

## Standard Paper

# Seven new species of *Verrucaria* from calcareous and siliceous rocks of Finland

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### Abstract

Seven new species of *Verrucaria* are described from Finland: *Verrucaria hakulinenii* sp. nov., *V. juumaensis* sp. nov., *V. linkolae* sp. nov., *V. lohjaensis* sp. nov., *V. norrlinii* sp. nov., *V. oulankajokiensis* sp. nov., and *V. vainioi* sp. nov. *Verrucaria linkolae* is also reported from the Czech Republic, Germany and the United Kingdom, *V. norrlinii* from Norway and *V. juumaensis* from Canada based on a previously unidentified soil sample. Based on ITS sequences, *V. hakulinenii* and *V. juumaensis* probably belong to the *Verrucaria hydrophila* group whereas *V. linkolae*, *V. norrlinii*, *V. oulankajokiensis* and *V. vainioi* are closely related to *V. hunsrueckensis* and *V. nodosa*. The new species are characterized by a thin brown or green thallus, rather small perithecia and a predominantly thin involucrellum reaching the exciple base level. *Verrucaria hakulinenii* is characterized by a thin thalline cover of the perithecia, a green thallus and fairly large spores (18–22 × 8–10 μm). *Verrucaria juumaensis*, *V. linkolae*, *V. norrlinii* and *V. vainioi* are characterized by a predominantly brown thallus, often with gonicyst-like units. *Verrucaria linkolae* has densely occurring perithecia (100–330 perithecia per cm<sup>2</sup>) whereas in *V. juumaensis*, *V. norrlinii* and *V. vainioi* perithecia occur more sparsely (40–160 perithecia per cm<sup>2</sup>). *Verrucaria juumaensis* and *V. vainioi* usually have a minute thallus. *Verrucaria juumaensis* differs from *V. vainioi* by slightly larger perithecia (0.18–0.27 mm diam.) and longer and wider spores. *Verrucaria lohjaensis* is characterized by a mosaically dark brown and white, small areolate thallus and conspicuous ostioles. *Verrucaria oulankajokiensis* has small perithecia that are often thinly covered by thalline tissue and a thallus partly surrounded by dark lines. Most species occur on calcareous rocks, but *V. vainioi* is restricted to siliceous rocks. *Verrucaria linkolae* and *V. norrlinii* are widely distributed both on calcareous, serpentine and siliceous rocks, preferring pebbles. Epiphytic occurrences of *V. linkolae* and *V. norrlinii* are confirmed. A key to the new species and species with a similar morphology in Finland is provided.

**Keywords:** Canada; Central Europe; ITS barcode; lichen; Norway; taxonomy

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### Introduction

*Verrucaria* Schrad. is one of the most difficult genera among lichens. Relatively simple morphology combined with plasticity in response to environmental conditions make delimitation of the species challenging (Orange & Chhetri 2022). Over 900 species have been described in the genus, but most species are considered synonyms or their taxonomic status is uncertain (Pykälä *et al.* 2018). The number of currently accepted species of *Verrucaria* varies from 200 (Orange 2013a) to 500–600 (Breuss & Berger 2010). Gueidan *et al.* (2009) showed that *Verrucaria* is restricted to its type, *V. rupestris* Schrad., and the remaining species are not related. However, the phylogenetic position and generic affiliation of most species are still unknown.

During the 1800s and the first half of the 1900s, a significant number of new *Verrucaria* species were described from Europe (see the monographs of Zschacke (1934) and Servit (1954)). Despite this, recent studies suggest that the species richness in

the genus is underestimated in Europe, mainly because several groups are poorly collected and understudied due to their small and inconspicuous habit (e.g. Orange 2004, 2013b, 2014, 2020; Breuss & Berger 2012; Thüs *et al.* 2015, 2018; Gasparyan & Aptroot 2016; Pykälä *et al.* 2017a, b, 2018, 2019, 2020).

Species with a thin green or brown thallus, rather small perithecia and a usually thin involucrellum reaching the exciple base level form an extremely difficult morphogroup. Previous studies have shown that such species occur widely among *Verrucariaceae* (Orange 2013b; Pykälä *et al.* 2018, 2019; Thüs *et al.* 2018). Here, we study the taxonomy of seven species belonging to this morphogroup. Despite long-lasting efforts to find names for these species, such attempts have failed. Altogether, type material of 434 *Verrucaria* species has been studied. Thus, we describe them here as new based on morphological and molecular characters.

### Material and Methods

#### Taxon sampling

This study is based on material collected by the first author during the years 2003–2021. The sampling was most extensive on

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calcareous rocks in Southern Finland (over 50% of all calcareous rock outcrops and lime quarries studied) (Pykälä *et al.* 2017a, b). Sampling of siliceous rocks has been far less intensive. Finnish bedrock is mainly acid, and siliceous rocks are frequent. Some sampling has also been made on serpentine rocks (mainly during 2020–2021), which are rare in Finland, most often occurring in eastern and northern parts of the country. Type material of morphologically similar *Verrucaria* species from herbaria B, H, H-NYL, M, PRM, S, UPS and VER was studied for comparison. This includes specimens with small perithecia (less than 0.3 mm), a thin brown or green thallus, an involucrellum mainly reaching the exciple base level and small to medium sized spores (10–25 × 5–12 µm).

### Morphology

Perithecia and thalli were hand-sectioned with razor blades. The sections were examined and measured in tap water. Asci and ascospores were also studied in squash preparations of perithecia mounted in water. Sections and squash preparations of old herbarium specimens were studied using 10% potassium hydroxide (KOH). Additionally, involucrellum characters and exciple colour and diameter were examined by cutting perithecia into two pieces and studying the pieces using a stereomicroscope. The range of spore size is indicated as arithmetic mean and standard deviation ( $\bar{x} \pm SD$ ) with minimum and maximum values given in parentheses, and  $n$  = the number of measurements made. The size of the perithecia (diam.) is given in surface view. The colour of the wall of the exciple was assessed from the basal parts.

Specimens were photographed with a Nikon Z50 camera attached to Leica Z16 and Leica DM 2500 microscopes. Layered images were taken manually from 6 to 8 points and stacked by using Corel PaintShop Pro 2023 software.

### DNA extraction and sequencing

Total genomic DNA was extracted from the perithecia (1–3) of 1–15-year-old herbarium specimens. We used two different techniques for extraction and sequencing. Most Finnish samples were sequenced during the research project ‘Finnish Barcode of Life’ between 2012–2021 (FinBOL; <https://finbol.org/>). The two Norwegian specimens were obtained from the Norwegian lichen DNA barcoding project (OLICH).

DNA samples were placed in 96-well microplates and sent to the Canadian Centre for DNA Barcoding (CCDB). CCDB’s standard protocols (documentation available at <http://ccdb.ca/resources>) were used for extraction, PCR and sequencing. Primers ITS1 and ITS4 (White *et al.* 1990) were used both for PCR and sequencing of the nuclear ribosomal ITS region. For the Norwegian specimens, the primers ITS5 and ITS4 (White *et al.* 1990) were used for PCR, and ITS1 and ITS4 for sequencing. The barcode sequences, their trace files, along with all relevant collection data and images of the voucher specimens were uploaded to the Barcode of Life Data Systems (BOLD; <https://www.boldsystems.org>) database.

DNA of the 11 specimens (Pykälä 27107, 29774, 29883, 31875, 36440, 54604, 56270, 57469, 58042, 58317, 59214) was extracted using the DNeasy® Blood & Tissue Kit by Qiagen, following the protocol described in Myllys *et al.* (2011). PCR reactions were prepared using PuReTaq Ready-To-Go PCR beads (GE Healthcare). The 25 µl reaction volume contained 19 µl of dH<sub>2</sub>O, 0.4 µM of each primer and 4 µl of extracted DNA. PCR

was run under the following conditions: initial denaturation for 5 min at 95 °C followed by five cycles of 30 s at 95 °C (denaturation), 30 s at 58 °C (annealing), and 1 min at 72 °C (extension); in the remaining 35 cycles, the annealing temperature was decreased to 56 °C; the PCR schedule ended with a final extension for 7 min at 72 °C. PCR products were purified and sequenced by MacroGen Inc. (Amsterdam, The Netherlands; [www.macrogen.com](http://www.macrogen.com)) or, alternatively, cleaned with ExoSAP (Affymetrix, Santa Clara, California, USA) and sequenced by FIMM Genomics (<https://www.helsinki.fi/en/infrastructures/genome-analysis/infrastructures/fimm-genomics>). The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) were used both for PCR amplification and sequencing of the ITS regions.

### Phylogenetic analyses

BLAST (Altschul *et al.* 1990) was used to search for the most similar ITS sequences available on GenBank. First, all sequences exceeding 97% similarity were selected. Seven GenBank sequences for *V. linkolae* and one *V. hunsrueckensis* Thüs *et al.* sequence for *V. vainioi* met this criterion. Second, if sequences exceeding this threshold were not found, one sequence each of the two most similar species were included in the phylogeny. These included ITS sequences of *V. hunsrueckensis*, *V. hydrophila* Orange, *V. nodosa* Orange (from type material), *V. phloeophila* Breuss, *V. placida* Orange, *V. rosula* Orange and *Verrucaria* spp. The similarities of these sequences varied from 96.3% to 92.4%. Sequences obtained from the holotype specimens of *V. hunsrueckensis*, *V. hydrophila* and *V. rosula* were included for comparison. In total, 21 ITS sequences representing seven *Verrucaria* species and eight unidentified *Verrucaria* specimens were selected and downloaded from GenBank. However, unidentified specimens from soil samples were not included in the analyses. *Verrucaria macrostoma* Dufour ex DC. and *V. nigrescens* Pers. were chosen as outgroups based on earlier results by Orange (2013a) and Thüs *et al.* (2018) on the systematic position of the selected *Verrucaria* species. A total of 73 ITS sequences were aligned with MUSCLE v. 3.8.31 (Edgar 2004) using EMBL-EBI’s freely available web service (<http://www.ebi.ac.uk/Tools/msa/muscle/>). The aligned data set was subjected to maximum likelihood analysis (ML). The analysis was performed with RAXML v. 8.1.15 (Stamatakis 2014) on the CSC – IT Center for Science server (<http://www.csc.fi>). The ITS region was divided into three partitions: ITS1, 5.8S and ITS2. These partitions were analyzed under the universal GTR-GAMMA model. Node support was estimated with 1000 bootstrap replications using the rapid bootstrap algorithm. Branches with bootstrap values ≥ 70% were considered strongly supported.

### Results and Discussion

We generated 50 new ITS sequences for this study (Table 1). In the ITS phylogeny, seven new lineages were observed (Fig. 1). These lineages, when represented by multiple samples, received high support values (86–100%). Since we did not find any existing names for these clades, we describe them as new species (see ‘The Species’).

*Verrucaria juumaensis* sp. nov. (represented by five specimens in our study) and *V. hakulinenii* sp. nov. (three specimens) form a strongly supported (98% bootstrap support) sister group. Together they cluster with *V. hydrophila*, *V. placida* and *V. phloeophila* with high confidence (100%). A single specimen of *V.*

**Table 1.** *Verrucaria* specimens with voucher information and GenBank Accession numbers, used in the phylogenetic analyses. New ITS sequences are in bold.

<b>Taxon</b>	<b>Country</b>	<b>Voucher specimen</b>	<b>GenBank Accession number</b>
<i>Verrucaria hakulinenii</i>	Finland	Pykälä 43448 (H)	<b>PP337728</b>
<i>V. hakulinenii</i>	Finland	Pykälä 43451 (H)	<b>PP337729</b>
<i>V. hakulinenii</i>	Finland	Pykälä 29774 (H)	<b>PP337730</b>
<i>V. 'hegetschweileri'</i>	Czech Republic	Vondrák (PRA)	OK333039
<i>V. 'hegetschweileri'</i>	Czech Republic	Vondrák & Palice (PRA)	OK333040
<i>V. hunsrueckensis</i>	Austria	Klüssendorf (STU)	MZ409491
<i>V. hunsrueckensis</i>	Finland	Pykälä 54551 (H)	OQ604673
<i>V. hunsrueckensis</i>	Germany	Fischer et al. (BM, holotype)	MG242446
<i>V. hydrophila</i>	Nepal	Orange & Chhetri 18537 (NMW)	OM228813
<i>V. hydrophila</i>	UK	Orange 16776 (NMW, holotype)	NR 137565
<i>V. juumaensis</i>	Finland	Pykälä 39204 (H)	<b>PP337731</b>
<i>V. juumaensis</i>	Finland	Pykälä 44700 (H)	<b>PP337732</b>
<i>V. juumaensis</i>	Finland	Pykälä 54852 (H)	<b>PP337733</b>
<i>V. juumaensis</i>	Finland	Pykälä 55391 (H)	<b>PP337734</b>
<i>V. juumaensis</i>	Finland	Pykälä 58317 (H)	<b>PP337735</b>
<i>V. linkolae</i>	Finland	Pykälä 27107 (H)	<b>PP337736</b>
<i>V. linkolae</i>	Finland	Pykälä 27347 (H)	<b>PP337737</b>
<i>V. linkolae</i>	Finland	Pykälä 29400 (H)	<b>PP337738</b>
<i>V. linkolae</i>	Finland	Pykälä 36440 (H)	<b>PP337739</b>
<i>V. linkolae</i>	Finland	Pykälä 37711 (H)	<b>PP337740</b>
<i>V. linkolae</i>	Finland	Pykälä 48795 (H)	<b>PP337741</b>
<i>V. linkolae</i>	Finland	Pykälä 52420 (H)	<b>PP337742</b>
<i>V. linkolae</i>	Finland	Pykälä 52423 (H)	<b>PP337743</b>
<i>V. linkolae</i>	Finland	Pykälä 52480 (H)	<b>PP337744</b>
<i>V. linkolae</i>	Finland	Pykälä 54003 (H)	<b>PP337745</b>
<i>V. linkolae</i>	Finland	Pykälä 54723 (H)	<b>PP337746</b>
<i>V. linkolae</i>	Finland	Pykälä 55040 (H)	<b>PP337747</b>
<i>V. linkolae</i>	Finland	Pykälä 55267 (H)	<b>PP337748</b>
<i>V. linkolae</i>	Finland	Pykälä 55547 (H)	<b>PP337749</b>
<i>V. lohjaensis</i>	Finland	Pykälä 58042 (H)	<b>PP337750</b>
<i>V. macrostoma</i>	UK	Orange 17825 (NMW)	JX848568
<i>V. nigrescens</i>	UK	Orange 17295 (NMW)	FJ664877
<i>V. nodosa</i>	UK	Orange 20660 (NMW, holotype)	JX848561
<i>V. norrlinii</i>	Finland	Pykälä 29883 (H)	<b>PP337751</b>
<i>V. norrlinii</i>	Finland	Pykälä 30542 (H)	<b>PP337752</b>
<i>V. norrlinii</i>	Finland	Pykälä 31030 (H)	<b>PP337753</b>
<i>V. norrlinii</i>	Finland	Pykälä 31875 (H)	<b>PP337754</b>
<i>V. norrlinii</i>	Finland	Pykälä 38854 (H)	<b>PP337755</b>
<i>V. norrlinii</i>	Norway	Pykälä 48271 (H)	<b>PP337756</b>
<i>V. norrlinii</i>	Norway	Pykälä 48279 (H)	<b>PP337757</b>
<i>V. norrlinii</i>	Finland	Pykälä 55480 (H)	<b>PP337758</b>
<i>V. norrlinii</i>	Finland	Pykälä 55483 (H)	<b>PP337759</b>
<i>V. norrlinii</i>	Finland	Pykälä 55698 (H)	<b>PP337760</b>

(Continued)

Table 1. (Continued)

Taxon	Country	Voucher specimen	GenBank Accession number
<i>V. norrlinii</i>	Finland	Pykälä 56243 (H)	PP337761
<i>V. norrlinii</i>	Finland	Pykälä 56259 (H)	PP337762
<i>V. norrlinii</i>	Finland	Pykälä 56270 (H)	PP337763
<i>V. norrlinii</i>	Finland	Pykälä 57469 (H)	PP337764
<i>V. oulankajokiensis</i>	Finland	Pykälä 36048 (H)	PP337765
<i>V. oulankajokiensis</i>	Finland	Pykälä 45173 (H)	PP337766
<i>V. phloeophila</i>	Austria	Vondrák 26149 (PRA)	OQ717640
<i>V. placida</i>	Norway	Orange & Tønsberg (NMW, holotype)	NR 137102
<i>V. placida</i>	UK	Orange 17493 (NMW)	FJ664880
<i>V. 'rosula'</i>	China	Niu 17-0716	MN103180
<i>V. rosula</i>	UK	Orange 16753 (NMW, holotype)	NR 137025
<i>V. vainioi</i>	Finland	Pykälä 52499 (H)	PP337767
<i>V. vainioi</i>	Finland	Pykälä 54604 (H)	PP337768
<i>V. vainioi</i>	Finland	Pykälä 54610 (H)	PP337769
<i>V. vainioi</i>	Finland	Pykälä 54650 (H)	PP337770
<i>V. vainioi</i>	Finland	Pykälä 55548 (H)	PP337771
<i>V. vainioi</i>	Finland	Pykälä 56981 (H)	PP337772
<i>Verrucaria</i> sp.	Czech Republic	Vondrák (PRA)	OK332900
<i>Verrucaria</i> sp.	Czech Republic	Palice (PRA)	OK332901
<i>Verrucaria</i> sp.	Czech Republic	Vondrák (PRA)	OL396617
<i>Verrucaria</i> sp.	UK	Orange 15780 (NMW)	FJ664851
<i>Verrucaria</i> sp.	Germany	Fischer et al. 200/2016 (STU)	MG242447
<i>Verrucaria</i> sp.	Iceland	Orange 17068 (NMW)	FJ664859
<i>Verrucaria</i> sp.	UK	Orange 16504 (NMW)	FJ667941
<i>Verrucaria</i> sp.	Czech Republic	Thüs & Vondrák (STU)	OL457961
<i>Verrucaria</i> sp.	Finland	Pykälä 28977 (H)	PP337773
<i>Verrucaria</i> sp.	Finland	Pykälä 35945 (H)	PP337774
<i>Verrucaria</i> sp.	Finland	Pykälä 47727 (H)	PP337775
<i>Verrucaria</i> sp.	Finland	Pykälä 55769 (H)	PP337776
<i>Verrucaria</i> sp.	Finland	Pykälä 59214 (H)	PP337777

*lohjaensis* sp. nov. groups with one *V. rosula* specimen collected in China (MN103180) and one *Verrucaria* specimen collected from the Czech Republic (OL457961) with moderately low support (62%). However, the Chinese collection is most probably a mis-identification since the ITS sequence obtained from the holotype of *V. rosula* does not group with this specimen. It is noteworthy that our 14 *V. linkolae* specimens form a strongly supported (100%) group with two *V. hegetschweileri* Körb., nom. illeg. non (Naegeli ex Hepp) Garov. specimens, three *Verrucaria* sp. specimens from the Czech Republic (OK332900, OK332901, OL396617), one *Verrucaria* sp. specimen from Germany (MG242447) and one *Verrucaria* sp. specimen collected in Great Britain (FJ664851). As discussed in the taxonomy section below, we suspect that all these specimens actually represent our new species *V. linkolae*. The species forms a moderately strongly supported group (76%) with *V. oulankajokiensis* sp.

nov. (two specimens), *V. vainioi* sp. nov. (six specimens), *V. norrlinii* sp. nov. (14 specimens), *V. hunsrueckensis*, *V. nodosa*, five *Verrucaria* sp. specimens collected in Finland (28977, 35945, 47727, 55769, 59214) by the first author, and two *Verrucaria* sp. specimens collected in Scotland (FJ667941) and Iceland (FJ664859) by Alan Orange. The last mentioned specimen forms a strongly supported group (90%) with *V. nodosa* and our new species, *V. vainioi* forms a strongly supported group (90%) with *V. hunsrueckensis* and the collection from Scotland, while *V. norrlinii* and *V. oulankajokiensis* group with *V. nodosa* and specimens collected from Finland and Iceland with moderately strong support (75%).

According to the phylogeny, the new species *V. juumaensis* and *V. hakulinenii* belong to an informal group referred to as the *Verrucaria hydrophila* group (see Pykälä et al. 2018; Thüs et al. 2018; Orange & Chhetri 2022). In the multi-locus phylogeny



**Figure 1.** Phylogenetic relationships of the studied *Verrucaria* species based on a maximum likelihood (ML) analysis of the ITS data set. Maximum likelihood bootstrap values > 50% are shown at nodes. Thickened lines indicate bootstrap values > 70%. New species described in this study are indicated with shadowed boxes and holotype specimens are shown in bold. GenBank Accession numbers for sequences downloaded from GenBank and collection numbers for the specimens sequenced for this study are shown after the taxon names. In colour online.



of Thüs *et al.* (2011), the *V. hydrophila* group is placed as a sister to *Trimmatothele* Norman ex Zahbr. Furthermore, our results suggest that *V. linkolae*, *V. norrlinii*, *V. oulankajokiensis* and *V. vainioi* are closely related to the *V. nodosa/hunsrueckensis* group (see Thüs *et al.* 2018).

Generally, *Verrucaria* species are considered to be either corticolous or epilithic (e.g. Lendemer & Breuss 2009). However, our studies show that *V. linkolae* and *V. norrlinii* (this study), and *V. hydrophila* (Pykälä *et al.* 2018) may occur on both substrata. All three of these species are chiefly epilithic, but are also rarely found on bark.

## The Species

All cited specimens of the new species are deposited in H.

### *Verrucaria hakulinenii* Pykälä & Myllys sp. nov.

Mycobank No.: MB 852433

Differing from *Verrucaria tenebrosa* Pykälä *et al.* by the paler and larger thallus and more immersed perithecia.

Type: Finland, Varsinais-Suomi, Pohja, Kuovila, 400 m SE of Kalkkuuninmäki, *Picea abies*-dominated OMT-forest, abandoned lime quarry, on E-facing wall, 46 m a.s.l., 60°08'N, 23°24'E, 12 October 2006, J. Pykälä 29774 (H9203803—holotype). GenBank Accession no.: PP337728.

(Figs 2A & 3A)

*Thallus* continuous, rimose to cracked-areolate, pale green, medium green to medium brownish green, after storage pale brownish grey, *c.* 0.05–0.1 mm thick, areoles 0.2–0.8 mm, algal cells *c.* 6–10 × 5–8 µm, medulla not differentiated, algal cells distributed throughout, cortex not clearly differentiated, *c.* 10–30 µm thick, cortical cells pale or brown. *Prothallus* not seen or brown, non-fimbriate.

*Perithecia* 0.13–0.26 mm diam., 1/2–3/4-immersed, not leaving pits, often with a thin thalline cover except at the apex, *c.* 110–200 perithecia per cm<sup>2</sup>. *Ostiole* tiny, pale to dark, plane or depressed, *c.* 10–30 µm wide. *Involucrellum* to the exciple base, *c.* 25–70 µm thick, appressed to the exciple or slightly diverging from it near the base. *Exciple* *c.* 0.15–0.24 mm diam, wall pale to medium brown, *c.* 22–25 µm thick. *Periphyses* *c.* 17–25 × 1–2 µm, branching. *Asci* 8-spored, *c.* 45–65 × 20–23 µm. *Ascospores* (16.8–)18.4–20.2–21.8(–24.7) × (6.5–)7.6–8.6–9.6(–11.1) µm (*n* = 98).

*Etymology.* Named after Rainar Hakulinen (1918–1991), an important link in the chain of Finnish lichenologists (see Ahti 1993).

*Ecology and distribution.* Only two localities are known: one in SW Finland and one in NW Finland, *c.* 1000 km apart. In inland SW Finland, the species grows on an E-facing wall of an abandoned lime quarry. In NW Finland, the species is known only from a dolomite rock outcrop on a river shore on a subarctic fell.

*Notes.* The two populations of *V. hakulinenii* have minor morphological differences. However, due to the high sequence similarity of the ITS regions (99%) they are included here in the same species. *Verrucaria hakulinenii* differs in several characteristics from the other species treated in this study. Of the species

belonging to the *V. hydrophila* group, it mostly resembles *V. tenebrosa* Pykälä *et al.* which, however, usually has a darker, often brown, more weakly developed thallus and less immersed and more sparsely occurring perithecia (Pykälä *et al.* 2018). *Verrucaria hakulinenii* also shares some morphological similarities with other, less closely related species. *Verrucaria tallbackensis* Pykälä *et al.* has less immersed perithecia, often pale ostioles with projecting papillae and smaller spores (Pykälä *et al.* 2019). The description of *V. floerkeana* Dalla Torre & Sarntheim by Breuss & Berger (2010) is rather similar to the description of *V. hakulinenii*. Based on Breuss & Berger, *V. floerkeana* has a larger exciple (0.2–0.3 mm), smaller spores (15–20(–22) × 6–9 µm), a thinner involucrellum (20–30 µm thick) and thicker periphyses (3 µm thick). The identity of *V. floerkeana* is not clear (several specimens cited in the protologue), and the species is in need of lectotypification.

*Additional specimens examined.* Finland: Enontekiön Lappi: Enontekiö, Porojärvet, Toskalharji, Toskaljärvi N, fell, brook, W-shore, dolomite rock outcrop, on N-slope, 710 m a.s.l., 69° 11'N, 21°26'E, 2011, J. Pykälä 43448, 43451.

### *Verrucaria juumaensis* Pykälä & Myllys sp. nov.

Mycobank No.: MB 852434

Differing from *Verrucaria hakulinenii* sp. nov. by a more reduced thallus, less immersed perithecia, narrower spores and the occurrence of goniocyst-like units.

Type: Finland, Koillismaa, Kuusamo, Juuma, Oulanka National Park, Hautaniitynuoma, gorge, dolomite rock outcrop, on high NE-facing wall, 190 m a.s.l., 66°15'N, 29°22'E, 21 August 2011, J. Pykälä 44700 (H9204984—holotype). GenBank Accession no.: PP337732.

(Figs 2B & 3B)

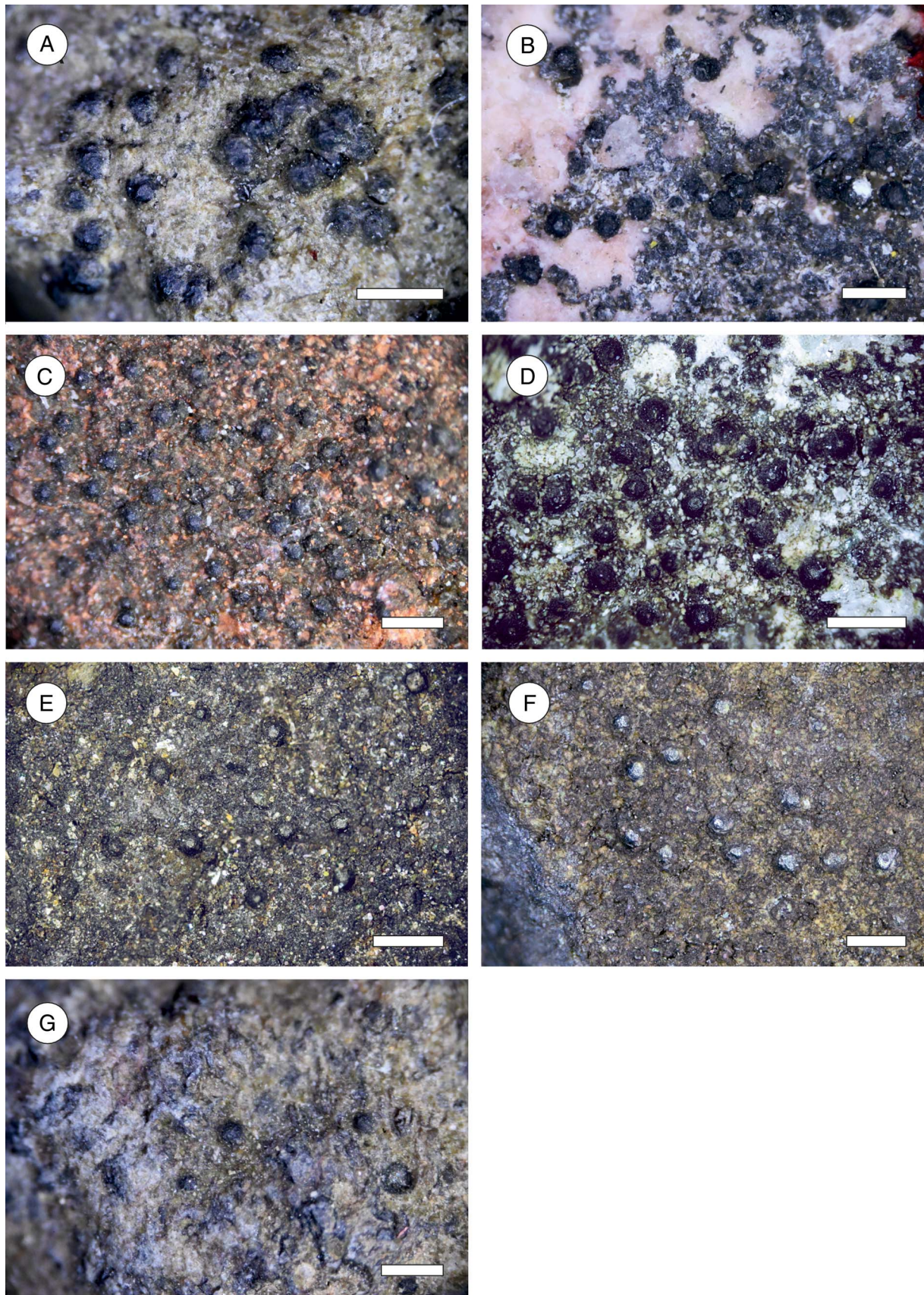
*Thallus* pale green, brownish green, medium greenish brown, dark brown, in one specimen with some grey pruina, fleck-like to rarely rimose, usually tiny flecks, *c.* 5–100 µm thick, partly consisting of goniocyst-like units, *c.* 40–80 µm, algal cells 5–8 µm, cortex pale brown to dark brown, often weakly differentiated. *Prothallus* absent or weakly developed, dark brown, fimbriate.

*Perithecia* 0.18–0.27 mm diam., 1/4–1/2-immersed to superficial, *c.* 40–160 perithecia per cm<sup>2</sup>. *Ostiole* inconspicuous, tiny, dark, rarely pale, plane to depressed, *c.* 10–30 µm wide. *Involucrellum* to the exciple base or more rarely enveloping the exciple, *c.* (20–)30–40 µm thick. *Exciple* 0.14–0.20 mm, wall pale to dark brown. *Periphyses* *c.* 15–20 × 2 µm. *Asci* 8-spored, *c.* 56–90 × 13–15 µm. *Ascospores* (15.5–)18.0–20.5–22.7(–24.9) × (5.8–)6.7–7.3–7.8(–8.5) µm (*n* = 66).

*Etymology.* The specific epithet refers to the village of Juuma in Kuusamo from where two specimens, including the type, originate.

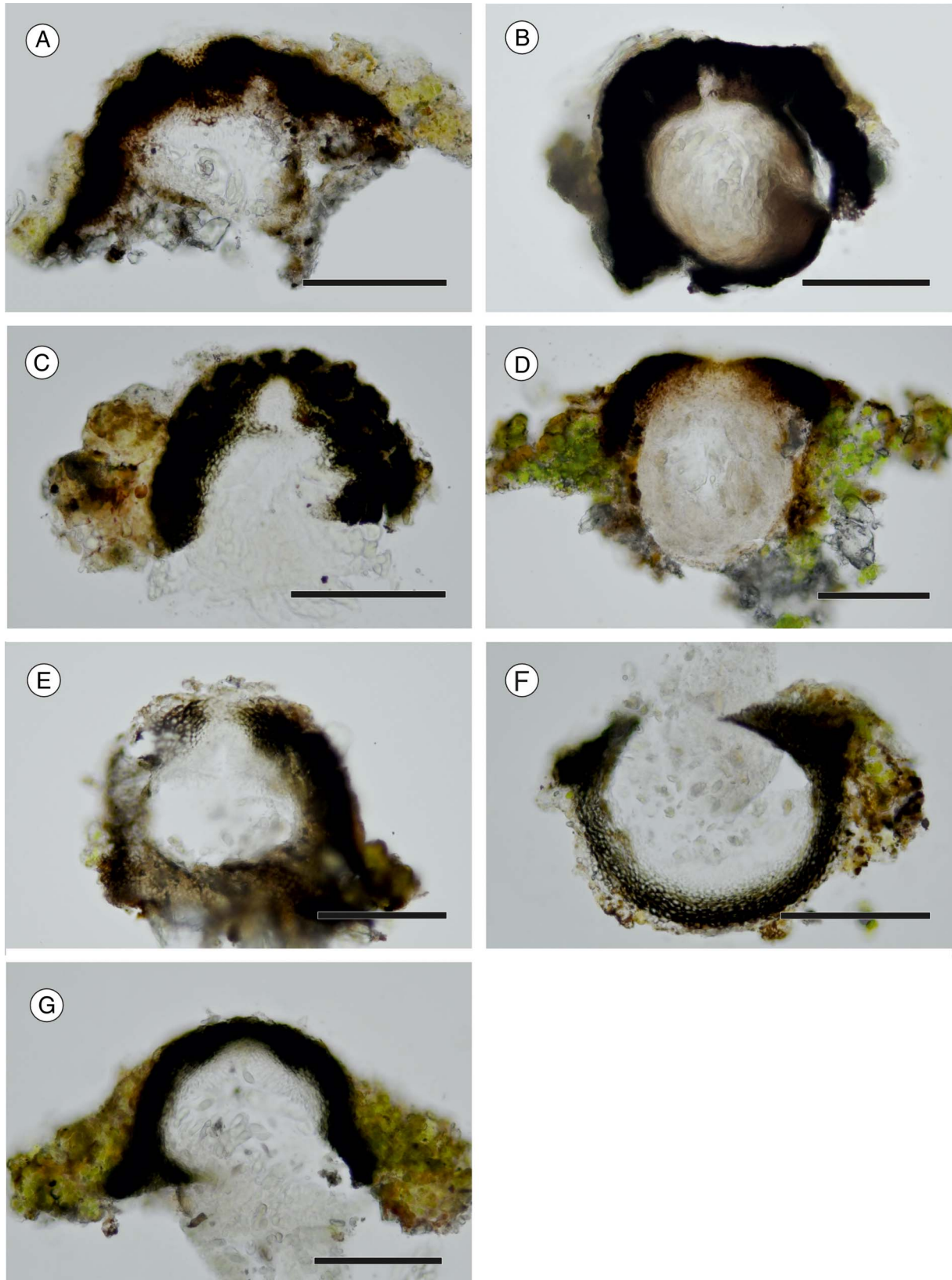
*Ecology and distribution.* *Verrucaria juumaensis* has been collected from dolomite and serpentine rocks, both on rock outcrops and on pebbles. All the localities are in Eastern Finland, in the biogeographical provinces of Kainuu and Koillismaa. The species may have been overlooked due to its small size and morphological similarity with several species of *Verrucaria*, but it is also apparently





**Figure 2.** A, *Verrucaria hakulinenii* (holotype). B, *V. juumaensis* (holotype). C, *V. linkolae* (holotype). D, *V. lohjaensis* (holotype). E, *V. norrlinii* (holotype). F, *V. oulankajokiensis* (holotype). G, *V. vainioi* (holotype). Scales = 0.5 mm. In colour online.





**Figure 3.** Sections of perithecia. A, *Verrucaria hakulinenii* (holotype). B, *V. juumaensis* (holotype). C, *V. linkolae* (holotype). D, *V. lohjaensis* (holotype). E, *V. norrlinii* (holotype). F, *V. oulankajokiensis* (holotype). G, *V. vainioi* (holotype). Scales = 0.1 mm. In colour online.



rare in Finland. It may belong to the north-eastern element that can be found in Finland only in the eastern region of the country. An unidentified sequence from a soil sample from Canada (KC 965589) (Timling *et al.* 2014) differs only in two bases in the ITS regions, and most probably belongs to *V. juumaensis*.

**Notes.** This species differs from its sister species *V. hakulinenii* by a more reduced thallus, less immersed perithecia and narrower spores. *Verrucaria floerkeana* has a paler and thicker thallus lacking goniocyst-like units, a larger exciple and thinner involucrellum (see the description in Breuss & Berger (2010)). *Verrucaria oulankajokiensis* has a pale exciple wall, a rimose to areolate thallus and smaller perithecia. *Verrucaria juumaensis* is difficult to separate from several other unrelated species of *Verrucaria*. *Verrucaria juankoskiensis* Pykälä & Myllys has a thicker involucrellum and slightly narrower spores (Pykälä *et al.* 2019). *Verrucaria kalenskyi* Servit and *V. raesaenenii* Pykälä & Myllys, both members of the *V. praetermissa* (Trevis.) Anzi group, have smaller spores, slightly smaller perithecia and thalli that lack goniocyst-like units (Pykälä *et al.* 2019). In *V. kalenskyi*, the prothallus is absent. *Verrucaria lapidicola* Orange from the same group has smaller perithecia (in the Finnish material up to 0.22 mm, but usually 0.11–0.17 mm) (Pykälä 2023). *Verrucaria infumata* Nyl. may be superficially a rather similar species but it has broader spores (*c.* 7–10 µm wide). Specimens with the involucrellum enveloping the exciple may be confused with *V. xyloxena* Norman. *Verrucaria xyloxena* lacks an involucrellum, ostioles often have pale, projecting papillae and the spores are smaller.

**Additional specimens examined. Finland:** *Koillismaa*: Salla, Oulanka National Park, Pikkuköngäs, shore of River Oulankajoki, high cliff, calciferous (dolomite) schistose rock outcrop, on overhanging SE-facing wall, 180 m a.s.l., 66°25'N, 29°09'E, 2010, *J. Pykälä* 39204; Kuusamo, Juuma, Myllyniemi SE, steep N-slope, dolomite rock outcrop on shore of Lake Yli-Juumajärvi, on 60 cm high N-facing wall, on dolomite pebbles, 225 m a.s.l., 66°15'N, 29°22'E, 2021, *J. Pykälä* 58317. *Kainuu*: Paltamo, Mieslahti, Mineraali, *Pinus sylvestris*-dominated, slightly paludified heath forest, ECT site type, on serpentine boulder, on N-facing wall, 158 m a.s.l., 64°19'N, 28°02'E, 2020, *J. Pykälä* 54852; Paltamo, Mieslahti, Heinimäki, calcareous rock outcrop, E-facing wall, crevice, on pebble, 143 m a.s.l., 64°20'N, 28°02'E, 2020, *J. Pykälä* 55391.

### *Verrucaria linkolae* Pykälä & Myllys sp. nov.

Mycobank No.: MB 852435

Differing from other European *Verrucaria* species with a brown thallus and goniocyst-like units by the smaller, occasionally 1-septate, spores.

**Type:** Finland, Varsinais-Suomi, Lohja, Paloniemi, S of Paloniemi, house ruins, on bricks, half-shady habitat, 43 m a.s.l., 60°16'N, 24°00'E, 18 July 2005, *J. Pykälä* 27347 (H9204043—holotype). GenBank Accession no.: PP337737.

(Figs 2C & 3C)

**Thallus** pale greyish brown, greenish brown to usually dark brown, fleck-like, granular or continuous, sometimes tiny flecks, goniocyst-like units often present, *c.* 25–50 µm, algal cells

*c.* (4–)5–7(–10) µm, cortex thin, cortical cells hyaline to brown, *c.* 3–5 µm. *Prothallus* absent or brown fimbriate.

*Perithecia* 0.11–0.19 mm diam., 1/4–1/2(–3/4)-immersed, *c.* 100–330 perithecia per cm<sup>2</sup>. *Ostiole* inconspicuous, tiny, dark, plane, depressed or projecting papillae, *c.* 10–30 µm wide. *Involucrellum* to the exciple base level, *c.* 15–30(–40) µm thick, rarely thickening to the base to *c.* 40–50 µm thick, appressed to the exciple or slightly diverging. *Exciple* 0.10–0.16 mm, wall pale to medium brown, 19–23 µm thick. *Periphyses* *c.* 10–18 × (1–)2–2.5(–3) µm. *Asci* 8-spored, *c.* 37–68 × 12–17 µm. *Ascospores* aseptate, in few specimens occasionally/predominantly 1-septate, (11.4–)12.6–13.9–15.2(–17.4) × (4.4–)5.0–5.7–6.4(–7.6) µm (*n* = 236).

**Etymology.** Named after Kaarlo Linkola (1888–1942), one of Finland's most eminent botanists, nature conservationists and lichenologists.

**Ecology and distribution.** *Verrucaria linkolae* seems to be common in Southern and Central Finland. It is common on siliceous rocks but may be rare on calcareous and serpentine rocks. It prefers pebbles and stones on the ground and also grows on bricks. Pebbles and stones can occur in herb-rich and heath forests as well as on road verges and lime quarry spoils. One locality is from an overhanging wall in a lime quarry. The species may prefer half-shady and shady habitats. However, some localities are rather sun-exposed. Most localities are rather dry but the species has also been collected on periodically wet stones by a brooklet. The species was once collected on exposed roots of *Betula* on a road bank (calcareous soil).

In GenBank, the specimens which are likely to belong to *V. linkolae* (over 98% similarity in the ITS regions; see below) are from the Czech Republic (OK332900, OK332901, OK333039, OK333040, OL396617), the United Kingdom (FJ664851) and Germany (MG242447).

**Notes.** Seven GenBank ITS sequences have over 98% similarity with those of *V. linkolae* and are likely to represent this species. The specimens have been previously identified as *Verrucaria* sp. and with the illegitimate name *V. hegetschweileri* Körb. ex Nyl. (two epiphytic specimens). *Verrucaria linkolae* fits rather well with the description of *V. hegetschweileri* (Körb. ex Nyl. (illeg.) non (Naegeli ex Hepp) Garov.) in Breuss (1998) and Lendemer & Breuss (2009), but the latter differs in the inclusion of specimens without an involucrellum, while all sequenced specimens seen so far from Finland do have an involucrellum. We considered *V. linkolae* as a replacement name for the illegitimate *V. hegetschweileri* Körb. ex Nyl. and studied the material used for its description (Baar pr. Zug ad fagorum radices, Hegetschweiler (H-NYL 3316!)), but based on our inspection, the morphology does not correspond to that of *V. linkolae*. The specimen has a grey to pale brown thallus, an involucrellum at the exciple base level (not enveloping the exciple) and larger spores (16–21 × 7–8 µm) compared to *V. linkolae*.

Most specimens of *V. linkolae* have only aseptate spores but in a small number of specimens, 1-septate spores are frequent. *Verrucaria linkolae* is difficult to separate from several species of *Verrucaria* with small perithecia, rather small spores and a predominantly brown thallus. Species with thalli consisting of goniocyst-like units, *V. hunsrueckensis*, *V. lapidicola*, *V. norrlinii* and *V. umbrinula* Nyl., may be the most similar. These species usually have larger spores but some specimens have a spore size similar to *V. linkolae*. *Verrucaria norrlinii* has, on average, more immersed

perithecia, which are less densely spaced and slightly larger. *Verrucaria umbrinula* has slightly larger perithecia (up to 0.22 mm) and a thicker thallus. *Verrucaria lapidicola* is restricted to calcareous rocks, the perithecia tend to be slightly larger (up to 0.22 mm) and 1-septate spores have not been reported (Pykälä 2023). *Verrucaria hunsrueckensis* is otherwise morphologically similar but has slightly larger spores ( $\bar{x}$  16.8 × 6.6 μm vs 13.9 × 5.7 μm in the Finnish specimens in this study).

*Verrucaria memnonia* auct. may be a similar species based on the description in Breuss & Berger (2010). We have examined the three syntypes of *V. memnonia* (Flot.) Arnold: Ad saxa granitica (rarius schistose) in sylvis umbrosis vallis Hirschbergensis Silesiae, *Körber*, *Körber*, *Lich. Sel. Germ.* 173 (M-01565311!); Sattler prope Hirsch bergam Silesiae 1854 *Körber* (VER!); Räuberberg im Sattler bei Hirschberg 3.6.1840 *Körber* (UPS!). However, none of these is identifiable to any lichen genus (putative perithecia are overmature, and it is uncertain whether the structures are even perithecia). Thus, unless better developed syntypes are found, *V. memnonia* auct. (*sensu* Breuss & Berger 2010) as well as *V. memnonia* (Flot.) Arnold should be treated as a species of unknown identity.

**Additional specimens examined. Finland:** *Varsinais-Suomi:* Lohja, Marttila, Savilahdensalmi, Seppälänsaari road, steep road bank, on exposed roots of *Betula*, 37 m a.s.l., 60°14'N, 23°51'E, 2005, *J. Pykälä* 27107; Nummi-Pusula, Hyönölä, Remo, clear-cut forest, abandoned lime quarry, on overhanging S-facing wall, 70 m a.s.l., 60°28'N, 23°57'E, 2006, *J. Pykälä* 29400; Salo (Kisko), Haapaniemi, Hauksuonlahti, clear-cut herb-rich forest, heap of calcareous stones of lime quarry spoil, on calcareous stones, 50 m a.s.l., 60°12'N, 23°30'E, 2010, *J. Pykälä* 37711; Lohja, Vappula, Haukkavuori 200 m NW, road verge, on siliceous pebbles, 55 m a.s.l., 60°13'N, 24°00'E, 2015, *J. Pykälä* 48795; Lohja, Maksjoki, former trotting track, young *Pinus sylvestris* forest, road verge, soil heap, on siliceous pebbles, 65 m a.s.l., 60°13'N, 24°02'E, 2019, *J. Pykälä* 52420; Lohja, Koikkala, Nälköönlampi N, former summer cottage, stony heath forest, on siliceous pebbles, 86 m a.s.l., 60°18'N, 24°12'E, 2019, *J. Pykälä* 52423; Karkkila, Haavisto, Koirakallio 400 m NW, brooklet, *Alnus incana*-dominated wet herb-rich forest, on siliceous stones, 76 m a.s.l., 60°29'N, 24°20'E, 2020, *J. Pykälä* 54723. *Pohjois-Savo:* Kuopio (Nilsjä), Nilsjä, Iso-Loutteinen E, abandoned gravel pit, field, young mixed forest, on bricks, 150 m a.s.l., 63°13'N, 28°02'E, 2020, *J. Pykälä* 55547. *Pohjois-Karjala:* Kuopio (Juankoski), Säyneiskylä, Lehtola, 150 m NE, clear-cut forest, top of serpentine boulder, on pebbles, 144 m a.s.l., 63°07'N, 28°39'E, 2019, *J. Pykälä* 54003. *Kainuu:* Paltamo, Melalahti, Melalahdentie, road bank, on siliceous pebbles, 157 m a.s.l., 64°24'N, 27°40'E, 2020, *J. Pykälä* 55040; Sotkamo, Jormaskylä, Raatteikonpuro E, *Pinus sylvestris*/*Picea abies*-dominated heath forest, EVT site type, on serpentine pebble, 215 m a.s.l., 63°57'N, 28°08'E, 2020, *J. Pykälä* 55267. *Koillismaa:* Kuusamo, Oulanka national park, Jäkälävuoma, gorge, steep SE-slope, sparse herb-rich forest, on pebbles, 215 m a.s.l., 66°15'N, 29°26'E, 2009, *J. Pykälä* 36440.

***Verrucaria lohjaensis* Pykälä & Myllys sp. nov.**

Mycobank No.: MB 852436

Differing from other species of the *Verrucaria hydrophila* group by the small areolate thallus mosaically dark brown and white, and by the more conspicuous ostioles.

Type: Finland, Varsinais-Suomi, Lohja, Lohja, Pitkäniemi industrial area, calcareous rock outcrop, on SW-slope, 40 m a.s.l., 60°15'N, 24°03'E, 23 July 2021, *J. Pykälä* 58042 (H9242535—holotype). GenBank Accession no.: PP337750.

(Figs 2D & 3D)

*Thallus* mosaically dark brown and white, areolate, *c.* 0.1–0.15 mm thick, sterile areoles 0.15–0.3 mm, algal cells *c.* 4–8 μm, cortex thin, cortical cells hyaline to brown. *Prothallus* not seen.

*Perithecia* 0.16–0.22 mm diam., 3/4-immersed in thallus, *c.* 80–160 perithecia per cm<sup>2</sup>. *Ostiole* pale, plane, *c.* 20–50 μm wide. *Involucrellum* covering half of the exciple or to the exciple base level, *c.* 30–40 μm thick, appressed to the exciple. *Exciple* *c.* 0.18–0.21 mm, wall brown. *Periphyses* *c.* 12–20 × 2 μm. *Ascospores* aseptate, (16.6–)17.7–19.9–22.1(–24.4) × (7.3–)7.5–7.9–8.3(–8.6) μm (*n* = 14).

**Etymology.** The species was named after the municipality of Lohja, where the only known collection is from. Lohja is among the hot spots of lichen diversity and red-listed lichens in Finland.

**Ecology and distribution.** It is known to have been found in only one locality: a sun-exposed calcareous rock outcrop in inland SW Finland.

**Notes.** The thallus morphology of *V. lohjaensis* is different from that of the other species treated in this study. However, other morphological characteristics fit in rather well with the other species treated here. Superficially, *V. lohjaensis* may resemble some species in the *Verrucaria nigrescens* Pers. complex or in *Placopyrenium* Breuss.

***Verrucaria norrlinii* Pykälä & Myllys sp. nov.**

Mycobank No.: MB 852437

Differing from *Verrucaria linkolae* sp. nov. by the usually less densely occurring and less immersed perithecia.

Type: Finland, Kainuu, Kuhmo, Vieksi, Kellojärvi, Näätäniemi, serpentine rock outcrop on shore of Lake Kellojärvi, under overhanging N-facing wall of serpentine boulder, on serpentine pebbles, 163 m a.s.l., 64°14'N, 29°01'E, 17 August 2020 *J. Pykälä* 56243 (H9223911—holotype). GenBank Accession no.: PP337761.

(Figs 2E & 3E)

*Thallus* medium brown to dark brown, fleck-like, granular or continuous, more rarely rimose to areolate, in which case the surface is granular, *c.* 10–250 μm thick, commonly invaded by algae, often composed of goniocyst-like units *c.* 20–35 μm, algal cells *c.* 5–8 μm, cortex absent to thin, cortical cells brown. *Prothallus* absent or brown fimbriate.

*Perithecia* 0.12–0.23 mm diam., 1/4–3/4-immersed in thallus, *c.* 60–160 perithecia per cm<sup>2</sup>. *Ostiole* inconspicuous, tiny, pale to dark, plane or projecting papillae, 10–30 μm wide. *Involucrellum* to the exciple base level or rarely enveloping the exciple, *c.* 15–30 μm thick, rarely 30–50 μm thick, appressed to the exciple or slightly diverging from it. *Exciple* 0.11–0.21 mm,

wall pale to brown, *c.* 20 µm thick. *Periphyses* *c.* 12–20 × 1.5–2.5 µm. *Asci* 8-spored, *c.* 40–59 × 15–23 µm. *Ascospores* aseptate, (10.2–)13.4–15.9–18.4(–25.5) × (4.8–)5.6–6.6–7.5(–10.8) µm (*n* = 168).

**Etymology.** Named after J. P. Norrlin (1842–1917), a Finnish plant geographer and lichenologist. Norrlin collected a rather high number of lichens in Finland, which W. Nylander described as new. Many of them are at present accepted species (Pykälä & Lommi 2021).

**Ecology and distribution.** The species may be rather common in Southern Finland. The most northern localities are in Kittilä and Kuusamo (in the southern part of the northern boreal vegetation zone), but the distribution area may extend further north. Two sequenced specimens are available from Norway. Thus, the species may be widely distributed in the boreal vegetation zone in Fennoscandia.

Habitats of the species are rather variable. It grows on calcareous, siliceous and serpentine rocks, particularly on pebbles. It also often occurs on road banks on pebbles. However, most localities are from calcareous and serpentine rocks. The species may be edaphically relatively demanding and uncommon on siliceous rocks. This is different from *V. linkolae*, which prefers siliceous rocks. Two collections are from the exposed roots of *Alnus* (one from *A. glutinosa* and one from *A. incana*) on lake shores. It is likely that the species has more epiphytic occurrences, but epilithic populations seem to be predominant. Further studies are needed to establish whether epiphytic occurrences are restricted to shores. Such occurrences on shores are unexpected since epilithic populations are usually found in rather dry habitats.

**Notes.** The species has a very high variation in the size of its spores which makes identification based on morphology somewhat difficult. Furthermore, five *Verrucaria* specimens which clustered outside of *V. norrlinii* (28977, 47727, 35945, 59214, 55769) were morphologically indistinguishable from our new species. *Verrucaria norrlinii* is also difficult to separate from, for example, *V. linkolae* and impossible to separate from *V. hunsrueckensis* (for a description of the latter species see Thüs *et al.* (2018) and Pykälä (2023)), but *V. linkolae* often has more densely occurring perithecia. *Verrucaria linkolae* has on average smaller spores, but in some specimens of *V. norrlinii* the spore size is similar to *V. linkolae*. Perithecia of *V. norrlinii* tend to be more immersed in the thallus compared to *V. linkolae*. *Verrucaria mauriza* Nyl. (type: [Russia,] Nyland, Hogland, vid Selkäpajanlahti 11.6.1870, *M. Brenner* (H!, TUR-V!, syntypes)) has more densely distributed perithecia (*c.* 160–200 perithecia per cm<sup>2</sup>) and a thicker involucrellum (*c.* 50–60 µm thick). *Verrucaria buellioides* Servit (type: Germania, Heidelberg, auf Porphyrfelsen bei Handschuhsheim, Zwackh-Holzhausen, Zwackh, *Lich. Exs.* 151 (M-0204053!, holotype, UPS!, isotype); An Porphyrfelsen im Tü... bei Handschuhsheim ... 151 / 1848 Zwackh (PRM-756818!, isotype)) differs in the more densely occurring perithecia (*c.* 200 perithecia per cm<sup>2</sup>). The spore size (*c.* 16–20 × 7(–9) µm) is also larger than usual in *V. norrlinii*.

**Additional specimens examined. Finland:** *Varsinais-Suomi:* Lohja, Lohja, 100 m S of Hiidensalmi bridge, stony shore of Lake Lohjanjärvi, on exposed thick root of dead *Alnus incana*,

32 m a.s.l., 60°16'N, 24°03'E, 2006, *J. Pykälä* 29883; Lohja, Lohja, Kiviniemi lime quarry, beneath N-facing wall, on boulder, 35 m a.s.l., 60°15'N, 24°03'E, 2007, *J. Pykälä* 30542; Lohja, Hermala, Kalkkimäki, 20 m E of Kekla lime quarry, flat calcareous rock outcrop, 65 m a.s.l., 60°13'N, 23°51'E, 2007, *J. Pykälä* 31030; Lohja, Piispala, Puntari, Kalvik, shore forest of Lake Lohjanjärvi, on shore, on exposed thick roots of *Alnus glutinosa*, 32 m a.s.l., 60°11'N, 23°52'E, 2007, *J. Pykälä* 31875; Kemionsaari (Hiittinen), Holma, Långholmen Island, siliceous rock outcrop on shore of the Baltic Sea, gentle W-slope, on narrow vein of calcite, 1 m a.s.l., 59°53'N, 22°22'E, 2010, *J. Pykälä* 38854. *Pohjois-Karjala:* Kuopio (Juankoski), Säyneiskylä, Pajumäki, beneath NE-facing wall of serpentine rock outcrop, on top of uprooted windfall *Picea abies*, on pebbles, 130 m a.s.l., 63°09'N, 28°36'E, 2020, *J. Pykälä* 55480, 55483. *Kainuu:* Kuhmo, Vieksi, Kellojärvi, Näätäniemi, Junkkiniemi 150 m S, young *Pinus sylvestris*-dominated heath forest, tiny serpentine rock outcrop, on serpentine pebbles, 175 m a.s.l., 64°14'N, 29°01'E, 2020, *J. Pykälä* 56259; Kuhmo, Vieksi, Kellojärvi, Perttilä 200 m S, abandoned soapstone quarry, on top of NE-facing wall, 172 m a.s.l., 64°15'N, 29°02'E, 2020, *J. Pykälä* 56270; Kuhmo, Vieksi, Kellojärvi, Kivihiekka E, mixed heath forest, VMT site type, serpentine rock outcrop, on W-facing wall, 172 m a.s.l., 64°17'N, 29°03'E, 2021, *J. Pykälä* 57469. *Koillismaa:* Kuusamo, Kurvinen, Pieni Rajakumpu 200 m SE, *Pinus sylvestris* heath forest, ECT site type, path, on serpentine stones, 245 m a.s.l., 65°35'N, 29°43'E, 2020, *J. Pykälä* 55698.—**Norway:** *Sør-Trøndelag:* Oppdal, Kongsvoll, S of Kongsvoll Fjeldstue, subalpine sparse *Betula pubescens* forest, stony path, on pebbles, 960 m a.s.l., 62°17'N, 9°36'E, 2015, *J. Pykälä* 48271; Oppdal, Kongsvoll, N-NE of Kongsvoll, main road, road cutting of a schistose rock outcrop, under overhanging wall, on pebbles, 890 m a.s.l., 62°18'N, 9°36'E, 2015, *J. Pykälä* 48279.

### *Verrucaria oulankajokiensis* Pykälä & Myllys sp. nov.

Mycobank No.: MB 852438

Differing from *V. kalenskyi* and *V. raesaeneni* by perithecia that are often thinly thalline covered, and a thallus that is partly surrounded by dark thalline lines.

Type: Finland, Koillismaa, Salla, Oulanka National Park, Pikkuköngäs, shore of River Oulankajoki, high cliff, calciferous (dolomite) schistose rock outcrop, on SW-facing wall, rather scarce, 178 m a.s.l., 66°25'N, 29°09'E, 10 August 2009, *J. Pykälä* 36048 (H9205776—holotype). GenBank Accession no.: PP337765.

(Figs 2F & 3F)

**Thallus** pale brown, medium greenish brown to dark brown, rimose to small areolate, areoles 0.1–0.25 mm, partly surrounded by dark thalline line, *c.* 20–100 µm thick, algal cells *c.* 7–11 µm, medulla not differentiated, cortex not clearly differentiated, cortical cells pale brown *c.* 3–5 µm.

**Perithecia** 0.15–0.19 mm diam., 1/2–3/4-immersed, often thinly thalline covered, *c.* 80–120 perithecia per cm<sup>2</sup>. **Ostiole** pale to dark, plane, *c.* 20–40(–60) µm wide. **Involucrellum** to the exciple base, *c.* 30–50 µm thick, appressed to the exciple to slightly diverging at the base, thickening towards the base. **Exciple** 0.13–0.22 mm, wall pale to rarely dark brown, *c.* 25 µm thick. **Periphyses** *c.* 15–25 × 2–2.5 µm. **Asci** 8-spored, *c.* 43–49 ×



19–24 µm. *Ascospores* (12.4–)13.5–14.7–15.9(–17.2) × (5.6–)5.7–6.4–7.1(–7.8) µm ( $n = 32$ ).

**Etymology.** Both known localities occur on rocks on the shores of the River Oulankajoki.

**Ecology and distribution.** The species grows on calcareous and calciferous rocks on shores of the River Oulankajoki in north-eastern Finland. Two localities are known which are *c.* 12 km apart. One locality is on calciferous schistose rock outcrop and the other is on dolomite stone.

**Notes.** The species is morphologically most similar to *V. norrlinii*. *Verrucaria norrlinii* usually has a thinner involucrellum (usually 15–30 µm thick), but a small number of specimens may have involucrella with a similar thickness to *V. oulankajokiensis*. Furthermore, dark thalline lines are not found in any studied specimen of *V. norrlinii*.

*Verrucaria oulankajokiensis* may also be confused with the species of the *V. kalenskyi*–*V. xyloxena* complex, particularly *V. kalenskyi* and *V. raesaenii* (see Pykälä *et al.* 2019). These species do not have dark thalline lines and their perithecia are usually not thalline covered. *Verrucaria dolosa* Hepp has a thinner involucrellum (15–30 µm thick) and ostioles with pale projecting papillae.

**Additional specimen examined. Finland:** Koillismaa: Kuusamo, Oulanka National Park, Mataraniemi, shore of Oulankajoki River, treeless stony river shore, on dolomite stones, 145 m a.s.l., 66°22'N, 29°20'E, 2011, *J. Pykälä* 45173.

### *Verrucaria vainioi* Pykälä & Myllys sp. nov.

MycoBank No.: MB 852439

Differing from *V. hunsrueckensis* by the thallus of tiny dots.

**Type:** Finland, Varsinais-Suomi, Lohja, Muijala, Mustalahdentie 100 m W, Lohjanharju esker, *Pinus sylvestris*-dominated heath forest, VT site type, path, on siliceous pebbles, 102 m a.s.l., 60°17'N, 24°12'E, 14 November 2020, *J. Pykälä* 56981 (H9223939—holotype; UPS—isotype). GenBank Accession no.: PP33772.

(Figs 2 G & 3G)

**Thallus** medium green, medium brown to dark brown, tiny dots, fleck-like, *c.* 5–30(–50) µm thick, *c.* 5–100 µm wide, goniocyst-like units usually present *c.* 15–35 µm, algal cells *c.* 5–8 µm, cortex absent to thin, cortical cells hyaline to brown. *Prothallus* dark brown, fimbriate, often weakly developed.

**Perithecia** 0.08–0.23 mm, 1/4–1/2-immersed in thallus, *c.* 40–160 perithecia per cm<sup>2</sup>. **Ostiole** tiny, pale to dark, plane to sometimes projecting papillae, *c.* 15–40 µm wide. **Involucrellum** to the exciple base, rarely covering only half of the exciple, *c.* 15–25 µm thick, appressed to slightly diverging near base. **Exciple** *c.* 0.12–0.15 mm, wall pale to brown. **Periphyses** *c.* 10–25 × 2–3 µm. **Asci** 8-spored, *c.* 47–61 × 13–19 µm. **Ascospores** aseptate, (12.3–)14.8–17.4–20.0(–24.6) × (4.8–)5.5–6.1–6.8(–7.7) µm ( $n = 89$ ).

**Etymology.** Named after Edvard August Vainio (1853–1929), a world-famous Finnish lichenologist (Alava 1998). Vainio also

published a monograph of pyrenocarpous lichens in eastern Fennoscandia (Vainio 1921).

**Ecology and distribution.** Six specimens are known from Southern and Central Finland. The species grows on siliceous pebbles and stones, with one locality on brick on the ground. *Verrucaria vainioi* may be a pioneer species and often grows on human-influenced sites such as road verges and paths, but it is also found in herb-rich forests without recent human activity. The species may prefer half-shady habitats. Habitats may be rather dry to periodically wet, with the latter potentially affected by some flooding.

**Notes.** The species is closely related to *V. hunsrueckensis* as well as to one unidentified specimen (Orange 16504 (NMW), FJ667941). *Verrucaria vainioi* is also morphologically rather similar to *V. hunsrueckensis*. However, *Verrucaria hunsrueckensis* usually has a better developed thallus which is usually rimose or areolate. Nevertheless, a DNA barcode is needed for unambiguous identification of the species. Morphologically, *V. vainioi* may be most difficult to separate from *V. norrlinii* but the latter species usually has a larger thallus and, on average, wider spores. *Verrucaria danica* Servit & M. S. Christ. has slightly smaller perithecia (up to 0.17 mm), an even more reduced thallus and it grows on calcareous rocks.

**Additional specimens examined. Finland:** Varsinais-Suomi: Lohja, Laakspohja, Eskolantie, abandoned road, road bank, on siliceous pebbles, 60 m a.s.l., 60°15'N, 24°08'E, 2019, *J. Pykälä* 52499; Lohja, Paloniemi, Lakimäki S, *Picea abies*-dominated herb-rich forest, brooklet, on siliceous pebbles, 47 m a.s.l., 60°17'N, 23°58'E, 2020, *J. Pykälä* 54604; Lohja, Paloniemi, Palomäki S, *Picea abies*-dominated herb-rich heath forest, close by a brooklet, on siliceous stone, 62 m a.s.l., 60°17'N, 23°58'E, 2020, *J. Pykälä* 54610. **Etelä-Häme:** Padasjoki, Vesijako Strict Nature Reserve, Hyödynmäki 200 m SE, *Picea abies*-dominated heath forest, small little-used road, between tracks, on siliceous pebbles, 150 m a.s.l., 61°20'N, 25°06'E, 2020, *J. Pykälä* 54650. **Pohjois-Savo:** Kuopio (Nilsä), Nilsä, Iso-Loutteinen E, abandoned gravel pit, field, young mixed forest, on bricks, 150 m a.s.l., 63°13'N, 28°02'E, 2020, *J. Pykälä* 55548.

### *Verrucaria* sp.

**Notes.** These specimens are morphologically similar to *V. norrlinii*. More genetic markers are needed before their status can be resolved.

**Specimens examined. Finland:** Pohjois-Karjala: Polvijärvi, Sotkuma, Repovaara, calciferous serpentine rock outcrop, on top, on stone, 100 m a.s.l., 62°47'N, 29°20'E, 2014, *J. Pykälä* 47727. **Koillismaa:** Kuusamo, Kurvinen, Hanhiharju, N of Vasseleenlampi, gravel pit, on serpentine pebbles, 245 m a.s.l., 65°34'N, 29°48'E, 2006, *J. Pykälä* 28977; Kuusamo, Liikanen, Oulanka National Park, Korvasvaara, 200 m NW of Kotilaisenlampi, springy brook running through rich fen, on stones, 250 m a.s.l., 66°21'N, 29°36'E, 2009, *J. Pykälä* 35945; Kuusamo, Kirkonkylä, Luhtalampi E, small road, ditch bank, on calcareous pebble, 281 m a.s.l., 66°03'N, 29°15'E, 2020, *J. Pykälä* 55769. **Kittilän Lappi:** Kittilä, Sirkka, Kuukerimaa, abandoned multi-metal ore mine, mine spoil heap, on pebbles, 193 m a.s.l., 67°48'N, 24°44'E, 2021, *J. Pykälä* 59214.


## Key to the studied species and species with a similar morphology in Finland

1	Thallus often/usually with goniocyst-like units	2
	Thallus without goniocyst-like units	11
2(1)	Mean spore length 18–23 $\mu\text{m}$	3
	Mean spore length 14–18 $\mu\text{m}$	4
3(2)	Thallus of tiny dots, involucrellum (20–)30–50 $\mu\text{m}$ thick, often thickening to base to 50–70 $\mu\text{m}$ thick	
	..... <b>V. juankoskiensis</b> Pykälä & Myllys	
	Thallus occasionally of tiny dots, usually fleck-like, involucrellum (20–)30–40 $\mu\text{m}$ thick	..... <b>V. juumaensis</b> Pykälä & Myllys
4(2)	Thallus of tiny dots	5
	Thallus fleck-like, rimose to areolate	6
5(4)	Perithecia 0.08–0.23 mm; siliceous rocks	..... <b>V. vainioi</b> Pykälä & Myllys
	Perithecia 0.11–0.17 mm; calcareous rocks	..... <b>V. danica</b> Servit & M. S. Christ.
6(4)	Thallus pale green to dark brown, ostiole often projecting papillae	7
	Thallus medium brown to dark brown, ostiole more rarely projecting papillae	9
7(6)	Involucrellum 15–30(–40) $\mu\text{m}$ thick	..... <b>V. lapidicola</b> Orange
	Involucrellum 30–50 $\mu\text{m}$ thick	8
8(7)	Prothallus absent, perithecia rarely leaving pits	..... <b>V. kalenskyi</b> Servit
	Fimbriate prothallus often present, perithecia usually leaving pits	..... <b>V. raesaenii</b> Pykälä & Myllys
9(6)	100–330 perithecia per $\text{cm}^2$ , aseptate spores occasionally present	..... <b>V. linkolae</b> Pykälä & Myllys
	60–230 perithecia per $\text{cm}^2$ , spores always aseptate	10
10(9)	60–160 perithecia per $\text{cm}^2$ ; calcareous and siliceous rocks	..... <b>V. norrlinii</b> Pykälä & Myllys
	80–230 perithecia per $\text{cm}^2$ ; siliceous rocks	..... <b>V. hunsrueckensis</b> Thüs <i>et al.</i>
11(1)	Spore length 11–17 $\mu\text{m}$	12
	Spore length mostly 18–25 $\mu\text{m}$	13
12(11)	Involucrellum 30–50 $\mu\text{m}$ thick, ostiole plane	..... <b>V. oulankajokiensis</b> Pykälä & Myllys
	Involucrellum 15–30 $\mu\text{m}$ thick, ostiole pale, projecting papillae	..... <b>V. dolosa</b> Hepp
13(11)	Thallus areolate, mosaically brown and white	..... <b>V. lohjaensis</b> Pykälä & Myllys
	Thallus fleck-like, rimose to more rarely areolate, pale green to dark brown	14
14(13)	Perithecia often thinly thalline covered except apex, thallus green to brown	15
	Perithecia not thalline covered, thallus brown	..... <b>V. infumata</b> Nyl.
15(14)	50–120 perithecia per $\text{cm}^2$ , perithecia 1/4–1/2-immersed	..... <b>V. tenebrosa</b> Pykälä <i>et al.</i>
	100–200 perithecia per $\text{cm}^2$ , perithecia 1/4–3/4-immersed	16
16(15)	Perithecia 1/2–3/4-immersed, ostiole plane to depressed	..... <b>V. hakulinenii</b> Pykälä & Myllys
	Perithecia 1/4–1/2-immersed, ostiole often pale, projecting papillae	..... <b>V. tallbackaensis</b> Pykälä <i>et al.</i>

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