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A new freshwater *Porina* (*Porinaceae*, *Ostropales*) from Great Britain

Alan ORANGE

Abstract: *Porina rivalis* Orange is described as new from rocks in streams in Great Britain. The involucrellum is yellow or orange within, but dark grey to purplish red at the surface; the ascospores are 3-septate, 13·0–18·5 × 4·0–5·5 µm.

Key words: acetone-insoluble pigments, ITS, lichen, mtSSU, streams, taxonomy, Wales

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Introduction

The family *Porinaceae* contains nearly 430 species, of which approximately 45% are corticolous, 35% are foliicolous, and 20% are saxicolous (McCarthy 2013). In recent decades most authors have accepted only two or three genera within the family, but four were accepted by Harris (1995) and five by Hafellner & Kalb (1995). Hafellner & Kalb defined their genera on the basis of characters including the presence of setae on the perithecium (*Trichothelium*), the type of acetone-insoluble pigment in the perithecial wall, and the presence or absence of a chitinoid ring structure in the ascus apex. However, these characters do not always agree with the authors placement of species. The newly-defined pigment Porina-yellow was said to be characteristic of *Porina* s. str., and absent in other genera, but it occurs as a minor pigment in several species placed in *Pseudosagedia*. *Pseudosagedia* was said to have a ring structure in the ascus, but one is absent in *P. guentheri* (Orange *et al*. 2009). Baloch & Grube (2006) analyzed 28 mostly foliicolous species, using mitochondrial SSU rDNA sequences. Their analysis revealed four main clades within the material studied, and *Trichothelium* was nested within *Porina*; the generic concepts used by Hafellner & Kalb as well as Harris were not supported. Nelsen *et al*. (2014), using mitochondrial SSU rDNA and nuclear LSU rDNA, and including some corticolous species of *Porina*, showed that the genus *Myeloconis* belongs in *Porinaceae*, and is sister to *Porina farinosa*. The genus *Porina* is well studied, with recent world keys to foliicolous species (Lücking 2004) and saxicolous species (McCarthy 2000).

A distinctive *Porina* has been known from streams in Wales for some years, but has been misidentified as *Porina lectissima*. It is described here as new, based on morphology and on mitochondrial SSU rDNA and nuclear ribosomal ITS sequences.

Materials and Methods

DNA was extracted from 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 two 20 µl tubes. The two internal transcribed spacer regions and the 5.8S region (ITS1-5.8S-ITS2) of the nuclear ribosomal genes, and part of the small subunit of the mitochondrial ribosomal DNA (mtSSU) were amplified, using the primers ITS1F, ITS2, ITS3, ITS4, and mrSSU1, mrSSU3R. 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

A. Orange: Department of Biodiversity and Systematic Biology, National Museum of Wales, Cathays Park, Cardiff CF10 3NP, UK. Email: alan.orange@museumwales.ac.uk

(College of Life Sciences, University of Dundee, www.dnaseq.co.uk) using Applied Biosystems Big-Dye Ver 3.1 chemistry on an Applied Biosystems model 3730 automated capillary DNA sequencer.

Alignment of assembled sequences 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. Ambiguously aligned regions were excluded from the analysis.

Phylogenetic relationships and support values based on mtSSU data were investigated using a Bayesian approach, and additional support values were obtained using Maximum Likelihood bootstrapping, as implemented in RaxML (Stamatakis 2006; Stamatakis *et al.* 2008); both programs were hosted on the CIPRES Science Gateway (Miller *et al.* 2010). The model of evolution for the Bayesian analysis (HKY + I + G) was selected using the Akaike Information Criterion (AIC) in MrModeltest 2.2 (Nylander 2004). Gaps were treated as missing data. Using MrBayes, two analyses of two parallel runs were carried out for 1 000 000 generations, with trees sampled every 100 generations. Stationarity was considered to have been reached when the mean standard deviation of split frequencies dropped to <0·01. A burn-in sample of 2500 trees was discarded from each run, respectively. Support values of ≥95% Bayesian posterior probabilities and ≥70% Maximum Likelihood bootstrapping were regarded as significant. ITS data were investigated using a similar Bayesian approach; models of evolution for the ITS1, 5.8S and ITS2 regions were HKY, K80 and GTR + I, respectively. A partitioned analysis was run for 500 000 generations, and a burn-in sample of 1250 trees was discarded.

Mitochondrial SSU sequences of saxicolous and corticolous species available on GenBank were included in the analysis, but not the numerous sequences of foliicolous species. No ITS sequences of *Porinaceae* were available from GenBank. Specimens used in the analyses are shown in Table 1.

Acetone-insoluble pigments in the perithecium were observed in water and in 10% KOH.

Results

Fifteen mtSSU sequences were prepared for the new species and for several additional taxa.

TABLE 1. *Specimens used in the phylogenetic analysis. Accession numbers refer to mtSSU sequences unless otherwise indicated. New sequences are in bold*

Species	Locality	Voucher	GenBank accession number
Belonia russula	-	-	AY648888
Gyalecta ulmi	-	-	AY300888
Porina aenea	Wales	*Orange* 17306 (NMW)	**KR108906**
P. aenea	-	-	DQ168410
P. aenea	-	-	DQ168411
P. aenea	-	-	HM244754
P. austroatlantica	Falkland Islands	*Orange* 22606 (NMW)	**KR108903**
P. byssophila	Wales	*Orange* 16444 (NMW)	**KR108909**
P. byssophila	Wales	*Orange* 17561 (NMW)	**KR108910**
P. byssophila	Wales	*Orange* 21213 (NMW)	**KR108912**
P. byssophila	Wales	*Orange*18349 (NMW)	**KR108911**
P. byssophila	Wales	*Orange* 21981 (NMW)	**KR108915; KT230857 (ITS)**
P. byssophila	-	-	HM244755
P. chlorotica	Wales	*Orange* 17307 (NMW)	**KR108907**
P. chlorotica	Ireland	*Orange* 18154 (NMW)	**KR108908**
P. guentheri var. *lucens*	England	*Orange* 16677 (NMW)	**KT230850 (ITS)**
P. interjungens	England	*Orange* 16686 (NMW)	**KT230851 (ITS)**
P. lectissima	Wales	*Orange* 16586 (NMW)	**KR108902**
P. lectissima	Wales	*Orange* 20441 (NMW)	**KT230852 (ITS)**
P. lectissima	Wales	*Orange* 20462 (NMW)	**KT230853 (ITS)**
P. lectissima	Wales	*Orange* 20855 (NMW)	**KR108904; KT230854 (ITS)**
P. lectissima	Ireland	*Orange* 21436 (NMW)	**KT230855 (ITS)**
P. lectissima	Wales	*Orange* 21663 (NMW)	**KR108905; KT230856 (ITS)**
P. lectissima	-	-	DQ168414
P. lectissima	-	-	HM244756
P. pacifica	British Columbia	*Orange* 22762 (NMW)	**KT230858 (ITS)**
P. pacifica	British Columbia	*Orange* 22770 (NMW)	**KT254300; KT230859 (ITS)**
P. rivalis	Wales	*Orange* 20628 (NMW)	**KR108913; KR108900 (ITS)**
P. rivalis	Wales	*Orange* 20644 (NMW)	**KR108914; KR108901 (ITS)**
Porina sp.	British Columbia	*Orange* 22778 (NMW)	**KT230860**

The ITS region was unexpectedly difficult to amplify, and a number of sequences obtained proved to be contaminants. Twelve ITS sequences were prepared.

The aligned mtSSU region comprised 1150 sites, of which 625 were removed as ambiguous. The 50% majority-rule consensus tree from the Bayesian analysis of mtSSU sequences is shown in Fig. 1. The lower nodes of the trees are poorly supported. Well-supported clades include: 1) *Porina lectissima* together with the single sequence of *P. austroatlantica*; 2) *P. aenea* and *P. chlorotica*; 3) the two sequences of *P. rivalis* together with one unidentified sequence; and 4) five sequences of *P. byssophila*. One sequence named as *P. byssophila* from GenBank clustered with *Porina aenea/chlorotica*, but is evidently based on a misidentified specimen.

The aligned ITS1-5.8S-ITS2 region comprised 583 sites, of which 230 were deleted as ambiguous. The unrooted 50% majority-rule consensus tree from the Bayesian analysis of the ITS region is shown in Figure 2, and supports the distinctness of the new species from *P. lectissima* and others.

The Species

Porina rivalis Orange sp. nov.

MycoBank No.: MB814184

Thallus thin; perithecia prominent, involucrellum with acetone-insoluble pigments Sagedia-red and Porina-yellow; ascospores 3-septate, 13·0–18·5 × 4·0–5·5 µm.

Type: Great Britain, Wales, Breconshire, near Llanwrtyd Wells, Nant Walch, 22/8551.4971, alt. 230 m, on stones submerged in stream, shaded; water pH 6·6, conductivity 43 µS cm^{-1}, 16 September 2011, *Alan Orange* 20628 (NMW – C.2015.005.8—holotype; GenBank accession nos: KR108900 (ITS), KR108913 (mtSSU)).

(Figs 3 & 4)

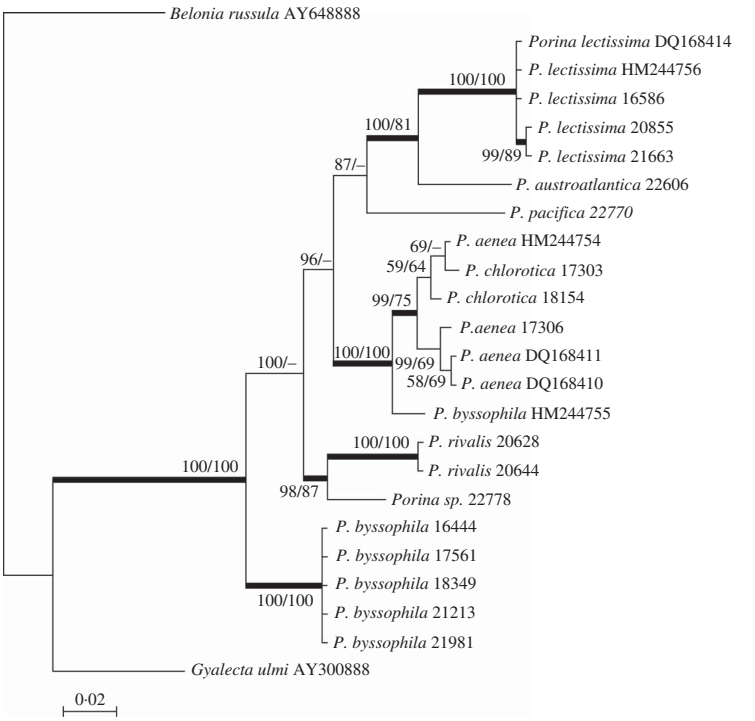

FIG. 1. Phylogenetic relationships of *Porina* species, based on a Bayesian analysis of the mitochondrial SSU. The tree was rooted using *Belonia russula* and *Gyalecta ulmi*. The two support branches associated with each branch are posterior probabilities (PP) and maximum likelihood bootstrap (MLb) values, respectively. Branches in bold indicate a support of PP ≥95% and MLb ≥70%.

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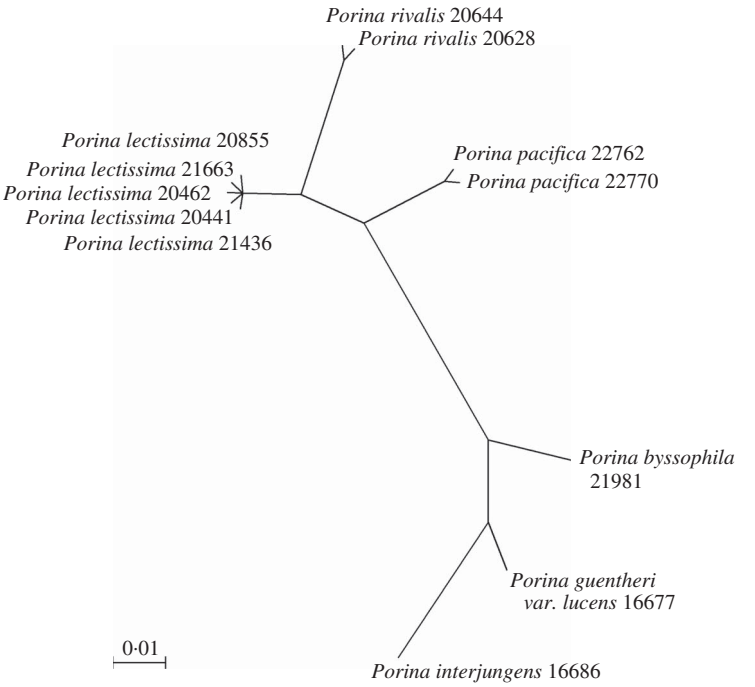

FIG. 2. Phylogenetic relationships of *Porina*, based on a Bayesian analysis of the ITS region. The tree is unrooted.

Prothallus brown, very thin (seen once). *Thallus* light orange-brown to grey-brown or dark grey (orange tints disappearing on storage), thin, 20–70 µm thick, continuous or with scattered cracks. *Photobiont* trentepohlioid. *Perithecia* prominent, 160–400 µm diam., dark brown or black, sometimes orange-brown or brown at extreme base. *Involucrellum* of isodiametric thick-walled cells, enclosing numerous photobiont cells, without crystals; inner part yellow to orange, K+ orange-red (Porina-yellow), near upper surface dark grey to purplish red, K+ dark grey or bluish grey (Sagedia-red at least in part), a small area adjacent to ostiole often dark dull violet. *Centrum* 185–295 µm diam.; *exciple* colourless or yellow (Porina-yellow). *Ascus* ± cylindrical, thin-walled, I−, with truncate apex, with a ring structure. *Ascospores* 3-septate, narrowly ellipsoid, 13·0–**15·3**–17·5(–18·5) × 4·0–**4·8**–5·5 µm, 2·3–**3·2**–4·1(–4·3) times as long as wide (based on 61 spores from 10 specimens).

Conidiomata up to 80 µm diam., occurring near junctions of conspecific thalli; *conidia* rod-shaped, aseptate, 4·0–4·5 × 1·2 µm.

Ecology and distribution. On frequently inundated siliceous rocks beside streams that are neither strongly acidified nor nutrient-enriched. The water chemistry at two sites in Mid-Wales was recorded on eight (calcium) to ten (others) occasions during 2011–2013 (mean values in bold):

Irfon: pH 5·8–**6·5**–7·2, conductivity 35–**42**–48 µS cm^{-1}, calcium 1·7–**2·1**–2·7 mg l^{-1};
Nant Walch: pH 5·1–**6·1**–7·0, conductivity 32–**44**–56 µS cm^{-1}, calcium 1·7–**2·4**–4·6 mg l^{-1}.

Associated species include *Dermatocarpon luridum*, *Ephebe lanata*, *Ionaspis lacustris*, *Rhizocarpon lavatum*, *Verrucaria consociata*, *V. rosula*, *V. sublobulata*, and the bryophytes *Heterocladium heteropterum*, *Hygrohypnum ochraceum*, *Hyocomium armoricum*, *Marsupella emarginata*, *Racomitrium aciculare* and

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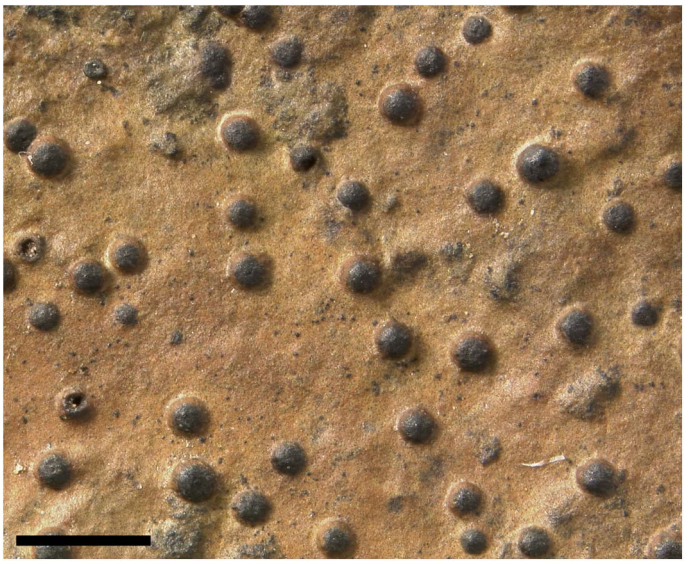

FIG. 3. *Porina rivalis* (holotype). Scale = 1 mm.

Scapania undulata. *Porina rivalis* has been recorded from at least 10 streams in Mid and North Wales, and one stream each in South-west England and North England.

Notes. Porina rivalis is best detected in the field by its distinctly semi-aquatic habit. In some specimens the perithecium is black above but concolorous with the thallus below, giving a distinctive appearance (Fig. 3), but in others it is entirely black. In section, the involucrellum has a cap of dark pigment over an inner layer containing yellow to brownish orange pigment. The amount of dark pigment is variable in extent, and it can be sparse in shaded habitats. The contrasting pigments are more easily seen in K, when the outer layer is grey, and the inner layer is often a rich orange-red (Fig. 4). *Porina rivalis* is likely to be confused with other saxicolous species with 3-septate spores. *Porina lectissima* has a thin to rather thick thallus, 40–200 µm thick, which is often cracked; the perithecia are somewhat larger, 320–500 µm diam. The perithecia of *P. lectissima* are often orange or orange-brown, at least when young or wet, but in a few specimens from exposed habitats they appear almost black when dry. In these cases, the pigment is still almost exclusively Porina-yellow. The ascospores of *P. lectissima* are considerably larger, (21·5–)22·0–**26·5**–31·0 × 4·5–**5·5**–6·5 µm. It is frequent on moist or flushed siliceous rocks, and while it can occur in places where it is submerged by high river flows, it is not a member of the distinctly semi-aquatic community of lichens and bryophytes. *Porina leptalea* has small perithecia, 240–320 µm diam., containing only Porina-yellow, and the ascospores are 14·5–23·0 × 3·5–5·0 µm. It is often found on bark, but can also occur on moist rocks. *Porina chlorotica* has a purplish brown pigment in the involucrellum, and Porina-yellow is absent or very localized, while *P. byssophila* has an involucrellum that is dominated by dark pigments, with only small amounts of Porina-yellow, and grows on non-aquatic rocks and bark. *Porina pacifica* Brodo (Brodo 2004) from western North America has the inner layer of the involucrellum containing Porina-yellow (best seen in K), but the thicker outer layer is dull purple-brown (colour only visible in very thin section). An unidentified collection from British Columbia (*Orange* 22778) on shaded stones has an involucrellum dominated by Porina-yellow, with a trace of darker pigment at the ostiole; the ascospores are slightly larger

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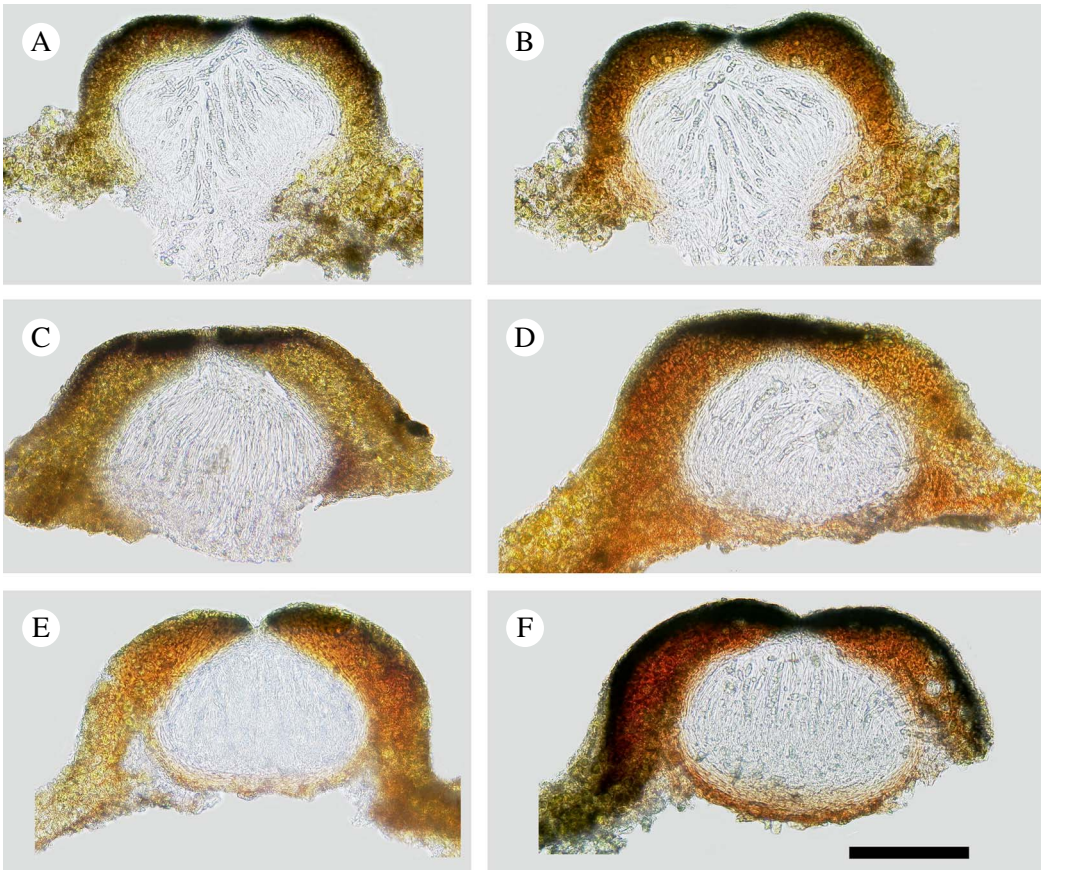

FIG. 4. *Porina rivalis*, sections of perithecia. A, *Orange* 20439 in water; B, *Orange* 20439 in K; C, holotype in water; D, holotype in K; E, *Orange* 20644 in K; F, *Orange* 20443 in K. Scale = 100 µm.

than in *P. rivalis*, 18–21(–23) × 4·5 µm. It is basal to *P. rivalis* in the mtSSU tree.

Additional selected specimens examined. **Great Britain:** *Wales*: **V.C. 48,** Merioneth: near Ganllwyd, Afon Gain near confluence with Afon Mawddach, 23/735.264, 1996, *A. Orange* 11137 (NMW – C97.35.184); above Llyn Celyn, Afon Tryweryn, 23/843.399, 1998, *A. Orange* 12098 (NMW – C.1999.011.74); near Llanfrothen, Dolfriog, near Corlwyni, 23/6146.4656, 2002, *A. Orange* 14153 (NMW – C.2003.002.141); Ganllwyd, Rhaeadr Mawddach, 23/7360.2757, 2011, *A. Orange* 20644 (NMW – C.2015.005.9); same locality and date, *A. Orange* 20649 (NMW – C.2013.001.126). **V.C. 49,** Caernarvonshire: Llanberis, Afon Hwch, 23/579.590, 1994, *A. Orange* 10058 (NMW – C95.38.121); east of Beddgelert, stream below Llyn Llagi, 23/6368.4890, 2011, *A. Orange* 20463 (NMW – C.2015.005.7); east of Beddgelert, Gelli Iago, 23/6334.4823, 2011. *A. Orange* 20443 (NMW – C.2015.005.6); same locality and date, *A. Orange* 20439 (NMW – C.2015.005.5). *England*: **V.C. 4,** North Devon: Belstone, River Okement, iv 1959, *A. E. Wade* (NMW 61.48.312). **V.C. 57,** Derbyshire: Goyts Clough, River Goyt, SK 01.73, xi 2010, *S. Price* (NMW–C.2015.005.10).

Discussion

The analysis of mtSSU sequences confirms that *Porina rivalis* is not closely related to *P. aenea*, *P. austroatlantica*, *P. byssophila*, *P. chlorotica*, *P. lectissima* or *P. pacifica*, some of the other saxicolous species with 3-septate ascospores, although it forms a well-supported clade with a single sequence from an unidentified collection from British Columbia. The analysis of the few available

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ITS sequences confirms that *P. rivalis* is not closely related to *P. byssophila*, *P. guentheri* var. *lucens*, *P. interjungens*, *P. lectissima* or *P. pacifica*. However, the phylogeny of the genus will only be resolved using a much broader sampling of taxa, and additional gene regions.

The acetone-insoluble pigments of the involucrellum are important in defining the new species. Such pigments have not been analyzed chemically, but a standard system of naming them, defined on the basis of their colour and colour changes in acids and alkalis, was proposed by Meyer & Printzen (2000). Four named pigments, two of them newly defined, were reported from *Porina* s. lat. by Hafellner & Kalb (1995), but the writer has found these difficult to apply, with the exception of Porina-yellow. It is also difficult to be certain whether a colour seen in a section is a 'different' pigment to adjacent areas, or the effect of a mixture of pigments. The pigments found in each species of *Porina* need to be described carefully, and the formal names used only with caution.

Key to saxicolous *Porina* species in Great Britain with 3-septate ascospores

1 Involucrellum in section predominantly yellow to orange, K+ darker orange to orange-red (Porina-yellow); dark pigments in shades of purple, brown and grey absent 2
Involucrellum in section with dark pigments (shades of purple, brown or grey); however, yellow to orange pigments often present as well 4

2(1) Isidia present (species is most frequent on bark) . . . . . **P. rosei**
Isidia absent 3

3(2) Perithecia 0·1–0·3 mm diam. (measured *in situ*), ascospores 14·5–23·0 × 3·5–5·0 µm . . . . . **P. leptalea**
Perithecia 0·22–0·50 mm diam., ascospores (21·5–)22·0–31·0 × 4·5–6·5 µm . . . . . **P. lectissima**

4(1) Involucrellum containing much Porina-yellow (best seen in K), dark pigment confined to a thin layer near the surface of the involucrellum or near the ostiole; on siliceous rocks in streams . . . . . **P. rivalis**
Involucrellum with Porina-yellow absent or confined to an area near the ostiole, dark pigments predominating 5

5(4) Involucrellum brown with a purplish, dull violet or bluish grey tinge, K+ dulling (no blue tints); on siliceous rock or on bark . . . . . **P. aenea** and **P. chlorotica**
Involucrellum at least in part dull purple, K+ dull blue to dark blue-grey; on slightly calcareous rocks or on limestone 6
(Rarely *P. byssophila* lacks a K+ bluish pigment, but the thallus is better developed and often paler than *P. chlorotica*, and it prefers calcareous rock)

6(5) Thallus endolithic, perithecia often immersed in pits in rock . . . . . **P. linearis**
Thallus superficial, well developed, perithecia not in pits . . . . . **P. byssophila**

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