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A new species of *Porpidia* from China

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Abstract: *Porpidia hypostictica* is described as a new species; it has a white to grey-white thallus with yellow, oxidized patches near the margin, and contains hypostictic acid as the only major compound. A phylogenetic analysis is provided which is based on ITS sequences using ML and Bayesian analyses. The phylogeny supported the separation of the new species from other species. A key to all known Chinese *Porpidia* species is provided.

Key words: phylogenetic analysis, saxicolous lichens, taxonomy

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Introduction

The lichen genus *Porpidia* Körb. (Körber 1855) has been the subject of a number of taxonomic studies in recent decades including Hertel (1975, 1977), Inoue (1983), Hertel & Knoph (1984), Knoph (1984), Schwab (1986), Gowan (1989*a*, *b*), Gowan & Ahti (1993), Buschbom & Mueller (2004), Fryday (2005) and Orange (2014). *Porpidia* generally displays few taxonomically useful characters and many of these are very variable. In addition, the homology of character states within and among groups is difficult to assess, consequently problems arise in the delimitation of species. There are also numerous unidentified collections which probably represent undescribed taxa. Molecular data have gained importance in lichen systematics and now have a significant impact on the classification and taxonomy of the genus *Porpidia*. Buschbom & Mueller (2004) investigated the phylogeny of the genus using nuclear ribosomal large subunit RNA and nuclear β-tubulin markers. The analyses indicated that the genus *Porpidia* could be divided into three infrageneric groups: the *P. macrocarpa* group, the *P. albocaerulescens* group and the *P. speirea* group. Orange (2014) investigated the phylogeny of the genus using the internal transcribed spacer (ITS) and nuclear ribosomal large subunit. The analyses separated *P. contraponenda* (Arnold) Knoph & Hertel and the new species *P. irrigua* Orange. These two molecular studies have greatly enhanced our understanding of the infrageneric relationships.

During our study on the lichen flora of Mt. Changbai in Jilin Province, China, a species of *Porpidia* new to science was found. We present a brief diagnosis, an extended description, and a phylogenetic analysis based on ITS-sequence data. The phylogeny will make it possible to re-evaluate morphological and chemical characters in the group, and to conduct detailed studies of species delimitations within the monophyletic subgroups.

The Changbai Mountain Range straddles the border between China and North Korea (41°41'–42°51'N, 127°43'–128°16'E). The range extends from the north-east Chinese provinces of Heilongjiang, Jilin and Liaoning to the North Korean provinces of Ryanggang and Chagang. Most peaks exceed 2000 m in height, with the highest being Paektu Mountain. Mt. Changbai sits in the

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temperate zone with a continental mountain climate and an annual average temperature of −7 °C to 3 °C.

Materials and Methods

The specimens studied were collected from Jilin Province, China, and are preserved in the Lichen Section of the Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU). The specimens were examined using standard microscopic techniques and hand-sectioned under a NIKONSMZ 645 dissecting microscope. Anatomical descriptions are based on observations of these preparations under a NIKON Eclipse E200 microscope. Sizes of the thallus, apothecium, hymenium and exciple were based on five measurements for each specimen. Dimensions of ascospores based on ten measurements per specimen are presented as the range with outlying values given in parentheses. Secondary metabolites of all the specimens were identified using TLC and solvent C as described by Orange *et al.* (2010). The medulla was tested for an amyloid reaction using IKI (10% aqueous potassium iodide).

DNA was extracted from frozen specimens using the SanPrep Column DNA Gel Extraction Kit, following the manufacturer's instructions. PCR amplification was carried out using Tiangen Taq in 50 ul tubes. The two internal transcribed spacer regions and the 5.8S region (ITS1-5.8S-ITS2) of the nuclear ribosomal genes were amplified, using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). The PCR thermal cycling parameters were: initial denaturation for 3 min at 94 °C, followed by 3 cycles of 30 s at 94 °C, 30 s at 52 °C, and 90 s at 72 °C, then 35 cycles of 30 s at 94 °C, 30 s at 52 °C and 90 s at 72 °C; and a final extension step at 72 °C for 10 min. PCR products were visualized on agarose gels stained with ethidium bromide. Sequencing was performed by Sangon Biotech Co. Ltd (Shanghai) with an ABI 3730 XL DNA Analyzer.

BLAST searches in GenBank were performed to ascertain that all sequences used in the phylogenetic analyses originated from the lichens and not from contaminating organisms such as parasymbiotic fungi. Contigs were assembled and edited using the program Geneious v6.1.2 (Biomatters Ltd, Auckland, New Zealand). Sequences were aligned using the program MAFFT v7. For ITS sequences, we used the L-ING-i alignment algorithm with the remaining parameters set

TABLE 1. *Specimens used in the phylogenetic analyses of* Porpidia *species. New sequences are in bold.*

Species	Country	Voucher	GenBank accession number
P. macrocarpa	Wales	*Orange* 16319 (NMW)	KJ162270
P. macrocarpa	Scotland	*Orange* 21043 (NMW)	KJ162269
P. macrocarpa	England	*Orange* 16225a (NMW)	KJ162267
P. flavocruenta	Norway	*Orange* 18941 (NMW)	KJ162275
P. crustulata	Turkey	–	HQ605941
P. islandica	Faroe Islands	*Orange* 17148 (NMW)	KJ162313
P. hypostictica	China	*L. Hu* 20141111 (SDNU)	**KR069079**
P. hypostictica	China	*L. Hu* 20141385 (SDNU)	**KR069081**
P. hypostictica	China	*L. Hu* 20141375 (SDNU)	**KR069080**
P. contraponenda	England	*Orange* 16220 (NMW)	KJ162296
P. contraponenda	Wales	*Orange* 20447 (NMW)	KJ162297
P. musiva	Turkey	–	HQ605939
P. cinereoatra	Ireland	*Orange* 21404 (NMW)	KJ162306
P. cinereoatra	Ireland	*Orange* 21426 (NMW)	KJ162307
P. cinereoatra	Wales	*Orange* 20432 (NMW)	KJ162305
P. irrigua	Wales	*Orange* 16321 (NMW)	KJ162299
P. irrigua	Wales	*Orange* 16494 (NMW)	KJ162301
P. irrigua	Wales	*Orange* 18014 (NMW)	KJ162302
P. striata	England	*Orange* 16227 (NMW)	KJ162314
P. hydrophila	Wales	*Orange* 16313 (NMW)	KJ162318
P. hydrophila	England	*Orange* 16218 (NMW)	KJ162317
P. hydrophila	Wales	*Orange* 17598 (NMW)	KJ162319
P. rugosa	Ireland	*Orange* 21403 (NMW)	KJ162321
P. rugosa	Faroe Islands	*Orange* 17159 (NMW)	KJ162320
P. albocaerulescens	USA	*Tripp* 2530 (NY)	KJ653475
P. albocaerulescens	USA	*Lendemer* 33291 (NY)	KJ653476
P. tuberculosa	Ireland	*Orange* 18291 (NMW)	KJ162322
P. melinodes	Norway	*Orange* 19209 (NMW)	KJ162327
P. flavicunda	Norway	*Orange* 18971 (NMW)	KJ162332
Immersaria iranica	China	*L. Hu* 20117623 (SDNU)	**KR061348**

to default values. Three *Porpidia* specimens representing the new species *P. hypostictica*, collected from Jilin Province in mainland China, were selected for the phylogenetic analysis. Sequences generated for this study were complemented with sequences from GenBank representing additional specimens or species. *Immersaria iranica* Valadb., Sipman & Rambold was selected as outgroup. Specimens used in the analyses are shown in Table 1.

Bayesian analyses were carried out using locus-specific model partitions (ITS1, 5.8s rDNA, ITS2) in MrBayes v3.2.3. The nucleotide substitution models of the three loci were selected using the Akaike Information Criterion in jModelTest v2.1.7. The Bayesian analysis was run for 10 000 000 generations with four independent chains and sampling every 1000th tree. All model parameters were unlinked. Two independent Bayesian runs were conducted to ensure that stationarity was reached and

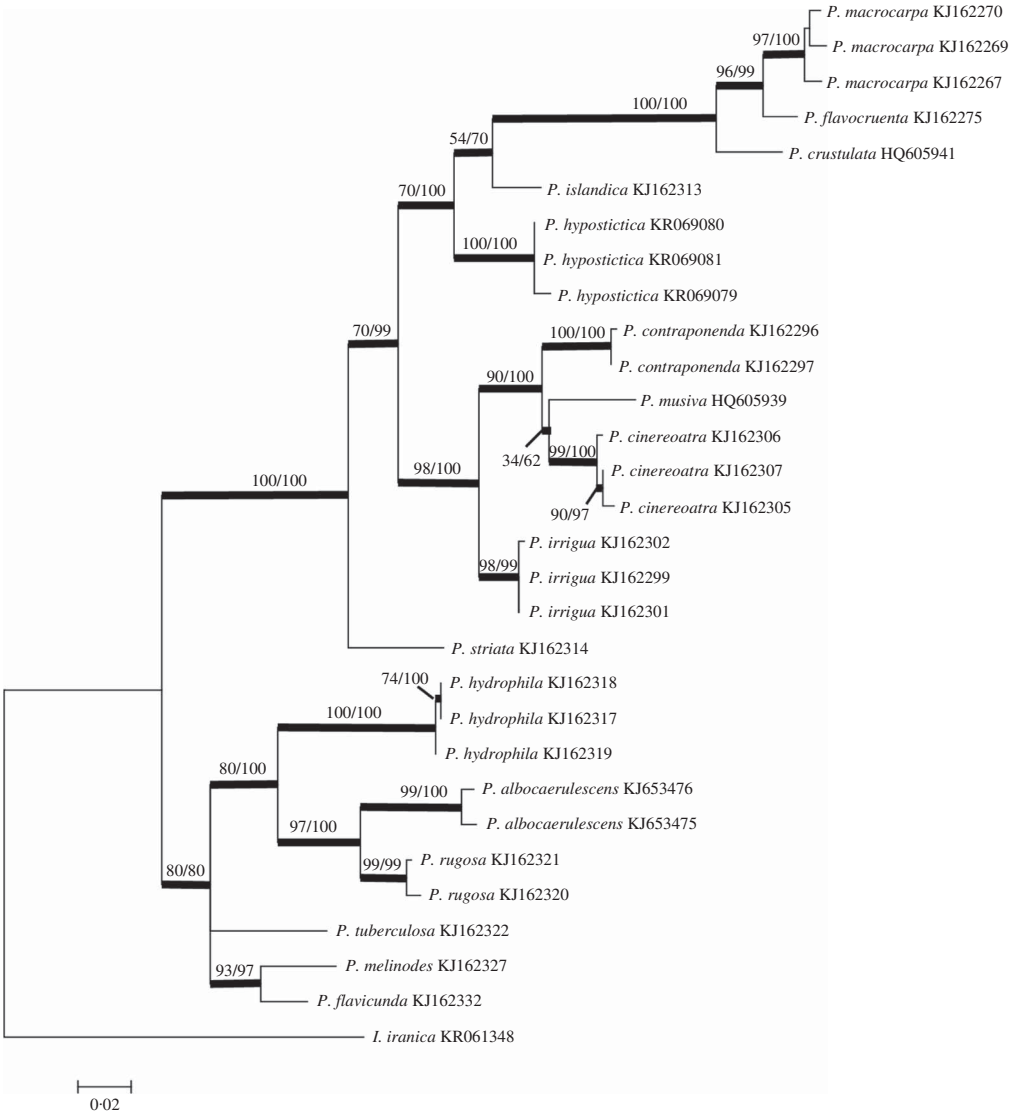

FIG. 1. Maximum likelihood phylogram for *Porpidia* species, based on the nuclear ribosomal ITS1-5.8S-ITS2 region. *Immersaria iranica* was used as outgroup. Posterior probabilities ≥95% and ML bootstrap values ≥70% are listed to the left and right of slashes respectively.

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the runs converged at the same log-likelihood level (verified by eye and with AWTY option). After discarding the burn-in, the remaining 7500 trees of each run were pooled to calculate a 50% majority-rule consensus tree. Maximum likelihood (ML) analyses were conducted in MEGA6 (Tamura *et al.* 2013). Phylogenetic relationships and support values were investigated using the Maximum Composite Likelihood method. The percentages of replicate trees (1000 replicates) in which the associated taxa clustered together in the bootstrap test (Felsenstein 1985) are shown next to the branches (Fig. 1). The clades that received a bootstrap support of 70% through ML and posterior probabilities of 0·95 were considered significant. Phylogenetic trees were visualized using FigTree v1.4.2.

Results and Discussion

Porpidia hypostictica L. Hu & Z. T. Zhao sp. nov.

MycoBank No.: MB 814973

Thallus white to grey-white, with yellow, oxidized patches near the margin; apothecia sessile, up to 2·3 mm diam., ascospores 17·5–25·0 µm long. Thallus containing hypostictic acid as the only major compound.

Type: China, Jilin Prov., Changbai Co., Mt. Changbai, 41°35'4·95"N, 127°51'51·69"E, alt. 1300 m, on rock, 25 July 2014, *Ling Hu* 20141385 (holotype—SDNU; GenBank: KR069081).

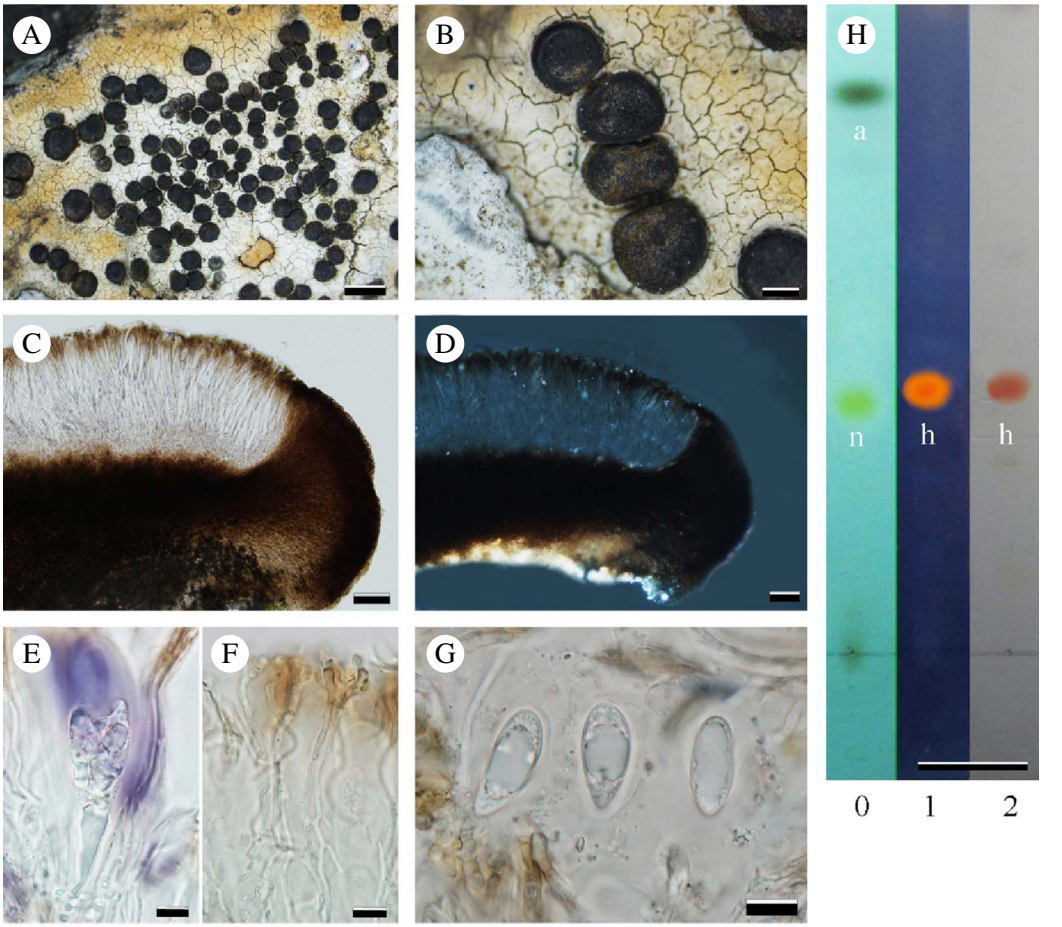

FIG. 2. *Porpidia hypostictica* (*Hu* 20141385, SDNU). A, thallus; B, apothecia; C, apothecium section; D, epihymenium and exciple without crystals; E, amyloid reaction of ascus; F, paraphyses; G, ascospores; H, chromatograms of whole thallus extracts (solvent C):0 = control extract from *Lethariella cladonioides* which contains atranorin and norstictic acid viewed in natural light; 1 & 2 = *P. hypostictica* (located using fluorescence under longwave (365nm) UV light (1) or natural light (2)). Compounds detected: a = atranorin; n = norstictic acid; h = hypostictic acid. Scales: A = 2 mm; B = 500 µm; C & D = 50 µm; E, F & G = 10 µm; H = 1 cm.

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Thallus continuous, saxicolous, usually rimose, sometimes areolate, 0·2–0·7 (–1·0) mm thick, surface white to grey-white with yellow, oxidized patches near the margin; medulla I−; *prothallus* inconspicuous; *soredia* and *isidia* absent.

Apothecia sessile, up to 2·3 mm diam., scattered or clustered in small groups; *disc* dark brown to black, plane to commonly convex, occasionally slightly orange pruinose; *margin* distinct when young, 90–140 µm wide, epruinose. *Exciple* with brown pigment, pigment dense at margin and dilute to moderately dense within, (100–)120–175 µm wide, without crystals; hyphae 5–8 µm diam. *Hypothecium* brown to dark brown, without crystals. *Hymenium* hyaline, 110–130(–150) µm high; *epihymenium* olive-brown to brown. *Asci* clavate, 8-spored, tholus with an I+ blue tube-structure; *ascospores* ellipsoid, simple, colourless, halonate, 17·5–23·0 (–25·0) × 7–10 µm.

Pycnidia usually present, sometimes frequent, black, slightly raised, orbicular to somewhat elongate, with raised and white pseudothalline margin; *conidia* simple, colourless, bacilliform, 10–14 × 0·7–1·2 µm.

Chemistry. Cortex and medulla K+ orange, C−, KC− . Hypostictic acid detected by TLC (Fig. 2).

Comments. The new species is similar to *Porpidia macrocarpa* (DC.) Hertel & A. J. Schwab in having a yellow oxidized surface, large apothecia, large ascospores, a wide exciple and olive-brown to brown epihymenium. However, *P. hypostictica* can be distinguished by the presence of stictic acid. This new species resembles *P. thomsonii* Gowan by having a similar thallus surface, dark exciple, and similar thick excipular cells. *Porpidia thomsonii*, on the other hand, has smaller apothecia, a lower hymenium, smaller ascospores, and a thinner thallus containing stictic acid. In the phylogenetic tree, *P. hypostictica* and other species clustered into different monophyletic clades, demonstrating that *P. hypostictica* is a distinct species.

Specimens examined. **China:** *Jilin Prov.*: Fusong Co., Mt. Changbai, 42°3'N, 127°47'E, 2300 m, on rock, 2014, *Ling Hu* 20141095, 20141097, 20141112 (SDNU), *Weicheng Wang* 20141074, 20141371, 20141111, 20141113 (SDNU); Changbai Co., Mt. Changbai, 41°35'N, 127°51E, 1300 m, on rock, 2014, *Feixiang Shi* 20141384, 20141383, 20141370 (SDNU), *Ling Hu* 20141385, 20141372, 20141374, 20141375 (SDNU).

Key to the species of *Porpidia* occurring in China

1 Thallus subsquamulose, brownish yellow, lacking secondary products. Epihymenium blue-green (Cinereorufa-green) . . . . . **P. squamosa**
Thallus crustose. Thallus upper surface, chemistry and epihymenium colour various 2

2(1) Thallus with soredia, apothecia present or absent 3
Thallus without soredia, apothecia present 4

3(2) Medulla I+ blue . . . . . **P. tuberculosa**
Medulla I−. On siliceous rocks. Soralia usually tuberculate . . . . . **P. soredizoides**

4(2) Medulla I+ blue, thallus with confluentic acid present 5
Medulla I−, thallus with confluentic acid or not 6

5(4) On basic rocks, thallus white, apothecia innate . . . . . **P. speirea**
On siliceous rocks, thallus grey, apothecia usually sessile . . . . . **P. grisea**

6(4) Exciple of apothecia in section with dark pigmented cortex and non-pigmented medulla composed of thin filamentous hyphae 2–4 µm wide (albocaerulescens-type) 7

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Exciple of apothecia with pigmented medulla, usually composed of thicker pseudoparenchymatous hyphae 3–8 µm wide; if hyphae thinner then medulla unpigmented 10

7(6) Epihymenium blue-green (Cinereorufa-green). Usually on siliceous rocks . . . . . **P. hydrophila**
Epihymenium brown (Arnoldiana-brown) or olivaceous (Macrocarpa-green), apothecia often pruinose 8

8(7) 2'-O-methylsuperphyllinic acid present . . . . . **P. carlottiana**
2'-O-methylsuperphyllinic acid absent, stictic acid or norstictic acid present. 9

9(8) Thallus and apothecium exciple containing stictic acid. Apothecia often heavily pruinose . . . . . **P. albocaerulescens var. albocaerulescens**
Thallus and apothecium exciple containing norstictic acid. Apothecia often slightly pruinose . . . . . **P. albocaerulescens var. polycarpiza**

10(6) Thallus orange, usually containing confluentic acid . . . . . **P. flavicunda**
Thallus white, grey, sometimes partly oxidized, chemistry various 11

11(10) On calcareous rocks 12
On siliceous rocks 14

12(11) Apothecia with a white pruinose outer rim to the proper margin. . . . **P. zeoroides**
Apothecia without a white pruinose outer rim to the proper margin 13

13(12) Epihymenium blue-green (Cinereorufa-green). Thallus endolithic, apothecia directly on rock. . . . . **P. shangrila**
Epihymenium and exciple with only orange-brown pigment (Superba-brown), exciple medulla dark orange-brown with dark brown cortex. Apothecia strongly constricted below, disc usually brown with a thick, raised margin. Ascospores usually >20 µm long. Thallus white, usually bullate, but occasionally smoother . . . . . **P. superba f. superba**

14(11) Apothecia usually innate. Thallus containing confluentic acid . . . . **P. cinereoatra**
Apothecia sessile 15

15(14) Stictic acid present or secondary products absent 16
Stictic acid absent, other secondary products present 19

16(15) Exciple thinner (<100 µm), spores shorter (10·0–17·5 µm) . . . . . **P. crustulata**
Exciple thicker (>100 µm), spores longer (16–25 µm) 17

17(16) Apothecia usually less than 1·2 mm diam., exciple hyphae wider, 5–8(–10) µm, spores ellipsoid, 17·5–22·5 × 7·5–9·0 µm . . . . . **P. thomsonii**
Apothecia up to 3 mm diam., exciple hyphae thinner, 3·0–4·5(–6·0) µm, spores ellipsoid, 17–25 × 6–11 µm 18

18(17) Exciple K− . . . . . **P. macrocarpa f. macrocarpa**
Exciple K+ red . . . . . **P. macrocarpa f. nigrocruenta**

19(15) Apothecia larger, 1·2–2·0 mm diam., exciple hyphae wider, 5–8 µm, hypostictic acid present . . . . . **P. hypostictica**
Apothecia 0·5–1·2 mm diam., exciple hyphae 3–5 µm. Thallus containing confluentic acid . . . . . **P. lowiana**

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References

Buschbom, J. & Mueller, G. (2004) Resolving evolutionary relationships in the lichen-forming genus *Porpidia* and related allies. *Molecular Phylogenetics and Evolution* **32:** 66–82.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39:** 783–791.

Fryday, A. M. (2005) The genus *Porpidia* in northern and western Europe, with special emphasis on collections from the British Isles. *Lichenologist* **37:** 1–36.

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.

Gowan, S. P. (1989*a*) The lichen genus *Porpidia* (*Porpidiaceae*) in North America. *Bryologist* **92:** 25–59.

Gowan, S. P. (1989*b*) A character analysis of the secondary products of the *Porpidiaceae* (lichenized Ascomycotina). *Systematic Botany* **14:** 77–90.

Gowan, S. P. & Ahti, T. (1993) Status of the lichen genus *Porpidia* in Eastern Fennoscandia. *Annales Botanici Fennici* **30:** 53–75.

Hertel, H. (1975) Beiträge zur Kenntnis der Flechtenfamilie *Lecidieaceae* VI. *Herzogia* **3:** 365–406.

Hertel, H. (1977) Gesteinsbewohnende Arten der Sammelgattung *Lecidea* (Lichenes) aus Zentral-, Ost- und Südasien. *Khumbu Himal, Ergebnisse des Forschungsunternehmens Nepal Himalaya* **6:** 145–378.

Hertel, H. & Knoph, J. G. (1984) *Porpidia albocaerulescens* eine weit verbreitete, doch in Europa seltene und vielfach verkannte Krustenflechte. *Mitteilungen der Botanischen Staatssammlung München* **20:** 467–488.

Inoue, M. (1983) Japanese species of *Huilia* (Lichenes) (1–3). *Journal of Japanese Botany* **58:** 113–128, 161–173, 225–236.

Knoph, J. G. (1984) *Vorarbeiten zu einer Monographie der euthallinen Arten der Flechtengattung* Porpidia (Porpidiaceae, Lecanorales) *Europas, mit besonderer Berücksichtigung des Alpengebietes*. München: Institut für Systematische Botanik der Universität München.

Körber, G. W. (1855) *Systema Lichenum Germaniae*. Breslau: Trewendt & Granier.

Orange, A. (2014) *Porpidia irrigua*, a new species related to *P. contraponenda*. *Lichenologist* **46:** 269–284.

Orange, A., James, P. W. & White, F. J. (2010) *Microchemical Methods for the Identification of Lichens 2nd edition*. London: British Lichen Society.

Schwab, A. J. (1986) Rostfärbene Arten der Sammelgattung *Lecidea* (*Lecanorales*) Revision der Arten Mittel- und Nordeuropas. *Mitteilungen der Botanischen Staatssammlung München* **22:** 221–476.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30:** 2725–2729.

White, T. J., Bruns, T., Lee, S. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. San Diego: Academic Press.