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Hidden in the dark under umbrellas: two new *Psilolechia* species (lichenized *Ascomycota*, *Lecanorales*) described from the Czech Republic

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Abstract. Recent lichenological fieldwork in the Czech Republic resulted in the discovery of two previously unrecognized entities in the genus *Psilolechia* which are described below as new species: the saxicolous *P. cretacea* and the lignicolous *P. torii*. Both taxa regularly produce a hyphomycetous anamorph in the form of erect, elongate conidiogenous cells on the thallus surface, and both contain unidentified specific secondary metabolites. The placement of the two new species within the genus is primarily based on morphology and chemistry. In addition, three barcodes were received for *P. cretacea* (ITS, mtSSU) and *P. torii* (ITS). A key to the five species of *Psilolechia* currently known from Europe is included.

Key words: ascomycetes, hyphomycetous lichens, *Lecanorales*, *Psilolechiaceae*, secondary chemistry, sterile lichens, taxonomy

Introduction

The crustose lichenized genus *Psilolechia* A. Massal. is morphologically a relatively well circumscribed group of small crustose microlichens forming visible extensive thalli. It is characterized by emarginate convex apothecia, asci showing an amyloid tubular structure (*Micarea*-/*Porpidia*-type) after IKI treatment, small simple dacryoid to elongate-ellipsoid ascospores usually not exceeding 7 × 2 µm in diam., and absence of pycnidia (Coppins & Purvis 1987; Czarnota & Kukwa 2008; Gilbert et al. 2009). Although pycnidia are unknown in the genus, a hyphomycetous anamorph has been described from one species, *P. clavulifera* (Coppins & Purvis 1987). All *Psilolechia* species are narrow niche specialists largely preferring rain-sheltered microhabitats. At the same time they are also shade-tolerant species occupying lichen-poor communities.

Psilolechia is the sole genus of the family *Psilolechiaceae* S. Stenroos, Miądl. & Lutzoni, a family recently established on the basis of phylogenetic multigene evidence (Miadlikowska et al. 2014). Based on their results (using two previously published sequences of the species), the authors tentatively suggested that '*Catillaria*' *erysiboides* also belonged to *Psilolechiaceae* as a member of a previously unrecognized genus, but at the same time pointed out that further sampling was needed as this relationship was not statistically supported.

Traditionally *Psilolechia* has been treated as a member of *Micareaceae* since the middle of the 1980s when that family was validly described (Hafellner 1984), and after merging of *Micareaceae* with *Pilocarpaceae*, *Psilolechia* was included in the latter family (Lumbsch & Huhndorf 2010). Previously Andersen & Ekman (2005) had shown that *Psilolechia* had fallen outside *Pilocarpaceae* based on a mtSSU phylogeny, but without any suitable alternative. Within *Lecanorales*, based on a multigene phylogeny, the family *Psilolechiaceae* appeared as the most basal clade within the suborder *Sphaerophorineae*, which otherwise includes quite distantly related families *Sphaerophoraceae*, *Psoraceae*, *Pilocarpaceae* and the more diverse *Ramalinaceae* s.lat. (Miadlikowska et al. 2014).

Currently, only four species are known in the genus *Psilolechia* worldwide (Lücking et al. 2017). Two of them (*P. lucida*, *P. clavulifera*) are widespread, subcosmopolitan lichens growing on a variety of substrates, but with different ecological preferences (see Coppins & Purvis 1987; Czarnota & Kukwa 2008). The third species (*P. leprosa*) is a rare substrate specialist occurring almost exclusively on copper-rich rocks (Coppins & Purvis 1987), but has been reported also from volcanic lahar flows in the Canary Isles and Kamchatka (Berger & Etayo 1998; Vereshchagin et al. 2023). The least known species is *P. purpurascens*, so far known only from its type specimen in Tasmania (Coppins & Purvis 1987).

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Methods

Standard lichenological techniques were used for the laboratory examination. Microscopic observations and measurements were made on hand-cut sections or as squashed preparations in tap water, using Olympus CX41 and SZ12 microscopes, the latter equipped with an Olympus DP70 camera for imaging. Habit images were made with a Olympus DP70 camera, using the extended depth-of-field module, Deep Focus. For better observations, a 5–10% aqueous solution of potassium hydroxide (K) and for amyloidity tests Lugol's solution were additionally applied. The spot reagents and thin layer chromatography procedures (using solvents A, B', C) followed Orange et al. (2010). In order to obtain higher yields of minor and trace compounds (terpenoids, UV+ substances), a repeated TLC in solvent C was carried out for improved interpretation. DNA from two samples were isolated and sequenced according to the methodology described by Vondrák et al. (2022, 2023). New ITS and mtSSU barcodes were deposited in the Genbank under the accession numbers given below in bold in the text. To compare our sequences with those deposited in Genbank and to find the closest matches, we used the megablast with default settings in the NCBI BLAST module. The vouchers are deposited in PRA, BG, and the private herbarium of J. Malíček.

Taxonomy

Psilolechia cretacea Palice, Svoboda & Vondrák, sp. nov. (Figs 1–4)

MycoBank MB 850194

Diagnosis: A sterile species similar to *Psilolechia leprosa* Coppins & Purvis, differing by having primarily non-sorediate, scurfy to unevenly cracked areolate thalli with conidiogenous cells on the surface, and by containing two types of unidentified UV+ substances and a range of terpenoids.

Type: Czech Republic, Northern Bohemia, Vysoká Lípa: National Park České Švýcarsko, W-facing rock outcrops just 0.2 km NNW from the point 'Na Tokáni', 50°52′44.9″N, 14°24′46.2″E, 450 m, on vertical to overhanging sandstone rock, NNW exposition, 3 Dec. 2015, Z. Palice 24761 (PRA holotype; BG – isotype).

Description. Thallus greyish white to almost chalky white, sterile, of dispersed or aggregated, relatively high granules to areoles (Fig. 1), ~0.05–0.8 mm in diam., up to 0.5–0.7(–1.5) mm high, with age coalescing into larger areoles and locally forming ± continuous rimose-areolate islets of several cm^2. Thallus surface matt, scurfy, finely tomentose due to the presence of numerous conidiogenous cells (Fig. 2). Cortex not evident or thin, up to 15 µm thick. Upper algal-free zone 15–50 µm thick, largely formed by a gelatinized layer (incorporating extraneous material, remnants of died hyphae and algal cells). Algal layer continuous, rich in algae, up to 200(–300) µm thick, almost completely filling younger areoles. Soredia-like, corticate granules (goniocysts) formed in peripheral parts of the algal layer (both in upper/outer and lower/inner part), 8–20 µm in diam., containing single or up to ~10 algal cells, enveloped by one layer of hyaline hyphae, 1.5 µm thick. Algae chlorococcoid, rounded or broadly ellipsoid (when larger), 5–15 µm diam., rarely up to 21 µm, often with a distinct parietal pyrenoid. Medulla in younger areoles thin, almost missing, formed by sporadic hyphae or by a loose mat of sparsely branched, narrowly luminate hyphae. In high areoles / thicker thalli the medulla may dominate. Higher areoles appear to be almost hollow in section because the medullary hyphae are sparse (Fig. 3). Hypothallus is not evident, or visible as a thin whitish mat of loose hyphae on the substrate surface, sometimes as a remnant of eroded areole. Medullary/hypothalline hyphae I– with Lugol's solution.

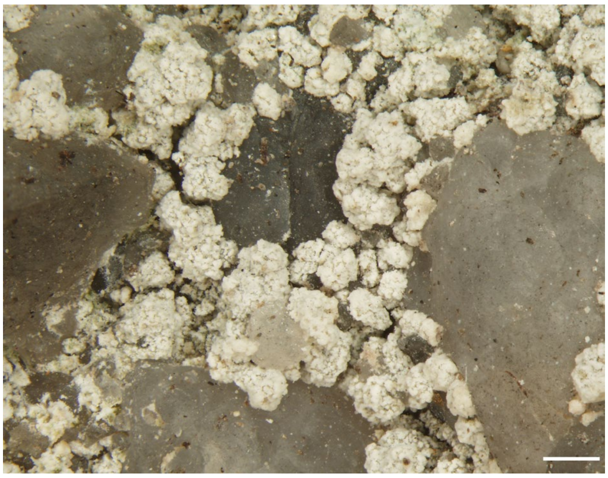

Figure 1. *Psilolechia cretacea* – habit; white areolate thalli among quartz grains, growing along fissures of more stable parts of a crumbling sandstone rock. Part of the holotype specimen (ZP 24761). Scale = 0.5 mm.

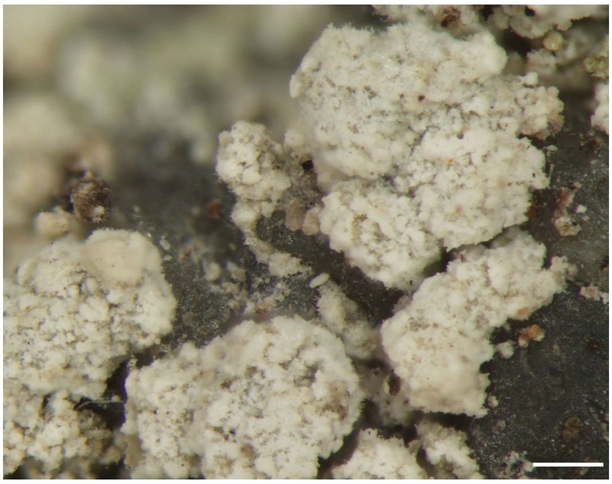

Figure 2. *Psilolechia cretacea* – a detail of scurfy / cracked areoles, numerous conidiogenous cells are visible on the lateral profiles of some of the areoles. Part of the holotype specimen (ZP 24761). Scale = 0.2 mm.

Anamorph hyphomycetous. Conidiogenous cells appearing on the thallus surface, conical to narrowly conical/cylindrical, 5–12(–18) × 2–3 µm (Fig. 4), broadened basally, individually formed, locally aggregated (sporodochia-like), but not coalescing into true sporodochia. Conidia ellipsoid to ovoid, pointed at one end, (2–)2.5–3(3.5) × 1.2–2 µm.

Chemistry. A complex chemistry of possibly three different chemical classes of secondary metabolites (two types of UV+ unknowns, both colorless in day light, and multiple terpenoids, visible after sulphuric acid treatment and charring; see Fig. 8). None of the substances could be identified based on the TLC results according to the relevant literature (Elix 2018). The UV+ ice blue substances include distinctly fluorescent compounds under UV (i) only after sulphuric acid treatment and charring and (ii) both before and after the treatment. None of the groups are visible in daylight on treated TLC plates (unlike e.g., micareic acid). We tentatively call the unknown substances of (i) as 'cretacea unknowns' as they were not detected in other *Psilolechia* species. Under UV light, they are visible as two (solvent C) or three spots (A, B') with lower Rf value [A(2–)3,3&3; B'2–3, C(2–)3&3]. Additional UV+ substances ('psilolechia unknowns') of (ii) include a range of three or more substances at positions between atranorin and norstictic acid (Rf: A5–6, B'4–5, C5–6–6). Part of these substances are shared with *P. clavulifera* and *P. torii*. The major compound from this group appears as the highest spot in solvent C and is slightly below the level of micareic acid (Fig. 7). Additionally, a range of at least 6 terpenoids (best displayed in solvent C in case of rich extracts) other than zeorin in various concentrations [Rf: A5–6, B'3–4, C4&(5–)6] were recorded on the TLC plates. Some of them are shared with *P. leprosa* or with *P. clavulifera* (see Fig. 8).

Etymology. The name refers to the white color of the thallus, which is almost chalky white, and to the fact that most of the localities are situated in the sandstone area in Northern Bohemia, once covered by the Cretaceous Sea and formed from its sediments.

Habitat and distribution. The species grows on shaded, vertical to overhanging faces of acidic rocks (sandstone, granite). It has been recorded on natural sandstone outcrops covered by a relic pine forest (type locality), but also on human-influenced sites (railway sandstone rock-cutting, granite mining gallery). So far, it has only been collected in Northern Bohemia, but it is likely to occur in the neighboring regions of Germany and Poland as well as in other areas, probably in similar localities as *Psilolechia lucida*.

Phylogeny. We received one ITS sequence (Genbank acc. n. OR581059) and one mtSSU (OR581060) of the recently collected specimen ZP 34542 (topotype). The ITS sequence has the closest NCBI BLAST hits with three sequences of *Psilolechia clavulifera* (OQ718036) and *P. leprosa* (MK811951, MK812436) with 91–92% identities. The mtSSU sequence has the closest NCBI BLAST with four sequences of *Psilolechia clavulifera* (OQ646408), *P. lucida* (KJ766473), *P. leprosa* (OP161957) and an unidentified *Psilolechia* sp. (OR490845) showing 96–97% identities.

Notes. Although this taxon is only known without apothecia, it is a distinctive species both chemically and morphologically. If not chemically tested, it could be misidentified as the sorediate species *Psilolechia leprosa*, a taxon almost restricted to copper-rich rocks (Coppins & Purvis 1987). However, visually *Psilolechia cretacea* forms non-sorediate thalli that are finely scurfy/hairy on the upper surface on closer inspection. Nonetheless, the uneven, sometimes deeply cracked surface can expose the algal layer and small propagules may be visible. Indeed, vegetative, soredia-like, corticate granules (goniocysts) have been observed on hand-cut microscopic sections of the areoles. We suspect that these structures are released more accidentally than typical soredia (in *Psilolechia leprosa*), e.g., in deeply cracked, older, eroded or broken thalli.

The species is probably overlooked because it may give the impression of juvenile or not well developed stages of more common crustose lichens (e.g. *Diploschistes scruposus*). In addition, it occurs in species-poor lichen communities of shade-tolerant crustose lichens dominated by *Psilolechia lucida* and *Lepraria* spp. Once microscopically and chemically examined, it is an identifiable lichen even without molecular evidence. There may be a closely related undescribed taxon, as suggested by TLC on a poorly developed sorediate saxicolous material from Slovakia (ZP 18088). This sample is distinguished by greenish (not white) more or less continuously sorediate

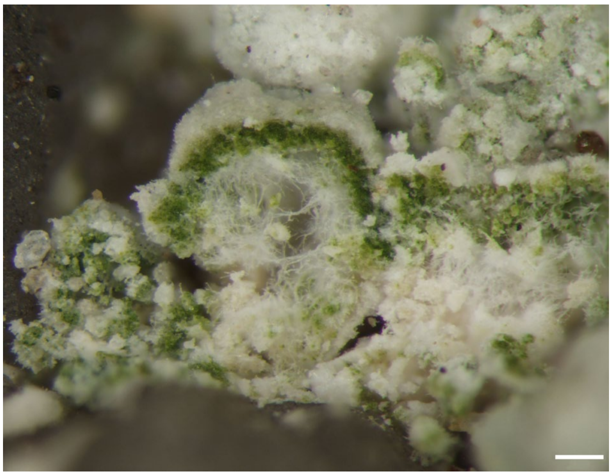

Figure 3. *Psilolechia cretacea* – a detail of sectioned larger areole (mid of the picture) – almost continuous cortex-like outer surface, green algal layer below and a thin medulla inside. Topotype specimen (ZP 34542). Scale = 0.1 mm.

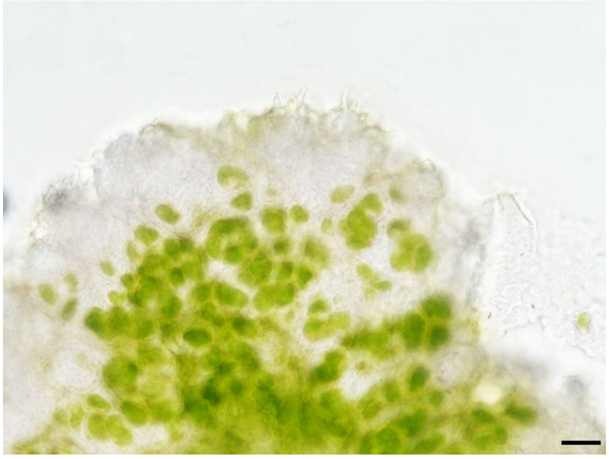

Figure 4. *Psilolechia cretacea* – sectioned areole under microscope, note a focused conical conidiogenous cell on the upper surface of the areole. Topotype specimen (ZP 34542). Scale = 20 μm.

thalli without conidiogenous cells. Chemically, it is similar to *P. cretacea* with both complexes of UV+ substances, but it seems to lack the range of terpenoids characteristic of the newly described taxon (see additional samples of other *Psilolechia* species examined for TLC).

Additional specimens examined (paratypes). CZECH REPUBLIC. Northern Bohemia, Vysoká Lípa: National Park České Švýcarsko, well-lit managed coniferous forest just E of the point 'Verdon', ~340 m NW of the settlement 'Na Tokáni', 50°52′46.4″N, 14°24′47.7″E, 465 m, on quite shaded, overhanging W-facing sandstone rock-face, 10 July 2013, Z. Palice 16377 (PRA); Northern Bohemia, Vysoká Lípa: National Park České Švýcarsko, W-facing rock outcrops just 0.2 km NNW from the point 'Na Tokáni', 50°52′44.9″N, 14°24′46.2″E, 450 m, on vertical to overhanging sandstone rock, NNW exposition, 29 Nov. 2022, Z. Palice 34542 (PRA); Northern Bohemia, distr. Česká Lípa, Sosnová: a grove between road n. 9 and railway near a small cemetery, N of the racing circuit 'Autodrom Sosnová', 50°39′44.1″N, 14°32′13.0″E, 260 m, on vertical sandstone rock by the railway, 23 Apr. 2018, Z. Palice 24945 & P. Uhlík (PRA); Northern Bohemia, distr. Děčín, Šluknov: abandoned village Fukov [Fugau], abandoned mining gallery overgrown by forest, 51°02′11.3″N, 14°29′56.2″E, 350 m, on shaded overhanging granite rock-wall, *Psilolechia lucida* associated, 11 Nov. 2021, Z. Palice 32099 & P. Uhlík (PRA).

Psilolechia torii Palice, Svoboda & Vondrák, sp. nov. (Figs 5–6)

MycoBank MB 850195

Diagnosis: A species similar to *Psilolechia clavulifera* (Nyl.) Coppins, but differing by having pale apothecia without green pigments, often inspersed with small oily granules internally, and by the absence of terpenoids detectable by TLC.

Type: Czech Republic, Western Bohemia, PLA Český les, distr. Tachov, Lesná, Mt Tetřeví vrch [815], just SW of the point Vašíček, managed spruce forest on a mild SSW-facing slope with occasional, beeches, 49°45'49.5"N, 12°26'57.5"E, 780 m, on dry shaded moribund wood of *Picea* stump, 25 Sept. 2020, Z. Palice 29649, J. Rydlo & J. Vondrák (PRA – holotype; BG – isotype).

Description. Thallus thin, up to 0.2–0.3 mm thick, non-stratified, wide-spreading, continuously granular to granular-areolate, or becoming farinose, ecorticate, green to greyish-whitish. Photobiont *Stichococcus*-like, forming small globose to more usually slightly elongated cells up to 6 µm in diam., sometimes in interrupted chains. Hypothallus indistinct. Thallus surface often more or less continuously covered by a white to greyish white hyphomycetous anamorph, more aggregated on elevated surfaces of convex granules or areoles, and then superficially resembling sporodochia.

Apothecia dispersed or locally abundant, sometimes aggregated or tuberculate, pallid, whitish to ochre-yellowish, ± glossy, plane to slightly convex, emarginate, 0.05–0.25 mm in diam., rarely wider up to 0.4 mm in tuberculate apothecia. Apothecia in section usually inspersed with small oily granules up to ~2 µm, exceptionally larger, partly dissolved in K. Hymenium unpigmented, 15–20(–25) µm high. Asci club-shaped, 12–18 × 4.5–5 µm in diam., sometimes with a stalked narrow basal part (then asci up to 25 µm long). Ascus apex poorly developed, an apical apparatus of the *Micarea*-/*Porpidia*-type only rarely observed in young asci after KI application. Ascospores elongate-ellipsoid to tear-shaped, 4–6 × 1.5–1.8 µm. Hamathecium of ± branched paraphyses, almost unthickened at apices, ~1–1.2 µm thick, rarely simple or sparsely branched, ~1.5 µm thick paraphyses intermingled, capitate at apices to ~2–2.5 µm. Hypothecium paraplectenchymatous, but usually hardly discernable due to the inspersion of oily granules, and also due to frequently incorporated non-symbiotic algae within apothecia. Excipulum receding, indistinctly delimited, composed of radiating hyphae about 1.5 µm thick, sometimes with free ends shortly protruding.

Anamorph hyphomycetous. Erect hyaline conidiogenous hyphae – conidiogenous phialidic cells cylindrical, slightly widening at the base, 10–18(–20) µm high and basally up to 2–2.5 µm wide. Conidia ellipsoid, pointed at one end, (2.5–)3–3.5(–4) × 1–1.2 µm in diam., formed singly or in chains.

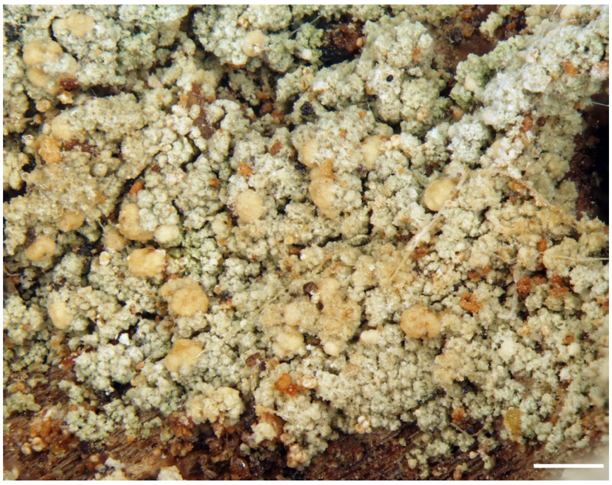

Figure 5. *Psilolechia torii* – overal habit of a richly fertile specimen. Paratype specimen (JM 13298). Scale = 0.5 mm.

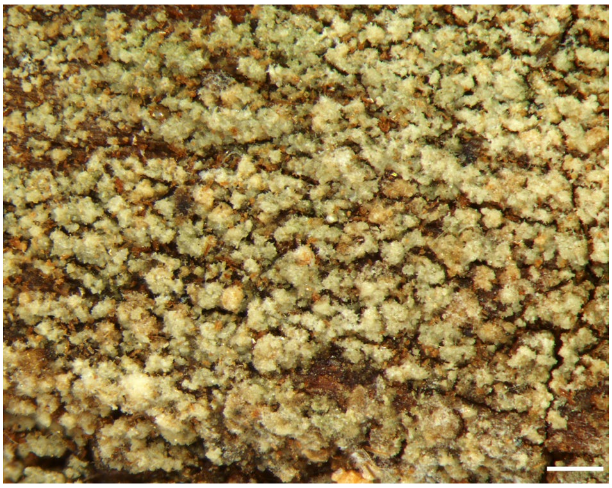

Figure 6. *Psilolechia torii* – detail of tomentose thalli, whitish hair-like conidiogenous cells visible in part of thalli. Part of the holotype specimen (ZP 29649). Scale = 0.2 mm.

Chemistry. 2–3 (or more) unidentified UV+ ice-blue substances ('psilolechia unknowns') both before and after sulphuric acid treatment and heating (Rf: A5–6, B'4–5, C6). Apparently, the same or similar substances occur in *P. cretacea*, but in different concentrations (Fig. 7), and in the latter species parts of spots are obscured by terpenoids on treated plates (Fig. 8). It is likely that the same substances are also present in *P. clavulifera*, but the yields of extracts made so far have been often too low for good interpretation of the TLC plates. A more sensitive method is needed to resolve this issue. No terpenoids were observed in *P. torii*, whereas in *P. clavulifera*, they were present on TLC plates when rich extracts of the lichen were loaded. These terpenoids are related to the terpenoids with higher Rf values detected in *P. cretacea* (see Fig. 8).

Etymology. The species name honors Tor Tønsberg, Professor Emeritus from Bergen, for his extraordinary contribution to the knowledge of lichens.

Habitat and distribution. This species has so far been recorded from a couple of localities in western and southern Bohemia, growing on rain-sheltered decaying soft coniferous wood of stumps in humid forests, where it inhabits dry and quite shaded microniches. The most suitable habitats are likely old-growth montane forests with higher volumes of decaying wood, but the type specimen was sampled in a middle-aged managed spruce forest. Associated species in the vouchers include *Chaenotheca furfuracea* and the moss *Tetraphis pellucida*.

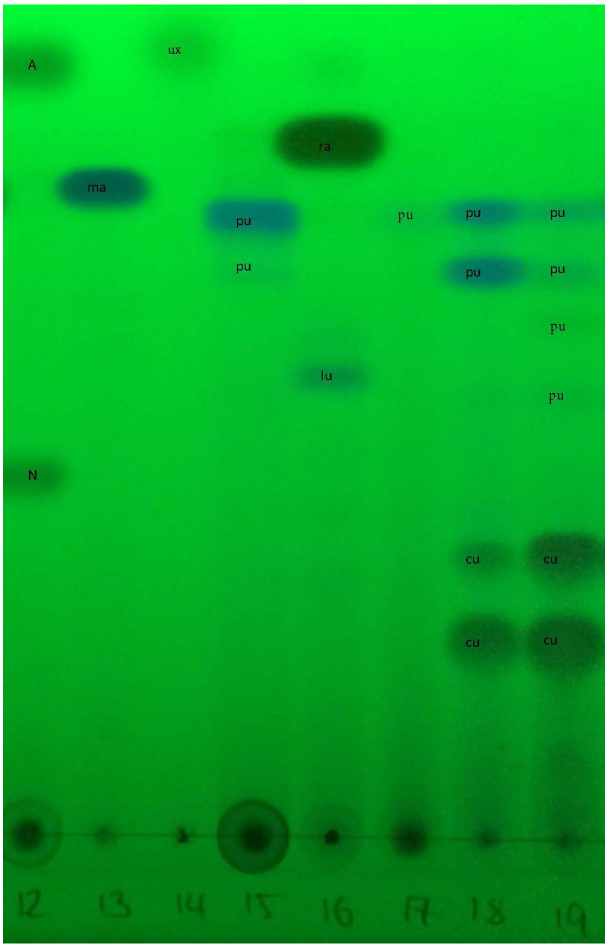

Figure 7. Part of the glass-silica TLC plate under SW UV before sulphuric acid treatment and charring – solvent C; A = atranorin, N = norstictic acid, ra = rhizocarpic acid, cu = 'cretacea unknowns', lu = 'lucida unknown', pu = complex of 'psilolechia unknowns', ux = unknown xanthone. Lane 12, controls (norstictic acid, atranorin). Lane 13, *Micarea soralifera* (Palice 33078). Lane 14, unidentified sterile lichen (Palice 34827). Lane 15, *Psilolechia torii* (Palice 35972). Lane 16, *P. lucida* (Palice 5528). Lane 17, *Psilolechia* sp. (Palice 31034). Lane 18, *P.* aff. *cretacea* (Palice 18006). Lane 19, *P. cretacea* (Palice 34542).

Phylogeny. We generated one ITS sequence (Vondrák et al. 2023, as *Psilolechia* sp., Genbank acc. n. OQ718035) of the holotype specimen ZP 29649. The closest NCBI BLAST hits are sequences of *Psilolechia leprosa* (JX171190) and *P. clavulifera* (OQ718036) showing 86–87% identities. An ITS sequence was also received from a morphologically and chemically similar specimen ZP 35972 lacking pallid apothecia (Vondrák et al. 2023, as *Psilolechia* sp., OR490825), which is however only 84% identical and may belong to another undescribed species.

Notes. *Psilolechia torii* may appear similar to the members of the *Micarea prasina* group s.lat. (Launis et al. 2019a, b) or to *Micarea farinosa* Coppins & Aptroot, an inhabitant of similar niches (Coppins & Aptroot 2014). These *Micarea* spp. differ by their usually larger ascospores and by having another photobiont than *Stichococcus*, and in most cases by the production of pycnidia. In addition, most members of the *Micarea prasina* group contain crystalline granules visible in apothecial sections under polarized light (see Launis et al. 2019a, b). Such Pol+ crystals were not observed in *Psilolechia torii*. A further difference compared to the *M. prasina* group is the presence of a hyphomycetous anamorph. The white erect conidiogenous cells on the thallus surface can make the impression of a dying "moldy" lichen. Indeed, the apparently fast-growing hyphomycetous anamorph may grow also on older apothecia, a phenomenon noted also in *Psilolechia clavulifera* (Coppins & Purvis 1987). A similar moldy growth form may presumably be shared by some members of the genus *Chaenotheca* (Honegger 1985; Tibell & Ryan 2004), and careful microscopic examination and TLC is recommended to distinguish between sterile specimens of these genera and saprobic or lichenicolous hyphomycetes.

Additional specimens examined (paratypes). CZECH REPUBLIC. Western Bohemia, Šumava Mts, Hamry: nature reserve Bílá strž, forest on the left bank of the brook Bílý potok above the waterfall, 49°11′23.8″N, 13°09′25.4″E, 940 m, on wood of dry *Picea* stump near the brook, 16 Sept. 2019, Z. Palice 27471 (PRA); Western Bohemia, PLA Český les, distr. Tachov, fragment of old-growth beech forest 3.7 km NW of Lesná, 49°46′18.7″N, 12°29′42.1″E, 730 m, on decaying stump of (?) *Picea* [*Fagus* on the label], 25 Oct. 2019, J. Malíček 13298 & J. Rydlo (hb. Malíček); Southern Bohemia, Šumava Mts, Prachatice, Záblatí: the canyon-like valley of Blanice, mixed forest on the right bank of the rivulet, N-NNW foothill of Mt. Panský vrch [834], 1.3 km SW-SSW of the settlement Hlásná Lhota, 48°58′19.0″N, 13°55′34.2″E, 671 m, on strongly rotten wood of stump of a conifer (?*Abies alba*), 12 July 2022, Z. Palice 30424 (PRA).

Additional specimens examined of other *Psilolechia* species (used for TLC).—*Psilolechia clavulifera*. CZECH REPUBLIC. Southern Bohemia, Šumava Mts, Volary, Černý Kříž: a channel of the Hučina brook, ~100 m up the stream of the confluence with the river Studená Vltava, 48°51′40″N, 13°52′15″E, 740 m, on hard wood of a pillar near the water,

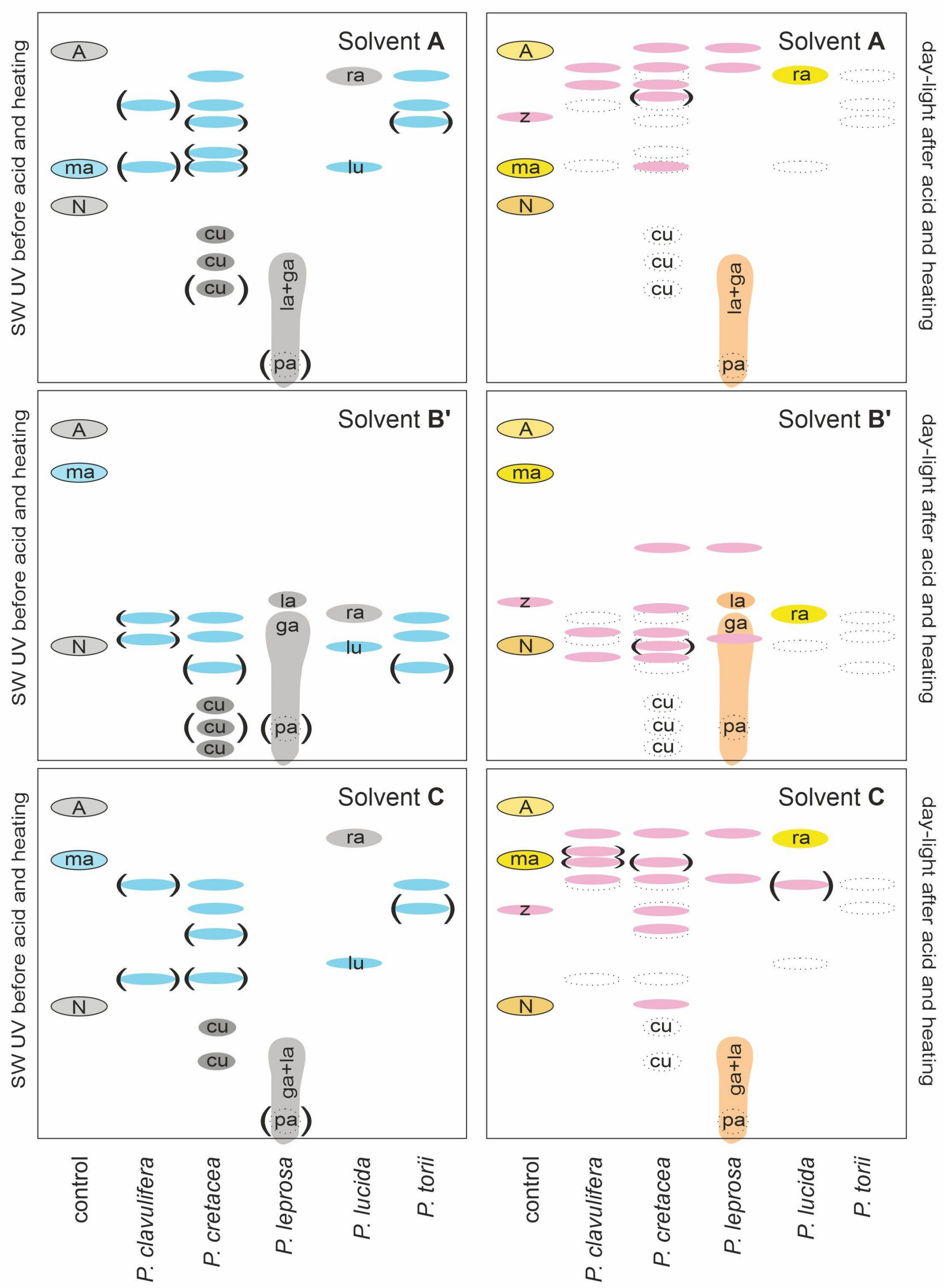

Figure 8. Schematic visualization of chromatograms with characteristic compounds detected in *Psilolechia*. Control compounds include atranorin (A), micareic acid (ma), zeorin (z) and norstictic acid (N). Unidentified class of UV+ ice-blue substances ('psilolechia unknowns') is displayed in blue color in first column (untreated plates under SW UV) and as colorless spots bordered by a dashed line (treated plates in day light). Unidentified terpenoids visible after treatments are displayed as purplish spots. Abbreviations of other substances (cu = 'cretacea unknowns', la = lecanoric acid, lu = 'lucida unknown', ga = gyrophoric acid, pa = porphyrilic acid, ra = rhizocarpic acid). Brackets indicate compounds in smaller concentration, often not detected on TLC plates. Trace LW UV+ compounds in *Psilolechia lucida* are not displayed here.

17 May 2003, Z. Palice 6323 (PRA); Ibid., nature reserve Mrtvý luh – spruce forest around a blind arm of the river Teplá Vltava, SE marginal part, 2.5 km E of the railway-station Černý Kříž, 48°51′42.4″N, 13°53′40.8″E, alt. 730 m, on sheltered root-plate (bark of roots) of eradicated *Picea abies*, 1 July 2023, Z. Palice 36010, 36016 (PRA); Northern Bohemia, Jizerské hory Mts, Mt. Na kneipě [1013], nature reserve 'Klečové louky', Malá klečová louka, boggy spruce forest at NW margin of the reserve, 50°50′19.5″N, 15°14′41″E, 972 m, on shaded roots of wind-blown *Picea*, 17 July 2028, Z. Palice 26201 (PRA).—*Psilolechia leprosa*. CZECH REPUBLIC. Northern Bohemia, Krkonoše Mts, Velký Kotel corrie – abandoned mining (crystalline limestone) minigallery, 50°45′06″N, 15°31′57″E, alt. 1350–1355 m, 31 August 2002, Z. Palice 12023 (PRA). Western Bohemia, distr. Sokolov, Slavkovský les, Horní Slavkov – "Jáma Hubert" mine 1 km S of town, 50°07′N, 12°48′E, alt. 600–650 m, on shady Cu-rich siliceous boulder, 18 April 2009, J. Malíček 1806 (PRA, dupl.).—*Psilolechia lucida*. CZECH REPUBLIC. Western Bohemia, Slavkovský les: Podhorní vrch hill near Mariánské lázně, a basalt scree on S slope, 810–820 m, on overhanging boulder, 25 April 1997, Z. Palice s.n. (PRA). Southern Bohemia, Šumava Mts, Záhvozdí: Mt. Černý les – W slope, an old-growth scree forest, ~48°50′N, 13°58′E, alt. 850 m, on overhanging silic. stone ar the forest-road-side,

14 April 2000, Z. Palice 5046 (PRA). Ibid., Volary: Mt. Stožec, nature reserve “Stožecká skála”, 48°52′25″N, 13°49′17″E, 940–950 m, on overhanging shaded granite rock-wall, 3 June 2001, Z. Palice 5528 (PRA) [TLC: rhizocarpic acid, unknown UV+ blue-white substance before sulphuric acid treatment and heating Rf A5, B’4–5, C5 (‘lucida unknown’), a terpenoid was observed in one solvent (Rf C6), traces of multiple long wave UV+ bluish and pinkish-substances visible before and after sulphuric acid treatment and charring, not shown on Fig. 8].—*Psilolechia* sp. (aff. *cretacea*). SLOVAKIA. Western Carpathians, distr. Poprad, Primovce, nature reserve Primovské skaly, N-facing steep shaded rock-outcrop above a brooklet (Tarnovský potok), 49°00′56.4″N, 20°22′56.5″E, 591 m, on overhanging shaded melaphyric rock, 16 June 2014, A. Guttová, A. Lackovičová, J. Liška & Z. Palice 18088 (PRA) [TLC: UV+ unknown substances as in *P. cretacea*, no terpenoids visible as purplish spots on treated plates].—*Psilolechia* sp. (aff. *torii*). CZECH REPUBLIC. Southern Bohemia, Šumava Mts, Volary, Jelení Vrchy: valley of a right-hand-side tributary of the Hučina brook, humid old-growth spruce-dominated forest, just NW of the settlement, 48°49′11″N, 13°52′12.8″E, 875 m, on sheltered hard wood of *Picea* stump, 4 July 2023, Z. Palice 35972 (PRA, with only one apothecium) [TLC: UV+ unknowns substances as in *P. torii*].

Remarks and discussion

The genus *Psilolechia* contains a spectrum of various metabolite substance classes (following Elix 2018): pulvinic acid derivatives (rhizocarpic acid), orcinol tridepsides (gyrophoric acid), dibenzofurans (porphyrilic acid), unidentified (tri-)terpenoids and β-orcinol depsides (atranorin) (Coppins & Purvis 1987; Yoshimura & Harada 2004; Czarnota & Kukwa 2008). Some of the unidentified compounds may belong to other substance classes. Curiously, the information on chemistry in the most widespread species, *P. lucida*, slightly differs among authors regarding the accessory substances that usually occur in trace amounts in addition to the major substance rhizocarpic acid giving the characteristic color to the lichen. Tønsberg (1992) noted traces of an unknown substance in corticolous Norwegian material without further specification. In Polish specimens of *P. lucida*, Czarnota & Kukwa (2008) detected at least 7 unknown accessory substances by TLC that they tentatively identified as terpenoids. Yoshimura & Harada (2004) reported the presence of atranorin in *P. lucida* (otherwise not known either from this species or the genus) from Japan, but using a more sensitive HPLC method.

Our preliminary TLC results suggest that all European representatives of *Psilolechia* contain secondary metabolites. However, for small species such as *P. clavulifera*, it may be difficult to find suitable and sufficient material large enough to obtain highly concentrated extracts for analysis, with limited amount of substrate contamination. The newly described saxicolous species *P. cretacea* contains three compound rich groups of substances and may prove to be a good reference species for future studies on *Psilolechia*. Some of these compounds may even be characteristic or specific for *Psilolechia* or *Psilolechiaceae*. Terpenoids detected previously or during this study in *P. clavulifera* and *P. leprosa* are likely to be closely related or the same as in *P. cretacea*. The same is true for the range of UV+ compounds found in most *Psilolechia* species, but in varying amounts and concentrations. For better orientation, we have prepared a simplified schematic overview (Fig. 8) of six chromatograms of the characteristic substances of the hitherto known European representatives of the genus *Psilolechia* in standard solvents before and after application of sulphuric acid and charring. Nonetheless, the complex chemistry of *Psilolechia* definitely requires further study.

The two most widespread species *P. lucida* and *P. clavulifera* are quite variable when it comes to the anatomy of the thallus. Both species may produce finely leprose-farinose or granular-areolate thalli. Coppins & Purvis (1987) mentioned a trebouxioid photobiont for *P. lucida*, but also noted populations with *Stichococcus*, while in *P. clavulifera*, only *Stichococcus* is known. In *Psilolechia lucida*, Tønsberg (1992) recorded only non-sorediate thalli in corticolous material with *Stichococcus*, whereas saxicolous specimens with trebouxioid algae were sorediate. According to our own observations, in *P. clavulifera* both morphotypes may occur even within a small specimen. It is likely a more complex problem, reflecting not only the nature of the photobiont, but also age and various environmental factors. Greater variability in thalline characters may also be expected in the lesser known members of the genus.

The presence of hyphomycetous anamorphs in lichens of rain-protected habitats is an underexplored topic. Apparently, the first observations of a hyphomycetous stage in lichens were made and illustrated as early as the 19th century by Neubner (1893) on a member of the genus *Chaenotheca*, but the formation of conidia was interpreted as fragmentation of hyphae (see Tibell 1997 for more details). R. Honegger documented a hyphomycetous anamorph and described conidiogenesis for the common species *Chaenotheca furfuracea* (Honegger 1985), both in culture and as a field observation. Coppins & Purvis (1987) compared erect phialidic conidiogenous cells of *Psilolechia clavulifera* and *Chaenotheca furfuracea* and suggested that their presense in these two species was a specific microhabitat adaptation. Later reports on anamorphic stages of the genus *Chaenotheca* were surprisingly few. Tibell (1993, 1997) successfully cultivated hyphomycetous anamorphs of eight species of *Chaenotheca* not observed by him in the herbarium material. Based on the morphology, this anamorph was considered close to the genus *Phialophora* (Tibell 1993). The presence of anamorphic hyphomycetous stage in *Chaenotheca* was later noted in the Lichen Flora of the Sonoran Desert (Tibell & Ryan 2004) for four common species that also occur in Europe (*C. chrysocephala*, *C. furfuracea*, *C. trichialis*, *C. xyloxena*), but without additional details whether this was also based on observations in the herbarium or in the field. In the case of the genus *Chaenotheca*, it is not unlikely that the lack or paucity of observation data on anamorphic stages in the herbarium or in the field is simply due to the fact that the co-occurrence of fruiting bodies and the anamorphic ‘mould-like’ stage may give the impression that dying/dead specimens are covered with alien saprobic fungi and therefore are not documented. From the recent field experience of the authors, it seems that in suitable microhabitats (e.g., wood in dry cavities of standing dead trees or snags in moist and shady locations), coatings of conidiogenous cells associated with fertile representatives of the genera *Chaenotheca* and *Psilolechia*, are not uncommon (but have not been studied in detail). However, we are well aware that there are similar saprophytic or lichenicolous fungi that may grow in the same habitats. Therefore, more attention needs to be paid to this challenging issue in the future, including the use of cultivation and molecular techniques.

Conclusions

Psilolechia is still a poorly known genus. The main aim of this paper was to describe previously unrecognized species in order to draw more attention to this neglected group of ecologically and morphologically highly adapted lichens. The paper also aims to draw attention to the possibility of omitting helpful morphological and chemical characters, such as the presence of hyphomycetous anamorphs, as well as unidentified accessory substances that may prove to be taxonomically important in the identification of sterile specimens and so far unrecognized members of *Psilolechiaceae*.

Identification key to *Psilolechia* species known from Europe

Note: The below preliminary key includes only described taxa of *Psilolechia* known to date from Europe. Sterile specimens (lacking apothecia or conidiogenous cells) morphologically, ecologically or chemically similar to *Psilolechia cretacea* or to *P. torii* may represent undescribed taxa and have not been included.

1(1) Thallus brightly yellow-green, bright yellow apothecia sometimes present, rhizocarpic acid as the major metabolite . . . . **P. lucida**

1(2) Thallus not yellow-green, other substances than rhizocarpic acid or seemingly without substances (by TLC) 2

2(1) Vividly green to gray thinner thallus (< 0.3 mm high) containing *Stichococcus*-like algae, often covered by a white sporodochium-like hyphomycete layer 3

2(2) White thicker thallus (> 0.3 mm high when well developed), with chlorococcoid algae 4

3(1) Apothecia dark with a green pigment, seemingly no lichen substances detectable by TLC or one to two UV+ substance(s) and terpenoid(s) other than zeorin (Fig. 8), wide substrate ecology, often on by soil-particle enriched organic substrates or on rock in rain-sheltered habitats . . . . **P. clavulifera**

3(2) Apothecia pale, inspersed with oily granules in section, two or more UV+ substances detectable by TLC (Fig. 8), terpenoids lacking, on decaying wood in rain-sheltered and shaded niches . . . . **P. torii**

4(1) Thallus C+ red, UV– or UV+ whitish (gyrophoric acid as major metabolite), soon forming a leprose crust lacking conidiogenous cells on the thallus surface, sometimes with pallid apothecia, saxicolous on copper-rich rocks and mortars, and / or volcanic lahars . . . . **P. leprosa**

4(2) Thallus C-, distinctly UV+ ice-blue, scurfy, covered by erect elongated conidiogenous cells with ovoid conidia, saxicolous on acidic rocks . . . . **P. cretacea**

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