

## Two new or misunderstood species related to *Verrucaria praetermissa* (Verrucariaceae, lichenized Ascomycota)

Alan ORANGE

**Abstract:** The taxonomy of species related to *Verrucaria praetermissa* is investigated using morphological and molecular data. *Verrucaria devensis* is closely related to *V. praetermissa* but distinguished by the darker colour, the thinner and more uneven thallus, and the ITS sequence. This species is based on *Leucocarpopopsis devensis* G. Salisb., which was originally described as the type species of a monotypic genus differing from *Verrucaria* by the absence of dark pigment. *Verrucaria lapidicola* is a new species from stones in non-aquatic situations; it is related to *V. praetermissa* and *V. elaeina* but differs in the very thin thallus and ITS sequence.

**Key words:** England, Estonia, freshwater, Germany, ITS, lichens, Scotland, taxonomy, Wales

Accepted for publication 23 February 2014

### Introduction

*Verrucaria praetermissa* (Trev.) Anzi is widespread in Europe in freshwater habitats; it is tolerant of shade and to some degree of silting and eutrophication (Thüs & Schultz 2009), and in Britain is often the only freshwater species of the genus present in suboptimal habitats. Orange (2000) compared it to *V. elaeina* Borrer, and noted that the thallus colour varied from pale greenish grey to dark brown. However, in recent years, observation of adjacent thalli differing in colour and other features has suggested the existence of two taxa within the current concept of *V. praetermissa*, and this hypothesis has been supported by DNA sequencing. A search for related taxa, using ITS sequences, revealed an additional species on calcareous stones in terrestrial habitats. This is described as new, and an unexpected published name is resurrected for the second freshwater taxon.

### Methods

#### DNA extraction and sequencing

DNA was extracted from ascoma and thallus tissue using the Qiagen DNeasy Plant Mini Kit. The two inter-

nal 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) (a proportion of specimens only) were amplified, using the primers ITS1F, ITS4, LR3, nu-LSU-155-5' and LR7 (Vilgalys & Hester 1990; Gardes & Bruns 1993; Döring *et al.* 2000). The PCR thermal cycling parameters were: initial denaturation for 5 min at 94°C, followed by 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 The Sequencing Service (College of Life Sciences, University of Dundee, [www.dnaseq.co.uk](http://www.dnaseq.co.uk)) using Applied Biosystems BigDye Ver 3.1 chemistry on an Applied Biosystems model 3730 automated capillary DNA sequencer, or by Macrogen Inc. The same primers used for amplification were also used for sequencing.

#### 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.

#### Phylogenetic analysis

##### Taxon selection

Material for possible inclusion in an analysis of the ITS region was initially selected by visual inspection of alignments of unidentified sequences generated by the author, and by using the BLAST search facility hosted by the National Center for Biotechnology Information website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Additional support for the close relationship of the selected

---

A. Orange: Department of Biodiversity and Systematic Biology, National Museum of Wales, Cathays Park, Cardiff CF10 3NP, UK.

Email: [alan.orange@museumwales.ac.uk](mailto:alan.orange@museumwales.ac.uk)

TABLE 1. *Specimens used in the ITS analysis of Verrucaria praetermissa and related species. New sequences are in bold.*

Species	Country	Voucher	GenBank accession numbers
<i>Staurothele areolata</i>	France	<i>C. Gueidan</i> 378 (MARSSJ)	EF643772
<i>S. fissa</i>	Wales	<i>Orange</i> 16265 (NMW)	FJ645265
<i>Verrucaria devensis</i>	Scotland	<i>Orange</i> 13178 (NMW)	<b>KF819515</b>
<i>V. devensis</i>	Wales	<i>Orange</i> 18804 (NMW)	<b>KF819516</b>
<i>V. devensis</i>	Wales	<i>Orange</i> 18814 (NMW)	<b>KF819517</b>
<i>V. devensis</i>	Wales	<i>Orange</i> 18869 (NMW)	<b>KF819518</b>
<i>V. devensis</i>	Wales	<i>Orange</i> 21331 (NMW)	<b>KF819519</b>
<i>V. devensis</i>	Wales	<i>Orange</i> 21502 (NMW)	<b>KF819520</b>
<i>V. elaeima</i>	England	<i>Orange</i> 16628 (NMW)	FJ664849
<i>V. lapidicola</i>	England	<i>Orange</i> 16690 (NMW)	FJ664850
<i>V. lapidicola</i>	Estonia	<i>Orange</i> 18112 (NMW)	<b>KF819512</b>
<i>V. lapidicola</i>	Wales	<i>Orange</i> 18790 (NMW)	<b>KF819513</b>
<i>V. lapidicola</i>	Wales	<i>Orange</i> 18847 (NMW)	<b>KF819514</b>
<i>V. praetermissa</i>	Wales	<i>Orange</i> 16257 (NMW)	FJ664881
<i>V. praetermissa</i>	Wales	<i>Orange</i> 18805 (NMW)	<b>KF819521</b>
<i>V. praetermissa</i>	Wales	<i>Orange</i> 18815 (NMW)	<b>KF819522</b>
<i>V. praetermissa</i>	Wales	<i>Orange</i> 18853 (NMW)	<b>KF819523</b>
<i>V. praetermissa</i>	Germany	<i>Orange</i> 19382 (NMW)	<b>KF819524</b>

taxa was gained by an analysis of the LSU of the taxa selected, together with available sequences of other *Verrucariaceae* (see below). Sequences used in the phylogenetic analysis are listed in Table 1.

#### LSU analysis

Relationships were investigated using Maximum Likelihood bootstrapping, as implemented in RaxML (Stamatakis 2006; Stamatakis *et al.* 2008), hosted on the CIPRES Science Gateway (Miller *et al.* 2010). Two sequences of *Verrucaria praetermissa* and sequences of the two newly distinguished taxa were analyzed together with most of the taxa used in a multigene analysis of the family by Gueidan *et al.* (2007), and with additional sequences produced by the author; a total of 85 sequences representing 82 species. No LSU sequence was available for *V. elaeima*, but this species is clearly related to the above species, based on BLAST searches and visual inspection of alignments of the ITS region.

#### ITS analysis

The ITS analysis was restricted to four species considered to be closely related following the investigations above. Two species of *Staurothele* were used as outgroup, suggested by the analysis of Gueidan *et al.* (2007). Other subclades within the *Staurothele* group of Gueidan *et al.* were omitted, partly due to alignment difficulties. The ITS alignment comprised 506 base pairs, of which a total of 40 base pairs in seven regions were considered ambiguous and were excluded from the analysis. Of the remaining positions, 342 were constant, 45 were variable and parsimony-uninformative, and 79 were variable and parsimony-informative. Models of evolution for the ITS1, 5.8S and ITS2 regions were selected using the Akaike

Information Criterion in MrModeltest 2.2 (Nylander 2004) and were HKY, JC and HKY+I respectively.

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 discarded from each run respectively. Additional support values were obtained using Maximum Likelihood bootstrapping, as implemented in RaxML, hosted on the CIPRES Science Gateway.

Support values of  $\geq 95\%$  Bayesian posterior probabilities and  $\geq 70\%$  Maximum Likelihood bootstrapping were regarded as significant.

## Results

### Phylogenetic analyses

#### LSU analysis

The analysis of LSU sequences (not shown) recovered a clade with bootstrap support of 59%, comprising *Verrucaria devensis*, *V. praetermissa* and *V. lapidicola*. *Verrucaria caerulea* was basal to this clade, and together they formed a clade with 36% support. All basal nodes in the tree were very poorly supported.

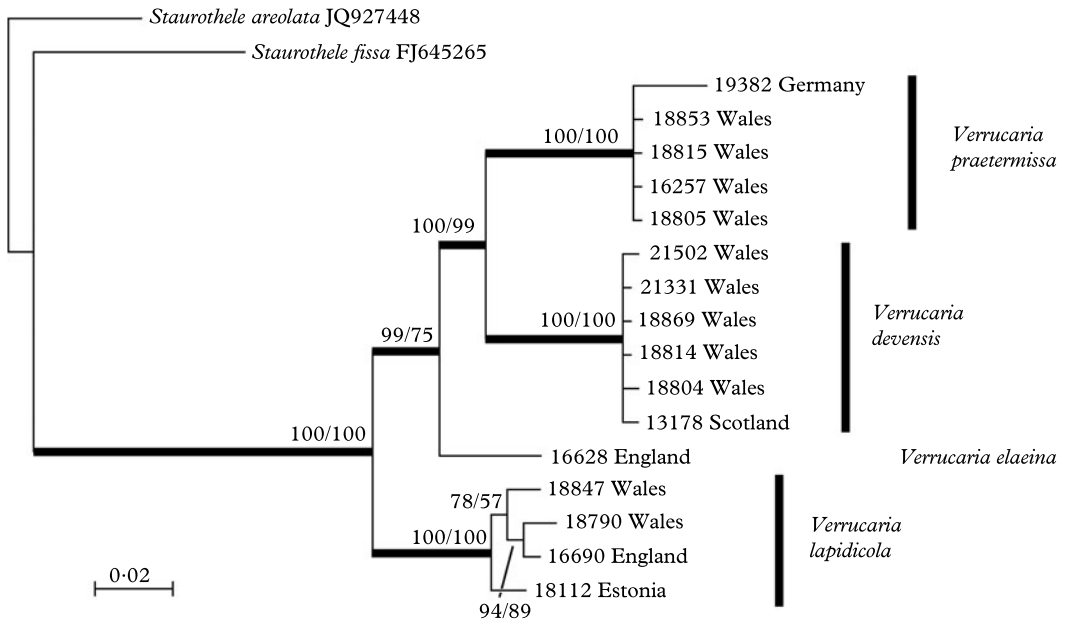


FIG. 1. Phylogenetic relationships of *Verrucaria praetermissa* and related species, based on a Bayesian analysis of the ITS region of the nuclear ribosomal DNA; additional support values were obtained from a Maximum Likelihood analysis as implemented in RaxML; the tree was rooted using two species of *Staurothele*.

### ITS analysis

Three well-supported clades were recovered, corresponding to *Verrucaria devensis*, *V. praetermissa* and *V. lapidicola*. The single sequence of *V. elaeina* was basal to *V. devensis* and *V. praetermissa* (Fig. 1).

### Morphology

In the following descriptions, a distinction is made between ‘primary’ and ‘secondary’ areoles. Primary areoles are considered to be independent lichenized units formed at the margin of the thallus; these usually become confluent with other primary areoles as the thallus grows. Secondary areoles are considered to be areas of thallus which are partially or completely surrounded by cracks which form (sometimes very early) as the thallus matures. The cracks may cut across the limits (where visible) of the primary areoles. The morphological differences between the species below are shown in Table 2.

### The Species

#### *Verrucaria devensis* (G. Salisb.) Orange comb. nov.

MycoBank No.: MB 808086

*Leucocarpopops devensis* G. Salisbury, *Nova Hedwigia* 26: 351 (1975); type: Wales, V.C. 48, Merioneth, Corwen, River Dee, 23 April 1974, G. Salisbury (BM 0000660945!); epitype (selected here): Wales, V.C. 42, Breconshire, 3 km south-east of Builth Wells, near Glanwye, River Wye, alt. 115 m, on unshaded stone at margin of river, with *Verrucaria praetermissa*, 22 April 2010, A. Orange 18814 (NMW – C.2011.014.68; GenBank accession no. KF819517).

(Figs 2–4)

*Prothallus* white, non-fimbriate. *Thallus* crustose, superficial, pale grey, dull grey-green or greenish grey, mid greyish brown or mid brown, smooth, almost subgelatinous, continuous or usually locally or extensively cracked; cracks partially or sometimes completely delimiting secondary areoles; thallus margin continuous, smooth, without visible

TABLE 2. *Morphological comparison of species in the Verrucaria praetermissa group.*

	Thallus thickness (µm)	Thallus colour	Thallus texture	Confluence of involucrella	Ascospore length (µm)	Ascospore width (µm)
<i>V. praetermissa</i>	(40–)100–240	pale greenish grey to dull pale brown	non-gelatinous	becoming confluent	(16·0–)18·0– <b>21·0</b> –25·0(–28·0)	(6·5–)7·0– <b>8·7</b> –10·0(–10·5)
<i>V. devensis</i>	40–70(–140)	pale grey to greenish grey or mid brown	almost subgelatinous	becoming confluent	(16·0–)18·5– <b>20·3</b> –22·0(–23·0)	(7·5–)8·5– <b>8·9</b> –9·5(–10·5)
<i>V. elaeina</i>	25–90(–105)	light grey-green to pale brownish green (rarely mid brown)	non-gelatinous	not confluent	(15·0–)16·5– <b>19·4</b> –22·5(–24·0)	(6·0–)7·0– <b>8·0</b> –9·0(–9·5)
<i>V. lapidicola</i>	25–35	dull grey-green to dull grey-brown	non-gelatinous	not confluent	(17·0–)18·0– <b>19·6</b> –21·5(–23·0)	6·5–7·4– <b>8·0</b> (–10·5)

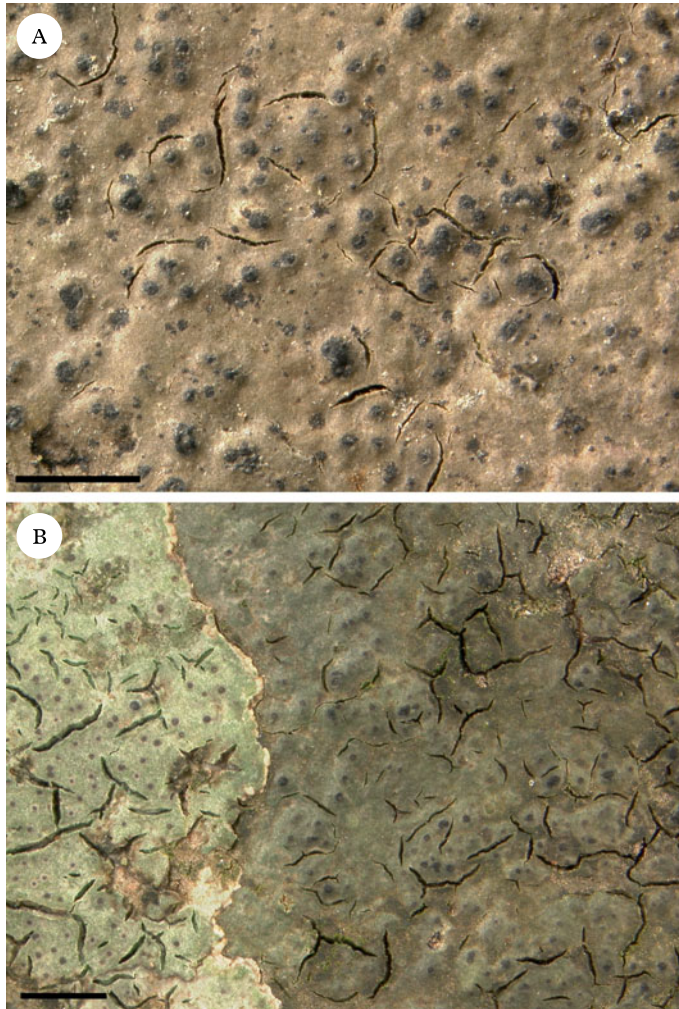


FIG. 2. *Verrucaria devensis* A, epitype; B, Orange 21243 (right), with *V. praetermissa* (left). Scales: A & B = 1 mm. In colour online.

primary areoles; secondary areoles 40–600  $\mu\text{m}$  diam., containing 1–7 perithecia, surface of areoles typically slightly convex or uneven; thallus 40–70  $\mu\text{m}$  thick away from perithecia, up to 140  $\mu\text{m}$  thick near perithecia; thallus cells 3.3–6.5  $\times$  3.3–5.0  $\mu\text{m}$ , irregularly arranged or in weak vertical columns, air spaces between cells absent or very few; surface a photobiont-free pseudocortex, the cell walls with a dilute or moderately dense brown pig-

ment; *epinecral layer* absent or thin, indistinct, up to 10  $\mu\text{m}$  thick, comprising a few dead, uncompressed cells; a dark basal layer present either locally or extensively. *Photobiont* cells 6.5–15.0  $\times$  4.0–6.5  $\mu\text{m}$ .

*Perithecia* immersed in the thallus, forming very low projections which are too ill-defined to measure, at first apex visible only by the ostiole which forms a grey dot 20  $\mu\text{m}$  diam., later the apex visible as a grey or black disc

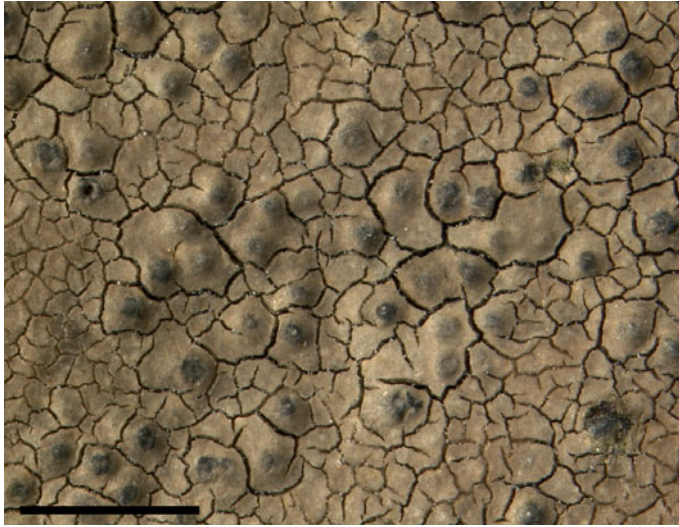


FIG. 3. *Verrucaria devensis*, Orange 13178. Scale = 1 mm. In colour online.

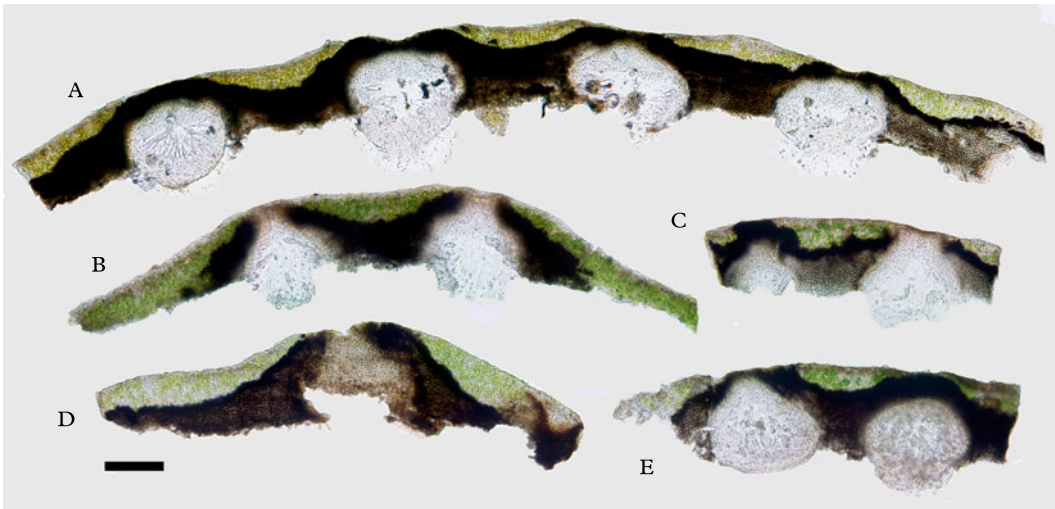


FIG. 4. *Verrucaria devensis*, sections of perithecia. A, epitype; B & C, Orange 21243; D & E, Orange 21502. Scale = 100  $\mu\text{m}$ . In colour online.

up to 180  $\mu\text{m}$  diam. *Involucrellum* conical at first, becoming very wide-spreading and confluent with other involucrella, forming the dark basal layer of the thallus; surface of involucrellum densely pigmented, below paler, with pigment largely confined to cell walls so that the cell outlines are often visible. *Exciple* 120–210  $\mu\text{m}$  diam., colourless at sides and base. *Periphysoids* c. 20–30  $\mu\text{m}$  long. *Asci*

8-spored. *Ascospores* colourless, oblong-ellipsoid, (16.0–)18.5–20.3–22.0(–23.0)  $\times$  (7.5–)8.5–8.9–9.5(–10.5)  $\mu\text{m}^1$ , (1.7–)2.2–2.3–2.4(–2.6) times as long as wide [40/5].

<sup>1</sup>Ascospore measurements are given as (min–)( $\bar{x}$ –SD)– $\bar{x}$ –( $\bar{x}$ +SD)(–max) where min, and max are extreme values and  $\bar{x}$  the arithmetic means and SD the corresponding standard deviation.

**Ecology and distribution.** On shaded or unshaded bedrock or cobbles beside streams and rivers or on flushed rocks, frequently to occasionally submerged; associated species include *Catillaria chalybeia* var. *chloropoliza*, *Placopyrenium cinereoatratum*, *Porina lectissima*, *Verrucaria cernaensis*, *V. margacea* and *V. praetermissa*. Specimens confirmed by sequencing are known from Wales and Scotland; additional specimens certainly referable to this species have been seen in Wales and Germany, but some unsequenced specimens could not be identified with certainty.

**Notes.** This species resembles *V. praetermissa* in the well-developed, often cracked thallus, the wide-spreading, often confluent involucrella, and the medium-sized ascospores. It differs from *V. praetermissa* in the more strongly pigmented thallus which is generally slightly thinner and often more uneven, and in the more compact, almost subgelatinous thallus structure. When the two species grow together, the differences are easily seen, even in the field. However, both are variable in appearance so that some individual specimens may be difficult to name. *Verrucaria devensis* often has a distinctly uneven surface, due partly to the tendency for the thallus to become thinner between the perithecia, but in some specimens also due to the occurrence of adjacent thinner, sterile and thicker, fertile areas of thallus. In *V. praetermissa* the thallus surface is often even, but some specimens are also uneven. In shade, *V. praetermissa* is typically pale grey-green, whereas *V. devensis* is darker, although it varies from pale grey-green to mid brown; in good illumination *V. praetermissa* can be dull pale brown, and here *V. devensis* is dull grey to mid brown. Both species can sometimes lack any cracks, even when material is fertile and healthy. The cracking in both species appears to be largely unrelated to the position of individual perithecia, so that the secondary areoles often contain more than one perithecium; however, in one sequenced specimen of *V. devensis* (Orange 13178), the areoles often contain only 1–2 perithecia, and small sterile areoles occur between the fertile areoles, giving a different appearance to many other specimens.

*Leucocaropsis devensis* was described as the type of a new monotypic genus by Salisbury (1975). This genus was said to lack dark pigment in the thallus and ascomata, but was otherwise similar to a species of *Verrucaria*. Purvis *et al.* (1992) considered it possible that it represented a decolourized morph of '*Verrucaria praetermissa*', which was said to be present on the same fragment of rock. Gilbert (1996) discussed the occurrence of rare, unpigmented morphs in otherwise darkly pigmented lichen species, and considered *L. devensis* to be merely an abnormal morph of *V. praetermissa*. This interpretation of *L. devensis* as an abnormal morph of a normally pigmented taxon is accepted here. On the fragment of rock which comprises the holotype, the unpigmented thallus is growing adjacent to a pigmented thallus which clearly belongs to the species described above, and not to *V. praetermissa*. Gilbert listed four taxa of *Verrucariaceae* in which 'albino' ascomata had been observed; in addition, the author has observed partly decolourized thalli (and their perithecia) in *Verrucaria aethiobola*, *V. hydrophila*, *V. margacea* and *V. praetermissa*. In view of this, and because unpigmented material of *L. devensis* is known only from the type, it is very likely that the decolourized thallus in the type specimen of *L. devensis* is conspecific with the normally pigmented material. It is unfortunate that this widespread species is represented by an abnormal type specimen, but adopting this name now may prevent a nomenclatural change in the future, when unpigmented material may be refound and sequenced.

**Additional specimens examined.** **Great Britain:** **Wales:** **V.C. 42,** Breconshire: 3 km south-east of Builth Wells, near Glanwye, River Wye, alt. 115 m, 2010, *Orange* 18804 (NMW – C.2011.014.67); south-east of Erwood, River Wye, alt. 100 m, 2010, *Orange* 18869 (NMW – C.2011.014.69); west of Brecon, near Fenni-fach, River Usk (north side), 32/0147.2846, alt. 140 m, 2012, *Orange* 21243 (NMW – C.2013.001.12); Llangynidr, River Usk, north side, east of bridge, 32/1570.20259, 2013, *Orange* 21333 (NMW – C.2013.001.44). **V.C. 48,** Merioneth: Talsarnau, Bryn Bwbach, Hendre Cerrig, 23/6492.3692, 2002, *Orange* 13926 (NMW – C.2004.002.308). **V.C. 50,** Denbighshire: south of Llanrwst, near Conwy Falls, Afon Conwy, 23/8109.5345, alt. 140 m, 2013, *Orange* 21502 (NMW – C.2013.001.151). **V.C. 88,** Mid Perthshire: south side of Loch Tay, near Shenlarich, 27/706.409, alt. 105 m,

2001, *Orange* 13178 (NMW – C.2001.024.203).—**Germany:** *Hessen:* Edertal, Ederbrinhausen, 1948, *O. Behr* 1173 (B 60 0143887).

### *Verrucaria elaeina* Borrer

In W. J. Hooker, *English Botany Supplement 1*: text to plate 2623 (fig. 2) (1830).

*Verrucaria guestphalica* Serv., *Ceskoslovenské Lisejníky Celedi Verrucariaceae* 146, Tab. IV, fig. 57 (1954); type: [Germany] An Dachziegel des alten Schlosses zu Bentheim in Westfalen, August 1880, *Lahm* [Arnold, *Lich. exs.* 861] (B 60 0143796, M-0059551, M-0059523—isosyntypes!).

A description of this species was given by Orange (2000), who showed that the name had been widely misunderstood. Servít (1954) described *V. guestphalica*, based on F. C. G. Arnold's *Lichenes exsiccati* no. 861 [misprinted as '867' in Servít]; the types in M (seen by Servít) and in B are clearly *Verrucaria elaeina* growing on fragments of tiles. Thüs (2002) and Krzewicka (2012) placed *V. guestphalica* in the synonymy of *V. praetermissa*, although Krzewicka noted that specimens named as *V. guestphalica* in Polish herbaria are in fact *V. elaeina*.

### *Verrucaria lapidicola* Orange sp. nov.

MycoBank No.: MB 807864

Thallus thin, uncracked, composed of a layer of goniocyst-like units; perithecia projecting, naked, 160–280 µm in diameter; ascospores (17.0–)18.0–21.5(–23.0) × 6.5–8.0(–10.5) µm.

Type: Great Britain, Wales, Glamorgan, north-west of Llancarfan, 31/0538.7081, alt. 25 m, on Liassic limestone on bank in woodland by pasture, 3 May 2010, *A. Orange* 18847 (NMW – C.2010.001.217—holotype; GenBank accession no KF819514).

(Figs 5–7)

*Prothallus* not detected. *Thallus* diffuse, very thin, 25–35 µm thick, dull grey-green to dull grey-brown, smooth, but often minutely and inconspicuously mottled or minutely uneven, both mottling and unevenness at a scale of c. 20–60 µm; uncracked (except where the substratum is cracking). *Thallus* in section paraplectenchymatous, comprising distinct or somewhat indistinct goniocyst-like units 18–33 µm diam., mostly in a single layer;

*thallus* cells c. 3.3–9.0 × 3.3–5.7 µm. *Photobiont* cells c. 7.5–12.5 × 5.0–8.2 µm.

*Perithecia* projecting, 160–280 µm diam., black, naked or occasionally with an incomplete thalline covering in lower two-thirds, apex rounded or slightly flattened, ostiolar region whitish to pale brown, plane or protruding as a low papilla, 30–40(–60) µm diam. *Involucrellum* conical-hemispherical, densely pigmented throughout, or densely pigmented at surface and hyaline at base next to exciple. *Exciple* 140–200 µm diam., colourless at sides and base. *Periphysoids* c. 12 µm long. *Asci* 8-spored, c. 85–90 × 23–24 µm. *Ascospores* colourless, ellipsoid, (17.0–)18.0–19.6–21.5(–23.0) × 6.5–7.4–8.0(–10.5) µm, (2.2–)2.5–2.6–2.8(–2.9) times as long as wide [25/4].

*Etymology.* From Latin, meaning living amongst stones.

*Ecology and distribution.* An early colonizer of small calcareous stones (including calcareous mudstone and limestone) shaded by trees or herbaceous vegetation, in woodland or beside tracks; confirmed records from Wales, England and Estonia. This is likely to be a widespread and common species.

*Notes.* This is a small species with few distinctive features. The thallus is thin, and composed of a layer of goniocyst-like units which are responsible for the minutely mottled or uneven surface. In the specimens studied, the ascospores may have been slightly immature. In *Verrucaria*, immature ascospores are generally narrower in outline than mature spores; at least the range of length of the spores measured is likely to be typical for the species.

This is one of several small species, not closely related, which have not been distinguished in recent years in Great Britain, and which have generally been called *Verrucaria murina* auct. (Orange *et al.* 2009). Some of these have larger ascospores than *V. lapidicola*, but until studies on these species are complete, the taxon needs to be confirmed by ITS sequence. *Verrucaria dolosa* Hepp differs in the slightly smaller spores (average



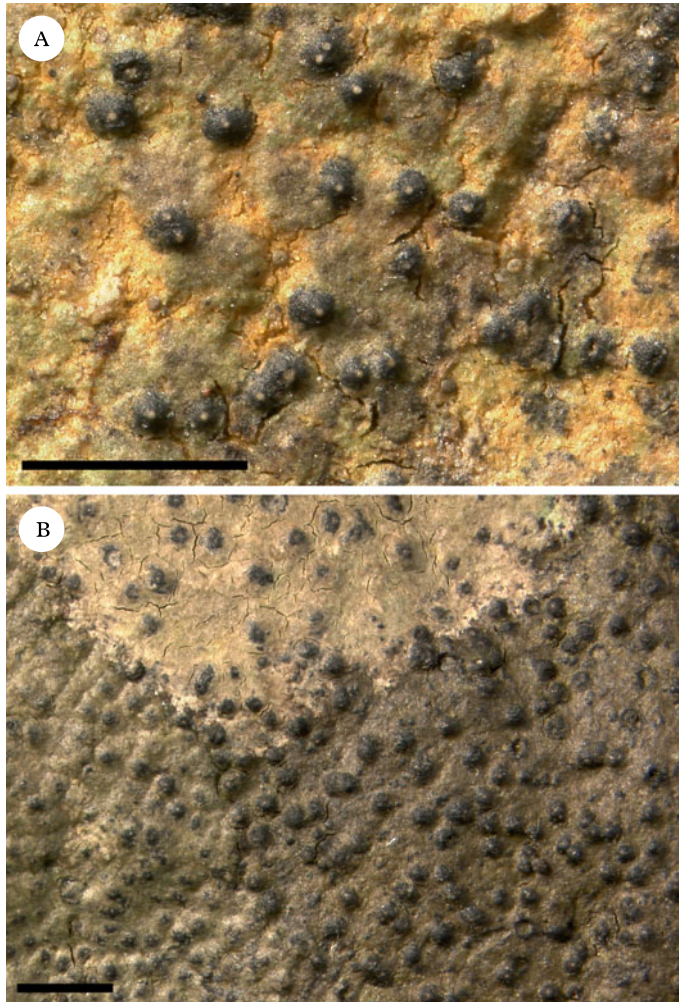


FIG. 5. *Verrucaria lapidicola*. A, holotype; B, Orange 18790 (right), with *Verrucaria elaeina* (top) and an unidentified *Verrucaria* species (left). Scales: A & B = 1 mm. In colour online.

size in four specimens sequenced  $15.9 \times 7.0$   $\mu\text{m}$ ), *V. elaeina* differs in the thicker, paler, often cracked thallus and the larger perithecia, and *V. myriocarpa* Hepp differs in the endolithic thallus and perithecia partly immersed in pits.

*Additional specimens examined.* **Great Britain:** *Wales:* V.C. 42, Breconshire: 2.5 km north-west of Erwood, near Tregaer, River Wye, 32/0792.4418, alt. 110 m, 2010, Orange 18790 (NMW—C.2013.001.169). *England:* V.C. 70, Westmorland: north-east of Coniston, Tarn Hows, 34/326.995, alt. 200 m, 2006, Orange 16690 (NMW—C.2005.001.645).—**Estonia:** *Saare maakond:* Saaremaa, south-west of Tagamõisa, near Kõruse, alt.

5 m, 58°28.375'N, 21°55.832'E, 2009, Orange 18112 (NMW—C.2013.001.168).

### ***Verrucaria praetermissa* (Trev.) Anzi (1864)**

A description of *V. praetermissa* was given by Orange (2000). That description is still valid, except that 'dark brown' specimens, and the specimen depicted in Fig. 5D, apparently belong to *V. devensis* or other species.

The typification and nomenclature of this species are complex but cannot be fully investigated at present, as the lectotype in L is

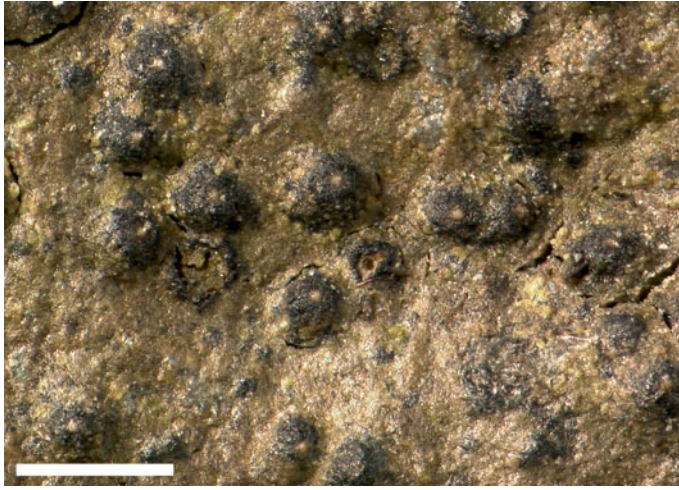


FIG. 6. *Verrucaria lapidicola*, Orange 18790. Scale = 500  $\mu\text{m}$ . In colour online.

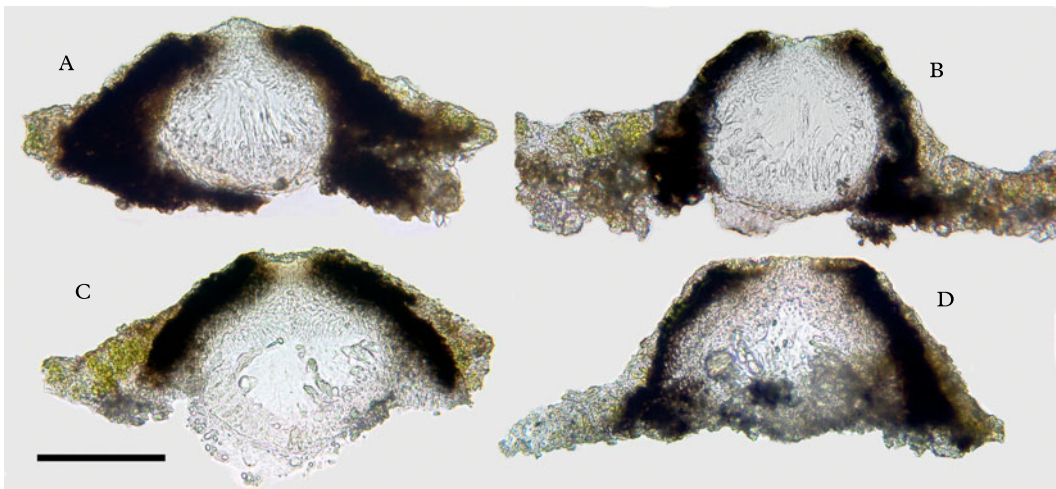


FIG. 7. *Verrucaria lapidicola*, sections of perithecia. A & B, holotype; C, Orange 16690; D, Orange 18790. Scale = 100  $\mu\text{m}$ . In colour online.

temporarily unavailable for study. However, available data suggest that this name almost certainly does not refer to *V. devensis*.

*Selected specimens examined.* **Great Britain:** *Wales:* **V.C. 42**, Breconshire: Builth Wells, Caer Beris, east side of Irfon, 32/0313.5071, 2005, Orange 16257 (NMW—C.2005.001.323); 3 km south-east of Builth Wells, near Glanwey, River Wye, 32/0657.4966, alt.

115 m, 2010, Orange 18805 [NMW, specimen not separable from Orange 18804 (*V. devensis*)]; 3 km south-east of Builth Wells, near Glanwey, River Wye, 32/0674.4871, alt. 115 m, 2010, Orange 18815 (NMW—C.2010.001.50); north-west of Erwood, near Tregaer, 32/0731.4476, alt. 125 m, on concrete in pasture, 2010, Orange 18853 (NMW—C.2010.001.89).—**Germany:** *Bayern:* Kempten, Göhlenbach, alt. 700 m, 47°43.74'N, 10°17.66'E, 2010, Orange 19382 (NMW—C.2010.001.88).

I would like to thank the curators of B, GLM, HBG, and M for the loan of specimens.

## REFERENCES

- Döring, H., Clerc, P., Grube, M. & Wedin, M. (2000) Mycobiont-specific PCR primers for the amplification of nuclear ITS and LSU rDNA from lichenized ascomycetes. *Lichenologist* **32**: 200–204.
- Gardes, M. & Bruns, T. D. (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.
- Gilbert, O. L. (1996) The occurrence of lichens with albino fruit bodies (ascmata) and their taxonomic significance. *Lichenologist* **28**: 94–97.
- Gueidan, C., Roux, C. & Lutzoni, F. (2007) Using a multigene phylogenetic analysis to assess generic delineation and character evolution in *Verrucariaceae* (*Verrucariales*, Ascomycota). *Mycological Research* **111**: 1145–1168.
- Huelsenbeck, J. P. & Ronquist, F. (2005) MrBayes 3.1.2: Bayesian inference of phylogeny. Available at <http://www.mrbayes.scs.fsu.edu/index.php>.
- Krzewicka, B. (2012) A revision of *Verrucaria* s.l. (*Verrucariaceae*) in Poland. *Polish Botanical Studies* **27**: 1–143.
- Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 November 2010, New Orleans, Louisiana, pp. 1–8.
- Nylander, J. A. A. (2004) *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Available at <http://www.abc.se/~nylander>.
- Orange, A. (2000) *Verrucaria elaeina*, a misunderstood European lichen. *Lichenologist* **32**: 411–422.
- Orange, A., Hawksworth, D. L., McCarthy, P. M. & Fletcher, A. (2009) *Verrucaria* Schrad. (1794). In *The Lichens of Great Britain and Ireland* (C. W. Smith, A. Aptroot, B. J. Coppins, A. Fletcher, O. L. Gilbert, P. W. James & P. A. Wolseley, eds): 931–957. London: British Lichen Society.
- Purvis, O. W., Coppins, B. J., Hawksworth, D. L., James, P. W. & Moore, D. M. (eds.) (1992) *The Lichen Flora of Great Britain and Ireland*. London: Natural History Museum Publications & British Lichen Society.
- Salisbury, G. (1975) *Leucocarpopis*, a new lichen genus. *Nova Hedwigia* **26**: 351–352.
- Servít, M. (1954) *Československé Lišejníky, Čeledi Verrucariaceae*. Praha: Československé Akademie Ved.
- Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A fast bootstrapping algorithm for the RAxML Web servers. *Systematic Biology* **57**: 758–771.
- Thüs, H. (2002) Taxonomie, Verbreitung und Ökologie silicoler Süßwasserflechten im außeralpinen Mitteleuropa. *Bibliotheca Lichenologica* **83**: 1–214.
- Thüs, H. & Schultz, M. (2009) Fungi. 1. Teil: Lichens. *Süßwasserflora von Mitteleuropa* **21**: 1–223.
- Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.