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***Verrucaria ahtii*, *V. oulankaensis* and *V. vitikainenii*,  
three new species from the *Endocarpon* group  
(*Verrucariaceae*, lichenized Ascomycota)**

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**Abstract:** Three new species of *Verrucaria* are described from calcareous and calciferous rocks in Finland based on morphology and ITS sequences. The species are all members of the *Endocarpon* group in the *Verrucariaceae*. *Verrucaria oulankaensis* sp. nov. is related to *V. cernaensis* but differing in its usually pruinose, small areolate thallus. It occurs in NE Finland on calcareous and calciferous rocks on river shores. *Verrucaria ahtii* sp. nov. and *V. vitikainenii* sp. nov. form a sister group in the ITS phylogeny. *Verrucaria vitikainenii* differs from *V. ahtii* in the darker and thinner thallus, absence of a prothallus and in the perithecia, which lack thalline cover. *Verrucaria ahtii* morphologically resembles *V. apomelaena* but differs in having a thinner involucrellum and a fimbriate prothallus. It has a southern distribution in Finland and prefers sun-exposed sites, particularly pebbles in lime quarries. The species is also reported from Lithuania and Russia. *Verrucaria vitikainenii* has an eastern distribution in Finland. The species is a strict calcicole, preferring half-shady habitats. *Verrucaria apomelaena* is excluded from the Finnish lichen flora.

**Key words:** calcareous rocks, Finland, ITS, lichens, Lithuania, Russia, taxonomy

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### Introduction

Members of *Verrucariales* grow mainly on rocks and are particularly diverse on calcareous substrata (Gueidan *et al.* 2007). In northern Europe *Verrucariales* has been understudied in these habitats, and *Verrucaria* and related species described from calcareous rocks in Fennoscandia are based on somewhat limited sampling. In his monograph of pyrenocarpous lichens, Vainio (1921) cites only 36 specimens representing 13 species of *Verrucaria* from calcareous rocks in Finland. In addition, Magnusson and Servít have described a small number of new species from calcareous rocks in Sweden and Norway (Magnusson 1946, 1948, 1952; Servít 1949, 1952).

During the study of lichens on calcareous rocks in Finland, it became evident that many species of *Verrucaria* and related genera do not fit any of the previously described species (Pykälä & Myllys 2016; Pykälä *et al.* 2017). In this paper, we describe three new *Verrucaria* species from the *Endocarpon* group (see Gueidan *et al.* 2007, 2009). These species are well defined by a combination of morphology and ITS sequences.

### Material and Methods

This study is based on material collected by the first author during a lichen inventory of calcareous rocks and lime quarries in Finland (see Pykälä *et al.* 2017). Type material of putatively related species from herbaria H, H-NYL, M, PRM, TUR-V, UPS and VER was studied for comparison.

### Morphology

Perithecia and thalli were hand-sectioned with razor blades. The sections were examined and measured in water. Asci and ascospores were also studied in squash preparations of perithecia mounted in water. Sections and squash preparations of old herbarium

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specimens were mounted in potassium hydroxide (KOH) solution. Additionally, involucrellum characters and exciple colour and diameter were studied by cutting perithecia into two pieces and studying the pieces using a binocular microscope.

Measurements for ascospores are given as  $\bar{x} \pm SD$ , with maximum and minimum values in parentheses. The sizes of the perithecia are given in surface view. The colour of the exciple wall was observed from the basal parts.

### DNA extraction and sequencing

Total genomic DNA was extracted from 1 to 3 perithecia of two to six-year-old herbarium specimens. All but 13 samples (see below) 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.php>) were used for extraction, PCR and sequencing. Primers ITS1-LM (Myllys *et al.* 1999) and ITS4 (White *et al.* 1990) were used both for PCR and sequencing of the nuclear ribosomal ITS barcode region. The barcode sequences and their trace files, along with all relevant collection data and photographs of the voucher specimens, were uploaded to the Barcode of Life Data Systems (BOLD, <http://www.boldsystems.org>) database. The sequences were deposited in GenBank (Table 1).

DNA of the specimens *Pykälä* 24961, 25113, 25794, 26951, 29868, 31224, 31495, 34775, 35076, 35631, 36134, 39726 and 39904 was extracted using DNeasy<sup>®</sup> Blood & Tissue kit by Qiagen following the protocol described in Myllys *et al.* (2011) (Table 1). PCR reactions were prepared using PuReTaq Ready-To-Go PCR beads (GE Healthcare). The 25  $\mu$ l reaction volume contained 19  $\mu$ l of dH<sub>2</sub>O, 0.4  $\mu$ M of each primer and 4  $\mu$ l 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 ending with a final extension for 7 min at 72 °C. PCR products were cleaned and sequenced by Macrogen Inc., South Korea ([www.macrogen.fi](http://www.macrogen.fi)). 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

Based on a BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) our new species belonged to the *Endocarpon* group as defined in Gueidan *et al.* (2007). Consequently, the phylogenetic analysis included 35 ITS sequences representing species of the *Endocarpon* group, in addition to 22 ITS sequences obtained from the new species (Table 1). *Dermatocarpon luridum* (With.) J. R. Laundon and *D. mimiatum* (L.) W. Mann

were used as outgroup taxa based on the studies of Gueidan *et al.* (2007) and Prieto *et al.* (2012). Altogether 34 new sequences were generated in this study and 25 sequences were loaded from GenBank.

A total of 59 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 parsimony analysis as implemented in TNT v.1.1 (Goloboff *et al.* 2008). The analysis was performed using Traditional search with random addition of sequences with 100 replicates and TBR branch-swapping algorithm. Ten trees were saved for each replicate and gaps were treated as missing data. Node support was estimated using the bootstrapping method (Felsenstein 1985) with 1000 replicates.

### Results and Discussion

The ITS data set consisted of 528 characters of which 175 were parsimony informative. In the strict consensus tree, all three of the new species were recovered as monophyletic (Fig. 1). *Verrucaria oulankaensis* sp. nov., represented by four specimens, is related to the freshwater species *V. cernaensis*. The two species are also morphologically similar, differing mainly in their thallus characters (see detailed discussion under species descriptions). *Verrucaria ahtii* sp. nov., 11 specimens of which were included in this study, and *V. vitikainenii* sp. nov., with seven specimens, are sister species with 84% support value and they form an unsupported group with *V. weddellii* and *V. viridula*. Our new species, however, do not resemble these two species morphologically. The monophyly of *V. vitikainenii* is relatively poorly supported (69%), but the species is morphologically well defined (see species descriptions).

### The Species

#### *Verrucaria ahtii* Pykälä, Launis & Myllys sp. nov.

Mycobank No.: MB 817958

Similar to *V. apomelaena* (A. Massal.) Hepp, but with a thinner involucrellum, (25%–)50–75%–immersed perithecia and a fimbriate prothallus.

Type: Finland, Varsinais-Suomi, Lohja, Lohja, NW of Tytyri lime processing factory, high road bank, on pebbles enriched by calciferous dust, 53 m, 60°15'N,

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

Species	Country	Voucher	GenBank Acc. No.
<i>Dermatocarpon luridum</i>	USA	<i>Amtoft</i> 2005 (NY)	EF014198
<i>D. miniatum</i>	UK	<i>Buck</i> 47331 (NY)	EF014192
<i>Endocarpon adscendens</i>	Switzerland	<i>Gueidan</i> 671 (DUKE)	KF959777
<i>E. adsurgens</i>	Finland	<i>Pykälä</i> 38336 (H)	<b>KX758063</b>
<i>E. pallidulum</i>	USA	<i>Joneson</i> 4028 (DUKE)	DQ826735
<i>E. petrolepideum</i>	USA	U-492F (DUKE)	KF959778
<i>E. psorodeum</i>	Estonia	<i>Gueidan</i> 684 (DUKE)	KF959779
<i>E. psorodeum</i>	Finland	<i>Pykälä</i> 34139 (H)	<b>KX758064</b>
<i>E. pusillum</i>	France	<i>Gueidan</i> 470 (MARSSJ)	JQ927447
<i>Neocatapyrenium rhizinosum</i>	Turkey	VV (LI)	KF959783
<i>N. rhizinosum</i>	Greece	<i>Breuss</i> (LI)	KF959782
<b><i>Verrucaria ahtii</i></b>	Finland	<i>Pykälä</i> 25794 (H)	<b>KX720563</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 26951 (H)	<b>KX720569</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 29868 (H)	<b>KX720568</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 32044 (H)	<b>KX720562</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 32349 (H)	<b>KX720561</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 34645 (H)	<b>KX720564</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 36696 (H)	<b>KX720570</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 37250 (H)	<b>KX720571</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 37273 (H)	<b>KX720567</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 41418 (H)	<b>KX720565</b>
<b><i>V. ahtii</i></b>	Finland	<i>Pykälä</i> 41875 (H)	<b>KX720566</b>
<i>V. cernaensis</i>	UK	<i>Orange</i> 16263 (NMW)	FJ645260
<i>V. cernaensis</i>	UK	<i>Orange</i> 16308 (NMW)	FJ664823
<i>V. funckii</i>	Germany	<i>Thüs</i> W1577 (BM)	KM243243
<i>V. macrostoma</i>	UK	<i>Orange</i> 17560 (NMW)	JX848567
<i>V. macrostoma</i>	UK	<i>Orange</i> 17825 (NMW)	JX848568
<i>V. nigrescens</i>	Estonia	<i>Orange</i> 18097 (NMW)	JX848569
<i>V. nigrescens</i>	Ireland	<i>Orange</i> (NMW)	JX848570
<b><i>V. oulankaensis</i></b>	Finland	<i>Pykälä</i> 35631 (H)	<b>KX720573</b>
<b><i>V. oulankaensis</i></b>	Finland	<i>Pykälä</i> 36091 (H)	<b>KX720574</b>
<b><i>V. oulankaensis</i></b>	Finland	<i>Pykälä</i> 36100 (H)	<b>KX720575</b>
<b><i>V. oulankaensis</i></b>	Finland	<i>Pykälä</i> 39726 (H)	<b>KX720572</b>
<i>V. polysticta</i>	Switzerland	<i>Gueidan</i> 689 (MARSSJ)	KF959785
<i>V. polysticta</i>	Finland	<i>Pykälä</i> 37580 (H)	<b>KX720583</b>
<i>V. rosula</i>	UK	<i>Orange</i> 16753 (NMW)	FJ664883
<i>V. viridula</i>	France	<i>Gueidan</i> 587B (MARSSJ)	KF959786
<i>V. viridula</i>	Sweden	<i>Savic</i> 3075 (UPS)	EU553510
<i>V. viridula</i>	UK	<i>Orange</i> 15145 (NMW)	FJ664822
<i>V. viridula</i>	Ireland	<i>Orange</i> 17893 (NMW)	JX848582
<i>V. viridula</i>	Finland	<i>Pykälä</i> 24961 (H)	<b>KX720591</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 25113 (H)	<b>KX720590</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 31224 (H)	<b>KX720589</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 31495 (H)	<b>KX720592</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 36134 (H)	<b>KX720588</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 37873 (H)	<b>KX720593</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 39904 (H)	<b>KX720586</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 41339 (H)	<b>KX720585</b>
<i>V. viridula</i>	Finland	<i>Pykälä</i> 46464 (H)	<b>KX720584</b>
<b><i>V. vitikainenii</i></b>	Finland	<i>Pykälä</i> 35076 (H)	<b>KX720576</b>
<b><i>V. vitikainenii</i></b>	Finland	<i>Pykälä</i> 36159 (H)	<b>KX720582</b>
<b><i>V. vitikainenii</i></b>	Finland	<i>Pykälä</i> 42415 (H)	<b>KX720580</b>
<b><i>V. vitikainenii</i></b>	Finland	<i>Pykälä</i> 44911 (H)	<b>KX720577</b>
<b><i>V. vitikainenii</i></b>	Finland	<i>Pykälä</i> 45416 (H)	<b>KX720581</b>
<b><i>V. vitikainenii</i></b>	Finland	<i>Pykälä</i> 45417 (H)	<b>KX720578</b>
<b><i>V. vitikainenii</i></b>	Finland	<i>Pykälä</i> 45435 (H)	<b>KX720579</b>
<i>V. weddellii</i>	France	<i>Gueidan</i> 460 (MARSSJ)	KF959787
<i>Willeya diffractella</i>	USA	<i>Harris</i> 56340 (NY)	KM371611
<i>W. laevigata</i>	Vietnam	<i>Gueidan</i> 1852 (BM)	KF959807

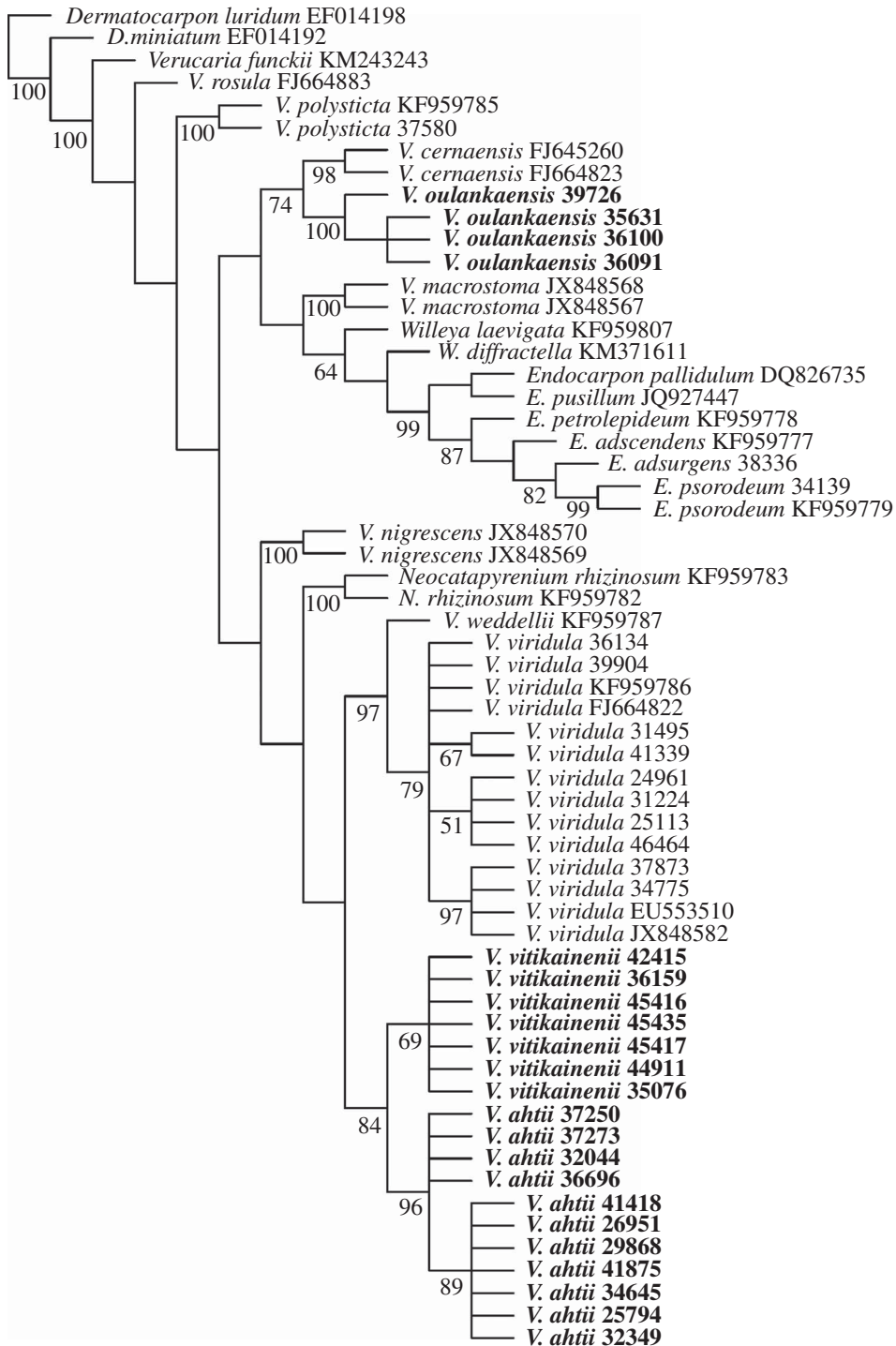


FIG. 1. Phylogenetic relationships of *Verrucaria ahtii*, *V. oulankaensis* and *V. vitikainenii*. Strict consensus tree based on ITS data set with bootstrap values (>50%) at nodes. New species described in this study are indicated in bold.



24°04'E, 6 February 2008, *J. Pykälä* 32349 (H—holotype). GenBank Accession number: KX720561 (ITS).

(Fig. 2A & B)

*Prothallus* medium brown or dark brown, fimbriate, usually rather inconspicuous. *Thallus* grey (rarely), pale brown or medium brown, fleck-like, continuous or rimose,

*c.* 0.02–0.15(–0.25) mm thick; thallus or thallus surface uneven, commonly consisting of tiny units of 0.03–0.08(–0.13) mm diam., algal cells *c.* 5–11  $\mu$ m, cortex *c.* 10–24  $\mu$ m thick, cortical cells brown.

*Perithecia* 0.15–0.32 mm diam., (25–)50–75%-immersed in thallus, not leaving pits, often with a thin thalline cover, thalline cover *c.* 11–24  $\mu$ m thick; perithecial density

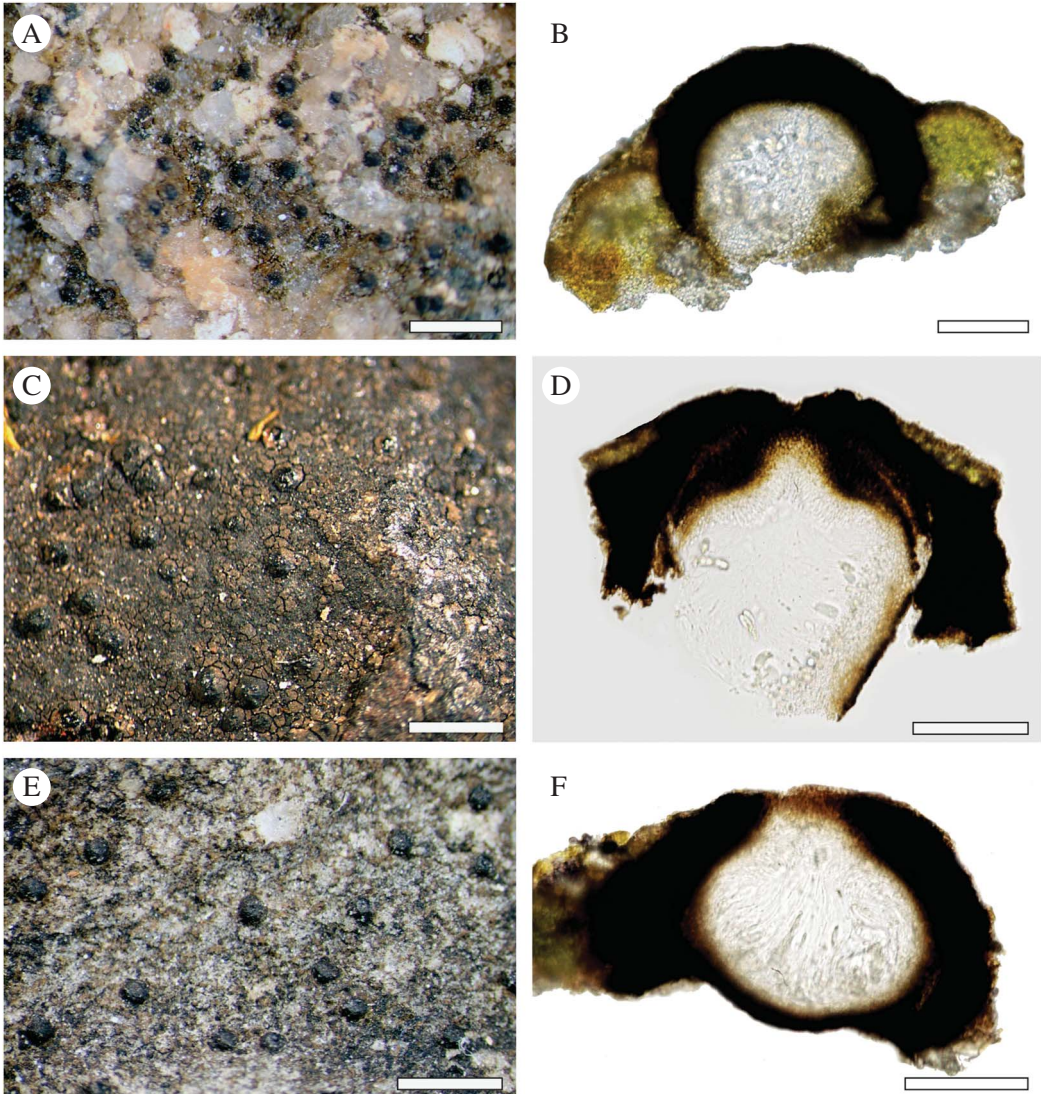


FIG. 2. A & B, *Verrucaria ahtii* (holotype); A, habitus; B, section through perithecium. C & D, *V. oulankaensis* (holotype); C, habitus; D, section through perithecium. E & F, *V. viitikainenii* (holotype); E, habitus; F, section through perithecium. Scales: A, C & E = 1 mm; B, D & F = 100  $\mu$ m. In colour online.

60–180(–220) cm<sup>-2</sup>. *Ostiole* inconspicuous, pale or usually dark, plane, depressed or often dark projecting papillae, *c.* 20–30–(50) µm wide. *Involucrellum* usually extending to the exciple base level, occasionally only exceeding half of the exciple, (20–)30–50 µm thick, in one specimen rarely increasing in thickness towards the base to 50–60 µm thick, appressed to the exciple or slightly diverging from it. *Exciple* 0.16–0.25(–0.32) mm diam., wall usually dark brown, in few specimens also pale exciple walls present, *c.* 13–18 µm thick. *Periphysoids* *c.* (20–)25–30(–40) × (1.5–)2.0–2.5(–3.0) µm. *Ascospores* (17.0–)20.6–23.1–25.5(–28.3) × (9.2–)10.2–11.3–12.4(–13.9) µm (*n* = 223), perispore absent.

*Etymology.* The species is named in honour of Professor Teuvo Ahti for his major contributions to lichenology.

*Habitat and distribution.* Known from Finland, Lithuania and Russia. The species prefers sun-exposed sites. It often grows on calcareous pebbles or on siliceous pebbles enriched by calcareous dust. It also grows in lime quarries on south-facing walls and on concrete and mortar but was not detected in natural calcareous habitats. However, the Lithuanian specimens are from calcareous pebbles in dry semi-natural grassland.

*Notes.* Based on the ITS phylogeny, *V. ahtii* is closely related to *V. vitikainenii*. For differences with *V. vitikainenii*, see that species (below). The first collections of *V. ahtii* were originally identified as *V. apomelaena* (Pykälä 2008) and *V. gudbrandsdalensis* Zschacke ex H. Magn. (Pykälä & Breuss 2008). Later some specimens in H were identified as *V. invenusta* H. Magn. Unfortunately, ITS sequences of *V. apomelaena* and *V. invenusta* are not available, but we detected some morphological differences between the taxa. Based on ITS sequences, *V. gudbrandsdalensis* is not closely related to *V. ahtii* (J. Pykälä, A. Launis & L. Myllys, unpublished data).

*Verrucaria apomelaena* (syntype: VER!) differs in a thicker involucrellum (40–70 µm thick), a larger exciple (0.20–0.35 mm wide) and the perithecia, which are 75%–(100%)-immersed

in thalline warts. The syntype specimen lacks a prothallus. *Verrucaria apomelaena* is excluded from the Finnish lichen flora.

*Verrucaria gudbrandsdalensis* (UPS-L-194855!, H!), syntypes) has larger perithecia (0.2–0.4 mm diam.), thinner periphysoids (1.5–2.0 µm thick), a usually grey (rather rarely pale brown) thallus and a prothallus is lacking.

The involucrellum of *V. invenusta* (syntypes: UPS-487169!, S-L72!) often thickens to the exciple base to *c.* 40–70 µm thick. The exciple wall is pale or pale brown. The prothallus was not seen. The type material is from a schistose rock outcrop in northern Sweden, whereas *V. ahtii* seems to occur in Finland only in the southern part of the country on calcareous habitats. Nevertheless, based on morphology, *V. invenusta* may be related to *V. ahtii*.

The identity of the Finnish specimen reported as *V. invenusta* in Pykälä & Breuss (2009) is uncertain. It does not belong to *V. ahtii*, and probably not to *V. invenusta*, but might represent another unidentified species of *Verrucaria*. The Lithuanian and Russian specimens erroneously reported as *V. invenusta* (Motiejūnaitė *et al.* 2012; Pykälä *et al.* 2012) represent *V. ahtii*.

*Additional specimens examined.* **Finland:** *Varsinais-Suomi:* Lohja, Pietilä, W of Lentoo, small dry grassland, house ruins, on mortar, 42 m, 60°13'N, 23°54'E, 2004, *Pykälä* 25794 (H); Lohja, Hermala, Kalkkimäki, 150 m NW of Kekla lime quarry, road cutting of calcareous rock outcrop, on pebbles, 53 m, 60°13'N, 23°51'E, 2005, *Pykälä* 26951 (H); Salo (Kiikala), Saari, Kalkkimäki, abandoned lime quarry, on SE-facing wall, 105 m, 60°25'N, 23°40'E, 2009, *Pykälä* 34645 (H); Länsi-Turunmaa (Parainen), Ersby, N of Sementitie Road, siliceous rock outcrop enriched by calcareous dust, on pebbles, 40 m, 60°17'N, 22°15'E, 2009, *Pykälä* 36696 (H); Länsi-Turunmaa (Parainen), Simonby, Gropen, abandoned lime quarry, road cutting of calciferous rock outcrop, on pebbles, 15 m, 60°16'N, 22°14'E, 2009, *Pykälä* 37250 (H); Länsi-Turunmaa (Parainen), Simonby, Gropen, abandoned lime quarry, road verge, on pebbles, 18 m, 60°16'N, 22°14'E, 2009, *Pykälä* 37273 (H). *Uusimaa:* Sipoo, Träsk, Kalkstrand, N of lime processing factory, flat siliceous rock outcrop enriched by calciferous dust, on path, on pebbles, 20 m, 60°15'N, 25°23'E, 2006, *Pykälä* 29868 (H); Sipoo, Träsk, Kalkstrand, lime quarry, E-slope, quarry spoil gravel on siliceous rock outcrop, on calcareous pebbles, 12 m, 60°15'N, 25°23'E, 2010, *Pykälä* 41418 (H); Helsinki (Vantaa), Västersundom, 400 m NW Vikkulla, abandoned lime quarry on shore of the Baltic Sea, on

SW-facing wall, 2 m, 60°13'N, 25°09'E, 2011, *Pykälä* 41875 (H).—**Russia:** *Leningrad Region:* Vyborg District, Berezovye Islands (Koiviston Saaret), Zapadny Berezovy Island (Tiurinsaari), 60°20'N, 28°31'E, on concrete of old military construction, 2004, *Alexeeva* (H) (not sequenced).—**Lithuania:** *Utena County:* Ignalina District, Aukštaitija National Park, Stripeikiai Village, close to Traditional Apiculture Museum, dry grassland, on calcareous pebbles, 55°24'N, 25°56'E, 2011, *Pykälä* 46085, 46087 (H) (not sequenced).

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

Mycobank No.: MB 817959

Similar to *V. cernaensis* Zschacke but differs in an often pruinose, small areolate (not rimose to areolate) thallus and narrower spores.

Type: Finland, Koillismaa, Salla, Oulanka National Park, Pikkuköngäs, shore of Oulankajoki River, high cliff, calciferous (dolomite) schistose rock outcrop, on SW-facing wall, 180 m, 66°25'N, 29°09'E, 10 August 2009, *J. Pykälä* 36100 (H—holotype). GenBank Accession number: KX720575 (ITS).

(Fig. 2C & D)

*Prothallus* dark brown, non-fimbriate. *Thallus* pale brown, brownish grey, medium brown or dark brown, usually grey pruinose, areolate, 0.1–0.2 mm thick, fertile areoles 0.2–0.7 mm diam., sterile areoles 0.1–0.4 mm diam., algal cells 5–10 µm, cortex 6–24 µm thick, cortical cells pale or brown, epinecral layer sometimes present, medulla not differentiated.

*Perithecia* 0.12–0.27 mm diam., (50–)75–100%—immersed in thallus or in thalline warts; perithecial density 80–160 cm<sup>-2</sup>; 1(–2) perithecia per areole. *Ostiole* inconspicuous, dark, depressed, *c.* 20–60 µm wide. *Involucrellum* covering half of the exciple or extending to the exciple base level, *c.* (40–)50–70(–80) µm thick, appressed to the exciple or slightly diverging from it. *Exciple* 0.15–0.25 mm diam., wall dark brown, 16–21 µm thick. *Periphysoids* 20–35 × (1.5–)2.0–2.5 µm. *Asci* *c.* 50–61 × 19–24 µm, 8-spored. *Ascospores* (15.2–)16.2–17.7–19.3(–22.4) × (7.2–)7.7–8.4–9.1(–10.3) µm (*n* = 62), perispore absent.

*Pycnidia* not seen.

*Etymology.* Most collections are from the Oulanka area, one of the lichenologically most important areas in Finland.

*Habitat and distribution.* Known from north-eastern Finland. All collections are from calcareous or calciferous rocks on river shores. The species occurs on the upper part of the geolittoral zone affected by spring flooding, often growing with *Staurothele areolata* (Ach.) Lettau. Calcareous rocks on river shores are very rare in Finland and are mostly found in the Oulanka area. It is possible that *V. oulankaensis* is a north-eastern species hardly reaching Fennoscandia and Europe. The biogeographical province Koillismaa (Ks), particularly the Oulanka area, is well known for very rare lichens in the European context, such as *Nephroma helveticum* Ach. and *Peltigera retifoveata* Vitik.

*Notes.* *Verrucaria oulankaensis* is usually easily identified by its pruinose small areolate thallus and rather short spores compared to their width. Based on the ITS phylogeny, *V. oulankaensis* is related to *V. cernaensis* (95% similarity in ITS). *Verrucaria oulankaensis* is also morphologically rather similar to the descriptions of *V. cernaensis* given by Zschacke (1927) and Orange (2013), as well as to the syntype of the species (M-0023497!). *Verrucaria cernaensis* and *V. oulankaensis* mainly differ in thallus characters. *Verrucaria cernaensis* has a non-pruinose thallus, which is rimose to cracked-areolate, whereas areoles of *V. oulankaensis* are mainly separated from each other. The thallus of *V. oulankaensis* is clearly thicker surrounding the perithecia, somewhat convex in fertile areoles, and the perithecia are commonly 75–100%—immersed in thalline warts and on a higher level than the surrounding sterile areoles. The colour of the exciple wall varies in *V. cernaensis* from pale to dark, whereas in *V. oulankaensis* only dark exciples were seen. Pycnidia have been reported as common in *V. cernaensis* (Orange 2013), but were not detected in *V. oulankaensis*. *Verrucaria cernaensis* also has broader spores: mean 10.6 µm (Orange 2013). It is possible that some specimens of *V. oulankaensis* (lacking pruina) might be difficult to separate from *V. cernaensis*.

*Verrucaria oulankaensis* may also resemble species of the *V. nigrescens* Pers. complex. These species, also members of the



*Endocarpon* group, lack pruina and have a fimbriate prothallus.

*Verrucaria obnigrescens* Nyl. (as observed from the syntypes H!, H-NYL 2625!) is superficially rather similar to *V. oulankaensis* as both species have a grey pruinose small areolate thallus. *Verrucaria obnigrescens*, however, differs from *V. oulankaensis* in having considerably larger spores (20–27 × 10–14 µm). Furthermore, *V. obnigrescens* is characterized by a usually shorter involucrellum and a fimbriate prothallus.

The syntypes of *V. mauriza* Nyl. (H!, TUR-V!, H-NYL 2824! not identifiable) also have a small, areolate grey pruinose thallus, but the species has a fimbriate prothallus, smaller perithecia (0.15–0.20 mm) and a pale exciple.

*Additional specimens examined.* **Finland:** *Koillismaa:* Kuusamo, Paljakka, E shore of Kuusinkijoki River, Kiukaankorva, nature reserve, dolomite rock outcrop, on SW-facing wall, with *Staurothele areolata*, 214 m, 66°11'N, 29°38'E, 2009, *Pykälä* 35631 (H); Salla, Oulanka National Park, Pikkuköngäs, shore of Oulankajoki River, high cliff, calciferous (dolomite) schistose rock outcrop, on overhanging SW-facing wall, 180 m, 66°25'N, 29°09'E, 2009, *Pykälä* 36091 (H); Kuusamo, Juuma, Oulanka National Park, Jyrävä, shore of Kitkajoki, cliff, calciferous (dolomite) schistose rock outcrop, on SW-facing wall, with *Staurothele areolata*, 206 m, 66°15'N, 29°26'E, 2010, *Pykälä* 39726 (H).

### ***Verrucaria vitikainenii* Pykälä, Launis & Myllys sp. nov.**

MycoBank No.: MB 817960

Similar to *V. ahtii*, but thallus darker brown, prothallus absent, perithecia lacking thalline cover, involucrellum thicker, exciple walls always dark and perithecia occurring more sparsely.

Type: Finland, Koillismaa, Salla, Hautajärvi, Kurtinniitykuru, cliff, dolomite rock outcrop, beneath W-facing wall, steep slope, on dolomite pebbles, 205 m, 66°26'N, 29°09'E, 29 August 2011, *f. Pykälä* 45435 (H—holotype). GenBank Accession number: KX720579 (ITS).

(Fig. 2E & F)

*Prothallus* absent. *Thallus* pale brown (rarely), medium brown or dark brown, fleck-like, sometimes continuous or granular, often poorly developed, *c.* 20–100 µm

thick, cortex absent or *c.* 11–17 µm thick, cortical cells brown, algal cells 5–9 µm.

*Perithecia* 0.20–0.36 mm diam., 25–50% immersed, not leaving pits, without thalline cover, occasionally irregular thin cover of dead cells; perithecial density 40–120 cm<sup>-2</sup>. *Ostiole* usually inconspicuous, pale brown to dark, plane or depressed, rarely projecting papillae-like, 20–50(–70) µm wide, ostiolar depression occasionally wide and up to 130 µm wide. *Involucrellum* to the exciple base level, occasionally enveloping the exciple, 30–60 µm thick, sometimes increasing in thickness towards the base to 60–80 µm thick, appressed to the exciple, more rarely slightly or moderately diverging from the exciple. *Exciple* 0.19–0.27 mm diam., wall rarely medium brown, usually dark brown or black, 14–21 µm thick. *Periphysoids* 15–40 × (1.0–)1.5–2.5 µm. *Asci* *c.* 65–82 × 25–30 µm, 8-spored. *Ascospores* (19.7–)21.9–23.7–25.6(–28.7) × (10.1–)10.9–11.8–12.8(–14.4) µm (*n* = 152), perispore absent.

*Etymology.* The species is named in honour of Dr Orvo Vitikainen who has with his modest style contributed significantly to the knowledge of taxonomy and biogeography of Finnish lichens.

*Habitat and distribution.* Known only from Finland. *Verrucaria vitikainenii* has a north-eastern distribution in Finland with only one isolated outpost known from south-west Finland. It occurs mainly in the Oulanka area in the biogeographical province Koillismaa (Ks) in north-east Finland. One specimen is from eastern Finland and one from south-west Finland. Outside the Oulanka area the species seems to be very rare. It is calcicolous growing on calcareous rock outcrops, stones and pebbles and might prefer half-shady habitats. We did not find any specimens occurring in limestone quarries. This suggests that the species might have declined due to mining of limestone.

*Notes.* The specimens of *V. vitikainenii* were first identified as *V. triglavensis* Servit (Pykälä 2013). However, examination of the holotype specimen of *V. triglavensis*



(PRM-756870!) revealed clear differences between the two species. *Verrucaria triglavensis* has perithecia leaving shallow to fairly deep pits, whereas the perithecia of *V. vitikainenii* do not leave pits. The spores of *V. triglavensis* are also larger: 24–30 × 13–15 µm.

*Verrucaria vitikainenii* may sometimes be difficult to separate from the closely related *V. ahtii*. *Verrucaria ahtii*, however, has a fimbriate prothallus, frequently a pale brown, thicker thallus and often thalline covered perithecia. Furthermore, *V. ahtii* may occasionally have pale exciples present and perithecia often occur more densely than in *V. vitikainenii*. The involucrellum of *V. ahtii* is also on average thinner than in *V. vitikainenii*. The two species have different ecology and distribution patterns: *V. vitikainenii* has an eastern or north-eastern distribution in Finland whereas *V. ahtii* is known only from southern Finland. *Verrucaria vitikainenii* has never been collected from artificial calcareous habitats whereas *V. ahtii* has not been collected from a natural calcareous habitat. *Verrucaria vitikainenii* prefers half-shady habitats whereas *V. ahtii* prefers sun-exposed sites.

All but one of the sequenced specimens of *V. vitikainenii* have identical ITS sequences. The specimen 42415 differs in nine bases, but it does not differ morphologically from the other specimens of *V. vitikainenii*.

*Additional specimens examined.* **Finland:** *Varsinais-Suomi:* Salo (Kisko), Haapaniemi, 200 m NW of Iso Sorronlahti, herb-rich heath forest, on calcareous stone, 56 m, 60°13'N, 23°30'E, 2009, *Pykälä* 35076 (H). *Koillismaa:* Kuusamo, Juuma, Oulanka National Park, Lammasvuoma, gorge, dolomite rock outcrop, on SW-facing wall, 220 m, 66°16'N, 29°26'E, 2009, *Pykälä* 36159 (H); Salla, Oulanka National Park, 1.4 km NE Savilampi, shore of Savinajoki River, cliff, dolomite rock outcrop, steep SE-slope, on dolomite pebbles, 190 m, 66°26'N, 29°11'E, 2011, *Pykälä* 44911 (H); Salla, Hautajärvi, Kurtinniittykuru, cliff, dolomite rock outcrop, beneath W-facing wall, steep slope, on dolomite pebbles, 205 m, 66°26'N, 29°09'E, 2011, *Pykälä* 45416 (H); Salla, Hautajärvi, Kurtinniittykuru, cliff, dolomite rock outcrop, on W-facing wall, 205 m, 66°26'N, 29°09'E, 2011, *Pykälä* 45417 (H). *Pohjois-Karjala:* Juuka, Polvela, Valkealampi, close by E-shore, *Pinus sylvestris*-dominated forest, calcareous rock outcrop, on W-facing wall, rather scarce, 175 m, 63°10'N, 29°07'E, 2011, *Pykälä* 42415 (H).

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