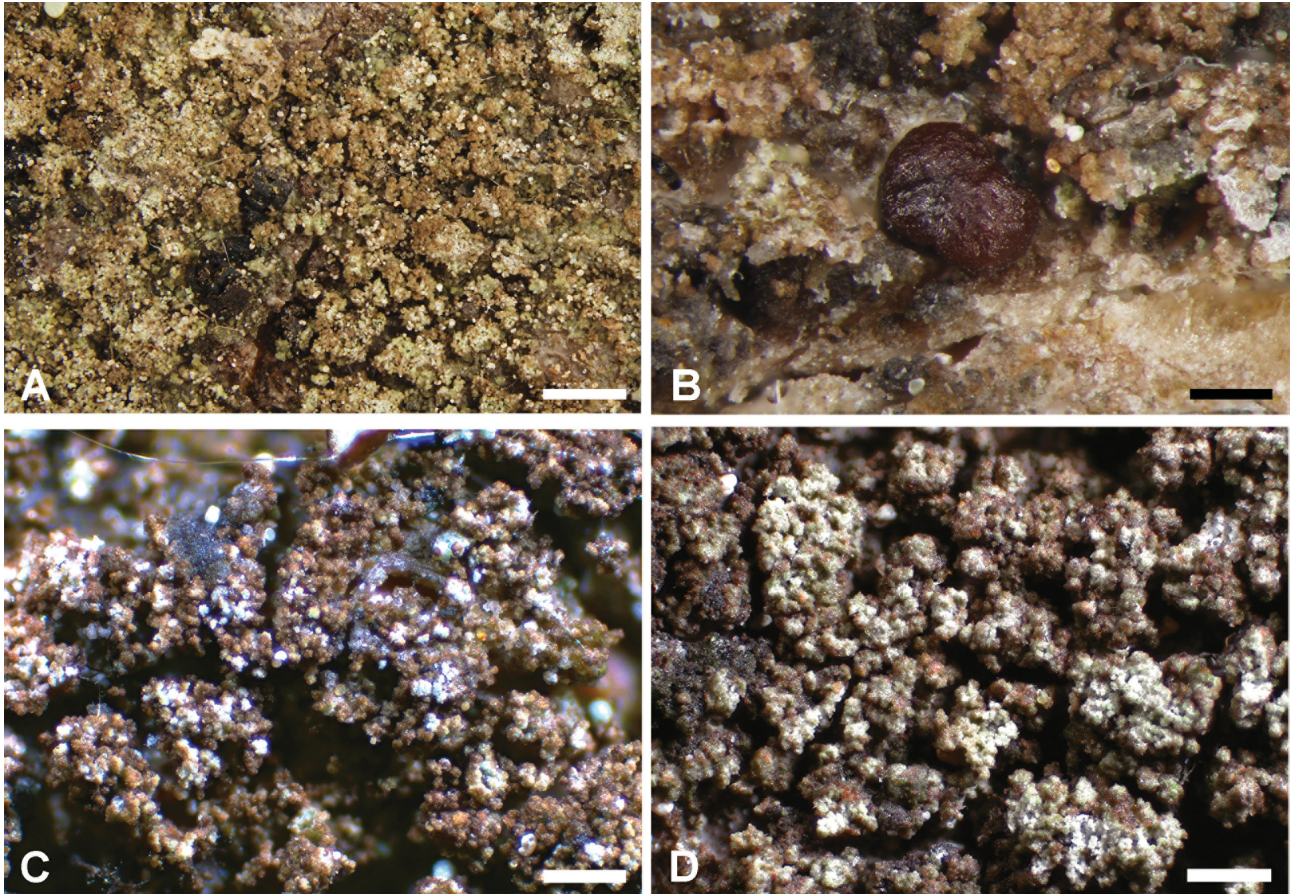


FIGURE 1. Morphology of *Japewia aliphatica*. A, thallus with blastidia developing soredia (PRA-Palice 18750); B, apothecium (PRA-Palice 25176); C, blastidiate thallus (PRA, holotype); D, soralia developing from blastidiate thallus (PRA-Palice 19029). Scales: 1 mm (A), 0,2 mm (B–D). Photo by J. Machač (A, B) and J. Malíček (C, D).

Apothecia very rare (known from two samples), lecideoid, convex, glossy, reddish-brown, epruinose, 0.4–0.5 mm in diam. Proper exciple colourless in inner part, reddish-brown in outer part, K+ brown, HNO_3^- , composed of radiating branched hyphae; cells 1.5–3.0 μm in diam. Hypothecium colourless, with oil droplets. Hymenium c. 100 μm high, with oil droplets; epihymenium reddish-brown, K+ brown, HNO_3^- , without granules (POL–); paraphyses surrounded by a gel coat, conglutinated, richly branched and anastomosing, 1.5–2.5 μm thick; tips swollen, up to 4 μm , in K with more or less distinct, brown caps. Asci broadly clavate, initially apparently 8-spored, but mature asci (1–)3–6-spored; ascospores broadly ellipsoid to subglobose, simple, colourless, (17–)18–23(–25) \times (12–)13–16(–17) μm (n=25), wall multilayered, 2–4(–6) μm thick. Pycnidia not observed.

Photobiont chlorococcoid, 5–9(–14) μm in diam., morphologically corresponds with the genus *Symbiochloris* (Škaloud *et al.* 2016), but one sequenced specimen (Vondrák 15548; MT022442) was identified as *Trebouxia* sp., using the BLASTN search (Altschul *et al.* 1990).

Chemistry: One or several fatty acids, which may belong to the apinnatic acid complex sensu Obermayer *et al.* (2018), detected by TLC. A major fatty acid (A4, B'5, C5) visible in all samples (n=27). A double fatty spot often visible in the A solvent. Spot reactions: K–, Pd–, C–, KC–; UV–.

Phylogeny: The new species as well as the genus *Japewia* are strongly supported as a distinct clade in the ITS and mtSSU phylogeny (Fig. 3). *Japewia subaurifera* and *J. tornoensis* are not monophyletic based on our results which may be caused by incomplete dataset of sequences (see Table 1) or a great variability among populations within individual continents. Current classification of Lecanoromycetes (Lücking *et al.* 2016) placed this genus in

the family Ramalinaceae. This contradicts our results as well as the phylogenetic studies by Schmull *et al.* (2011) and Miadlikowska *et al.* (2014). Therefore, the genus *Japewia* should be placed in Lecanoraceae.

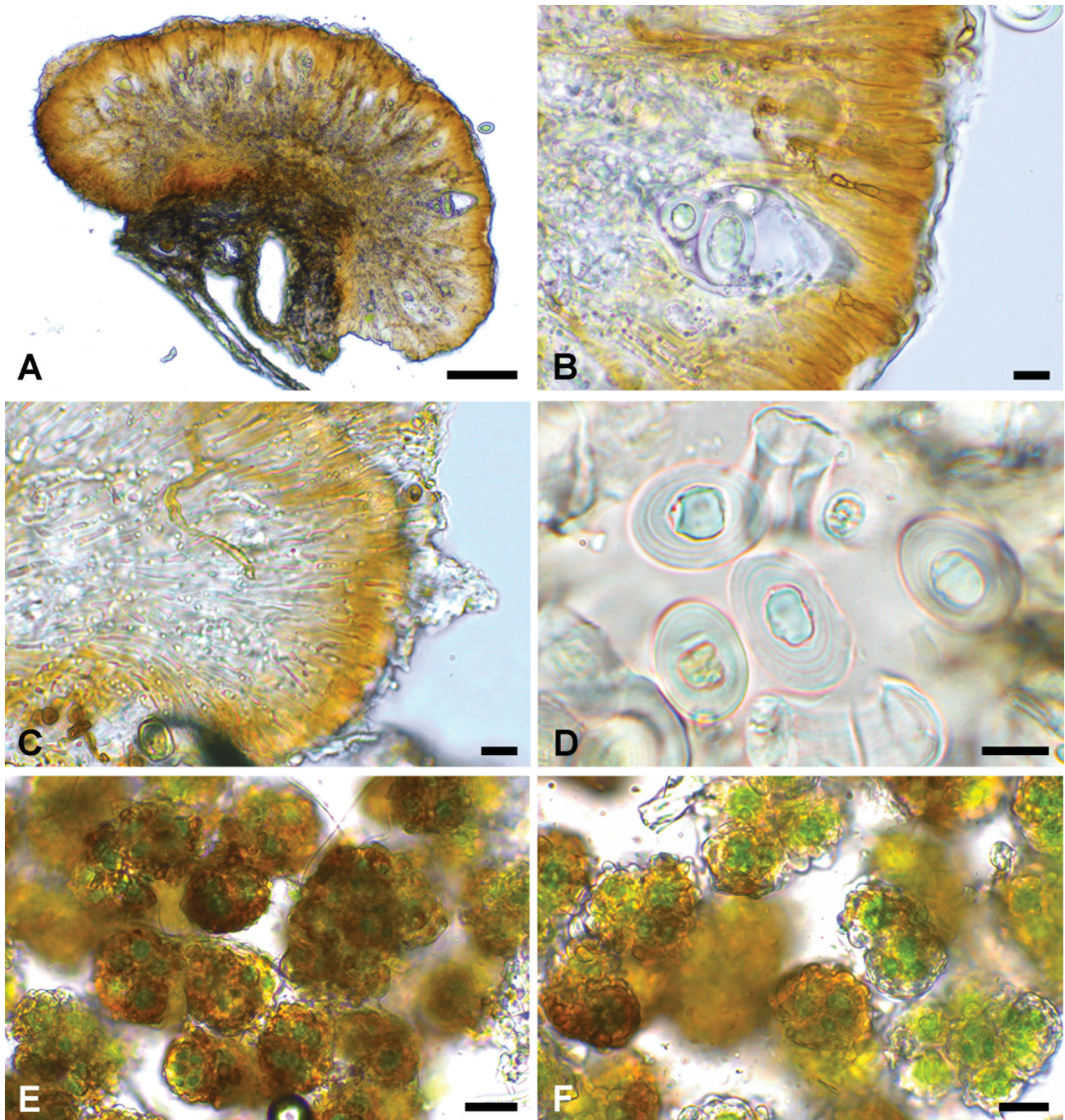


FIGURE 2. Anatomy of *Japewia aliphatica*. A, apothecial section (PRA-Palice 27112); B, hymenium and ascus (PRA-Palice 27112); C, exciple (PRA-Palice 27112); D, ascospores in KOH (PRA-Palice 27112); E–F, soredia (PRA-Palice 24323). Scales: 0,1 mm (C), 10 μ m (D–F, H) 20 μ m (H). Photo by J. Malíček.

Etymology: The name refers to the diagnostic chemical constituent being an aliphatic acid.

Distribution and ecology: The new species is known from Austria, the Czech Republic, Germany, Norway, Russia, Slovakia and Ukraine. It has already been published under provisional names *Placynthiella* aff. *dasaea*, *Japewia* sp. or *Japewia dasaea* ined. from the Eastern Carpathians in Slovakia and Ukraine (Vondrák *et al.* 2015, 2018, Malíček *et al.* 2018), the Czech Republic (Malíček *et al.* 2019) and as *J. aliphatica* from the Caucasus Mts (Urbanavichus *et al.* 2020).

Japewia aliphatica grows in various types of montane forests, where it prefers well-lit sites. In Norway, it has been found on *Alnus glutinosa* in a boreo-nemoral rainforest close to sea-level. In Central Europe, it occurs mainly above 1100 m elev. Rarely, it has been collected in river valleys at lower elevations (700–900 m). It prefers smooth

acidic bark on trunks of broad-leaved trees. *Fagus sylvatica* is the most frequent phorophyte, followed by *Sorbus aucuparia* and *Alnus incana*. More rarely it was collected on *Acer pseudoplatanus*, *Alnus glutinosa*, *Betula* sp. and *Picea abies*.

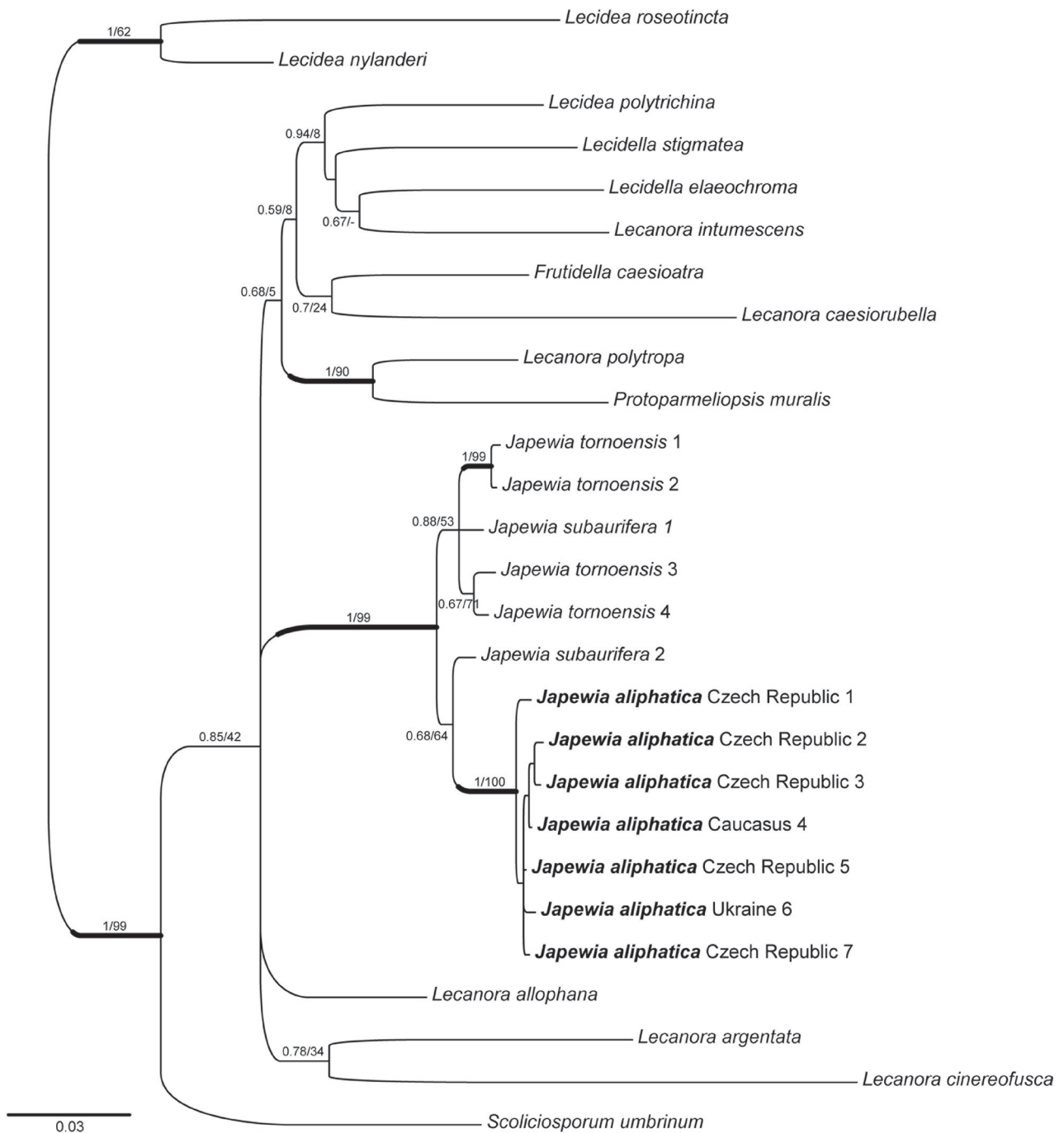


FIGURE 3. Phylogeny of the genus *Japewia* with selected members of the family Lecanoraceae. This is a Bayesian phylogenetic reconstruction based on nrITS and mtSSU sequences. The new species, *Japewia aliphatica*, is indicated in bold. Branches with >0.95 Bayesian posterior probability values are indicated by thicker lines. Bayesian posterior probabilities (first value) and maximum likelihood bootstrap percentages (second value) are indicated.

The most commonly co-occurring species are *Parmeliopsis ambigua* (Wulfen) Nyl., *Buellia griseovirens* (Turner & Borrer ex Sm.) Almb., *Lecanora pulicaris* (Pers.) Ach., *Hypogymnia physodes* (L.) Nyl., *Lepraria elobata* Tønsberg, *Parmelia saxatilis* agg. and *Platismatia glauca* (L.) W.L. Culb. & C.F. Culb. Other associated lichens include e.g. *Biatora efflorescens* (Hedl.) Räsänen, *Candelariella efflorescens* agg., *Lepraria rigidula* (B. de Lesd.) Tønsberg, *Melanelixia glabrata* (Lamy) Sandler & Arup, *Toensbergia leucococca* (R. Sant.) Bendiksby & Timdal and *Violella fucata* (Stirt.) T. Sprib.

Notes: Although the new species is usually sterile, it can be recognized by the brown, often areolate thallus composed of tiny blastidia and/or sorediate blastidia; and chemically by a fatty acid(s) as the diagnostic substance.

Japewia aliphatica and *Placynthiella dasaea* are usually morphologically indistinguishable. Chemically, however, they are easy to hold apart containing aliphatic acids (C⁻) versus gyrophoric acid (C⁺ red), respectively. It may also recall *Japewia subaurifera* but it is distinct in never having brightly coloured soralia ranging from yellow-, orange-brown to chocolate-brown colour due to the presence of pigments (Elix & Tønsberg 1999). That species differs further by the accessory presence of lobaric acid (Tønsberg 1992). The blastidiate thallus of *J. aliphatica* may also resemble specimens of *Scoliciosporum chlorococcum* with a granular to partly sorediate thallus but that species is usually more green and lacks secondary metabolites.

Additional specimens examined. AUSTRIA. Tyrol: Kitzbüheler Alpen, Kelchsau, Kurzer Grund, near river Kurzer-Grund-Ache, along the tributary river Trattenbach, 1150–1160 m elev., 47°20'N, 12°09'E, on *Alnus incana*, 31 August 1996, *T.Tønsberg* 24336 (BG, FR, GZU, O, TRH, UPS), 24349, 24355 (BG); *ibid.*: 1110–1140 m elev., on a river bank, on *Alnus incana*, 31 August 1996, *T.Tønsberg* 24380 (BG); Stubai Alpen, Stigleith (Oberperfluss) – Roskogelhütte, in a swampy spruce forest, 1600 m elev., 47°14.5'N, 11°11.7'E, on twig of *Picea abies*, 29 August 1996, *T.Tønsberg* 24317 (BG).

CZECH REPUBLIC. Western Bohemia: Šumava Mts, WNW of Železná Ruda, Revier Eisenstein, valley of brook Svarožná (Büchel-Bach), NE of the brook, 100–500 m E of German-Czech border, *Picea abies* forest, 900–970 m elev., 49°09'N, 13°11'E, on *Fagus sylvatica*, 12 October 1999, *T.Tønsberg* 28148 (BG); Šumava Mts, S of Železná Ruda, just E of forest road from Debrník to Ferdinand's valley, 0.5–1 km S of Debrník, along the brook, ±swampy bank of stream with *Alnus incana* and *A. glutinosa*, 720–750 m elev., 49°07.1'N, 13°14.1'E, on *Alnus glutinosa* and *A. incana*, 12 October 1999, *T.Tønsberg* 28186a, 28191a (BG); Železná Ruda, Mt Jezerní hora [1343], top plateau of W subsummit, remnants of spruce forest just NE of open boulder scree, 1327 m elev., 49°10'22.5"N, 13°10'28.2"E, on bark of *Sorbus aucuparia*, 18 September 2019, *Z.Palice* 27466 & *P.Uhlik* (PRA); Prášily, SW slope of Mt Ždánidla, 1180 m elev., 49.0983908°N, 13.3497794°E, on *Fagus sylvatica*, 14 September 2017, *J.Vondrák* 18729 (PRA); *ibid.*: 1130 m elev., 49°05'48.1"N, 13°21'12.5"E, on *Fagus sylvatica*, 18 September 2017, *J.Vondrák* 18554 (PRA); *ibid.*: 1120 m elev., 49.0979886°N, 13.3429339°E, 14 September 2017, *J.Vondrák* 18728 (PRA); Southern Bohemia: Šumava Mts, Nová Pec, managed beech forest on NE-facing slope of Mt Studničná (1160 m), 1130 m elev., 48°45'24"N, 13°54'10"E, on *Fagus sylvatica*, 16 June 2017, *J.Maliček* 11274 & *J.Vondrák* 18750 (herb. Maliček, PRA); Nová Pec, Mt Hraničník – NE slope, remnants of montane mixed forest, 1184 m elev., N48°45'07.2", E013°54'33.1", on branch of recently wind-broken *Fagus*, 28 October 2014, *Z.Palice* 18307, 19029 (PRA); *ibid.*: N slope, old-growth montane mixed forest with dominating *Fagus*, N48°45'14", 1165 m elev., E013°54'16.5", on bark of *Sorbus aucuparia*, 12 August 2017, *Z.Palice* 24455 (PRA); *ibid.*: 1170 m elev., 48.75364°N, 13.90472°E, on *Fagus sylvatica*, 15 June 2017, *J.Vondrák* 18753, 18754 (PRA); *ibid.*: managed forest with beech predominant, 1130 m elev., 48°45'24"N, 13°54'10"E, on bark of *Sorbus aucuparia* and *Fagus sylvatica*, 2 August 2017, *Z.Palice* 24323, 24381 (PRA); Nová Pec, Mt Plechý, NNW slope, 1 km NW from the top, a belt of young spruce forest in valley of Ježový potok brook, 1285 m elev., 48°46'40.5"N, 13°50'51"E, on bark of *Sorbus aucuparia*, 21 August 2018, *Z.Palice* 25176 (PRA); *ibid.*: 21 August 2019, *Z.Palice* 27112 (PRA); N Moravia: E Sudetes, Jeseníky Mts, Mt Červená hora [1333], well-lit spruce forest at S-facing slope just SE–SSE from the top, beneath a red-marked tourist trail, 1230 m elev., 50°08'23.5"N, 17°08'19.6"E, on bark of *Sorbus aucuparia*, 18 August 2015, *Z.Palice* 19222 & *J.Vondrák* (PRA); Eastern Moravia: Beskydy Mts, Frenštát pod Radhoštěm, Kněhyně–Čertův mlýn National Nature Reserve, SW-facing slope of Mt Čertův mlýn (1206 m), old-growth beech-spruce forest, 1150 m elev., ca. 49°29'08"N, 18°18'10"E, 28 September 2013, *J.Maliček* 6110, 6112 & *J.Vondrák* 11664 (herb. Maliček, PRA); *ibid.*: Mt Kněhyně, fragment of old-growth spruce forest with some beech and sycamore, E-facing slope, 1190 m elev., 49°29'47.8"N, 18°18'56.1"E, on bark of *Fagus*, 31 August 2016, *J.Maliček* & *Z.Palice* 21798 (PRA); *ibid.*: managed spruce forest at E facing slope, 1100 m elev., 49°29'47"N, 18°19'07.5"E, on bark of *Fagus*, 1 September 2016, *J.Maliček* & *Z.Palice* 21838 (PRA).

GERMANY. Niederbayern: Böhmerwald, Regen, Mt Zwercheck [1334], open spruce forest 315 m E-ESE of the top, just few metres from the state border with CR, near the saddle with Mt Jezerní hora [1343], 1317 m elev., 49°10'24.8"N, 13°10'14.7"E, on bark of *Sorbus aucuparia*, 18 September 2019, *Z.Palice* 27533 & *P.Uhlik* (PRA) [distributed also to other herbaria within the multiplicate by W. Obermayer: *Dupla Graecensia Lichenum*].

NORWAY. Hordaland: Lindås, along the brook W of Eidatræet, 1–10 m elev., 60°38'N, 5°28'E, on *Alnus glutinosa*, 5 September 1987, *T.Tønsberg* 10611 (BG).

RUSSIA. Republic of Adigea: Maykop, Guzeripl, protected area Kavkazskiy zapovednik, plateau Lagonaki, 1840 m elev., 44.07777°N, 40.01400°E, on *Betula*, 12 June 2016, *J.Vondrák* 15548 (PRA).

SLOVAKIA. Poloniny Mts: Ulič, Nová Sedlica, protected area Stuzica, 800 m elev., 49°4'20"N, 22°32'6"E, on *Fagus sylvatica*, 5 June 2014, *J.Šoun & J.Vondrák 12187* (PRA), *ibid.*: 1150 m elev., 49°5'24"N, 22°32'57"E, 7 June 2014, *J.Šoun & J.Vondrák 12186* (PRA).

UKRAINE. East Carpathians: Khust, Velyka Uhol'ka, old-growth beech forest on a ridge 1.5 km SW of Mt Manchul (1500 m), 6.5 km E of Zabrid, 1200 m elev., 48°17'52"N 23°39'59"E, on *Fagus sylvatica*, 17 May 2015, *J.Maliček 8238, Z.Palice 19774 & J.Vondrák 14134* (herb. Maliček, PRA); Shirokyi luh, old-growth beech forest on a ridge 1.5 km E–ESE of Mt Menchul [1501], 5.5 km NW–NNW of Prihid' village, 1250 m elev., 48°18'40"N, 23°41'59"E, on bark of old *Fagus*, 27 May 2019, *J.Maliček, Z.Palice 26915 & J.Vondrák 21652* (herb. Maliček, PRA); Gorgany Mts, Nadvirna, Bystrytsia, c. 5 km SE of village, 1050 m elev., 48.43071°N, 24.32381°E, on *Fagus sylvatica*, 23 August 2019, *J.Vondrák 21903* (PRA).

Acknowledgements

Pavel Škaloud and Ondřej Peksa checked the identity of the photobiont. Ivana Černajová kindly provided a sequence of the algal partner. Jiří Machač contributed with two photos of the new species. Both reviewers helped to improved the manuscript. JM, ZP and JV have been supported by the long-term research development project RVO 67985939.

References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
[https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 175: 40–52.
<http://doi.org/10.1093/oxfordjournals.molbev.a026334>
- Czarnota, P. (2009) *Japewia tornoenensis* and further localities of *J. subaurifera* found in the Carpathians. *Acta Mycologica* 44: 259–264.
<https://doi.org/10.5586/am.2009.024>
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
<https://doi.org/10.1038/nmeth.2109>
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.M. & Gascuel, O. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* 36: 465–469.
<http://doi.org/10.1093/nar/gkn180>
- Elix, J.A. & Tønsberg, T. (1999) Notes on the chemistry of some lichens from Norway. *Graphis Scripta* 10: 4–6.
- Ferencová, Z., Rico, V.J. & Hawksworth, D.L. (2017) Extraction of DNA from lichen-forming and lichenicolous fungi: a low-cost fast protocol using Chelex. *Lichenologist* 49: 521–525.
<https://doi.org/10.1017/S0024282917000329>
- Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes. Application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
<https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.
<https://doi.org/10.1093/bioinformatics/17.8.754>
- Kantvilas, G. (2011) The lichen genera *Japewia* and *Japewiella* in Australia. *Muelleria* 29: 99–103.
- Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33: 511–518.
<https://doi.org/10.1093/nar/gki198>
- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
<https://doi.org/10.1093/molbev/mst010>

- Lücking, R., Hodkinson, B.P. & Leavitt, S.D. (2016) The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *Bryologist* 119: 361–416.
<https://doi.org/10.1639/0007-2745-119.4.361>
- Maliček, J., Palice, Z., Acton, A., Berger, F., Bouda, F., Sanderson, N. & Vondrák, J. (2018) Uholka primeval forest in the Ukrainian Carpathians – a keynote area for diversity of forest lichens in Europe. *Herzogia* 31: 140–171.
<https://doi.org/10.13158/099.031.0110>
- Maliček, J., Palice, Z., Vondrák, J., Kostovčík, M., Lenzová, V. & Hofmeister, J. (2019) Lichens in old-growth and managed mountain spruce forests in the Czech Republic: assessment of biodiversity, functional traits and bioindicators. *Biodiversity and Conservation* 28: 3497–3528.
<https://doi.org/10.1007/s10531-019-01834-4>
- Miadlikowska, J., Kauff, F., Högnabba, F., Oliver, J.C., Molnár, K., Fraker, E., Gaya, E., Hafellner, J., Hofstetter, V., Gueidan, C., Otálora, M.A., Hodkinson, B., Kukwa, M., Lücking, R., Björk, C., Sipman, H.J., Burgaz, A.R., Thell, A., Passo, A., Myllys, L., Goward, T., Fernández-Brime, S., Hestmark, G., Lendemmer, J., Lumbsch, H.T., Schull, M., Schoch, C.L., Sérusiaux, E., Maddison, D.R., Arnold, A.E., Lutzoni, F. & Stenroos, S. (2014) A multigene phylogenetic synthesis for the class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric taxa, 317 genera and 66 families. *Molecular Phylogenetics and Evolution* 79: 132–168.
<https://doi.org/10.1016/j.ympev.2014.04.003>
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In: Proceedings of the Gateway Computing Environments Workshop (GCE)*. 14 Nov. 2010. New Orleans Convention Center, New Orleans, LA, pp. 1–8.
<https://doi.org/10.1109/GCE.2010.5676129>
- Moya, P., Chiva, S., Molins, A., Jadrná, I., Škaloud, P., Peksa, O. & Barreno, E. (2018) *Myrmecia israeliensis* as the primary symbiotic microalga in squamulose lichens growing in European and Canary Island terricolous communities. *Fottea* 18: 72–85.
- Obermayer, W., Witzmann, M. & McCune, B. (2018) *Hypogymnia caperatica*, a new species from the Hengduan Shan area (easternmost Tibetan region), with notes on some rare taxa from the greater Tibetan region and Bhutan. *Herzogia* 31: 677–694.
<https://doi.org/10.13158/hea.31.1.2018.677>
- Orange, A., James, P.W. & White, F.J. (2010) *Microchemical Methods for the Identification of Lichens*. British Lichen Society, London, 101 pp.
- Øvstedal, D.O. & Lewis Smith, R.I. (2001) *Lichens of Antarctica and South Georgia*. Cambridge University Press, Cambridge, 424 pp.
- Printzen, C. (1999) *Japewiella* gen. nov., a new lichen genus and a new species from Mexico. *Bryologist* 102: 714–719.
<https://doi.org/10.2307/3244257>
- Ronquist, F., Teslenko, M., Van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
<https://doi.org/10.1093/sysbio/sys029>
- Schull, M., Miadlikowska, J., Pelzer, M., Stocker-Wörgötter, E., Hofstetter, V., Fraker, E., Hodkinson, B.P., Reeb, V., Kukwa, M., Lumbsch, H.T., Kauff, F. & Lutzoni, F. (2011) Phylogenetic affiliations of members of the heterogeneous lichen-forming fungi of the genus *Lecidea* sensu Zahlbruckner (Lecanoromycetes, Ascomycota). *Mycologia* 103: 983–1003.
<https://doi.org/10.3852/10-234>
- Simmons, M.P. & Ochoterena, H. (2000) Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.
<https://doi.org/10.1093/sysbio/49.2.369>
- Škaloud, P., Friedl, T., Hallmann, C., Beck, A. & Dal Grande, F. (2016) Taxonomic revision and species delimitation of coccooid green algae currently assigned to the genus *Dictyochloropsis* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology* 52: 599–617.
<https://doi.org/10.1111/jpy.12422>
- Stamatakis, A. (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* 30: 1312–1313.
<https://doi.org/10.1093/bioinformatics/btu033>
- Tønsberg, T. (1990) *Japewia subaurifera*, a new lichen genus and species from north-west Europe and western North America. *Lichenologist* 22: 205–212.
<https://doi.org/10.1017/S0024282990000226>
- Tønsberg, T. (1992) The sorediate and isidiate, corticolous, crustose lichens in Norway. *Sommerfeltia* 14: 1–331.
- Urbanavichus, G., Vondrák, J., Urbanavichene, I., Palice, Z. & Maliček, J. (2020) Lichens and allied non-lichenized fungi of virgin forests in the Caucasus State Nature Biosphere Reserve (Western Caucasus, Russia). *Herzogia* 33: 90–138.
<https://doi.org/10.13158/hea.33.1.2020.90>

- Vondrák, J., Malíček, J., Šoun, J. & Pouska, V. (2015) Epiphytic lichens of Stučica (E Slovakia) in the context of Central European old-growth forests. *Herzogia* 28: 104–126.
<https://doi.org/10.13158/heia.28.1.2015.104>
- Vondrák, J., Malíček, J., Palice, Z., Bouda, F., Berger, F., Sanderson, N., Acton, A., Pouska, V. & Kish, R. (2018) Exploiting hot-spots; effective determination of lichen diversity in a Carpathian virgin forest. *PLoS ONE* 13 (9): e0203540.
<https://doi.org/10.1371/journal.pone.0203540>
- White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: Innes, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) PCR protocols: a Guide to Methods and Applications*. Academic Press, New York, pp. 315–322.
- Zhao, X., Leavitt, S.D., Zhao, Y.T., Zhang, L.L., Arup, U., Grube, M., Pérez-Ortega, S., Printzen, C., Šliwa, L., Kraichak, E., Divakar, P.K., Crespo, A. & Lumbsch, H.T. (2016) Towards a revised generic classification of lecanoroid lichens (Lecanoraceae, Ascomycota) based on molecular, morphological and chemical evidence. *Fungal Diversity* 78: 293–304.
<https://doi.org/10.1007/s13225-015-0354-5>
- Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplifications of mitochondrial small subunit ribosomal DNA of lichen-forming Ascomycetes. *Lichenologist* 31: 511–516.
<https://doi.org/10.1006/lich.1999.0220>