

## *Porpidia irrigua*, a new species related to *P. contraponenda*

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**Abstract:** *Porpidia irrigua* is described as a new species; it has a white thallus, sessile apothecia, contains the depside methyl 2'-*O*-methylmicrophyllinate, and grows on damp siliceous rocks. It was previously included in a wide concept of *P. contraponenda*, but that species differs in the thicker thallus, partly immersed apothecia, and the possession of an unidentified depside in addition to methyl 2'-*O*-methylmicrophyllinate, which can be separated by thin-layer chromatography using Solvent System G.

**Key words:** Great Britain, lichens, Norway, nuclear ribosomal DNA, thin-layer chromatography.

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### Introduction

The genus *Porpidia* has been the subject of a number of taxonomic studies in recent decades, including Hertel & Knoph (1984) (Central Europe), Gowan (1989) (North America), Gowan & Ahti (1993) (eastern Fennoscandia) and Fryday (2005) (N Europe, especially the British Isles). However, problems remain in the delimitation of species, and there are numerous unidentified collections which probably represent undescribed taxa. Buschbom & Mueller (2004) investigated the phylogeny of the genus using nuclear ribosomal large subunit RNA and nuclear  $\beta$ -tubulin markers. They found that while *Porpidia sensu lato* formed a strongly supported group, the genus as currently circumscribed was not monophyletic, with *Amygdalaria*, *Cecidomia* and *Lecidea sensu stricto* nested within it. With the exception of studies in the *P. flavicunda*-*P. melinodes* group (Buschbom & Mueller 2006, Buschbom & Barker 2006), little other molecular work has been carried out to date.

*Porpidia contraponenda* belongs to Group I of Buschbom & Mueller (2004), together

with *Amygdalaria* spp., *P. cinereoatra*, *P. lowiana*, *P. macrocarpa*, *P. superba*, *Stenhamarella turgida* and others. According to recent authors *P. contraponenda* is most easily distinguished from related species by the presence of the depside methyl 2'-*O*-methylmicrophyllinate, which has been considered as almost confined to this species. However, Gowan (1989) distinguished two chemically similar species, *P. contraponenda* and *P. diversa*, on the basis of type of epihymenial pigment, apothecium size, and geographical distribution. *Porpidia diversa* was said to have a usually aeruginose epihymenial pigment and smaller apothecia than *P. contraponenda*. The thallus of both species was said to be 100–500  $\mu\text{m}$  thick, continuous or dispersed, and sometimes rimose-areolate, and the apothecia were described as soon becoming sessile. However, Gowan noted that the type of *P. contraponenda* (from Austria) had “an abnormally thick, rimose thallus”. Gowan & Ahti (1993) described the thallus of *P. contraponenda* from Finland as thin and verruculose to finely cracked, and the apothecia as sessile. They considered that *P. diversa* was not present in eastern Fennoscandia, on the basis of apothecial pigmentation. They noted that specimens chemically similar to Finnish *P. contraponenda* but with a thicker thallus and generally larger apothecia occur in the Alps and western North America. Fryday

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TABLE 1. Data for ITS and LSU alignments

	ITS1	5.8S	ITS2	LSU
Total number of aligned sites	212	160	171	1290
Number of ambiguous regions	12	1	7	17
Total length of ambiguous regions	82	1	42	5
Number of constant characters	43	143	43	1157
Number of variable, parsimony-uninformative characters	18	6	23	59
Number of variable, parsimony-informative characters	68	10	62	57
Model selected	TrN+G	K80+I	TrN+G	GTR+I+G

(2005) considered that the distinction of *P. contraponenda* and *P. diversa* on the basis of epihymenial pigmentation and apothecial size was untenable, and synonymised the two species. He noted that the holotype of *P. diversa* had a brown epihymenial pigment, and that the apothecia are immature. Fryday reported unidentified sorediate collections which also contained methyl 2'-*O*-methylmicrophyllinate. Fryday *et al.* (2009) described the apothecia of *P. contraponenda* as sessile.

In Great Britain, it has been known for at least 10 years that fertile non-sorediate specimens containing methyl 2'-*O*-methylmicrophyllinate belong to two separate species, differing in morphology and chemistry. More recently, DNA sequencing has supported the separation of the two species, which are described below. One is conspecific with *P. contraponenda sensu stricto*, but the other is apparently undescribed and is here described as the new species *P. irrigua*.

## Materials and Methods

Thin-layer chromatography was carried out using Solvent Systems A and G, using standard methods (Orange *et al.* 2010). Fragments of thallus and/or apothecia which were extracted with acetone for TLC were used for subsequent DNA analysis. Ascospore measurements are given as (min)-(x̄-SD)-x̄-(x̄+SD) (-max) where min. and max. are extreme values and x̄ the arithmetic means and SD the corresponding standard deviation.

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 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, LR3, nu-LSU-155-5' and LR7 (Döring *et al.* 2000, Gardes & Bruns 1993, Vilgalys & Hester 1990). 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) using Applied Biosystems Big-Dye Ver 3.1 chemistry on an Applied Biosystems model 3730 automated capillary DNA sequencer, or by Macrogen Inc.

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. The length of the alignments, and number of informative sites, are shown in Table 1.

Phylogenetic relationships and support values were investigated using a Bayesian approach. Additional support values were obtained 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 datasets were analysed separately: the ITS1-5.8S-ITS2 region (numerous specimens), and the ribosomal LSU (selected specimens only). The few relevant sequences of *Porpidia* in GenBank were included in the analyses. Models of evolution for the Bayesian analyses were selected using the Akaike Information Criterion (AIC) in MrModeltest 2.2 (Nylander 2004) (Table 1). Gaps were treated as missing data. Using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2005), two analyses of two parallel runs were carried out for 2 000 000 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 5 000 trees was discarded from each run, respectively. Support values of ≥95% Bayesian posterior probabilities and ≥70% Maximum Likelihood bootstrapping were regarded as significant.

Specimens used in analyses are shown in Table 2.

TABLE 2. Specimens used in the phylogenetic analyses of *Porpidia* species. New sequences are in **bold**

Species	Country	Voucher	GenBank accession number (and gene region)
<i>Bellemerea alpina</i>	–	<i>Hafellner</i> 39225 (GZU)	AF332116 (ITS)
<i>Cecidomia umbonella</i>	Sweden	<i>Buschbom</i> 21.08.2001-9b (F)	AY532990 (LSU)
<i>Lecidea atrobrunnea</i>	–	–	HQ650657 (ITS)
<i>Lecidea confluens</i>	Austria	<i>Tuerk</i> 39641 (hb. Tuerk)	EU263921 (ITS)
<i>L. fuscoatra</i>	–	<i>Arup</i> L02894	HQ650662 (ITS)
<i>L. lapicida</i> var. <i>lapicida</i>	Norway	<i>Orange</i> 19146 (NMW)	<b>KJ162333 (ITS)</b>
<i>L. lapicida</i> var. <i>pantherina</i>	–	<i>Hafellner</i> & <i>Hafellner</i> 42081 (GZU)	AF332119 (ITS)
<i>P. cf. austrosheilandica</i>	Kerguelen	<i>R. Poulsen</i> 547 (C)	AY532939 (LSU)
<i>P. cinereoatra</i>	Wales	<i>Orange</i> 20432 (NMW)	<b>KJ162305 (ITS-LSU)</b>
<i>P. cinereoatra</i>	Ireland	<i>Orange</i> 21426 (NMW)	<b>KJ162307 (ITS)</b>
<i>P. cinereoatra</i>	Ireland	<i>Orange</i> 21404 (NMW)	<b>KJ162306 (ITS)</b>
<i>P. cinereoatra</i>	Wales	<i>Orange</i> 21655 (NMW)	<b>KJ162308 (ITS)</b>
<i>P. contraponenda</i>	England	<i>Orange</i> 16217 (NMW)	<b>KJ162293 (ITS-LSU)</b>
<i>P. contraponenda</i>	England	<i>Orange</i> 16220 (NMW)	<b>KJ162296 (ITS-LSU)</b>
<i>P. contraponenda</i>	Wales	<i>Orange</i> 20447 (NMW)	<b>KJ162297 (ITS-LSU)</b>
<i>P. contraponenda</i>	Austria	<i>Orange</i> 21127 (NMW)	<b>KJ162298 (ITS)</b>
<i>P. crustulata</i>	Turkey	–	HQ605941 (ITS)
<i>P. crustulata</i>	Sweden	<i>J. Buschbom</i> 19.8.2001-2a (F)	AY532943 (LSU)
<i>P. diversa</i>	USA	<i>J. Buschbom</i> 2953 (F)	AY532944 (LSU)
<i>P. flavicunda</i>	Norway	<i>Orange</i> 18971 (NMW)	<b>KJ162332 (ITS)</b>
<i>P. flavocruenta</i>	Norway	<i>Orange</i> 17051 (NMW)	<b>KJ162273 (ITS)</b>
<i>P. flavocruenta</i>	Norway	<i>Orange</i> 18941 (NMW)	<b>KJ162275 (ITS)</b>
<i>P. flavocruenta</i>	Norway	<i>Orange</i> 19236 (NMW)	<b>KJ162274 (ITS)</b>
<i>P. flavocruenta</i>	Sweden	<i>J. Buschbom</i> 25.08.2001-41 (F)	AY532959 (LSU)
<i>P. hydrophila</i>	England	<i>Orange</i> 16218 (NMW)	<b>KJ162317 (ITS)</b>
<i>P. hydrophila</i>	Wales	<i>Orange</i> 16313 (NMW)	<b>KJ162318 (ITS)</b>
<i>P. hydrophila</i>	Wales	<i>Orange</i> 17598 (NMW)	<b>KJ162319 (ITS)</b>
<i>P. irrigua</i>	Norway	<i>Ekman</i> 3183 (NMW)	AF429267 (ITS)
<i>P. irrigua</i>	Wales	<i>Orange</i> 16321 (NMW)	<b>KJ162299 (ITS-LSU)</b>
<i>P. irrigua</i>	Wales	<i>Orange</i> 16494 (NMW)	<b>KJ162301 (ITS)</b>
<i>P. irrigua</i>	Wales	<i>Orange</i> 17372 (NMW)	<b>KJ162334 (LSU)</b>
<i>P. irrigua</i>	Wales	<i>Orange</i> 18014 (NMW)	<b>KJ162302 (ITS)</b>
<i>P. irrigua</i>	Wales	<i>Orange</i> 20448 (NMW)	<b>KJ162303 (ITS-LSU)</b>
<i>P. irrigua</i>	Wales	<i>Orange</i> 20712 (NMW)	<b>KJ162300 (ITS)</b>
<i>P. islandica</i>	Faroe Islands	<i>Orange</i> 17148 (NMW)	<b>KJ162313 (ITS)</b>
<i>P. lowiana</i>	Finland	<i>J. Buschbom</i> 31.8.2001-14 (F)	AY532952 (LSU)
<i>P. lowiana</i>	Finland	<i>J. Buschbom</i> 30.8.2001-1 (F)	AY532941 (LSU)
<i>P. macrocarpa</i>	Turkey	–	HQ605940 (ITS)
<i>P. macrocarpa</i>	Finland	<i>J. Buschbom</i> 30.8.2001-12 (F)	AY532953 (LSU)
<i>P. macrocarpa</i>	Sweden	<i>J. Buschbom</i> 4.09.2001-1 (F)	AY532964 (LSU)
<i>P. macrocarpa</i>	England	<i>Orange</i> 16216 (NMW)	<b>KJ162266 (ITS)</b>
<i>P. macrocarpa</i>	England	<i>Orange</i> 16225a (NMW)	<b>KJ162267 (ITS)</b>
<i>P. macrocarpa</i>	Wales	<i>Orange</i> 16319 (NMW)	<b>KJ162270 (ITS)</b>
<i>P. macrocarpa</i>	Wales	<i>Orange</i> 16490 (NMW)	<b>KJ162271 (ITS)</b>
<i>P. macrocarpa</i>	Ireland	<i>Orange</i> 17906 (NMW)	<b>KJ162272 (ITS)</b>
<i>P. macrocarpa</i>	Scotland	<i>Orange</i> 21043 (NMW)	<b>KJ162269 (ITS)</b>
<i>P. melinodes</i>	Wales	<i>Orange</i> 17370 (NMW)	<b>KJ162324 (ITS)</b>
<i>P. melinodes</i>	Wales	<i>Orange</i> 17603 (NMW)	<b>KJ162323 (ITS)</b>
<i>P. melinodes</i>	Scotland	<i>Orange</i> 17636 (NMW)	<b>KJ162325 (ITS)</b>
<i>Porpidia melinodes</i>	Norway	<i>Orange</i> 18967 (NMW)	<b>KJ162326 (ITS)</b>
<i>P. melinodes</i>	Norway	<i>Orange</i> 19209 (NMW)	<b>KJ162327 (ITS)</b>
<i>P. melinodes</i>	Norway	<i>Orange</i> 19212 (NMW)	<b>KJ162328 (ITS)</b>
<i>P. melinodes</i>	Norway	<i>Orange</i> 19214 (NMW)	<b>KJ162329 (ITS)</b>
<i>P. melinodes</i>	Norway	<i>Orange</i> 19230 (NMW)	<b>KJ162330 (ITS)</b>
<i>P. melinodes</i>	Norway	<i>Orange</i> 19234 (NMW)	<b>KJ162331 (ITS)</b>
<i>P. musiva</i>	Turkey	–	HQ605939 (ITS)

TABLE 2. *Continued*

Species	Country	Voucher	GenBank accession number (and gene region)
<i>P. rugosa</i>	Faroe Islands	Orange 17159 (NMW)	<b>KJ162320 (ITS)</b>
<i>P. rugosa</i>	Ireland	Orange 21403 (NMW)	<b>KJ162321 (ITS)</b>
<i>P. soredizodes</i>	Great Britain	<i>B. Coppins</i> 21.8.2001 (F)	AY532965 (LSU)
<i>P. striata</i>	England	Orange 16227 (NMW)	<b>KJ162314 (ITS)</b>
<i>P. striata</i>	Wales	Orange 16320 (NMW)	<b>KJ162315 (ITS)</b>
<i>P. striata</i>	Wales	Orange 17584 (NMW)	<b>KJ162316 (ITS)</b>
<i>P. superba</i>	Canada	<i>f. Buschbom</i> 16.7.2000-64 (F)	AY532972 (LSU)
<i>P. tuberculosa</i>	Ireland	Orange 18291 (NMW)	<b>KJ162322 (ITS)</b>
<i>P. zeoroides</i>	France	<i>V. Reeb</i> VR 9-VII-98/38 (F)	AY532976 (LSU)
<i>Porpidia</i> sp.	USA	<i>Cagle</i> 000810-2 (WTU)	AY532942 (LSU)
<i>Porpidia</i> sp.	USA	<i>Harris</i> 52962 (NY 01518605)	<b>KJ162312 (ITS)</b>
<i>Porpidia</i> sp.	USA	<i>Harris</i> 55558 (NY 01103898)	<b>KJ162311 (ITS)</b>
<i>Porpidia</i> sp.	Austria	<i>f. Buschbom</i> 14.9.2001-10	AY532968 (LSU)
<i>Porpidia</i> sp.	Austria	<i>f. Buschbom</i> 14.9.2001-9 (F)	AY532967 (LSU)
<i>Porpidia</i> sp.	Sweden	<i>f. Buschbom</i> 26.8.2001-9	AY532969 (LSU)
<i>Porpidia</i> sp.	USA	<i>f. Buschbom</i> 3161 (F)	AY532966 (LSU)
<i>Porpidia</i> sp.	USA	<i>Lendemer</i> 29720 (NY 01228628)	<b>KJ162304 (ITS)</b>
<i>Porpidia</i> sp.	England	Orange 16225b (NMW)	<b>KJ162268 (ITS)</b>
<i>Porpidia</i> sp.	Faroe Islands	Orange 17149 (NMW)	<b>KJ162294 (ITS)</b>
<i>Porpidia</i> sp.	Faroe Islands	Orange 17168 (NMW)	<b>KJ162281 (ITS)</b>
<i>Porpidia</i> sp.	Faroe Islands	Orange 17174 (NMW)	<b>KJ162295 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 18914 (NMW)	<b>KJ162276 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 18926 (NMW)	<b>KJ162288 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 18945 (NMW)	<b>KJ162278 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 18951 (NMW)	<b>KJ162280 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 18972 (NMW)	<b>KJ162286 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 18981 (NMW)	<b>KJ162291 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 19067 (NMW)	<b>KJ162277 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 19086 (NMW)	<b>KJ162287 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 19095 (NMW)	<b>KJ162289 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 19096 (NMW)	<b>KJ162292 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 19097 (NMW)	<b>KJ162290 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 19098 (NMW)	<b>KJ162279 (ITS)</b>
<i>Porpidia</i> sp.	Norway	Orange 19347 (NMW)	<b>KJ162282 (ITS)</b>
<i>Porpidia</i> sp.	Ireland	Orange 21414 (NMW)	<b>KJ162283 (ITS)</b>
<i>Porpidia</i> sp.	Ireland	Orange 21416 (NMW)	<b>KJ162284 (ITS)</b>
<i>Porpidia</i> sp.	Ireland	Orange 21417 (NMW)	<b>KJ162285 (ITS)</b>
<i>Porpidia</i> sp.	USA	<i>Tripp</i> 3453 (NY 1685056)	<b>KJ162310 (ITS)</b>
<i>Porpidia</i> sp.	USA	<i>Tripp</i> 3688 (NY 1684837)	<b>KJ162309 (ITS)</b>
<i>Porpidia</i> sp.	Austria	<i>Tuerk</i> 39740	EU263923 (ITS)

## Results

### Thin-layer chromatography

The following substances were detected in material of *P. contraponenda* and *P. irrigua* (numbered as in Fig. 1):

1. Unknown A: spot almost colourless, UV<sup>-</sup>, UV<sup>+</sup> blue-violet; trace amounts in *P.*

*contraponenda* and *P. irrigua*, not always present.

2. Unknown B: colourless spot, UV<sup>-</sup>, after heating UV<sup>-</sup> (greyish); usually a major compound in *P. contraponenda*, absent or in trace amounts in *P. irrigua*.
3. Methyl 2'-O-methylmicrophyllinate: spot almost colourless, UV<sup>-</sup>, after heating UV<sup>+</sup> blue-violet; a major compound in *P. contraponenda* and *P. irrigua*.

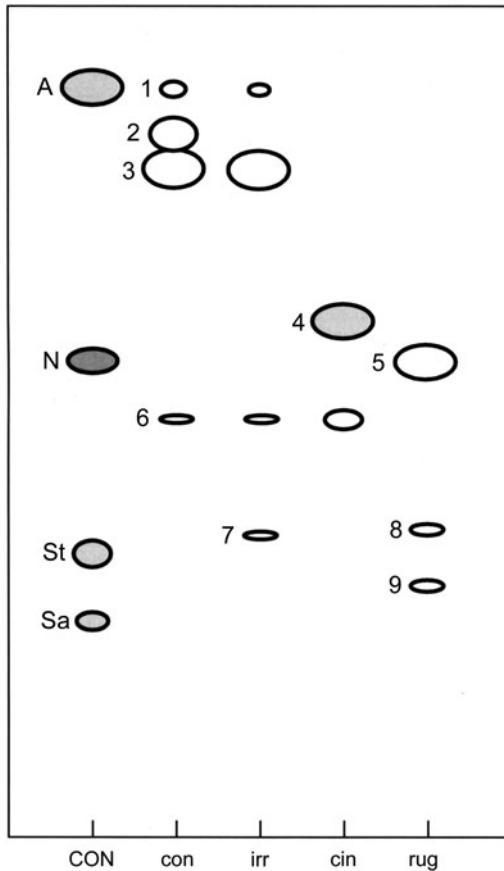


FIG. 1. Chromatogram of selected species of *Porpidia* in Solvent system G (diagrammatic). Samples: CON = controls; con = *P. contraponenda*; cin = *P. cinereoatra*; irr = *P. irrigua*; rug = *P. rugosa*. Substances: A = atranorin, N = norstictic acid, Sa = salazinic acid, St = stictic acid, 1 = unknown A, 2 = unknown B, 3 = methyl 2'-*O*-methylmicrophyllinate, 4 = confluent acid, 5 = 2'-*O*-methylsuperphyllinic acid, 6 = 2'-*O*-methylmicrophyllinic acid, 7–9 = unidentified.

6. 2'-*O*-methylmicrophyllinic acid: spot almost colourless, UV–, after heating UV+ blue-violet; trace amounts in *P. contraponenda* and *P. irrigua*
7. Unknown; trace amounts in *P. irrigua*.

In addition, the two species share other unidentified compounds in trace amounts (not shown in Fig. 1). Unknown B is usually

clearly present in British material of *P. contraponenda* as a major compound, and is absent or in trace amounts in *P. irrigua*. It was present in the two syntypes of *P. contraponenda* examined from BM and M respectively, but only in small quantities. However, only very small samples of these specimens could be used for TLC, so the true concentration in these specimens may be higher than suggested by the TLC plate. Unknown B was present as a major compound in the epitype and topotypes of *P. contraponenda*, collected in 2012. Unknown A may be overlooked until the plate has been heated, when it is visible by its UV fluorescence.

The compounds above are readily separated in Solvent System G. However, Solvent System A does not separate Unknown B and methyl 2'-*O*-methylmicrophyllinate, and thus is not suitable for differentiation of the two species.

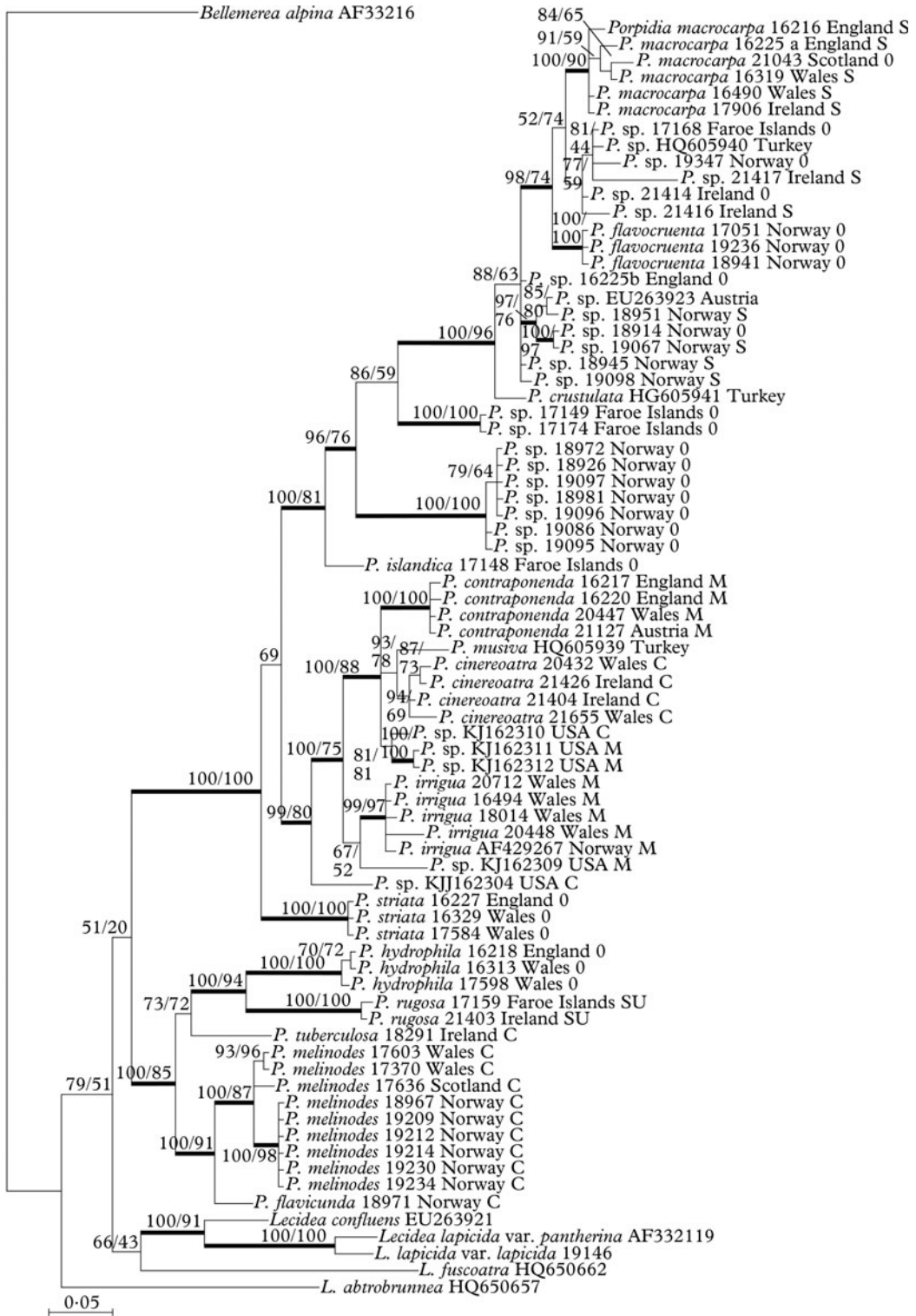
### ITS analysis

The aligned ITS1–5.8S–ITS2 region comprised 543 sites, of which 125 were removed as ambiguous; 140 sites were parsimony-informative (Table 1).

The ITS tree shows (among others) four well-supported clades, one of which (Clade A) consists of two, well-supported subgroups (Fig. 2):

A. The *Porpidia macrocarpa* and *P. cinereoatra* subgroups, together corresponding to Group I of Buschbom & Mueller (2004). *Porpidia striata* is basal to these two subgroups, and they and *P. striata* together form a well-supported clade.

A1: *Porpidia macrocarpa*, *P. flavocruenta*, *P. crustulata*, *P. islandica*, and at least three well-supported clades representing unidentified or undescribed species. For instance, seven specimens forming a well-supported clade are united by a uniformly rusty-coloured thallus, lack of lichen substances, and occurrence near water in northern Norway.



A2: Specimens containing methyl 2'-*O*-methylmicrophyllinate form three well-supported clades, which correlate with features of the morphology and chemistry of the specimens. The clades are attributed here to *Porpidia contraponenda sensu stricto*, the new species *P. irrigua*, and an unidentified taxon from North America, originally named as *P. contraponenda*. In the voucher specimens of the unidentified North American taxon (Harris 55558 [NY – 01103898] and Harris 52962 [NY – 01518605]) the apothecia are small, 0.50–1.14 mm diameter, and semi-immersed to sessile; the ascospores are 14–18 µm in length, and the presence of methyl 2'-*O*-methylmicrophyllinate and Unknown A was confirmed by TLC. Four sequences of *P. cinereoatra* from Wales and Ireland, and one sequence of *P. musiva* from Turkey also form a clade, but without strong support.

- B. *Porpidia hydrophila* and *P. rugosa*; the latter formed part of Group III of Buschbom & Mueller, *P. hydrophila* was not included in their analyses but Fryday (2005) considered it to belong to the same infra-generic group as *P. rugosa*.
- C. *Porpidia flavicunda* and *P. melinodes*, corresponding to Group IV of Buschbom & Mueller.
- D. *Lecidea confluens* and *L. lapicida*, corresponding to Group II of Buschbom & Mueller.

The position of *P. tuberculosa* (Group IV) and *Lecidea fuscoatra* and *L. atrobrunnea* (Group II) was not resolved.

## LSU analysis

The LSU tree is shown in Fig. 3. Specimens containing methyl 2'-*O*-methylmicrophyllinate occurred in four clades; two clades represent *P. contraponenda* and *P. irrigua* respectively, and the other two comprise a single sequence each, originating from North American specimens. One is basal to *P. contraponenda* (Fig. 3), the other is basal to *P. lowiana* (Fig. 3). The voucher specimen (Buschbom 2953; originally identified as *P. diversa*) is not present in F and could not be traced. The voucher specimen (Cagle 00810-2 [WTU L-19693], originally identified as *P. contraponenda*) has sessile apothecia 1.1–2.0 mm diameter and ascospores 19–21 µm long, and much resembles *P. irrigua* in appearance; it contained methyl 2'-*O*-methylmicrophyllinate and Unknown A by TLC.

## The Species

### *Porpidia contraponenda* (Arnold) Knoph & Hertel

In Hertel & Knoph, *Mitt. Bot. Staatssamml. München* 20: 477 (1984).—*Lecidea contraponenda* Arnold, *Verh. K. K. zool.-bot. Gesellsch. Wien* 36: 79 (1886); type: [Austria] Auf kleinen Gneissblöcken am Fusswege zwischen Kühtai [Kühtai] und den Finsterthaler Seen, Tirol, 16 Juli 1884, F.C.G. Arnold (Arnold, *Lich. exs.* 1055) (BM 764657 – syntype!; M – 0025569 & 0024172 – syntypes!); epitype (selected here): Austria, Tyrol, Kühtai, Finstertal, 47°12.43'N, 11°01.44'E, alt. 2025 m, on stones in flush, 11 August 2012, A. Orange 21126 (NMW – C.2012.002.116).

(Figs 4, 6A)

*Prothallus* black, usually inconspicuous but sometimes extensive. *Thallus* white to pale

FIG. 2. Phylogenetic relationships amongst *Porpidia* species, based on a Bayesian analysis of the nuclear ribosomal ITS1-5.8S-ITS2 region. The tree was rooted using *Bellemeria alpina*. The two support values 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%. If a node of the Bayesian tree was not recovered by ML bootstrapping, the ML value is replaced by a dash. Chemosyndromes of specimens (where known) are indicated: C = confluent acid, M = 2'-*O*-methylmicrophyllinate, S = stictic acid, SU = 2'-*O*-methylsuperphyllinic acid, 0 = no depside or depsidone secondary products.

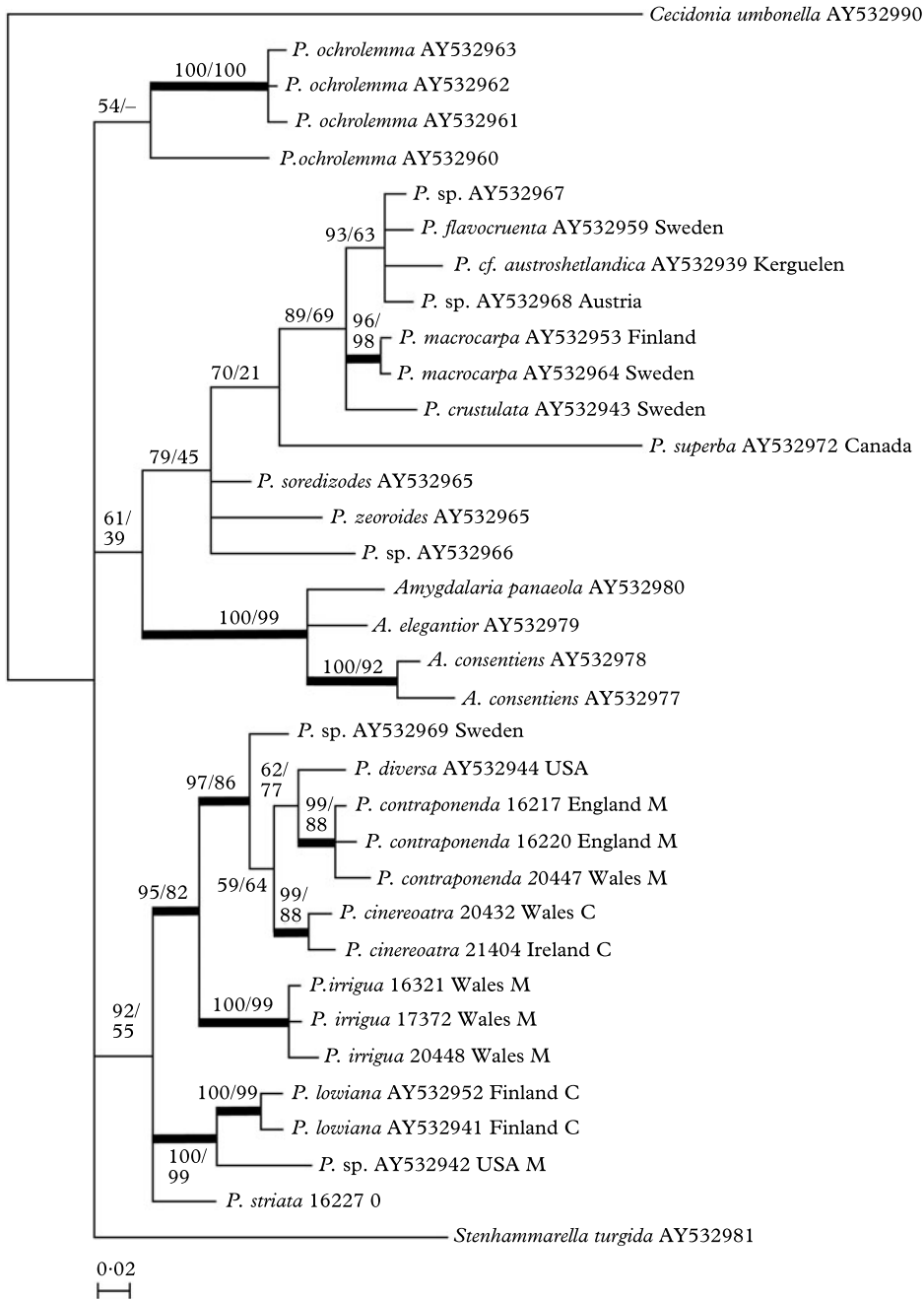


FIG. 3. Phylogenetic relationships amongst *Porpidia* species, based on a Bayesian analysis of the nuclear ribosomal LSU region. The tree was rooted using *Cecidonia umbonella*. The two support values associated with each branch are posterior probabilities (PP) and maximum likelihood bootstrap (MLb) values, respectively. Branches in bold indicate a support of PP  $\geq 95\%$  and MLb  $\geq 70\%$ . If a node of the Bayesian tree was not recovered by ML bootstrapping, the ML value is replaced by a dash. Chemosyndromes of specimens (where known) are indicated: C = confluent acid, M = 2'-O-methylmichrophyllinate, O = no depside or depsidone secondary products.



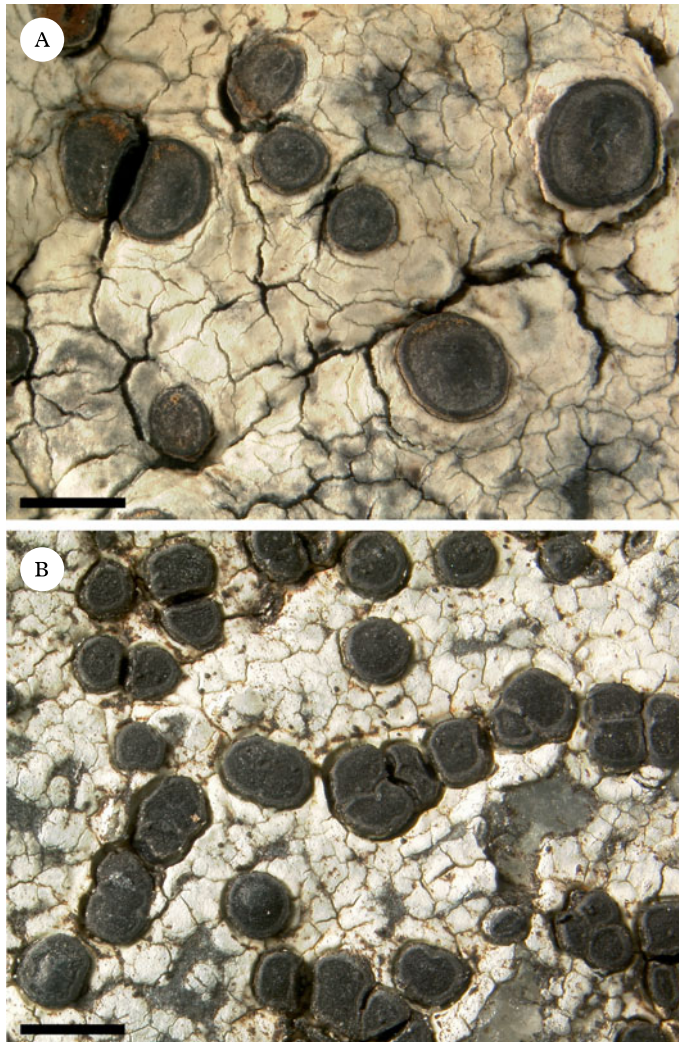


FIG. 4. *Porpidia contraponenda*; A, Orange 16228, note thick thallus and mostly immersed apothecia; B, Orange 21126 (epitype), note moderately thick thallus and semi-immersed apothecia. Scales: A & B = 1 mm. In colour online.

cream, sometimes locally blue-grey, rarely tinged rusty orange; young areoles arising on the prothallus (when visible), soon coalescing and forming a cracked crust 100–800  $\mu\text{m}$  thick; low rugose galls caused by *Cecidonia xenophana* frequently present.

*Apothecia* half-immersed to completely immersed when young, when mature usually one quarter to  $\pm$  completely immersed in thallus, occasionally more or less sessile, at least in thin areas of thallus; up to 1.6 mm

diameter; margin 80–160  $\mu\text{m}$  wide, smooth, sometimes flattened when young; disc slightly concave to plane or convex; pruina often present on young disc and on inner edge of young margin, present or absent on mature disc. *Exciple* at surface with dense pigment which is dull greenish to dull brown, sometimes with bluish tinges, brown within, translucent to opaque in sections 10–20  $\mu\text{m}$  thick, hyphae 3.7–4.1  $\mu\text{m}$  diameter. *Hypothecium* brown to reddish brown. *Hymenium* 100–170  $\mu\text{m}$  high.

*Epithymenium* dull brown to dull greenish brown, K–. *Paraphyses* 1.2–1.6 µm wide in centre of hymenium, widening to 3.7 µm above, tip with colourless to brown wall, but most pigment extracellular. *Ascospores* simple, colourless, ellipsoid, halonate, (14.0–)15.5–17.0–18.5(–20.5) × (8.0–)8.5–9.4–10.5(–13.0) µm, (1.2–)1.6–1.8–2.1(–2.3) times as long as wide (30 spores measured, from 6 specimens).

*Pycnidia* occasionally present. *Conidia* not seen.

**Chemistry.** Methyl 2'-*O*-methylmicrophyllinate (major), Unknown B (major), Unknown A (trace, ±), 2'-*O*-methylmicrophyllinic acid (trace) (Fig. 1). Unknown B is readily seen under short-wave UV on TLC plates, but Unknown A is present only in trace amounts and may be detectable only by its fluorescence in long-wave UV after heating the plate. Thallus K–, PD–, C–; microscopic preparations in K not exuding minute droplets. Solvent System G is recommended for separating the major compounds, which are not separated in Solvent System A.

**Ecology and distribution.** In Great Britain the species is known from damp siliceous rock, including flushed rock faces, boulders, fine scree, and stones in snow beds, at altitudes of 100–1140 m. Numerous associated species are recorded, including the lichens *Amygdalaria consentiens*, *Ionaspis lacustris*, *I. odora*, *Pilophorus strumaticus*, *Porpidia macrocarpa*, *P. tuberculosa*, *Rhizocarpon hochstetteri*, *R. lavatum* and *R. sublavatum*, and the bryophytes *Andreaea alpina*, *A. rothii* subsp. *falcata*, *A. rupestris*, *Kiaeria falcata*, *Marsupella adusta*, *M. emarginata* and *Racomitrium sudeticum*. Two collections were also associated with *Porpidia irrigua*. Examples of relevés containing *P. contraaponenda* are shown in Table 3. At the type locality, visited in 2012, the species occurred on stones on damp ground in flushes. It is known from North Wales, the English Lake District, western Scotland, and from Austria. Other records are uncertain due to confusion with *P. irrigua*.

**Typification.** There are two specimens of Arnold, *Lichenes exsiccati* 1055 in F.C.G. Arnold's herbarium in M. The specimen numbered M0025569 was annotated as 'holotype' by H. Hertel in 1969, who was probably unaware of the second specimen. The specimen might be better regarded as a lectotype, but this is not proposed here as there is no evidence that the exsiccata is heterogeneous. M0025569 has a well-developed whitish to pale grey thallus; the young apothecia are at most half-immersed, later immersed only at base, and no more than one-quarter immersed when mature. M0024172 comprises one fragment with a thinner thallus than M0025569 with more black prothallus visible, and a second fragment with a well-developed thallus. The syntype in BM has the same details as the exsiccati specimens, but the label is handwritten. This specimen and M0025569 contain the unknown substance above methyl 2'-*O*-methylmicrophyllinate (unknown B) on TLC plates. Several specimens (topotypes) collected from the type locality in 2012 agree with the syntypes in morphology and chemistry, and one is proposed here as an epitype.

**Notes:** There is considerable variation in the thallus thickness; specimens from apparently more extreme environments tend to have a thinner thallus and consequently more prominent apothecia, also a more extensive prothallus and more blue-grey thallus pigment. In some British specimens the apothecia are more or less immersed in the thallus (Fig. 4A) and thus strikingly different to *P. irrigua*, but in others they are more prominent and more closely resemble the type of *P. contraaponenda*, for instance Orange 14499 (Fig. 4B). Although *Cecidonia* galls are frequent, they tend to be lower and less conspicuous than in *P. irrigua*.

**Selected specimens examined.** **Great Britain:** *Wales:* V.C. 48, Merioneth, Cader Idris, near Llyn y Gafr [23/71.14], 1960, Wade (NMW – 60.494.107); Cadair Idris, near Llyn Arran, 23/7424.1420, 2003, Orange 14611 (NMW – C.2005.001.413). V.C. 49, Caernarvonshire, Nantgwynant, Afon Cwm Llan, 23/6267.5142, 2011, Orange 20447 (NMW – C.2011.014.9). V.C. 50, Denbighshire, Llanarmon, above Swch-cae-rhiw, [33/13.35], 1938, W. Watson (NMW – 38.859.15). *England:*

TABLE 3. Community ecology of *Porpidia contraponenda*. Examples of relevés from Great Britain.

	Relevé number															
	2005– 184	2005– 187	2004– 16	2005– 169	2003– 280	2006– 44	2003– 489	2003– 186	2003– 217	2003– 220	2003– 223	2003– 228	2003– 230	2003– 231	2003– 234	2003– 233
<i>Porpidia contraponenda</i>	5	6	4	4	5	4	5	5	5	4	4	5	5	5	6	5
<i>Rhizocarpon lavatum</i>	1	2		7	6				5	6	5		4	1	1	1
<i>Ionaspis odora</i>				5	5	4			1	5	4	4	4	4	6	
<i>Racomitrium sudeticum</i>			5		2	2	4	2	5	1	4	5	5	1		
<i>Andreaea rupestris</i>				4	1	2	3	4		1	1	2	1	4		2
<i>Rhizocarpon hochstetteri</i>				2	1	2	2	2		1	1	5			1	1
<i>Porpidia tuberculosa</i>	4	2		4				1			4				1	4
<i>Stereocaulon vesuvianum</i>							5		2			4	5	2	2	
<i>Andreaea alpina</i>			4							1	1	2				6
<i>Marsupella adusta</i>								3		1		2	3	4		
<i>Amygdalaria consentiens</i>			1		1	5					5					
<i>Kiaeria falcata</i>								5		4	1		1			
<i>Marsupella alpina</i>			4				1								5	5
<i>Porpidia macrocarpa</i>	1		1			4		5								
<i>Racomitrium lamuginosum</i>							1							1	2	
<i>Rhizocarpon sublavatum</i>						5				6	2					
<i>Ionaspis lacustris</i>					1	1										
<i>Andreaea rothii</i>		6		5												
<i>Campylopus atrovirens</i>			6												4	
<i>Porpidia striata</i>	6										4		5			
<i>Marsupella emarginata</i>								2								
<i>Lecidea lithophila</i>				1									5			
<i>Racomitrium aquaticum</i>	4		1													
<i>Protothelenella corrosa</i>															6	
<i>Porpidia lowiana</i>					5											
<i>Miriquidica griseoatra</i>							4									
<i>Miriquidica leucophaea</i>							4									
<i>Porpidia superba</i>		4														
rock	5	6	4	4	2	4	?	5	4	4	4	3	2	5	4	4
altitude	695	725	685	940	890	990	1145	890	880	935	930	780	790	800	800	820
vice-county	69	69	69	70	88	88	97	98	98	98	98	98	98	98	98	98
grid ref.	35/ 3428.1563	35/ 3429.1559	35/ 3449.1389	35/ 2167.0724	27/ 2681.2646	27/ 4151.3761	27/ 1905.7413	27/ 1360.5466	27/ 1420.5456	27/ 1412.5448	27/ 1415.5447	27/ 1428.5475	27/ 1426.5473	27/ 1424.5472	27/ 1422.5476	27/ 1419.5474
date	14 Sep 2005	14 Sep 2005	19 Apr 2004	12 Sep 2005	21 Jun 2003	9 Jun 2006	17 Sep 2003	19 May 2003	22 May 2003	22 May 2003	22 May 2003	23 May 2003	23 May 2003	23 May 2003	23 May 2003	23 May 2003

Relevés recorded on stands homogeneous in terms of vegetation. Cover expressed by the Domin scale. Species listed in order of frequency and cover; those recorded only as Domin 1, 2 or 3 omitted from table.

**V.C. 69**, Westmorland, Helvellyn, Brown Cove, 35/3428.1563, 2005, *Orange* 16224 (NMW – C.2005.001.305). **V.C. 70**, Cumberland, rocks by Stickle Beck below the Tarn, [35/28.07], 1916, *Wheldon* (NMW – 25.146.4776); near Eskdale, Lingcove Beck, 35/2356.0453, 2005, *Orange* 16217 (NMW – C.2005.001.301); near Eskdale, Lingcove Beck. 35/2340.0437, 2005, *Orange* 16220 (NMW – C.2005.001.304); Hopedale Head, Hobcarton Crags, 35/1886.2209, 2004, *Orange* 15490 (NMW – C.2005.001.632). *Scotland*: **V.C. 88**, Mid-Perthshire, Beinn Heagsarnich, NW of Stob an Fhir-Bhogha, 27/4151.3761, 2006, *Orange* 16545 (NMW – C.2005.001.583). **V.C. 97**, Westernness, Ben Nevis, Coire na h-Urchaire, 27/1509.7159, 2003, *Orange* 14829 (NMW – C.2004.002.373); Ben Nevis, Coire Leis, 27/1719.7167, 2003, *Orange* 15323 (NMW – C.2004.002.389). **V.C. 98**, Main Argyll, Glen Coe, Coire nam Beitheach, 27/1360.5466, 2003, *Orange* 14499 (NMW – C.2004.002.162).—**Austria**: *Tyrol*: Kühltal, Finstertal, 47°12.48'N, 11°01.43'E, alt. 2025 m, 11 August 2012, *Orange* 21123 (NMW – C.2012.002.115); same locality and date, 47°12.43'N, 11°01.44'E, alt. 2025 m, *Orange* 21127 (NMW – C.2012.002.117); same locality and date, *Orange* 21129 (NMW – C.2012.002.118).

### **Porpidia irrigua Orange sp. nov.**

MycoBank no: MB807961

Thallus white or pale grey, apothecia sessile, to 2 mm diam., ascospores 15–21.5 µm long, containing 2'-O-methylmicrophyllinate as the only major compound.

Type: Great Britain, Wales, Breconshire, Glyntawe, Nant y Llyn, national grid reference 22/8452.2068, 51°52.34'N, 3°40.65'W, altitude 495 m, 8 November 2011, on Old Red Sandstone, on unshaded, flushed, gently sloping bedrock in *Nardus*-rich grassland, with *Rhizocarpon lavatum*, *Andreaea rothii* subsp. *falcata*, *A. Orange* 20712 (NMW – C.2013.001.218 – holotype, MSC – isotype; GenBank accession no: KJ162300).

(Figs 5, 6B)

*Prothallus* blue-black to black. *Thallus* white or pale grey, occasionally light blue-grey, areoles arising on prothallus when visible, soon coalescing and cracking; thallus more or less plane, or gently convex between cracks, 100–400 µm thick; very frequently with raised rugose galls caused by *Cecidonia xenophana*.

*Apothecia* sessile when mature, becoming sessile as soon as young disc begins to expand; up to 2.0 mm diameter, margin smooth, rarely faintly striate, 60–220 µm wide, eventually ± excluded in old apothecia; disc plane to gently to strongly convex when mature; pruina sometimes present on inner edge of

young apothecial margin, present or absent on mature disc. *Exciple* densely pigmented at surface, brown to dull green-brown or dark dull greenish blue, within paler, brown throughout; hyphae 3.5–8.0(–12) µm wide. *Hypothecium* brown to red-brown. *Hymenium* 100–140 µm high. *Epihymenium* dull green-brown or brown, K–. *Paraphyses* c. 1.5–2.0 µm wide in centre of hymenium, tips 2.9–3.3 µm wide, walls colourless or dilute brown, surrounded by pigment (epihymenial pigment mostly extracellular). *Asci* clavate, 8-spored, tholus with an I+ blue tube-structure. *Ascospores* ellipsoid, simple, colourless, halonate, (15.0–)16.5–18.3–20.5(–21.5) × (7.5–)8.0–9.3–10.5(–12.5) µm, (1.4–)1.7–2.0–2.2(–2.5) times as long as wide (33 spores measured, from 7 specimens).

*Pycnidia* frequent; up to 560 µm diam. in surface view, multilocular, with several ostioles, apex uneven, black. *Conidia* simple, colourless, straight, 8–11 × 0.8 µm.

*Chemistry*. Methyl 2'-O-methylmicrophyllinate (major), Unknown A (±, trace), 2'-O-methylmicrophyllinic acid (trace), unknown (trace) (Fig. 1). Thallus K–, PD–, C–; microscopic preparations in K not exuding minute droplets.

*Ecology and distribution*. On damp siliceous rock, frequently where seasonally flushed, on bedrock and boulders, occasionally on fine scree but usually only in small quantities, at altitudes of 215–950 m. Associated species include *Cladonia diversa*, *C. subcervicornis*, *Ephebe lanata*, *Pilophorus strumaticus*, *Porpidia tuberculosa*, *Rhizocarpon hochstetteri*, *R. lavatum* and the bryophytes *Andreaea alpina*, *A. rothii*, *A. rupestris* and *Racomitrium sudeticum*. *Andreaea rothii* ssp. *falcata* is a particularly frequent associate on seasonally flushed surfaces. The species is apparently more tolerant of seasonal drought than *P. contraponenda*. Examples of relevés containing *P. irrigua* are shown in Table 4. Although the known distribution of the two species is quite similar, *P. irrigua* is the more frequent in southerly and lowland sites. The two can be found growing together in the same community. Recorded from North and South Wales,

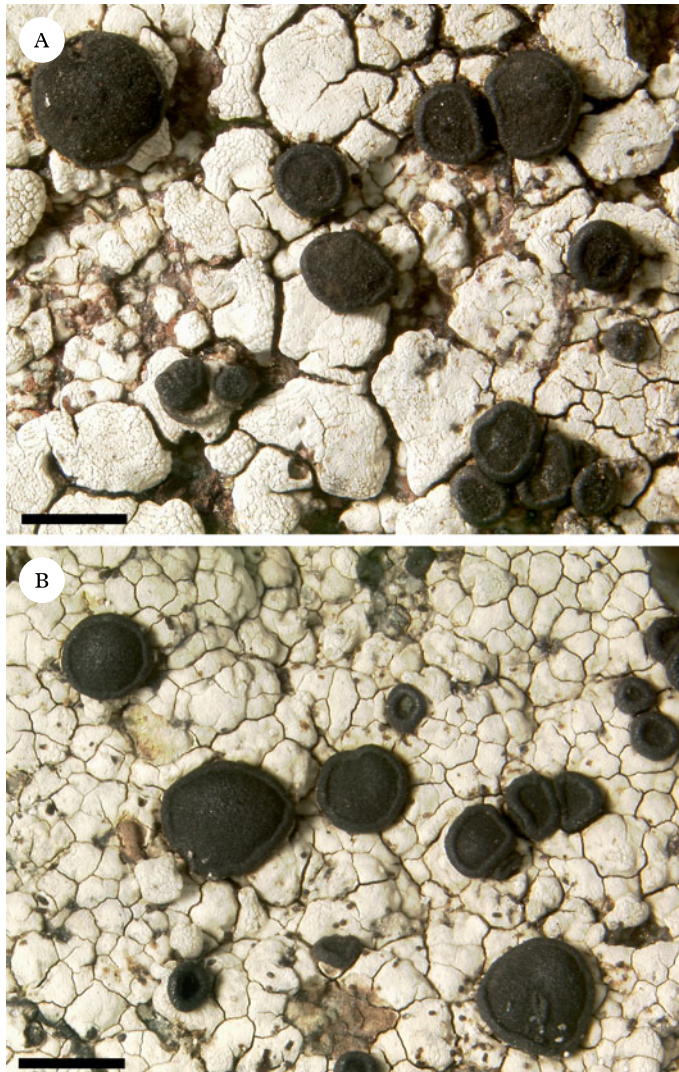


FIG. 5. *Porpidia irrigua*; A, holotype, note sessile apothecia and rugose galls of *Cecidonia*; B, Orange 17372. Scales: A & B = 1 mm. In colour online.

North-west England, West and Central Scotland, and Norway. Distribution outside Britain poorly known due to confusion with *P. contraponenda*.

*Notes.* *Porpidia irrigua* differs from *P. contraponenda* in the generally thinner thallus (although the ranges of thickness overlap greatly), the apothecia which are sessile from a very early stage, and methyl 2'-*O*-methyl-

microphyllinate as the only major compound. In contrast, the apothecia in *P. contraponenda* are at least partly immersed in the thallus, and in some specimens they are completely immersed; in addition, there is a second unidentified depside as a major compound in addition to methyl 2'-*O*-methylmicrophyllinate.

Besides *P. contraponenda*, the only previously described species in the genus which contains 2'-*O*-methylmicrophyllinate is *P.*

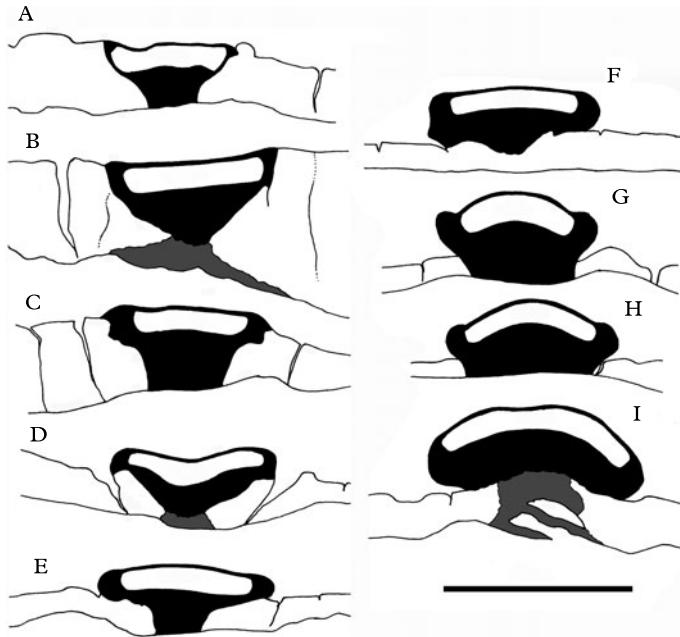


FIG. 6. *Porpidia* species, sections of thalli and apothecia (semi-diagrammatic); A – E, *P. contraponenda* (A, Orange 15490; B & C, Orange 20447; D & E, Orange 14499); F – I, *P. irrigua* (F, Orange 16494; G, Orange 12542; H, Orange 17372; I, Orange 15772) Scale = 1 mm.

*diversa* (Lowe) Gowan, described from eastern North America. This species was regarded as a synonym of *P. contraponenda* by Fryday (2005). Specimens with a similar morphology and chemistry to *P. irrigua* occur in North America, but cannot currently be identified (see discussion).

*Selected specimens examined.* **Great Britain:** *Wales:* V.C. 41, Glamorgan, Rhondda Fach, Ynyshir, Mynydd Troed-y-rhiw, 31/012.934, 2006, Orange 16494 (NMW – C.2005.001.542). V.C. 42, Breconshire, Brecon Beacons, Corn Du, alt. 860 m, 32/0079.2130, 2002, Orange 13737 (NMW – C.2001.024.570); Glyntawe, Nant y Llyn, 22/8452.2068, alt. 495 m, 2005, Orange 16321 (NMW – C.2005.001.311; topotype). V.C. 43, Radnorshire, near Llanelwedd, Carneddau, Caer Fawr, [32/05.53], 1959, Wade (NMW – 60.139.122). V.C. 45, Pembrokeshire, near Fishguard, Newport, Mynydd Carn Ingli, Carn Ingli, 22/0644.3733, 2007, Orange 17372 (NMW – C.2007.001.143). V.C. 48, Merioneth, north-west of Dinas Mawddwy, Nant y Graig-wen, 23/828.175, 1998, Orange 12144 (NMW – C1999.011.72); Ganllwyd, Gwynfynydd, 23/7336.2802, 2009, Orange 18014 (NMW – C.2010.001.22). V.C. 49, Caernarvonshire, Nantgwynant, Afon Cwm Llan, 23/6235.5159, 2011, Orange 20448 (NMW – C.2011.014.10). *England:* V.C. 69, Westmorland, Teesdale, Falcon Clints,

35/8175.2840, 2005, Orange 16166 (NMW – C.2005.001.201). V.C. 70, Cumberland, Derwent Fells, north of Dale Head, 35/2293.1663, 2004, Orange 15486 (NMW – C.2005.001.628). *Scotland:* V.C. 88, Mid Perthshire, Ben Lui, [27/26.26], 1911, Wilson & Wheldon (NMW – 25.146.4613). *Ireland:* V.C. H1, South Kerry, 13 km SE of Kenmare, Slaheny river, 10/028.659, 1998, Orange 11947 (NMW – C98.8.78). *Norway:* Sogn og Fjordane, Førde, N of lake Åsvatnet, Nordbø, 61°26.3'N, 6°01.7'E, 32V LP 415 152, 1998, Ekman 3183 (BG – L-38157).

## Discussion

*Porpidia contraponenda* has been interpreted broadly in Great Britain, with the name encompassing two species differing in subtle morphological characters, chemistry, and ITS sequence. One of these has been shown to correspond to the type of *P. contraponenda*, and the other is described here as new.

Although *P. contraponenda* has been reported from North America (Gowan 1989, Fryday 2005), further work is necessary to confirm whether or not the species occurs there. In the present study, the ITS and

TABLE 4. Community ecology of *Porpidia irrigua*. Examples of relevés from Great Britain.

	Relevé number																	
	2002– 105	2002– 117	2002– 186	2005– 230	2008– 7	2003– 379	1998– 86	2002– 359	2002– 394	2002– 421	2005– 87	2005– 106	2004– 34	2004– 216	2004– 157	2005– 166	2006– 12	
<i>Porpidia irrigua</i>	6	5	6	7	5	4	5	5	5	4	5	5	6	7	5	7	5	
<i>Andreaea rothii</i>	5	5	5		5	6					5		4	5		7		
<i>Cladonia diversa</i>	4		2		2		1		1			1	2	1				
<i>Porpidia tuberculosa</i>			2	1	2		1		4			1	1		4			
<i>Racomitrium sudeticum</i>		1				4	5		5				4		1		1	
<i>Stereocaulon vesuvianum</i>					1		5		5	1		2			2		2	
<i>Cladonia subcervicornis</i>			2		1	2	4	2						2			1	
<i>Andreaea rupestris</i>							4		4	2		4		1	2		4	
<i>Rhizocarpon lavatum</i>				4		5			6			1		1		1		
<i>Micarea lignaria</i> var. <i>lignaria</i>	3		1			2	2			1								
<i>Lepraria caesia</i> alba	2		1				1			4								
<i>Rhizocarpon geographicum</i>									1								2	
<i>Andreaea alpina</i>								8		5				2				
<i>Rhizocarpon hochstetteri</i>											1				5		1	
<i>Racomitrium fasciculare</i>		4					1						4					
<i>Lecanora polytropa</i>		4	1						1									
<i>Lecanora soralifera</i>											1	2	4					
<i>Ephebe lanata</i>								5		6								
<i>Pilophorus strumaticus</i>						4					5							
<i>Miriquidica leucophaea</i>					2							5						
<i>Racomitrium aquaticum</i>														2		5		
<i>Trapelia coarctata</i>	1										5							
<i>Marsupella emarginata</i>						2		4										
<i>Marsupella alpina</i>																	7	
<i>Ionaspis lacustris</i>														5				
<i>Miriquidica pycnocarpa</i> f. <i>soralifera</i>												5						
<i>Porpidia cinereoatra</i>					4													
<i>Pseudophebe pubescens</i>													4					
<i>Racomitrium heterostichum</i>									4									
rock	5	5	6	5	4	2	5	5	4	5	4	4	4	4	7	5	4	
altitude (m)	820	860	465	495	310	600	500	730	970	660	440	410	670	530	660	950	920	
vice-county	42	42	42	42	45	48	49	49	49	49	66	69	69	69	70	70	88	
grid ref.	32/	32/	22/	22/	22/	23/	23/	23/	23/	23/	35/	35/	35/	35/	35/	35/	27.4220.3891	
	0055.2072	0079.2130	9628.2199	8452.2068	0626.3717	7371.1381	640.589	6714.6319	6808.6424	6050.5569	8175.2840	7424.2574	3411.1578	1888.2209	2291.0850	2161.0708		
date	23 Apr 2002	24 Apr 2002	19 Jun 2002	6 Oct 2005	15 Feb 2008	25 Jul 2003	7 May 1998	6 Sep 2002	17 Sep 2002	20 Sep 2002	17 Jun 2005	19 Jun 2005	22 Apr 2004	4 Sep 2004	1 Jul 2004	12 Sep 2005	6 June 2006	

Relevés recorded on stands homogeneous in terms of vegetation. Cover expressed by the Domin scale. Species listed in order of frequency and cover; those recorded only as Domin 1, 2 or 3 omitted from table.

LSU analyses distinguished either two or three species in North America which contain 2'-O-methylmicrophyllinate (the possibility that the taxon from which the ITS sequences were derived is conspecific with one of the taxa from which the LSU sequences were derived cannot be ruled out), and none of these are conspecific with sequenced specimens from Europe. Examination of three North American voucher specimens (the fourth was not found) for the ITS and LSU sequences suggests that these taxa resemble either *P. contraponenda* or *P. irrigua* in morphology. A resolution of these, and confirmation of the identity of *P. diversa*, will require a wide-ranging molecular study of *Porpidia* in North America. Thus, it is not possible at present to identify *P. irrigua* with any described or undescribed American taxon, and it is consequently described as a new species.

The ITS analysis demonstrates that there is a great deal of undescribed diversity in species related to *P. macrocarpa*. Fryday (2005) described *P. flavocruenta* and *P. islandica* as new taxa within the *P. macrocarpa* group; these are supported by the present analyses of ITS data, but further undescribed taxa appear to be present. Some of the well-supported clades in the ITS analysis (Fig. 2) may correspond to described species, but more detailed studies are needed.

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