A remarkable new freshwater Verrucaria from Europe

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Abstract: *Verrucaria madida* is described as a new species with a green, subgelatinous thallus, and small ascospores; it is unique in the genus in having 4-spored asci. Three other small-spored freshwater species, *V. aquatilis*, *V. rheitrophila* and *V. scabra*, are compared. The type of pigment in the thallus cortex is a useful taxonomic character in the genus.

Key words: Verrucaria, freshwater lichens, Europe

Introduction

The genus *Verrucaria* contains approximately 200 currently accepted species, but is still poorly known taxonomically. The thallus and ascomata are morphologically simple and provide few clear-cut characters for identification of the species. Up to now, only 8-spored asci have been reported, although 1- to many-spored asci are known in other genera of the family *Verrucariaceae*. An unidentified freshwater species has been collected by the author on three occasions, and has proved to possess predominantly 4-spored asci; it is described here as new, and compared to three other species with subgelatinous thalli and small ascospores.

Materials and Methods

Sections of thalli and perithecia were prepared by hand. Ascospore measurements were made in a mixture of 10% KOH and 1% Congo Red. Ascospore measurements are expressed as the average (in bold), plus and minus one standard deviation, with the extremes in parentheses. The letter *n* indicates the number of spores measured/number of specimens from which spores were measured.

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The Species

Verrucaria madida Orange sp. nov.

Thallus atrovirens vel atrogriseus, tenuis, subgelatinosus; cortex pigmentum viridem vel ex parte brunneum continens. Perithecia prominentiae conicohemisphaericae formans. Involucrellum conicum. Asci (3-)4(-5) sporae continens. Ascosporae aseptatae, hyalinae, $(9-)10\cdot5-12\cdot1-13\cdot5(-15)\times(5\cdot5-)6-6\cdot6-7$ $(-7\cdot5)$ µm.

Typus: France, Cantal, west of Murat, 1·8 km west of Fraisse-Haute, 45°6′N, 2°44′E, alt. *c*. 1000 m, shallowly submerged on sloping rocks in stream in woodland, lightly shaded, with *Verrucaria rheitrophila*, 16 May 1990, *A. Orange* 7959 (NMW.C90.13.74—holotypus; BERN, UPS—isotypi).

(Figs 1 & 2)

Prothallus not seen. Thallus dark green or dark greenish grey, thin, $40{\text -}60~\mu\text{m}$ thick, smooth, uncracked, subgelatinous; cells weakly arranged in columns; cortex with dull green pigment which is K – , HCl+brownish, and then dull greenish but faded with addition of further K, or in part with brownish pigment; medulla absent.

Perithecia forming low to moderate conical-hemispherical projections 200–420 μm diam., covered by a layer of thallus at first, this layer sometimes partly lost later; apex of perithecium rounded to slightly flattened, black when thalline layer has been eroded. Exciple 140–290 μm diam., colourless or brown below; ostiole with dull green pigment similar to that in thallus.

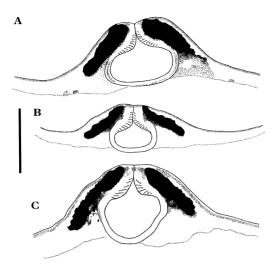


Fig. 1. Verrucaria madida, perithecia in section. A, holotype; B, Orange 8194; C, Orange 9986. Scale= 200 um.

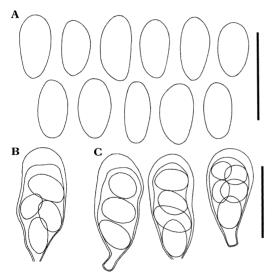


FIG. 2. *Verrucaria madida*. A, ascospores (holotype); B, ascus (holotype); C, asci (*Orange* 9986). Scales: A, B & C=20 μm.

Involucrellum conical, or somewhat spreading at sides and then curved down slightly. Asci (3–)4(–5)-spored. Ascospores ellipsoid, without perispore, (9–)10·5–12·1–13·5(–15) \times (5·5–)6–6·6–7(–7·5) μ m, length/width ratio (1·4–)1·6–1·8–2·1(–2·6) [n=67/3].

Conidiomata not detected.

Ecology and distribution. On frequently immersed siliceous rocks in streams, rare in South Wales (Brecon Beacons), Norway (Hordaland), France (Cantal). The detection of this species amongst relatively small numbers of specimens collected in Norway and France suggests that it is likely to be much overlooked. However, only one British site is known, and the species was not found again on visits to this site in 2003 and 2004.

Notes. Distinguished by the green, subgelatinous thallus (with greenish pigment), conical involucrellum, and small ascospores. Remarkable for the occurrence of 4-spored asci; this character was not noticed until the specimens had been united on other features, and is apparently unique in the genus. In Orange 9986, of 15 asci examined, one had three spores, another five spores, and the remainder four spores. Verrucaria aquatilis differs in the usually blackish thallus and smaller perithecia and ascospores, V. rheitrophila differs in the immersed perithecia and presence of punctae in the thallus, and V. scabra differs in the presence of immersed punctae and a dark basal layer, and in the larger, oblong ascospores.

Additional specimens examined. Great Britain: Wales: V.C. 42, Breconshire: Brecon Beacons, west of Beacons Reservoir, by Nant Pennig, 22/977190, alt. 510 m, in calcareous streamlet on rocky bank on Old Red Sandstone, with Verrucaria rheitrophila, V. aquatilis, Palustriella commutata, Philonotis fontana, Brachythecium rivulare, Bryum pseudotriquetrum, 1994, Orange 9986 (NMW.C.1999.011.159).—Norway: Hordaland: Kvam, Tørvikbygd, Kolltveit, near Øyjordsvatn, 32VLM410848, 60°16′N, 6°7′E, alt. c. 70 m, on stone in stream by fields near woodland, with V. rheitrophila, Verrucaria sp., 1990, Orange 8194b (NMW.C90.32.173).

Verrucaria aquatilis Mudd

(Fig. 3)

Prothallus very inconspicuous, perhaps brown. Thallus thin, 20–55 µm thick, diffuse, dark greyish brown or dark greenish brown to brownish black (blackish with the

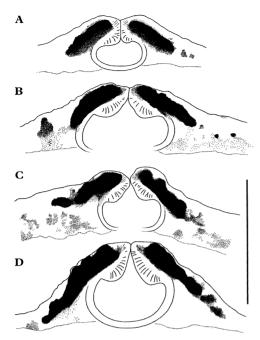


FIG. 3. Verrucaria aquatilis, sections of perithecia. A, B, Orange 6976; C, Orange 6726; D, Orange 10355. Scale=200 µm.

unaided eye), uncracked or cracks very few, surface smooth or slightly roughened; upper layer of thallus (and sometimes also elsewhere) with greenish brown to dull brown pigment; lower part of thallus unpigmented, or with brown pigment sparse to extensive, but not forming a well-defined basal layer; rarely with a few intensely pigmented punctae within thallus.

Perithecia forming low to moderate conical-hemispherical projections 100–240 μm diam., more or less concolorous with thallus, covered at least when young by a layer of thallus; ostiole inconspicuous, sometimes visible as a minute pit up to 20 μm wide. Exciple 120–160 μm, colourless at sides and base, ostiole often dull blue-grey or blue-green. Involucrellum conical, often not reaching base of thallus, but sometimes merging into pigment in lower part of thallus; pigment in involucrellum red-brown, K+ grey. Ascospores broadly ellipsoid, without perispore, (6·5–)7·5–8·2–9(–10) × (4·5–)

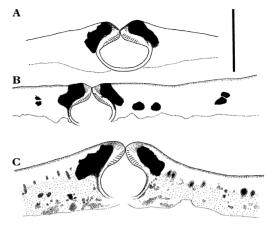


FIG. 4. Verrucaria rheitrophila, sections of perithecia. A, Orange 9679; B, Orange 10483; C, Orange 12410 p.p. Scale=200 µm.

5·5–6·2–6·5(–8) μ m, (1·1–)1·2–1·3–1·5(–1·8) times as long as wide [n=134/13].

Conidiomata not detected.

Ecology and distribution. On long-submerged rocks, widespread in N and W Britain; throughout Europe; Himalayas, Siberia, New Zealand.

Notes. Usually easily identified by the thin blackish thallus and the very small, broadly ellipsoid ascospores. Verrucaria rheitrophila is green or brown, the perithecia are immersed, the ascospores are slightly larger, and the thallus usually contains black punctae. Verrucaria madida differs in the larger perithecia and ascospores, and the dark green thallus. The thin blackish crust of V. aquatilis could be overlooked as a dead lichen, even under the dissecting microscope.

Selected specimens examined. Great Britain: Wales: V.C. 46, Cardiganshire: Cwm Rheidol, Coed Simdde-lwyd, 1988, Orange 6976 (NMW.C.2004.002.70). V.C. 52, Anglesey: 2 km north-east of Amlwch, Aber Cawell, 23/460935, 1995, Orange 10355 (NMW.C95.38.162). England: V.C. 4, North Devon: Lee, Borough Valley, 21/481458, 1988, Orange 6726 (NMW.C88.26.56). Scotland: V.C. 104, North Ebudes: Isle of Skye, near Carbost, Fiskavaig, 18/322344, 1985, Orange 3115 (NMW.C88.16.183).

Verrucaria rheitrophila Zschacke

(Fig. 4)

Prothallus whitish, not fimbriate; contiguous conspecific thalli often separated by thin black lines. Thallus greyish green to pale brownish green to dark brown, welldeveloped, 60–95 μm thick, subgelatinous, usually not cracked, but sometimes with sparse or numerous cracks which probably form only after collection; thallus surface smooth or with sparse to numerous black punctae 20-40 µm diam. at the surface. Thallus in section composed of more or less distinct columns of cells; cortex poorly defined, cell walls with brown pigment (dilute or absent in shade). Lower part of thallus often forming an ill-defined medulla; in this zone living algal cells are often few, and the fungal cells contain oil drops; this zone is colourless to dilute brown, locally with intensely pigmented patches near its upper edge, often appearing as more or less discrete punctae in section.

Perithecia immersed in the thallus or forming very low projections which are usually too indistinct to measure; apex appearing at the thallus surface only as a small black dot, or more usually as a black disc up to 220 µm diam., often roughened or surrounded by a few punctae. Exciple 110-210 µm diam., sides and base colourless or partly brown. Involucrellum present, well-developed in the upper half of the exciple, often spreading outwards and downwards and grading into the punctae of the upper layer of the medulla. Ascospores ellipsoid, (8.5-)11-12.7-14(-16) \times (6–)6·5–7·4–8(–9·5) µm, length/width ratio (1.3-)1.5-1.7-1.9(-2.3), without perispore [n=112/8].

Ecology and distribution. On long-submerged rocks, frequent in Britain. Known from Europe, N. America, and alpine Australia and New Zealand.

Notes. This species can usually be recognized in the field by the immersed perithecia visible as black dots, and often by the black punctae which give the thallus a scabrid

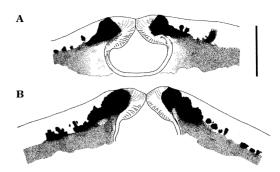


FIG. 5. Verrucaria scabra, sections of perithecia. A, Orange 12410; B, Orange 9710 Scale=200 μm.

appearance. Punctae are most easily seen in thin thalli, but are immersed in thicker thalli.

Selected specimens examined. Great Britain: Wales: V.C. 46, Cardiganshire: Pentre-cwrt, Afon Teifi, Orange 10483 (NMW.C.2004.002.69). England: V.C. 5, South Somerset, 5 km south-west of Porlock, Wilmersham Common, alt. 335 m, 1993, Orange 9679 (NMW C.1999.011.164). V.C. 69, Westmorland: Helvellyn, Brown Cove Tarn, 1998, Orange 12410 p.p. (NMW.C.1999.011.162, sub V. scabra).

Verrucaria scabra Vězda

(Fig. 5)

Prothallus whitish, not fimbriate; contiguous conspecific thalli often separated by thin black lines. Thallus dull green to dark greygreen or dark green (sometimes blackish to unaided eye), well-developed, 35–150 µm thick, subgelatinous, uncracked (rarely with fine splits probably created on drying for the herbarium), surface smooth to slightly scabrid with concolorous papillae, occasionally with very local black punctae c. 30 μ m diam. Thallus in section composed of more or less distinct columns of cells; cortex poorly defined, cell walls with dull green pigment (dilute or absent in shade). Lower part of thallus often forming an ill-defined medulla, ranging from a zone with illdefined areas of brown pigment, to a pigmented zone forming an extensive dark basal layer to the thallus; upper part of medulla often with small, upwardly projecting areas of intensely pigmented cells, appearing as more or less discrete punctae in section.

Perithecia usually immersed in the thallus, forming at most very low projections which are too indistinct to measure, or (when the thallus is thin) forming low to moderate projections 300-650 µm diam.; apex appearing at the thallus surface either as a whitish ring 120–150 µm diam., or more usually as a black disc 200-300 µm diam., often roughened or surrounded by a few punctae, rarely radially fissured. Exciple 275-290 µm diam., sides and base colourless or partly brown. Involucrellum present, well-developed in the upper half of the exciple, often spreading outwards and downwards and grading into the punctae of the upper layer of the medulla; sometimes (where medulla is thin), involucrellum conical and reaching base of thallus. Ascospores ellipsoid to (mostly) oblongellipsoid, $(11-)14-15\cdot7-17\cdot5(-21\cdot5)\times(7-)$ $7.5-8.4-9(-10.5) \mu m$, length/width ratio $(1\cdot4-)1\cdot7-1\cdot9-2\cdot1(-2\cdot4)$ [n=116/6], without perispore.

Conidia straight or slightly curved, $3.5-5 \times 1.2 \mu m$.

Ecology and distribution. In Great Britain, recorded on permanently or frequently submerged siliceous rocks in streams and lakes, shaded or unshaded, at altitudes of 225–660 m, in SW England, Wales and N England; associated species include Dermatocarpon meiophyllizum, Verrucaria aquatilis, V. funckii, V. rheitrophila, Staurothele fissa, the hepatic Jungermannia exsertifolia and the alga Hildenbrandia rivularis. Recorded from Slovakia (Vězda 1970), Germany (Thüs 2002), and Switzerland.

Notes. Differs from V. rheitrophila in the green (rather than brown) thallus pigment, the slightly larger and more oblong ascospores, the larger, more widely spaced and more projecting perithecia, and the tendency for the medulla to be more darkly pigmented. However, the basic morphology is very similar, with a mainly apical involucrellum which is often continued laterally as a line of punctae. The differences are striking in mixed collections from well-lit sites (for example Orange 11528 and 12410). In

shade, the thallus pigment is poorly developed, and there may be little difference between the colour of the two species. This species has been overlooked in Britain and has probably been misidentified as V. rheitrophila. For instance, Gilbert & Giavarini (1993) described submerged pebbles in Brown Cove Tarn as "completely covered with V. rheitrophila", but V. scabra appeared to be the dominant species here in 1998, although both were present. The green thallus of V. scabra externally resembles that of V. pachyderma (Arnold) Arnold, but that species has larger ascospores, (15–)16–19·1– $22(-23.5) \times (6-)6.5-7.7-9 \text{ }\mu\text{m} \text{ } [n=160/10],$ and a different involucrellum; thalli superficially resemble V. praetermissa (Trevis.) Anzi in the green thallus and dark basal layer, but in that species the thallus is non-gelatinous and the ascospores are $(16-)19-21\cdot 0-23(-28) \times (6\cdot 5-)8-$ 8.7–9.5 (-10.5) μ m [n=355/23].

Selected specimens examined. Great Britain: Wales: V.C. 49, Caernarvonshire: north of Llyn Ogwen, Ffynnon Lloer, alt. 660 m, 1997, Orange 11528 (NMW.C97.35.503). England: V.C. 4, North Devon: Malmsmead, East Lyn River, 21/788.485, alt. 225 m, 1993, Orange 9710 (NMW.C94.10.111). V.C. 69, Westmorland: Helvellyn, Brown Cove Tarn, 1998, Orange 12410 (NMW.C.1999.011.162).—Switzerland: Kanton Graubünden, Davos, Flüelatal Müller Tälli, alt. 2350 m, 1987, C. Keller 1277 (NMW.C93.12.12).

Discussion

Thallus colour can be a useful character in the identification of Verrucaria species, but it is also very variable, and readily modified by factors including degree of shading, and the age of the thallus. It depends not only on the presence of pigment in the thallus, but on the consistency and thickness of the thallus. It is remarkable that the thallus of V. aquatilis is black, although it is thin and not obviously more densely pigmented than other species. The type of pigment within the thallus does not appear to have been used previously as a taxonomic character. Cortical pigments are difficult to study since they are usually dilute in comparison to ascomatal pigments, but a brown and a dull green pigment can be found within the cortex of European freshwater species. These pigments can intergrade in some specimens of some species, but the possession of green pigment in some or all specimens appears to be characteristic of certain species, including $V.\ madida,\ V.\ scabra$ and $V.\ pachyderma$, but not $V.\ rheitrophila$ or $V.\ funckii$ (Spreng.) Zahlbr. The thallus of such species is usually dark green when dry (in well-lit habitats). The type of thallus pigment helps to confirm the distinctness of $V.\ pachyderma$ and $V.\ funckii$; these were regarded as synonymous by Thüs (2002), but they also differ in ascospore size, $(15-)16-19\cdot1-22(-23\cdot5)\times (6-)6\cdot5-7\cdot7-9$ µm in $V.\ pachyderma$ and

 $(19.5-)22.5-24.1-26(-30) \times (8-)9-10.1-11$ (-13) µm [n=226/13] in V. funckii. The type of pigment is not helpful in specimens from shady habitats, as here cortical pigment may be absent.

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