

Four new species of *Verrucaria* (Verrucariaceae, lichenized Ascomycota) from freshwater habitats in Europe

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Abstract: *Verrucaria nodosa* sp. nov. and *V. rosula* sp. nov. each have a thallus composed of initially discrete units which coalesce and overlap with age, forming an uneven crust. They are related species and similar in appearance, but the thallus units are coarser in *Verrucaria nodosa*, and the involucrellum is typically more regularly thickened than in *V. rosula*. *Verrucaria placida* sp. nov. has a smooth, subgelatinous thallus; it is related to *V. hydrophila* sp. nov. but differs in the larger ascospores and perithecia. It is not closely related to *V. andesiatica* but is morphologically similar, differing mainly in the narrower ascospores. *Verrucaria hydrophila* has been widely known as *V. hydrela* auct., non Ach. or *V. denudata* Zsch. nom. illeg. *Verrucaria laevata* Ach. is considered to be a *nomen dubium*.

Key words: Germany, ITS, lichen, mtSSU, Norway, taxonomy, Wales

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Introduction

Damp or regularly submerged rocks are an important habitat for species of the genus *Verrucaria*. Orange *et al.* (2009) reported 16 *Verrucaria* species in freshwater habitats in Great Britain and Ireland (including three since transferred to *Hydropunctaria* or *Placopyrenium*), and Thüs & Schultz (2009) reported 17 species of *Verrucaria* (and 2 of *Hydropunctaria*) from Central Europe. The latter authors listed a further 25 published names as ‘uncertain species’, reflecting the poor taxonomic knowledge of this group. Difficulties in studying *Verrucaria* arise from the simple structure and consequent scarcity of good morphological distinguishing features between species, the apparent variability of some taxa, and the large number of published names of doubtful application.

Three species below were first suspected as being undescribed taxa on morphological grounds, but DNA sequencing has provided

greater confidence that they represent taxa distinct from those already known.

Materials and Methods

Fresh or herbarium material was sectioned by hand. Ascospores were measured in *c.* 5% KOH. Perithecial density was estimated for subjectively chosen areas of thallus by using a stereomicroscope fitted with a drawing tube, to allow simultaneous viewing of an area of thallus and of a square drawn on a sheet of paper, the square representing an area of 25 mm² at the magnification in use. The number of perithecia which had their ostioles within the square was counted.

DNA extraction and sequencing

DNA was extracted from thallus or ascoma tissue of recently collected or frozen specimens, using the Qiagen DNeasy Plant Mini Kit; the manufacturer’s instructions were followed except that warm water was used for the final elution. PCR amplification was carried out using Bioneer AccuPower PCR Premix in 20 µl tubes. The two internal transcribed spacer regions and the 5.8S region (ITS1–5.8S–ITS2) of the nuclear ribosomal genes, and the 5’ end of the nuclear ribosomal large subunit (LSU) were amplified, using the primers ITS1F, ITS4, LR3, nu-LSU-155-5’ and LR7 (Vilgalys & Hester 1990; Gardes & Bruns 1993; Döring *et al.* 2000). Part of the mitochondrial ribosomal small subunit (mtSSU) was amplified using the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999). The PCR thermal cycling parameters were: initial denaturation for 5 min at 94°C, followed by

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5 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C, then 30 cycles of 30 s at 94°C, 30 s at 52°C and 1 min at 72°C. PCR products were visualized on agarose gels, stained with ethidium bromide, and purified using the Sigma GenElute PCR Clean-Up Kit. Sequencing was performed by Macrogen Inc.

Sequence editing and alignment

Sequences were assembled and edited using DNASTar Lasergene software (<http://www.dnastar.com/products/lasergene.php>). Alignment was carried out using BioEdit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>); ClustalW was used to create an initial alignment, which was edited manually. All newly generated sequences were deposited in GenBank (Table 1).

Phylogenetic analysis

Three gene regions were analyzed, the ribosomal LSU (selected taxa), the ITS1+ITS2 region (all taxa treated below), and the mitochondrial SSU (selected taxa). Sequences used in analyses are listed in Table 1.

1. Ribosomal LSU analysis

Sixty sequences of *Verrucariaceae* from GenBank, and four sequences representing each of *Verrucaria hydrophila*, *V. nodosa*, *V. placida*, and *V. rosula*, were analyzed to assist in the recognition of taxa likely to be related to the three new species, and in the selection of suitable outgroups for the ITS analyses. Phylogenetic relationships and support values were investigated using Maximum Likelihood bootstrapping, as implemented in the RaxML Black Box, hosted by the Cipres Web Portal (http://www.phylo.org/sub_sections/portal/; Miller *et al.* 2010), using the default settings.

2. ITS analysis

Two groups of taxa, suggested by the results of the LSU analysis, were analyzed separately, to allow a more successful alignment of ITS sequences:

- Verrucaria hydrophila* and *V. placida*, together with *V. dolosa*.
- Verrucaria rosula*, together with *V. macrostoma*, *V. nigrescens*, and *V. viridula*. *Verrucaria nodosa* (for which no LSU was available) was also included, due to strong similarities between the ITS of this species and *V. rosula*.

In both groups of taxa the 5.8S region was invariable and was omitted from the analysis. Analysis was undertaken using a Bayesian approach. Models of evolution for the ITS1 and ITS2 regions were selected using the Akaike Information Criterion in MrModeltest 2.2 (Nylander 2004). Gaps were treated as missing data. Using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2005), two analyses of two parallel runs were carried out on a partitioned dataset for 1 000 000 generations, with trees sampled every 100 generations. Stationarity was considered to have been reached when the average standard deviation of split frequencies dropped to <0.01, and the values for the Potential Scale Reduction Factor were close to 1. A burn-in sample of 2500 trees was dis-

carded from each run, respectively. Support values of $\geq 95\%$ Bayesian posterior probabilities were regarded as significant.

3. mtSSU analysis

Mitochondrial SSU sequences from the first group of taxa of the ITS analysis were also analyzed using a Bayesian approach, using the same procedure as for the ITS.

Results

Phylogenetic analyses

The LSU analysis (not shown) recovered with good support a clade containing *Verrucaria rosula*, together with *Endocarpon* spp., *V. macrostoma* and *V. nigrescens*, *V. polysticta* and *V. viridula* (species belonging to the *Endocarpon* clade of Gueidan *et al.* 2007, 2009), and *V. funckii* (not included in the analysis by Gueidan *et al.*). *Verrucaria dolosa*, *V. hydrophila* and *V. placida* also formed a well-supported clade, but their position in relation to other sequences was unresolved.

The ITS analysis of *Verrucaria placida* and related taxa recovered three well-supported clades, here regarded as corresponding to *V. placida*, *V. hydrophila*, and *V. dolosa* (Fig. 1A). *Verrucaria placida* sequences showed little variation, but within *V. hydrophila* and *V. dolosa* some subclades received good support. Analysis of available mtSSU sequences of the same taxa also recovered clades corresponding to *V. placida*, *V. hydrophila* and *V. dolosa* (Fig. 1B). The five sequences of *V. hydrophila* formed a well-supported clade. The two available sequences of *V. placida* formed a clade, but with low support. In *V. dolosa*, an mtSSU sequence was only available from one of the specimens used in the ITS analysis; this formed a poorly-supported clade with two mtSSU sequences from GenBank named as *V. dolosa*, suggesting that this species as understood by recent authors may be heterogeneous.

ITS1 of *Verrucaria placida*, *V. hydrophila* and *V. dolosa* also contains a short unalignable region, 8–12 bases long at approximately position 123–134 of the ITS. This region is distinctive for each species, though there is variation within *V. hydrophila*.

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

Species	Country	Voucher	GenBank accession numbers
<i>Verrucaria dolosa</i>	Wales	Orange 16777 (NMW)	FJ664847 (ITS–LSU) JX848583 (mtSSU)
<i>V. dolosa</i>	Estonia	Orange 18068 (NMW)	JX848553 (ITS)
<i>V. dolosa</i>	Estonia	Orange 18082 (NMW)	JX848554 (ITS)
<i>V. dolosa</i>	Wales	Orange 18810 (NMW)	JX848555 (ITS)
<i>V. dolosa</i>	Wales	Orange 20544 (NMW)	JX848556 (ITS)
<i>V. dolosa</i>	Germany	Thiis W0956	EF105148 (mtSSU)
<i>V. dolosa</i>	Germany	Thiis W0965	EF105147 (mtSSU)
<i>V. elaeina</i>	England	Orange 16628 (NMW)	FJ664849 (ITS–LSU)
<i>V. funckii</i>	Wales	Orange 18454 (NMW)	JN638250 (ITS–LSU)
<i>V. hydrophila</i>	Wales	Orange 16290 (NMW)	FJ664841 (ITS–LSU) JX848584 (mtSSU)
<i>V. hydrophila</i>	England	Orange 16612 (NMW)	FJ664842 (ITS–LSU)
<i>V. hydrophila</i>	England	Orange 16621 (NMW)	FJ664843 (ITS–LSU) JX848585 (mtSSU)
<i>V. hydrophila</i>	Wales	Orange 16771 (NMW)	JX848562 (ITS)
<i>V. hydrophila</i>	Wales	Orange 16776 (NMW)	FJ664844 (ITS–LSU) JX848586 (mtSSU)
<i>V. hydrophila</i>	Wales	Orange 18314 (NMW)	JX848563 (ITS)
<i>V. hydrophila</i>	Wales	Orange 18455 (NMW)	JX848564 (ITS–LSU) JX848587 (mtSSU)
<i>V. hydrophila</i>	Wales	Orange 18793 (NMW)	JX848565 (ITS) JX848588 (mtSSU)
<i>V. hydrophila</i>	Wales	Orange 18873 (NMW)	JX848566 (ITS)
<i>V. hydrophila</i>	Wales	Orange 20490 (NMW)	JX848578 (ITS)
<i>V. hydrophila</i>	Wales	Orange 20642 (NMW)	JX848579 (ITS)
<i>V. hydrophila</i>	Wales	Orange 20829 (NMW)	JX848580 (ITS)
<i>V. hydrophila</i>	Wales	Orange 20874 (NMW)	JX848581 (ITS)
<i>V. hydrophila</i>	Austria	Orange 21125 (NMW)	KC242614 (ITS)
<i>V. macrostoma</i>	Wales	Orange 17560 (NMW)	JX848567 (ITS–LSU)
<i>V. macrostoma</i>	Wales	Orange 17825 (NMW)	JX848568 (ITS–LSU)
<i>V. nigrescens</i>	Estonia	Orange 18097 (NMW)	JX848569 (ITS–LSU)
<i>V. nigrescens</i>	Ireland	Orange 18137 (NMW)	JX848570 (ITS)
<i>V. nodosa</i>	Wales	Orange 20455 (NMW)	JX848558 (ITS)
<i>V. nodosa</i>	Wales	Orange 20458 (NMW)	JX848559 (ITS)
<i>V. nodosa</i>	Wales	Orange 20648 (NMW)	JX848560 (ITS)
<i>V. nodosa</i>	Wales	Orange 20660 (NMW)	JX848561 (ITS)
<i>V. placida</i>	Norway	Orange 17209 (NMW)	JX848571 (ITS)
<i>V. placida</i>	Norway	Orange 17211 (NMW)	JX848572 (ITS)
<i>V. placida</i>	Norway	Orange 17212 (NMW)	JX848573 (ITS)
<i>V. placida</i>	Wales	Orange 17426 (NMW)	FJ664879 (ITS)
<i>V. placida</i>	Wales	Orange 17493 (NMW)	FJ664880 (ITS–LSU) JX848589 (mtSSU)
<i>V. placida</i>	Germany	Orange 19380 (NMW)	JX848574 (ITS) JX848590 (mtSSU)
<i>V. rosula</i>	Scotland	<i>A. Acton</i> [E916] (hb. Acton)	JX848557 (ITS)
<i>V. rosula</i>	Wales	Orange 16285 (NMW)	FJ664880 (ITS–LSU)
<i>V. rosula</i>	Wales	Orange 16753 (NMW)	FJ664883 (ITS–LSU)
<i>V. rosula</i>	Wales	Orange 17241 (NMW)	FJ664884 (ITS–LSU) JX848591 (mtSSU)
<i>V. rosula</i>	Wales	Orange 17489 (NMW)	JX848575 (ITS)
<i>V. rosula</i>	Wales	Orange 18876 (NMW)	JX848576 (ITS)
<i>V. rosula</i>	Wales	Orange 20542 (NMW)	JX848577 (ITS)
<i>V. sublobulata</i>	Norway	Orange 17206 (NMW)	FJ664885 (ITS–LSU)
<i>V. viridula</i>	Scotland	Orange 15145 (NMW)	FJ664822 (ITS–LSU)
<i>V. viridula</i>	Ireland	Orange 17893 (NMW)	JX848582 (ITS)

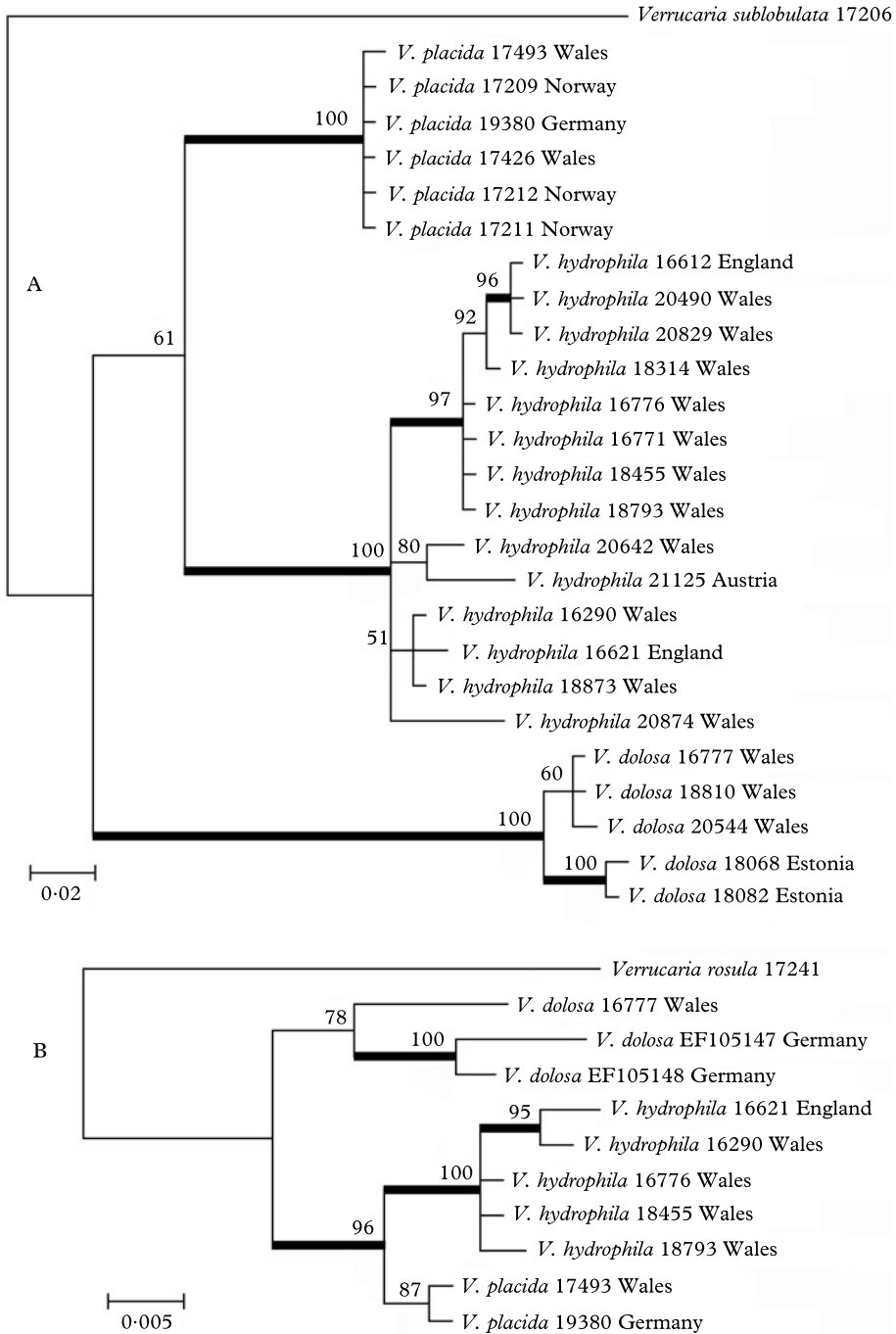


FIG. 1. Phylogenetic relationships of *Verrucaria placida* and related species, based on a Bayesian analysis. Branches in bold indicate a support of PP \geq 95%. A, Bayesian analysis of the ITS1+ITS2 regions of the nuclear ribosomal DNA; the tree was rooted using *Verrucaria sublobulata*; B, Bayesian analysis of the mitochondrial SSU; the tree was rooted using *Verrucaria rosula*.

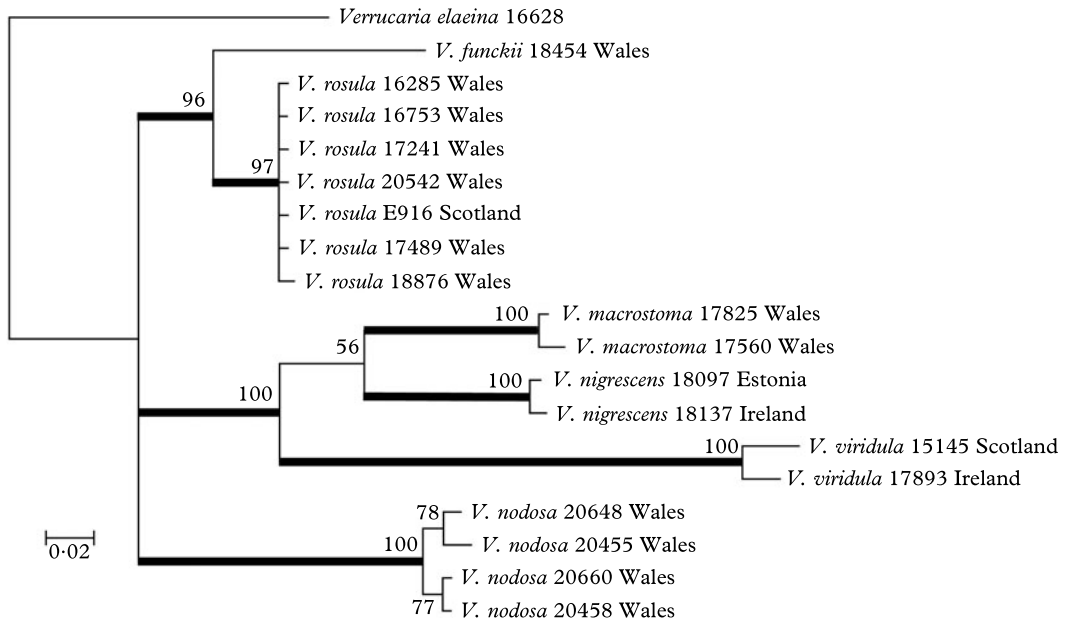


FIG. 2. Phylogenetic relationships of *Verrucaria rosula* and related species, based on a Bayesian analysis of the ITS1+ITS2 regions of the nuclear ribosomal DNA; the tree was rooted using *Verrucaria elaeina*. Branches in bold indicate a support of PP $\geq 95\%$.

The ITS analysis of *V. rosula* and related taxa recovered three well-supported clades corresponding to *V. rosula* (seven sequences with little variation), *V. macrostoma* together with *V. nigrescens* and *V. viridula*, and *V. nodosa* (Fig. 2).

The results support the integrity of the four species *Verrucaria hydrophila*, *V. nodosa*, *V. placida* and *V. rosula*, which are described below as new.

The Species

Verrucaria hydrophila Orange sp. nov.

Mycobank No.: MB802488

Verrucaria hydrela auct., non Ach., *Syn. meth. lich.*: 94 (1814).

Verrucaria denudata Zsch., *Hedwigia* 67: 74 (1927); nom. illeg., non Nyl. (1858), *Mém. Soc. Acad. Maine-et-Loire* 4: 49.

Thallus tenuis, 25–60 μm crassus, laevis, continuus. Perithecia prominentias conico-hemisphaericas formantia, prominentiae 240–400 μm latae, ab thallo pro parte maxima obtectae. Involucrellum conicum vel conico-

hemisphaericum. Excipulum 145–225 μm latum. Ascospores ellipsoideae, aseptatae, hyalinae, (15.0–)19.5–21.1–23.0(–26.0) \times (6.5–)8.0–8.9–9.5(–11.0) μm . Pycnidia nulla.

Typus: Great Britain, Wales: V.C. 50, Denbighshire, Bontuchel, Coed y Fron-wyllt, Nant Melin-dŵr, 53.10°N, 3.37°W, national grid reference 33/0825.5713, on shaded stone by stream in woodland, 26 April 2007, *A. Orange* 16776 [NMW – C.2007.001.11; GenBank accession nos FJ664844 (ITS–LSU), JX848586 (mtSSU)].

(Figs 3 & 4)

Thallus thin, 25–60 μm thick, smooth, continuous or sparsely cracked, grey-green to mid-brown, more or less translucent when fresh and wet; cells irregularly arranged, at most with a very weak vertical arrangement; cells coherent, with no air spaces between them; cortical pigment, when present, brown; adjacent conspecific thalli never separated by dark lines.

Perithecia forming conical-hemispherical mounds 240–400 μm wide, at first covered to the apex by a layer of thallus, later the black apex of the involucrellum sometimes



FIG. 3. *Verrucaria hydrophila* (holotype). Scale = 1 mm. In colour online.

exposed; 29–42–64 perithecia in an area of 25 mm² (11 areas measured, on 5 sequenced specimens); ostiolar area inconspicuous, plane or convex, appearing as a pale dot 15–50 μm wide. *Involucrellum* conical, reaching to substratum, densely pigmented on upper side, often becoming almost colourless adjacent to base of exciple; pigment dark brown to dark reddish brown, K+darker brown to grey-brown. *Exciple* 145–225 μm diam., colourless except at apex. *Ascospores* (15.0–)19.5–21.1–23.0(–26.0) × (6.5–)8.0–8.9–9.5(–11.0) μm, (1.7–)2.2–2.4–2.6(–3.3) times as long as wide [473/34], perispore not detected.

Pycnidia not detected.

Ecology and distribution. On rocks and stones in streams, streamlets, and seepages. Associated species include *Hydropunctaria rheitrophila*, *Verrucaria andesiatica*, *V. aquatilis*, *V. margacea*, *V. pachyderma*, *V. rosula* and *V. sublobulata*. Throughout the British Isles. Widespread in Europe.

Notes. Distinguished by the smooth, thin thallus, small perithecia, the conical involucrellum which is covered by a thin layer of thallus, and rather small ascospores. *Verrucaria placida* and *V. andesiatica* (*sensu* Orange *et al.* 2009) differ in the larger and more widely spaced perithecia and the larger

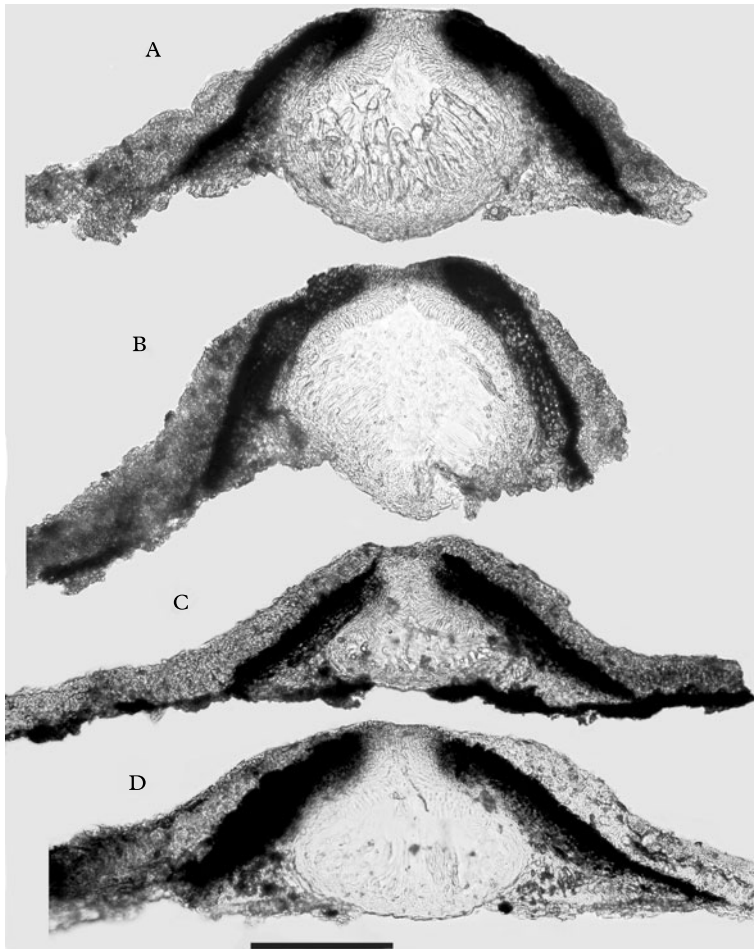


FIG. 4. *Verrucaria hydrophila*, sections of perithecia. A, holotype; B, Orange 18455; C, Orange 16612; D, Orange 16290. Scale = 100 μm .

ascospores (Table 2). *Verrucaria dolosa* differs in the distinctly smaller ascospores ($15\text{--}18 \times 6\text{--}8 \mu\text{m}$), smaller, more crowded perithecia, and lack of a regular thalline covering to the mature perithecium. The young perithecium in *V. hydrophila* is typically covered to its apex by a layer of thallus, but the dark apex of the involucrellum may be exposed later. The exposure of the involucrellum is sometimes caused by abrasion in the stream environment, but can also be seen in specimens from tiny flushes where there is minimal sediment load and a very gentle current.

This species has been widely known as *Verrucaria hydrela* (Santesson *et al.* 2004;

Thüs & Schultz 2009) but is not conspecific with the type of that species (H-ACH 682A!), which has a cracked thallus with slightly convex areoles, unlike any morph seen of the present species. The name *Verrucaria denudata* Zschacke has also been widely used for this species (e.g. Krzewicka 2012), but is unfortunately illegitimate.

The type specimen of *Verrucaria subhydrela* Serv. [(Czech Republic) Bohemia, (montes Krušné hory), Rudohoří, Hassenbach s.v. ad Volyne, 650 m, 1950, *Servit* (PRM 758626—holotype!)] is similar in external appearance to this species, and the single available section shows a spreading, conical

TABLE 2. *Morphological differences between Verrucaria andesiatica, V. placida and V. hydrophila*

	<i>V. hydrophila</i>	<i>V. placida</i>	<i>V. andesiatica</i> †
Density of perithecia*	29–42–64	13–21–27	12–16–26
Perithecial projections, diameter (µm)	240–400	400–600	280–680
Centrum diameter (µm)	145–225	250–310	(100–)235–330
Spore length	(15.0–)19.5–21.1–23.0(–26.0)	(19.0–)21.5–24.0–26.5(–30.5)	(19–)23–25.2–27(–32)
Spore width	(6.5–)8.0–8.9–9.5(–11.0)	(8.0–)9.0–9.9–10.5(–12.0)	(9.5–)11.5–12.4–13.5(–16.5)

* number of perithecia in an area 25 mm².

† *Verrucaria andesiatica sensu* Orange *et al.* (2009)

involucrellum. Despite this, Servít (1951) distinguished *V. subhydrella* from *V. demudata* by the involucrellum not spreading sideways (and by the presence of a thalline covering to the perithecium). Servít's drawing shows a thin, appressed involucrellum unlike any examples seen in *V. hydrophila*. This disparity between the type specimen and protologue provides a poor basis for a name, and there is the possibility that the description is based on discordant elements: although the two small fragments of the holotype appear to be uniform, other parts of the collection may exist, as Servít cites a duplicate sent to A. H. Magnusson.

The opportunity is taken here to describe this common and widespread but unnamed taxon as a new species, based on a type specimen for which ITS, and partial LSU and mtSSU sequences are available. The available ITS sequences for the species show some variation, and it is possible that a more intensive survey over a wider geographical area would delimit additional taxa within this group. In this case, sequenced specimens would be invaluable in order to fix the application of the new name with confidence.

Selected specimens examined. **Great Britain:** *Wales:* **V.C. 41**, Glamorgan: St Fagans, Museum of Welsh Life, 31/1133.7711, 2009, *Orange* 18314 (NMW – C.2010.001.123). **V.C. 42**, Breconshire: 2.5 km north-west of Erwood, near Tregaer, River Wye, 32/0795.4416, 2010, *Orange* 18793 (NMW – C.2010.001.128). **V.C. 43**, Radnorshire: Llanstephan, Bach Howey, 32/105.428, 1993, *Orange* 9746 (NMW – C.2003.002.44). **V.C. 44**, Carmarthenshire: near Rhandirmwyn, Nant y Gelynen, 22/7484.4577, 2012, *Orange* 20874 (NMW – C.2012.002.58). **V.C. 46**, Cardiganshire: Devil's Bridge, west side of Mynach,

22/741.772, 1998, *Orange* 12515 (NMW – C.2003.002.40); south of Ponterwyd, Ystumtuen, south of Penrhiw, Afon Tuen, 22/7381.7793, 2010, *Orange* 18873 (NMW – C.2010.001.117). **V.C. 48**, Merioneth: Talsarnau, Bryn Bwbach, 23/6393.3685, 2005, *Orange* 16290 (NMW – C.2005.001.347); Dolgellau, near Hendre-gefeilliad, 23/7582.1815, 2009, *Orange* 18455 (NMW – C.2009.002.66); Ganllwyd, Rhacadr Mawddach, 23/7360.2757, 2011, *Orange* 20642 (NMW.C.2011.014.97); Ganllwyd, Dolmelynllyn Hall, 23/7264.2976, 2011, *Orange* 20490 (NMW – C.2011.014.27); west of Dolgellau, Coedydd Abergwynant, 23/6799.1695, 2012, *Orange* 20829 (NMW – C.2012.002.57). **V.C. 50**, Denbighshire: Bontuchel, Coed y Fron-wyllt, Nant Melin-dŵr, 33/0825.5713, 2007, *Orange* 116771 (NMW – C.2010.001.225). *England:* **V.C. 70**, Cumberland: Keswick, Threlkeld, 35/2994.2548, 2006, *Orange* 16612 (NMW – C.2005.001.553); Keswick, Threlkeld, Glenderaterra Beck, 35/296.251, 2006, *Orange* 16621 (NMW – C.2005.001.559). *Scotland:* **V.C. 97**, Westernness: near Fort William, Loch Linnhe, 27/0507.6715, 2004, *Orange* 15018 (NMW – C.2004.002.399). – **Austria:** *Tyrol:* Kühtai, Finstertal, 47°12.46'N, 11°01.44'E, alt. 2025 m, 2012, *Orange* 21125 (NMW – C.2012.002.131).

Verrucaria nodosa Orange sp. nov.

MycoBank No.: MB802489

Thallus epilithicus, cinereo-viridis vel brunneus, inaequalis, ex areolis imbricatis constans. Perithecia prominentias formans, raro immersa, prominentiae 220–460 µm latae, nuda vel inferne ad thallo obiectae. Involucrellum anguste conicum vel ad excipulum adpressum. Excipulum 190–310 µm latum. Asci 8 sporae continens. Ascosporae aseptatae, hyalinae, (17–)20.5–22.2–24.0(–28) × (8.0–)9.0–9.7–10.5(–11.5) µm.

Typus: Great Britain, Wales: V.C. 48, Merioneth, north-west of Llanuwchllyn, Afon Lliw, 52.88°N, 3.76°W, national grid reference 23/8153.3268, alt. 350 m, on unshaded rock in stream, 28 September 2011, *A. Orange* 20660 (NMW – C.2012.002.127—holotypus; GenBank accession no JX848561).

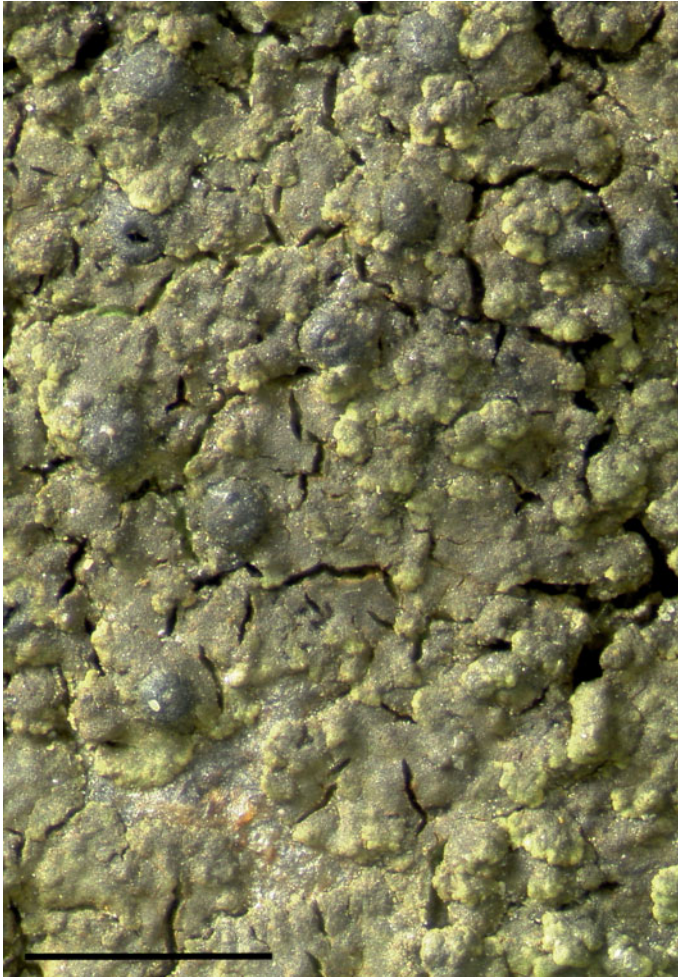


FIG. 5. *Verrucaria nodosa* (holotype), part of thallus lightly shaded by moss. Scale = 1 mm. In colour online.

(Figs 5–7)

Prothallus not detected. *Thallus* superficial, grey-green (in shade) to dark brown, 70–500 μm thick, initially comprising discrete, dorsiventrally flattened units which become crowded and overlapping, forming an uneven crust with sparse secondary cracks, sometimes overtopping each other to make a thick uneven crust. Surface of thallus uneven; thallus in section divided into units of variable size, 30–325 μm thick, paraplectenchymatous; cells isodiametric to shortly oblong, *c.* 5–9 \times 4–8 μm , polygonal and mostly coherent, or of somewhat rounded outline,

and with numerous air spaces between the cells; upper (and sometimes also lower) surface of units with brown-pigmented walls. *Photobiont* cells 5.5–9.0 \times 4.0–6.5 μm .

Perithecia forming low to moderately convex conical-hemispherical projections 220–460 μm diam., sometimes only the lower 0.2–0.3 of perithecium height is immersed in the thallus, the projecting part naked or with an irregular thalline cover below, or sometimes perithecium partly or almost completely overtopped by thallus units. *Ostiole* inconspicuous or appearing as a pale dot 20–40 μm wide, plane or slightly projecting.

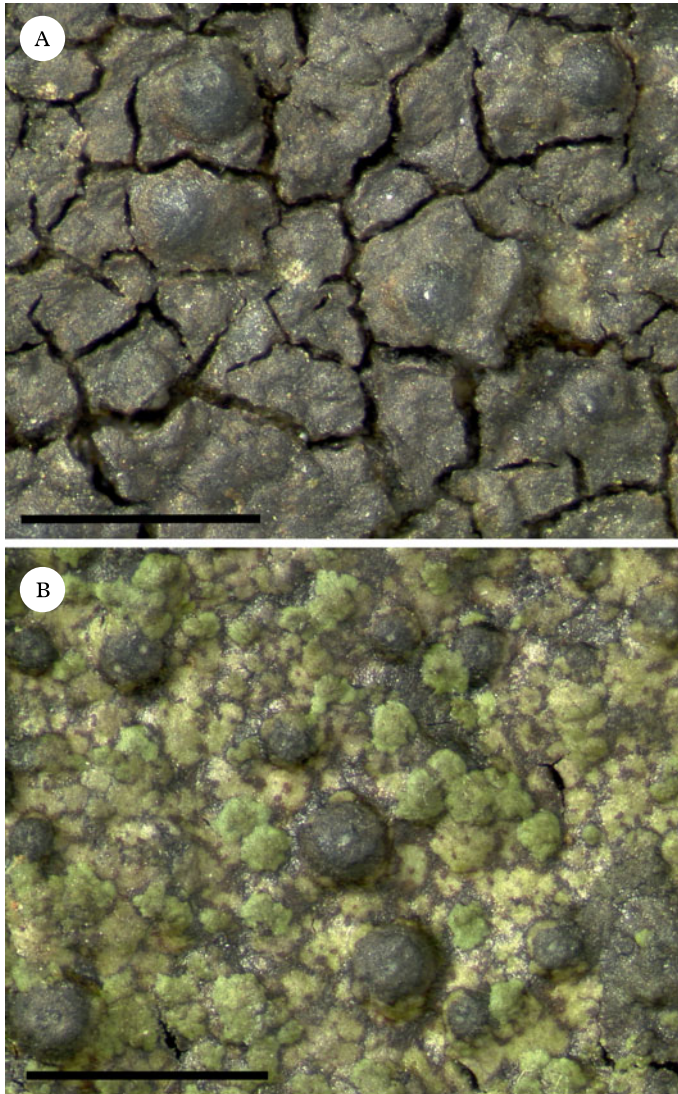


FIG. 6. *Verrucaria nodosa*. A, Orange 20458; B, Orange 20648. Scales = 1 mm. In colour online.

Involucrellum appressed to exciple above, usually becoming broader below, rather steeply conical-hemispherical in outline, densely pigmented throughout, or with a pale area adjacent to base of exciple; pigment dark brown to dark reddish brown, ostiolar area often with dark green pigment. *Exciple* 190–310 μm diam., colourless or the outer layer pigmented throughout. *Periphyses* c. 25–40 μm long (measured *in situ*). *Ascospores*

colourless, ellipsoid, simple, (17–)20.5–22.2–24.0(–28) \times (8.0–)9.0–9.7–10.5(–11.5) μm , (2–)2.1–2.3–2.5(–3) times as long as wide [75/4].

Pycnidia not detected.

Ecology and distribution. On rocks beside streams, unshaded or lightly shaded, in open woodland or unimproved grassland; associated species include *Ionaspis lacustris*, *Rhizocarpon lavatum*, *Porpidia hydrophila*,

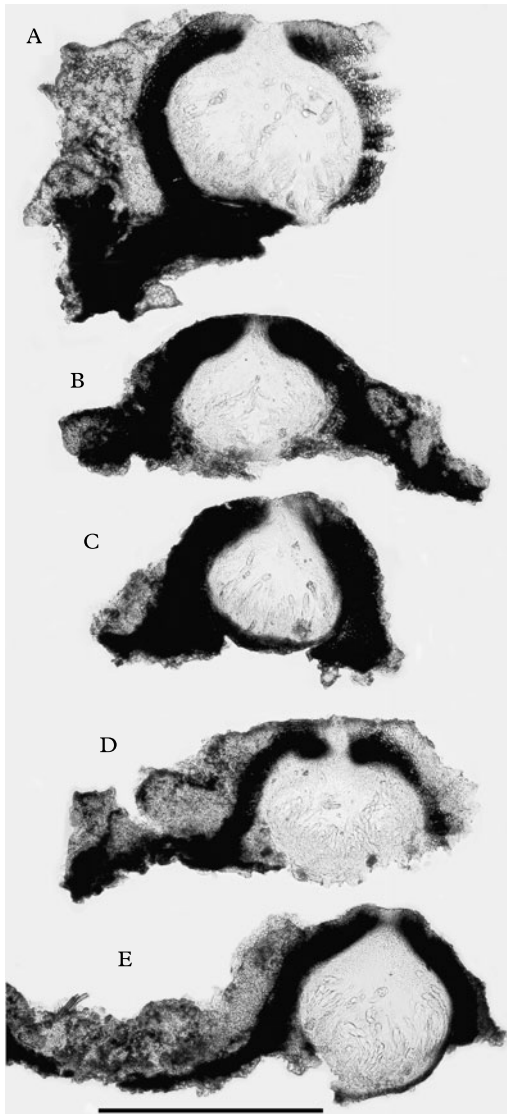


FIG. 7. *Verrucaria nodosa*, sections of perithecia. A, holotype; B, *Orange* 20455; C & D, *Orange* 20458; E, *Orange* 20648. Scale = 300 μ m.

Sporodictyon cruentum, *Trapelia coarctata*, and the bryophytes *Racomitrium aciculare* and *Scapania undulata*. So far recorded only from three streams in North Wales.

Notes. This species resembles *Verrucaria rosula* in the uneven thallus composed of initially discrete units which coalesce and overlap with age. The external appearance

varies considerably, probably due to differences in moisture and exposure of the different collecting sites. The initially discrete units are less finely crenulate than in *V. rosula*, and the surface is more coarsely uneven. In section, the thallus of *V. nodosa* is less clearly divided into small units than in *V. rosula*, and the cells frequently have air spaces between them. The involucrellum in *V. nodosa* is more uniform in thickness than in *V. rosula*. However, it is possible that some of these differences are due in part to habitat differences: the available specimens of *V. nodosa* are mostly from sunny rocks which are not permanently damp. It is likely that poorly developed specimens of *V. nodosa* and *V. rosula* will be difficult to distinguish by morphology.

Selected specimens examined. **Great Britain:** *Wales:* V.C. 48, Merioneth: Ganllwyd, Rhaeadr Mawddach, 23/7358.2756, alt. 140 m, 2011, *Orange* 20648 (NMW – C.2012.002.128). V.C. 49, Caernarvonshire: east of Beddgelert, stream below Llyn Llagi, 23/6396.4886, alt. 200 m, 2011, *Orange* 20455 (NMW – C.2011.014.23); *ibid.*, 23/6436.4870, alt. 315 m, 2011, *Orange* 20458 (NMW – C.2011.014.24).

Verrucaria placida Orange sp. nov.

Mycobank No.: MB802490

Thallus tenuis, 40–65 μ m crassus, laevis, continuus. Perithecia prominentias conico-hemisphaericas formantia, prominentiae 400–600 μ m latae, ab thallo pro parte maxima obtectae. Involucrellum conicum. Excipulum 250–310 μ m latum. Ascospores ellipsoideae, aseptatae, hyalinae, (19.0–)21.5–24.0–26.5(–30.5) \times (8.0–)9.0–9.9–10.5(–12.0) μ m. Pycnidia nulla.

Typus: Norway, Hordaland, Modalen, S of Moelvi river, by Bjørkehaugbekken, 60°50.69'N, 05°58.34'E, national grid 32 V LN 35342 49329, alt. 120 m, on stone in shaded streamlet in woodland, with *Verrucaria sublobulata*, 27 June 2007, A. *Orange* 17212 & T. *Tønsberg* (NMW – C.2010.001.116—holotypus; GenBank accession no JX848573).

(Figs 8–10)

Thallus thin, 26–65 μ m thick, subgelatinous, translucent when fresh and wet; smooth, more or less matt or slightly glossy, continuous, grey-green to mid brown; contiguous conspecific thalli not separated by dark lines; cells irregularly arranged or in weakly defined columns, coherent, without air spaces between cells; *cortex* poorly differentiated, comprising a thin layer with few or no photo-

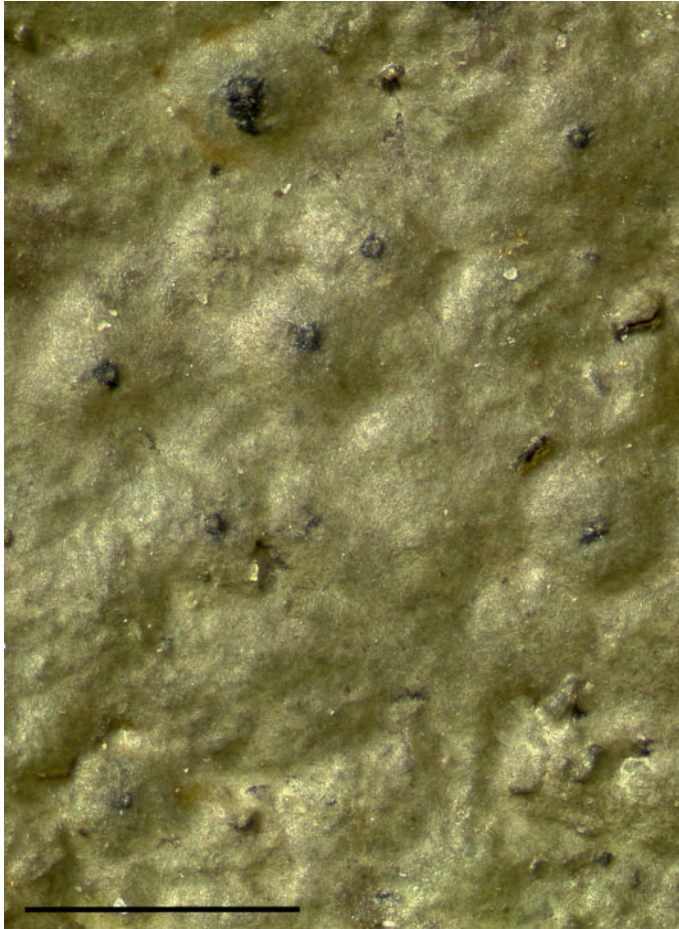


FIG. 8. *Verrucaria placida* (holotype). Scale = 1 mm. In colour online.

biont cells (a pseudocortex *sensu* Gueidan *et al.* 2007), cortical pigment, when present, brown; thallus without a dark basal layer.

Perithecia forming conical-hemispherical mounds 400–600 μm wide, at first covered by thallus up to apex, later sometimes eroded to expose black apex, 13–21–27 in an area of 25 mm^2 (18 areas measured, on 6 specimens). *Exciple* 250–310 μm wide. *Involucrellum* conical, reaching to substratum, pigment dark brown, K+dark grey. *Periphyses* 20–45 μm long. *Asci* 8-spored. *Ascospores* simple, hyaline, ellipsoid, (19.0–)21.5–24.0–26.5 (–30.5) \times (8.0–)9.0–9.9–10.5(–12.0) μm , length/breadth ratio (2.0–)2.2–2.4–2.7(–3.0)

[133/8], perispore apparently absent in mature spores.

Pycnidia not detected.

Etymology. The epithet (from Latin ‘*placida*’: quiet, peaceful) was suggested by the smooth, unbroken thallus and the characteristic but unstriking appearance of this lichen.

Ecology and distribution. On shaded siliceous rocks and stones in small streams in woodland. Associated species include *Ionaspis lacustris*, *Verrucaria rosula* and *V. sublobulata*. In Great Britain, so far detected in a semi-natural *Quercus petraea* woodland (Coed yr Allt-gôch) in Mid Wales at an altitude of

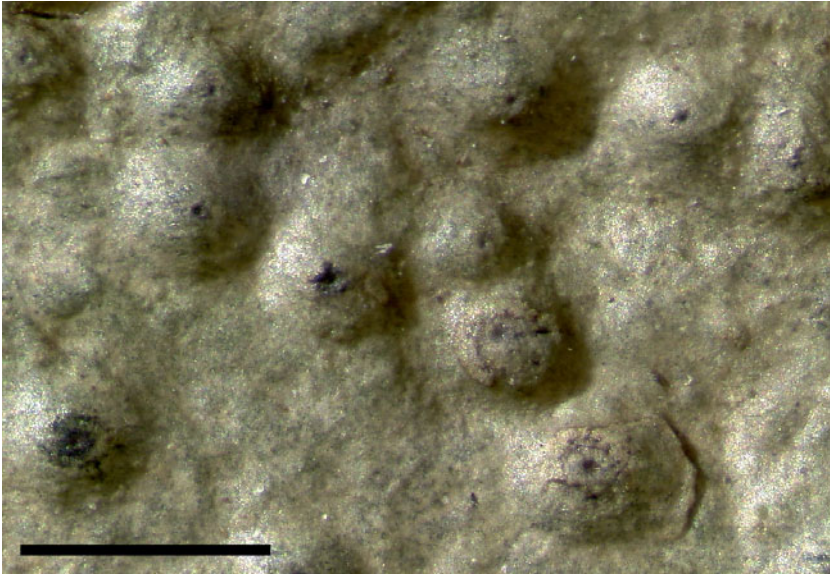


FIG. 9. *Verrucaria placida*, Orange 19830. Scale = 1 mm. In colour online.

300–350 m; also known from streams in forest in Norway and southern Germany.

Notes. This species is related to *Verrucaria hydrophila*, but the ITS sequence shows considerable differences. *Verrucaria hydrophila* differs in the smaller, more crowded perithecia and smaller ascospores. However, *V. placida* is more likely to be confused with *V. andesiatica* (*sensu* Orange *et al.* 2009), which is morphologically similar but not closely related (it is more closely related to *V. funckii*). *Verrucaria andesiatica sensu* Orange *et al.* (2009) differs in the wider ascospores; in addition, pycnidia are sometimes present, adjacent thalli may be separated by dark lines, the thallus is typically even smoother and more glossy, and the perithecia are usually a little less densely spaced, 12–16–26 in an area of 25 mm² (19 areas measured, on 5 specimens) (Table 2).

Additional specimens examined. **Great Britain:** *Wales:* V.C. 43, Radnorshire: Elan Valley, Penygarn Reservoir, Coed yr Allt-gôch, 22/902.680, 1991, *Orange* 9118 (NMW – C.2003.002.42); *ibid.*, 1991, *Orange* 9122 (NMW – C.2004.002.314); *ibid.*, 22/9027.6809, 2008, *Orange* 17426 (NMW – C.2007.001.266); *ibid.*, 2008, *Orange* 17493 (NMW – C.2007.001.269).—**Norway:** *Hordaland:* Modalen, S of Moelvi river, by Bjørkehaugbekken stream, 60°50'69"N, 05°58'34"E, national grid

32 V LN 35342 49329, alt. 120 m, 2007, *Orange* 17209 & *Tønsberg* (NMW – C.2010.001.114); same locality and date, *Orange* 17211 & *Tønsberg* (NMW – C.2010.001.115).—**Germany:** *Baden-Württemberg:* Schwarzwald, west of Bärenthal, 47°52'00"N, 08°03'67"E, alt. 980 m, 2010, *Orange* 19380 (NMW – C.2010.001.111).

Verrucaria rosula Orange sp. nov.

MycoBank No.: MB802491

Thallus epilithicus, cinereo-viridis vel brunneus, inaequalis, ex granulis parvulis similis gonicystis constans. Perithecia prominentias formans, prominentiae 240–400 µm latae, inferne ab thallo obtectae, superne nudaе. Involucrellum conicum vel ad excipulum adpressum. Excipulum 180–305 µm latum. Asci 8 sporae continens. Ascospores aseptatae, hyalinae, (20.5–)22.5–24.8–27.0(–32.0) × (7.5–)9.0–10.0–11.0(–14.0) µm.

Typus: Great Britain, *Wales:* V.C. 42, Breconshire, Brecon Beacons, Cwm Dringarth, Nant y Gwair, 51°87'N, 3°52'W, national grid ref. 22/9539.2042, alt. 470 m, on unshaded rock in flush, 4 April 2007, *A. Orange* 16753 (NMW – C.2007.001.4—holotypus; GenBank accession no FJ664883).

(Figs 11–13)

Prothallus absent. *Thallus* superficial, grey-green (in shade) to brown, 40–200 µm thick, initiated by very small granules, first visible as isodiametric structures *c.* 20–60 µm wide,

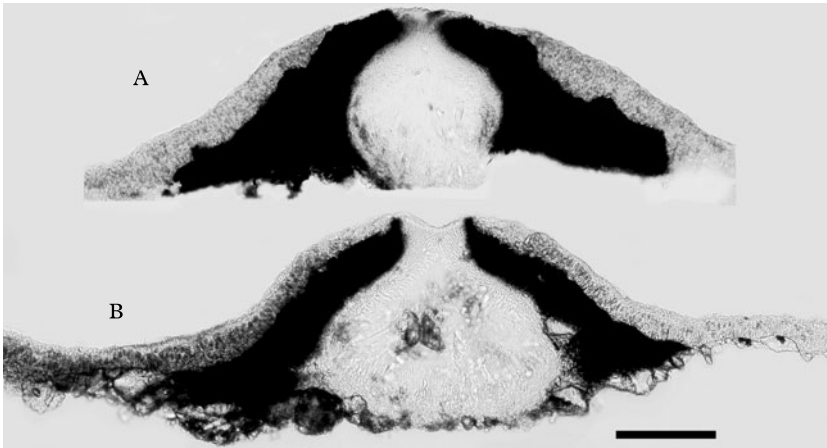


FIG. 10. *Verrucaria placida*, sections of perithecia. A, holotype; B, Orange 19830. Scale = 100 μm .

these increasing in size to form dorsiventrally flattened, more or less circular, rosette-like patches with a minutely crenulate margin; patches coalescing with neighbouring ones to form a diffuse crust which is uncracked or has sparse secondary cracks; healthy layers of thallus often overlying older layers, which are partly dead or which contain abundant brown pigment. Surface of thallus minutely uneven at a scale of *c.* 20–50 μm ; in section usually divided into units *c.* 30–80 μm wide; paraplectenchymatous, fungal cells more or less isodiametric, 4–10 \times 3–6 μm , walls thin or slightly thickened, *c.* 0.5–0.8 μm thick, cells at surface (except in shade) with brown-pigmented walls. *Photobiont* cells 4–10 \times 3.5–7.5 μm .

Perithecia forming projections 240–400 μm diam., often with an irregular covering of thallus below, but naked above, ostiolar region often conspicuous, visible as a pale brown or whitish plane or convex dot 40–80 μm wide. *Exciple* 180–305 μm diam., unpigmented except for dilute brown pigment near ostiole. *Involucrellum* variable, thin to well developed, often appressed to exciple, or more or less conical and reaching to the substratum. *Asci* 8-spored. *Ascospores* simple, colourless, oblong-ellipsoid, mostly widest above the middle, (20.5–)22.5–24.8–27.0(–32.0) \times (7.5–)9.0–10.0–11.0(–14.0) μm , length/breadth ratio (2.0–)2.3–2.5–2.7(–3.1) [159/10], perispore absent.

Pycnidia rare or overlooked (detected on two specimens); *conidia* *c.* 5.0 \times 1.2 μm .

Etymology. From the Latin ‘*rosula*’, suggested by the rosette-like areas of new thallus growth.

Ecology and distribution. On damp siliceous rocks and stones in or near streams, or on flushed ground. Associated species include *Ionaspis lacustris*, *Thelidium pluvium*, *Verrucaria cernaensis*, *V. hydrophila*, *V. sublobulata* and *V. margacea*. Recorded up to an altitude of 470 m in Great Britain (SW England, Wales, Scotland), and at an altitude of *c.* 640 m in France.

Notes. The mature thallus is an aggregation of dorsiventrally flattened units, expanding at their margins, which coalesce and overlap each other. In turn these are composed of much smaller units, *c.* 30–80 μm wide, which usually appear as more or less discrete, slightly convex regions in section, and which are responsible for the minutely uneven surface of the thallus (often clearly seen under the dissecting microscope, sometimes more obscure). The perithecia are often overlain by patches of thallus below, but lack a regular and complete thalline covering. The ostiolar region is often rather conspicuous as a pale convex dot; this is a minor but rather characteristic feature of this species.

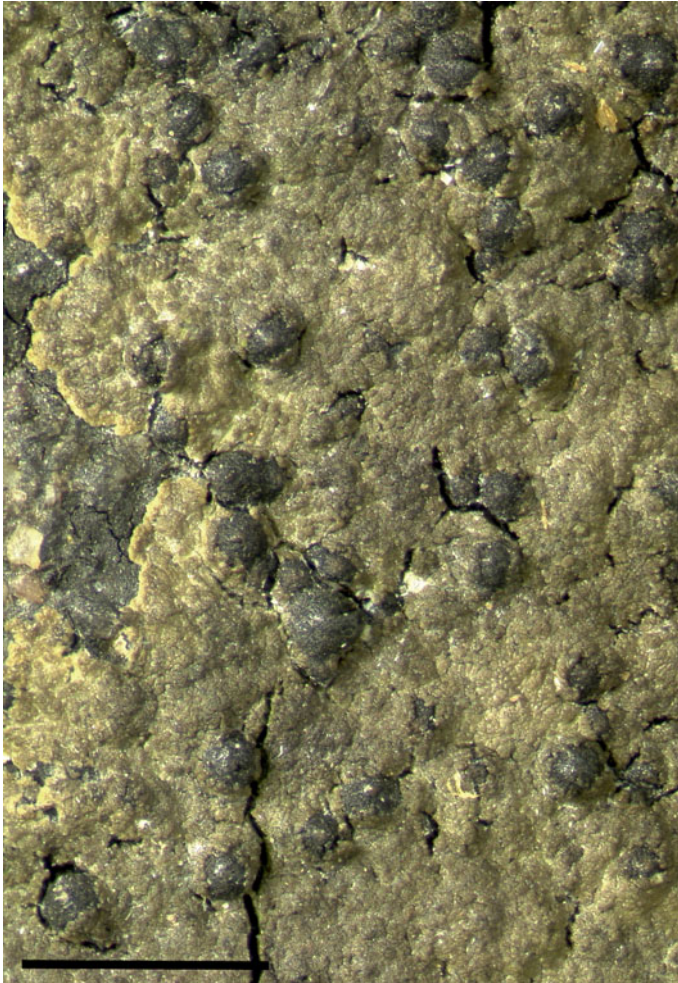


FIG. 11. *Verrucaria rosula* (holotype). Scale = 1 mm. In colour online.

Although this is a locally frequent species in damp habitats, it appears to have been overlooked, and no published names for this taxon have been found. *Verrucaria consociata* Serv. [Czech Republic, Rudohoří, Suniperk, 1950, coll. M. Servít (PRM 756809—holotype!)] also has a thallus composed of goniospores, but the perithecia and ascospores are smaller, and the thallus is weakly developed. Only one specimen of *V. rosula* has been detected in a herbarium (Watson, BM), originally identified as *Verrucaria margacea*.

Selected specimens examined. **Great Britain:** *Wales:* **V.C. 42**, Breconshire: north of Fan Nedd, Blaen Senni, 22/9143.1982, alt. 320 m, 2007, *Orange* 17241 (NMW –

C.2007.001.102); Llanwrtyd Wells, Irfon, 22/8673.4726, alt. 215 m, 2011, *Orange* 20542 (NMW – C.2012.002.129). **V.C. 43**, Radnorshire: Penygareg Reservoir, Coed yr Allt-gôch, 22/905.676, 1991, *Orange* 9131 (NMW – C.2005.001.62, M); *ibid.*, 22/9060.6770, alt. 350 m, 2008, *Orange* 17489 (NMW – C.2007.001.283). **V.C. 46**, Cardiganshire: south of Ponterwyd, Ystumtuen, south of Penrhiw, Afon Tuen, 22/7381.7793, alt. 220 m, 2010, *Orange* 18876 (NMW.C.2010.001.118). **V.C. 48**, Merioneth: west of Penmaenpool, Coedydd Afon Gwynant, 23/681.166, 1991, *Orange* 8570 (NMW – C.2005.001.66); Talsarnau, Afon y Glyn, 23/6239.3583, 2002, *Orange* 13919c (NMW – C.2005.001.701); Ganllwyd, Afon Wen, 23/739.230, 1998, *Orange* 11988 (NMW – C.2005.001.699); Talsarnau, Bryn Bwbach, Coed Caerwch, 23/6418.3683, alt. 150 m, 2005, *Orange* 16285 (NMW – C.2005.001.700). **V.C. 50**, Denbighshire:

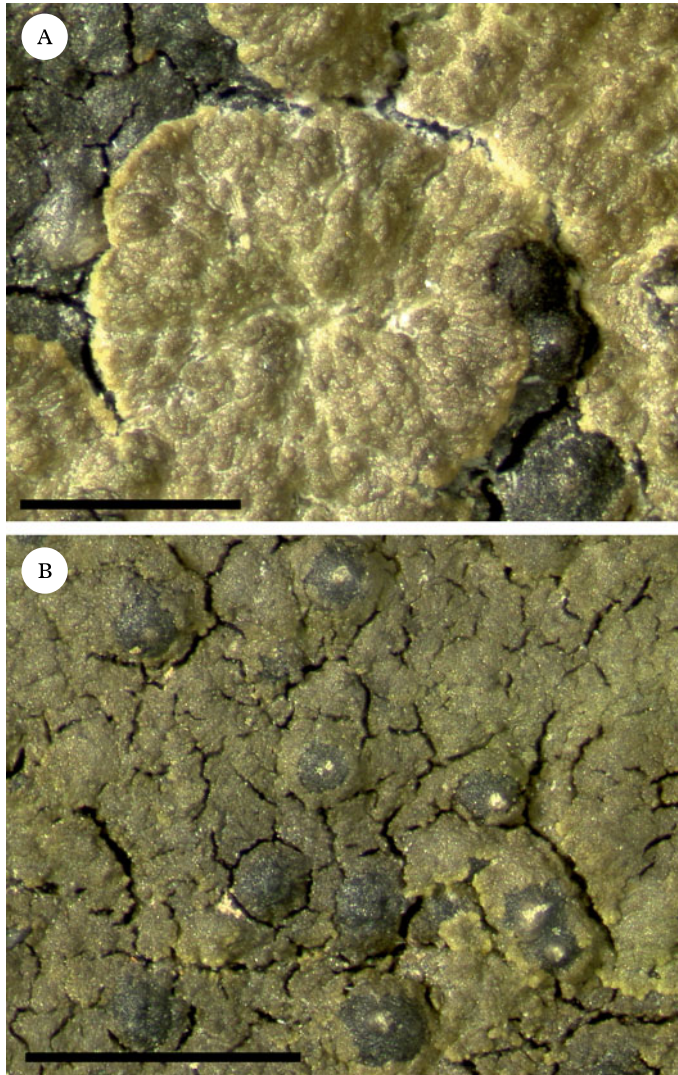


FIG. 12. *Verrucaria rosula*. A, rosette-like area of growth composed of small subunits (holotype); B, *Orange* 17241. Scales: A = 500 μ m; B = 1 mm. In colour online.

Llanrhaeadr-ym-Mochnant, Afon Iwrch, 33/124.280, 1998, *Orange* 12119 (NMW – C.2005.001.697). *England*: V.C. 5, South Somerset: Treborough, Cold Harbour, 1916, *W. Watson* (BM); 5 km SW of Porlock, Wilmersham Common, 21/859.426, 1993, *Orange* 9680 (NMW – C.2005.001.63). *Scotland*: V.C. 97, West-ernness: Achnacarry, River Arkaig, 27/17602.88118, 2011, *A. Acton* (hb. A. Acton).—*France*: *Cantal*: 5 km SE of Mur-de-Barrez, 0.4 km W of Truyère, 44°49'N, 2°43'E, 1990, *Orange* 7969 (NMW – C90.13.90).

Names considered inapplicable to the taxa treated above

Verrucaria hydrela Ach.

Syn. meth. lich.: 94 (1814); type: [Sweden] 'Svecia Vest-mannia' (H–ACH 682 A!).

The herbarium specimen H–ACH 682 comprises two fragments of rock pasted onto a slip of paper, and bearing different species.

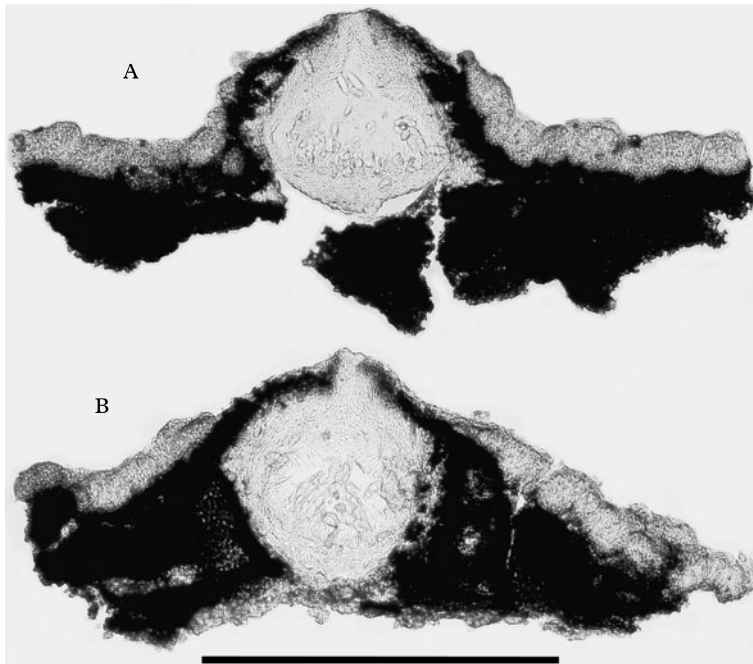


FIG. 13. *Verrucaria rosula*, sections of perithecia. A, holotype; B, Orange 17489. Scale = 300 μm .

The left fragment (H-ACH 682 A, labelled 'Svecia') has a *Verrucaria* species with a pale brown thallus which is cracked into somewhat convex areoles; the ascospores are (17.5–)18.5–20.4–22.0(–25.5) \times 7.5–8.2–9.0(–10.0) μm , length/breadth ratio (2.1–)2.3–2.5–2.7(–3.1) [35 measured]. No other lichens or bryophytes are present on this fragment of rock. The right fragment (H-ACH 682 B, without locality) carries a lichen with a thin, faintly cracked thallus, with prominent black perithecia 80–160 μm diam.; in the one perithecium examined microscopically, no hymenium was present. It is possible that the perithecia belong to a lichenicolous fungus.

The description in the protologue is not detailed enough to determine with certainty which specimen was the basis of Acharius' description; however, H-ACH 682 A is labelled 'Svecia', which is in accordance with the protologue, and the description of the thallus as '*verruculosa*' also fits this fragment better than fragment B, which furthermore is without locality. The lichen on fragment

A does not correspond to any taxon known to the writer, but it is not the same taxon to which the name *V. hydrela* has been applied by recent authors. Probably this species is known only from the type locality in Sweden.

On the other hand, the type material of *V. hydrela* (H-ACH 682A!) strongly resembles the alleged type material of *V. laevata* (H-ACH 683A!), a taxon which was described four years earlier (see below).

***Verrucaria laevata* Ach.**

Lichenogr. univ.: 284 (1810); type: [Germany] 'ad saxa fluminis Queis Lusatae' [Lusatia] [*Mosig*] [material apparently lost].

A packet in Acharius' herbarium in H (H-ACH 683) contains a slip of paper to which two fragments of rock are pasted, and which is annotated '*Verrucaria laevata*' by Acharius. The left fragment (H-ACH 683 A) is annotated 'Lusatia', a locality that agrees with the protologue. However, it is very likely that this is not the fragment originally attached here. The current fragment bears a lichen very similar in appearance to H-ACH

682 A (the type of *Verrucaria hydrela*). In addition, the paper slip to which H-ACH 682 A is attached bears a glue mark which corresponds in size to the fragment currently in the position of H-ACH 683 A, suggesting that this mark indicates the true position of the fragment. Vainio also believed that the fragment currently labelled H-ACH 683 A was a misplaced piece of *V. hydrela*, attached in error (specimen annotated '*V. hydrela* Ach. errore haec positam' by Vainio). Vainio was not determining a type of *V. laevata*, as supposed by Swinscow (1968). Thus the original specimen of *V. laevata* appears to have been detached and lost.

The right-hand fragment in H-ACH 683 (H-ACH 683 B) is labelled 'Anglia' and 'Harriman', and carries an undetermined *Verrucaria* species, growing with *Eiglera flavida*. It is likely that the specimen is not from a truly freshwater habitat. This fragment was incorrectly ascribed to *V. aethiobola* by Thüs (2002). Thüs designated this fragment as a lectotype of *V. laevata*, but this is not a suitable choice, as there is no evidence that it is conspecific with the type of *V. laevata*.

The description of *V. laevata* in the protologue is too vague to suggest which species might have been intended. As the original material is lost, *Verrucaria laevata* should be regarded as a *nomen dubium*.

I am grateful to Andy Acton for submitting a specimen of *Verrucaria rosula*, to Tor Tønsberg for making fieldwork possible in Norway in 2007, the curators of H and PRM for granting loans, and to two anonymous reviewers for their helpful comments.

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