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Standard Paper

Coenogonium nimisii – a new isidiate epiphytic lichen similar to *Porina rosei*

Jiří Malíček[1], Brian Coppins[2], Zdeněk Palice[1], Lucie Vančurová[1], Jan Vondrák[1,3] and Neil Sanderson[4]

[1]Institute of Botany of the Czech Academy of Sciences, CZ-252 43 Průhonice, Czech Republic; [2]Royal Botanic Garden Edinburgh, Edinburgh EH3 5LR, UK; [3]Faculty of Biological Sciences, University of South Bohemia, CZ-370 05 České Budějovice, Czech Republic and [4]Green Close, Woodlands, Southampton SO40 7HU, UK

Abstract

Our floristic work in British ancient forests resulted in a description of a frequently reported but misidentified species, *Coenogonium nimisii*. Its thallus is very similar to *Porina rosei*, but the apothecia and pycnidia correspond with *C. luteum*. Sterile collections are not easy to distinguish but the new species differs from *P. rosei* in several microscopic characters of the isidia. *Coenogonium nimisii* is so far known from bark and epiphytic bryophytes, rarely mossy rocks, in ancient humid forests of Great Britain and Ireland. The genus *Coenogonium* is poorly represented by molecular data in the GenBank database. Our preliminary results revealed distinct genetic lineages within two traditionally circumscribed species, *C. luteum* and *C. pineti*, which may represent cryptic species.

Keywords: ancient forests; cryptic species; Great Britain; phylogeny; taxonomy; *Trentepohliaceae*

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Introduction

Coenogonium (*Ostropales*, *Coenogoniaceae*) is a large genus of crustose or filamentous lichenized fungi, currently including *c.* 90 species (Rivas Plata *et al.* 2006; Lücking *et al.* 2017). It is distributed almost worldwide with its centre of diversity in the tropics (Kantvilas *et al.* 2018). In Europe, three species are so far known: *C. luteum* (Dicks.) Kalb & Lücking, *C. pineti* (Ach.) Lücking & Lumbsch and *C. tavaresianum* (Vězda) Lücking *et al.* In addition, five tropical species have been reported from Macaronesia: *Coenogonium frederici* (Kalb) Kalb & Lücking (Aptroot & Rodrigues 2005; as *Dimerella frederici*), *Coenogonium implexum* Nyl. (Rodrigues & Aptroot 2005), *C. interplexum* (Tavares 1952), *C. luteolum* (Kalb) Kalb & Lücking (Kalb & Hafellner 1992; as *D. luteola*) and *C. subluteum* (Rehm) Kalb & Lücking (Follmann 1990; as *D. epiphylla*).

During our fieldwork in British ancient forests, we collected isidiate thalli of '*Porina rosei*' with apothecia or pycnidia of '*Coenogonium luteum*'. These strange specimens were sequenced and we revealed an undescribed *Coenogonium* species. Subsequently, this taxon was mentioned in the *Revisions of British and Irish Lichens* (Vol. 3) under the provisional name *C. confusum* Malíček & Sanderson *ined.* (Cannon *et al.* 2021). Finally, we decided to describe it here formally as *C. nimisii*.

Corresponding author: Jiří Malíček; Email: jmalicek@seznam.cz

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Material and Methods

Sampling, morphology and chemistry

Collected specimens are deposited in PRA and the personal herbaria of J. Malíček and N. Sanderson. Microscopic descriptions are based on hand-cut sections mounted in water or 10% KOH for observations of the isidia surface. Lichen secondary metabolites were identified using thin-layer chromatography (TLC) in solvents A, B′ and C (Orange *et al.* 2010). The images were captured using 1) an Olympus SZX 12 stereomicroscope with an Olympus DP 70 (resolution 12.5 Mpx) cooled colour digital camera with the software QuickPHOTO MICRO 3.0 (Promicra), using an extended depth of field module Deep Focus, and 2) an Olympus BX 43 microscope with a Promicra 3–5CP (resolution 5 Mpx) colour digital camera with the same software.

DNA extraction, PCR amplification and sequencing

The Invisorb Spin Plant Mini Kit (Invitek) and Chelex protocol (Ferencová *et al.* 2017) were used for DNA extractions. The fungal ITS rDNA (henceforth ITS) and mitochondrial SSU (mtSSU) were amplified with the following primers: ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), mrSSU1, mrSSU2R and mrSSU3R (Zoller *et al.* 1999). The mycobiont from three samples of *Porina rosei* was amplified with ITS-A (Blattner 1999; Škaloud & Rindi 2013) and ITS4. PCR reactions of ITS and mtSSU were prepared for a 20 µl final volume, containing 14 µl double-distilled water, 4 µl MyTaq polymerase reaction buffer, 0.2 µl MyTaq DNA polymerase, 0.4 µl of each of the 25 mM primers, and 1 µl of the sample. Amplifications of both loci consisted of an initial 1 min denaturation at 95 °C, followed by 35

cycles of 30 s at 95 °C, 30 s at 56 °C, 30 s at 72 °C, and a final extension of 7 min at 72 °C. For the combination ITS-A and ITS4, a cycle was programmed with an initial 1 min denaturation at 94 °C, followed by 30 cycles of 25 s at 94 °C, 30 s at 54 °C, 45 s at 72 °C, and a final extension of 10 min at 72 °C. The PCR products were visualized on a 0.8% agarose gel and cleaned with ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific), according to the manufacturer's protocols. The algal ITS and *rbcL* sequences were amplified as described by Černajová *et al.* (2022). In total, 19 new ITS, 19 mtSSU and 9 *rbcL* sequences were generated (Table 1). Sequences of the *Porina rosei* mycobiont, photobiont and *Coccomyxa* ITS were not included in the phylogenetic analysis.

Sequence alignment and phylogenetic analysis

The newly produced sequences were edited in BioEdit v. 7.2.5 (Hall 1999). The final analyses of the mycobiont included the 28 newly generated sequences and nrITS and mtSSU sequences of the genus *Coenogonium* available in the GenBank database. *Gyalecta jenensis* and *G. russula* were selected as outgroups because they form a sister clade to *Coenogonium* (see Miadlikowska *et al.* 2014) and both ITS and mtSSU sequences were available in GenBank. The ITS and mtSSU regions were aligned separately using MAFFT v. 7 (Katoh & Standley 2013) with the L-INS-i method (Katoh *et al.* 2005). The ITS alignment contained 531 positions and 20 sequences; the mtSSU alignment had 719 positions and 25 sequences. Both regions were analyzed as single-locus datasets (see Supplementary Material Figures S1 & S2, available online). For the final tree, we created a concatenated dataset of 27 sequences, containing 462 ITS positions and 719 mtSSU positions. Ambiguous positions (i.e. > 50% of missing data) were excluded from the final analysis. Support values on nodes were checked against single-gene trees and no conflict among well-supported branches (maximum likelihood bootstrap percentages > 0.7) was detected.

The photobiont analysis was based on seven newly generated *rbcL* sequences and 88 sequences retrieved from GenBank (16 reported as *Coenogonium* photobionts, 10 as *Porina* photobionts and 62 other reference sequences, both lichen photobionts and free-living algae). The *rbcL* alignment contained 87 unique sequences and 752 positions.

Phylogenetic trees were inferred with Bayesian inference (BI) using MrBayes v. 3.2.7a (Ronquist & Huelsenbeck 2003; Ronquist *et al.* 2012), maximum likelihood (ML) analysis using GARLI v. 2.0 (Zwickl 2006), and maximum parsimony (MP) analysis using PAUP v. 4.0b10 (Swofford 2003). BI and ML analyses were carried out on a partitioned dataset to differentiate among ITS1, 5.8S and ITS2 rDNA, and mtSSU regions for mycobionts. For the photobiont *rbcL* tree, the dataset was partitioned according to particular codon positions. Substitution models TN93 + G (ITS1), JC + G (5.8S), K2 + I (ITS2), T92 + G (mtSSU), and JC + G + I, T92 + G and K2 + G + I (*rbcL*) were selected using the Bayesian information criterion (BIC) as implemented in jModelTest2 (Guindon & Gascuel 2003; Darriba *et al.* 2012). Two parallel MCMC runs, with four chains, were carried out for 10 million generations. Trees and parameters were sampled every 100 generations. Finally, the burn-in values were determined using the 'sump' command. The ML analysis was carried out using default settings, five search replicates, and the automatic termination set at 5 million generations. The MP analysis was performed using heuristic searches with 1000 random sequence addition replicates and random addition of sequences (the number was limited to 10^4 per replicate). ML and MP bootstrap support values were obtained from 100 and 1000 bootstrap replicates, respectively. Only one search replicate was applied for ML bootstrapping. The alignment used in this study is publicly available on Zenodo as doi: 10.5281/zenodo.7627869.

Taxonomy

Coenogonium nimisii Malíček & Sanderson sp. nov.

MycoBank No.: MB 847489

Similar to *Coenogonium fruticulosum* L. Ludw., so far known from New Zealand, but different in several anatomical characters, such as the higher hymenium (80–110 µm), and larger photobiont cells (10–18 µm) and ascospores (9–11 µm). The isidia of *C. nimisii* are 25–70 µm thick, glossy in fresh material, with one or rarely more layers of thin periclinal hyphae. Apothecia and pycnidia rarely present, ±identical with *C. luteum*.

Type: Great Britain, England, V.C. 11, by Penderley Lodge, Stubbs Wood, New Forest, veteran *Quercus robur* in pasture woodland, 2020, *N. Sanderson* 2744 (PRA 21373—holotype). GenBank Accession nos: OQ366546 and OQ366530.

(Fig. 1)

Thallus crustose, thin, up to 40 µm thick, pale (green-)grey to whitish, without prothallus, often with yellow/ochre tinge, with abundant isidia, forming dense mounds away from the apothecia, more discrete near apothecia. *Isidia* coralloid, moniliform (i.e. with frequent constrictions), green and glossy in fresh material, pale grey-green, grading to ochre-orange where well lit, up to 0.5 mm high, 25–70 µm diam., filled with very abundant photobiont cells with 3–5 cells per isidium width in optical view, cortex poorly defined and composed of one or rarely more layers of colourless periclinal hyphae which are more visible in fresh material or KOH. *Photobiont* trentepohlioid, ±globose cells (7–)10–18 µm diam.

Apothecia rare, (0.5–)1–1.5 mm diam., sessile on the thallus (rarely formed on isidia), distinctly constricted at the base. *Disc* yellow-orange to dark orange, with creamy to yellow, sometimes flexuose margins. *Exciple* colourless, outermost part yellow-brown in dark-pigmented apothecia, composed of branching, conglutinated, 5–8 µm thick hyphae. *Hypothecium* colourless. *Hymenium* 80–110 µm tall, colourless, I+ brownish orange to locally pale blue, K/I+ blue; *epihymenium* pale yellow-brown in dark-pigmented apothecia; *paraphyses* 1.5–2.0 µm thick, upper cells sometimes moniliform, the apical cell up to 4.5 µm diam. *Asci* narrowly cylindrical, *c.* 30 × 4 µm, without an amyloid ring around the pore, 8-spored, uniseriate. *Ascospores* colourless, fusiform-ellipsoid, 1-septate, straight or rarely one cell slightly curved, (7–)9–11(–14) × 2.5–3(–3.5) µm (Supplementary Material Figure S3, available online).

Pycnidia rare, ±globose, 0.3–0.5 mm diam., sessile to shallowly immersed in the thallus and substratum, beige, pycnidia wall colourless in section, 15–30 µm thick, composed of branched hyphae of mostly parallel orientation. *Conidia* aseptate, colourless, ellipsoid, 3.5–5 × 2 µm.

Chemistry. No lichen compounds detected by TLC. Spot reactions: C−, K−, KC−, Pd−, UV− or UV+ whitish grey or ochre (parts with the orange/ochre pigment).

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Table 1. GenBank Accession numbers and voucher information for sequenced specimens of *Coenogonium*. Sequences in bold are newly produced.

Taxon	Source - Specimen	Mycobiont ITS	Mycobiont mtSSU	Algal ITS	Algal *rbcL*
Coenogonium isidiatum	Russia, Sakhalin, Shikotan Island, *S. Chesnokov* 172 (LE-L-14438)	MH179135	MH179140	–	–
C. isidiatum	Russia, Sakhalin, Iturup Island, *L. Konoreva* 606 (LE-L-14436)	MH179137	MH179142	–	–
C. isidiatum	Russia, Sakhalin, Iturup Island, *L. Konoreva* 618 (LE-L-14437)	MH179136	MH179141	–	–
C. isidiatum	Russia, Sakhalin, Sakhalin Island, *L. Konoreva* 198 (LE-L-14441)	MH179138	–	–	–
C. isidiatum	Russia, Kamchatka, *I. Stepanchikova* Nik-1790 (LE-L-14442)	–	MH179143	–	–
C. luteolum	Portugal, Madeira, São Jorge, *J. Malíček* 14105 (hb. J. Malíček)	**OQ366541**	**OQ366534**	–	–
C. luteum s. lat.	Austria, Niederösterreich, Rothwald, *J. Vondrák* 26140 et el. (PRA)	**OQ366542**	**OQ366535**	–	–
C. luteum s. lat.	Great Britain, Scotland, Lochgilphead, *J. Malíček* 9141 et al. (hb. J. Malíček)	–	**OQ366536**	–	–
C. luteum s. lat.	Russia, Caucasus, *J. Malíček* 10624 et al. (hb. J. Malíček)	–	**OQ366537**	–	–
C. luteum s. lat.	USA, California, Santa Rosa Island, *B. Ryan* 31430 (ASU)	HQ650710	AY584699	–	–
C. luteum s. str.	Great Britain, Scotland, Lochgilphead, *J. Malíček* 12550 et al. (hb. J. Malíček)	–	**OQ366526**	–	–
C. luteum s. str.	Great Britain, Scotland, Oban, *J. Malíček* 12390 et al. (hb. J. Malíček)	–	**OQ366527**	–	–
C. luteum s. str.	Portugal, Madeira, Ponta do Sol, *J. Malíček* 14095 (hb. J. Malíček)	**OQ366548**	**OQ366528**	–	–
C. nimisii	Great Britain, England, New Forest, *J. Vondrák* 19844 (PRA)	**OQ366545**	**OQ366532**	**OQ371695**	**OQ400977**
C. nimisii	Great Britain, England, New Forest, *J. Vondrák* 19912 (PRA)	**OQ366544**	**OQ366529**	–	**OQ400978**
C. nimisii	Great Britain, England, New Forest, *M. Powell* 4685 (hb. J. Malíček)	**OQ366543**	**OQ366531**	**OQ371694**	**OQ400974**
C. nimisii	Great Britain, England, New Forest, *N. Sanderson* 2744 (PRA-00021373, holotype)	**OQ366546**	**OQ366530**	–	**OQ400975**
C. nimisii	Great Britain, Scotland, Oban, *J. Malíček* 12373 et al. (hb. J. Malíček)	**OQ366547**	**OQ366533**	**OQ371693**	**OQ400973**
C. pineti	Canada, Ontario, Waterloo Region, *R. T. McMullin* (BIOUG24047-G12)	KT695346	–	–	–
C. pineti	Czech Republic, Southern Bohemia, Šumava Mts, *J. Vondrák* 23798 & *S. Svoboda* (PRA)	**OQ366550**	**OQ366538**	–	–
C. pineti	Czech Republic, Central Bohemia, Český kras, *Z. Palice* 32241 (PRA)	**OQ366551**	**OQ366539**	–	–
C. pineti	Czech Republic, Southern Bohemia, Šumava Mts, *Z. Palice* 32447 (PRA)	**OQ366552**	**OQ366540**	–	–
C. pineti	Germany, Nordrhein-Westfalen, Solingen, *Z. Palice* 15531 (PRA)	–	AY300884	–	–
C. pineti	USA, Alaska, Glacier Bay, *T. Spribille* 39097 (GZU)	–	KR017337	–	–
C. tavaresianum	Great Britain, England, New Forest, *J. Vondrák* 19776 et al. (PRA)	**OQ366549**	**OQ407486**	–	–
Gyalecta jenensis	Canada, British Columbia, Selkirk Mts, *T. Spribille* s. n. (GZU)	KR017099	KR017330	–	–
G. russula	Sweden, Dalarna, *J. Hermansson* 14140 (UPS)	HM244759	HM244735	–	–
Porina rosei	Czech Republic, Šumava Mts, *J. Malíček* 12126 & *Z. Palice* (hb. J. Malíček)	–	**OQ407485**	**OQ371692**	**OQ400972**
P. rosei	Russia, Caucasus, *J. Vondrák* 15450 (PRA)	**OQ407480**	**OQ407483**	–	**OQ400976**
P. rosei	Russia, Caucasus, *J. Vondrák* 22903 (PRA)	**OQ407482**	**OQ407484**	–	**OQ400979**
P. rosei	Russia, Caucasus, *J. Vondrák* 22938 (PRA)	**OQ407481**	–	–	**OQ400980**

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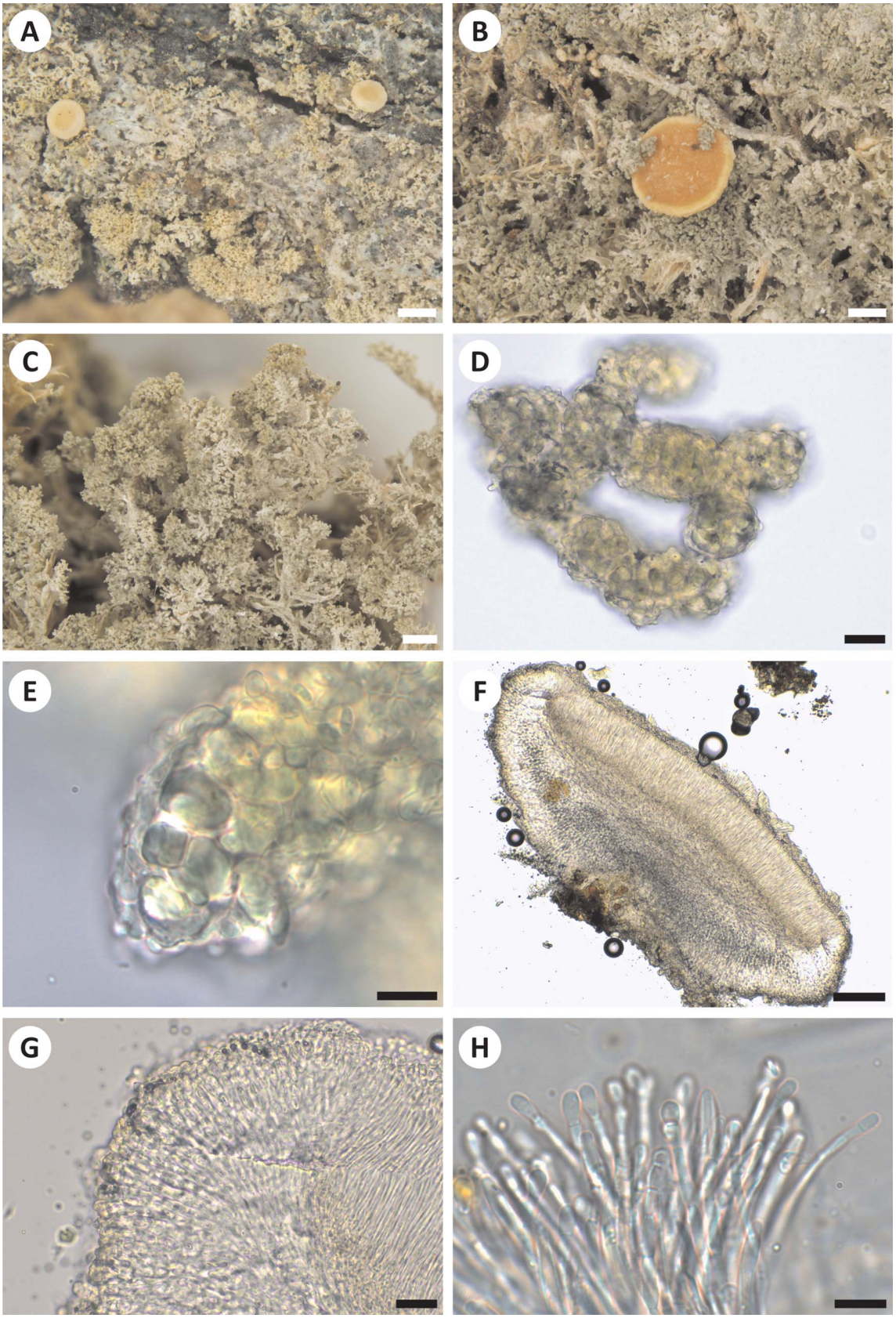

Figure 1. Habitus of *Coenogonium nimisii*. A, holotype (PRA). B, apothecium (*Sanderson* 436). C, isidia (*Sanderson* 436). D, isidia in water (*Malíček* 12373). E, isidia in KOH (*Malíček* 12373). F, apothecial section in water (holotype, PRA). G, exciple in water (holotype, PRA). H, paraphyses in water (*Sanderson* 436). Scales: A–C = 0.5 mm; D & G = 20 μm; E & H = 10 μm; F = 100 μm. In colour online.

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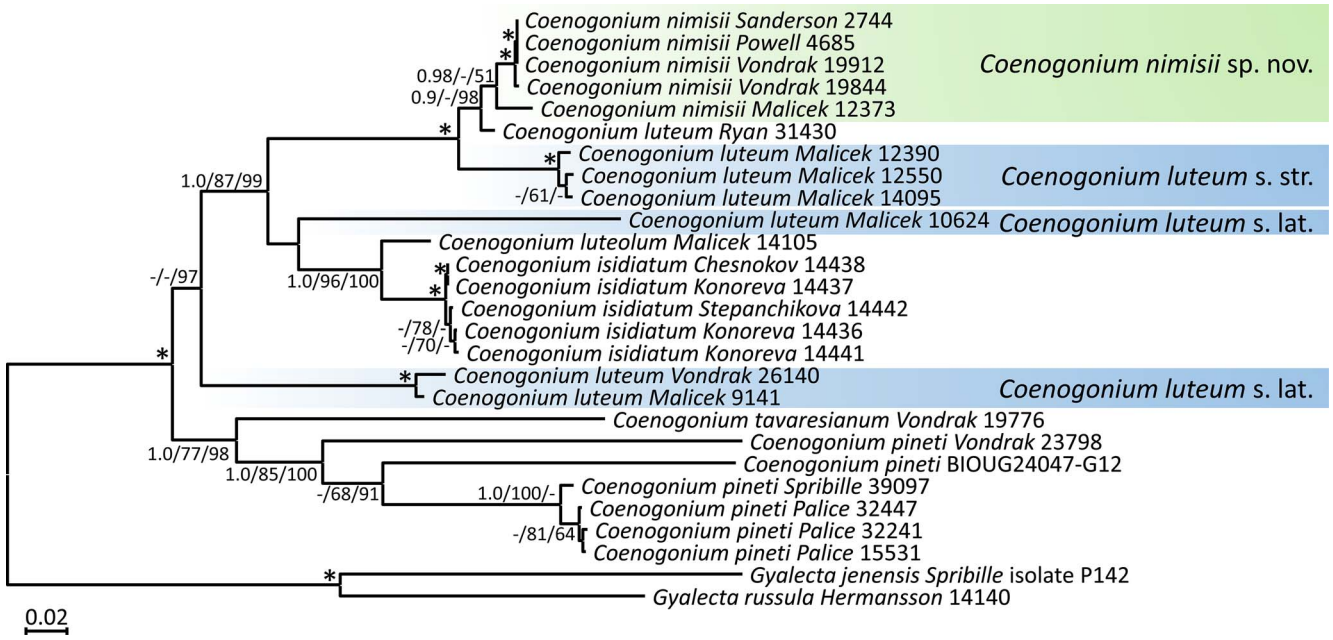

Figure 2. Phylogenetic hypothesis of *Coenogonium* resulting from the Bayesian analysis of combined ITS rDNA and mtSSU sequences. Values at nodes indicate the statistical supports of Bayesian posterior probability (left), maximum-likelihood bootstrap (middle) and maximum parsimony bootstrap (right). Fully supported branches (1.0/100/100) are marked with an asterisk. Scale bar shows the estimated number of substitutions per site. In colour online.

Phylogeny. The new species forms a well-supported clade in the phylogenetic tree (Fig. 2). The sequences of *C. nimisii* are very uniform with only one change in the mtSSU region. However, unexpectedly large variability was observed in the Scottish specimen (*Malíček* 12373), which differs in 31 ITS positions and four mtSSU positions. The new species is very closely related to the North American specimen of *C. luteum* (*Ryan* 31430), which could theoretically represent a non-isidiate form of *C. nimisii*. Nevertheless, the original collection has been examined only as a picture.

Etymology. The specific epithet honours Pier Luigi Nimis, the famous Italian lichenologist, who celebrates his 70th birthday this year.

Habitat and distribution. On a wide range of tree species with base-rich bark, especially on *Quercus robur*, frequently overgrowing epiphytic mosses and liverworts; rarely also on mossy rocks. *Agonimia octospora* Coppins & P. James, *Lepraria finkii* (B. de Lesd.) R.C. Harris and *Thelotrema lepadinum* (Ach.) Ach. are examples of associated species. *Coenogonium nimisii* is a typical species of ancient oceanic woodlands, so far known only from Great Britain and Ireland. It is widespread but local, known from SW England, Wales, SW Scottish Highlands and Ireland.

Notes. In Great Britain and Ireland *C. nimisii* is very rarely fertile and has been long mistaken for the very similar looking but much rarer *Porina rosei* (Fig. 3A). True *P. rosei* is also often sterile and isidia are very variable in colour, size and shape in both species. However, the two species differ mainly in microscopic characters of their isidia. The poorly defined cortex in *C. nimisii* is formed by periclinal hyphae, in contrast to a thick layer of irregular rounded cells in *P. rosei*, as demonstrated by Sérusiaux (1991) and Orange *et al.* (2020). In poorly developed isidia, we recommend observing this character carefully in KOH. Additionally, isidia in *P. rosei* are more slender (20–50 µm thick), dull in fresh material, thin isidia contain only one or two layers of photobiont cells (Fig. 3B), they are squarrose when richly branched and contain an unknown pigment which is K+ pale brown directly after application and pale purple after several hours (Fig. 3B & C).

Coenogonium nimisii and *Porina rosei* also differ in their trentepohlioid photobionts despite their morphological similarity. *Porina rosei* photobionts in our phylogenetic hypothesis, based on the *rbcL* gene (Supplementary Material Figure S4, available online) were recovered in clade 1 *sensu* Nelsen *et al.* (2011), together with all previously published *Porina* photobionts regardless of their geographical origin (including the tropics). The photobionts of *Porina rosei* originating from the Caucasus and Czech Republic were identical or closely related to those of *Porina leptalea* (Durieu & Mont.) A.L. Sm. from France in *rbcL* sequences (Borgato *et al.* 2022). *Phycopeltis*, which has been repeatedly mentioned as the photobiont of *Porina* spp. (Grube *et al.* 2017; McCarthy & Kantvilas 2017), could be placed within clade 1 (Zhu *et al.* 2015). However, the genera *Trentepohlia*, *Printzina* and *Phycopeltis* are polyphyletic (Škaloud *et al.* 2018); their taxonomic revision is beyond the scope of this study and we therefore maintain the clade nomenclature introduced by Nelsen *et. al.* (2011). *Coenogonium nimisii* photobionts were recovered in clade 2 together with the majority of previously published *Coenogonium* photobionts (Supplementary Material Figure S4). According to the BLAST search, the algal ITS sequences retrieved from both lichen species are congruent with *rbcL* sequences in terms of the closest relatives. From one sample of both lichen species, we obtained a sequence of *Coccomyxa* sp. and also observed its colonies (Fig. 3D) several times. The observations indicate the colonies are probably free-living but in a close association with the lichens.

Apothecia of *C. luteum* s. lat. are almost identical to those of *C. nimisii*. We observed only a slightly lower hymenium (up to

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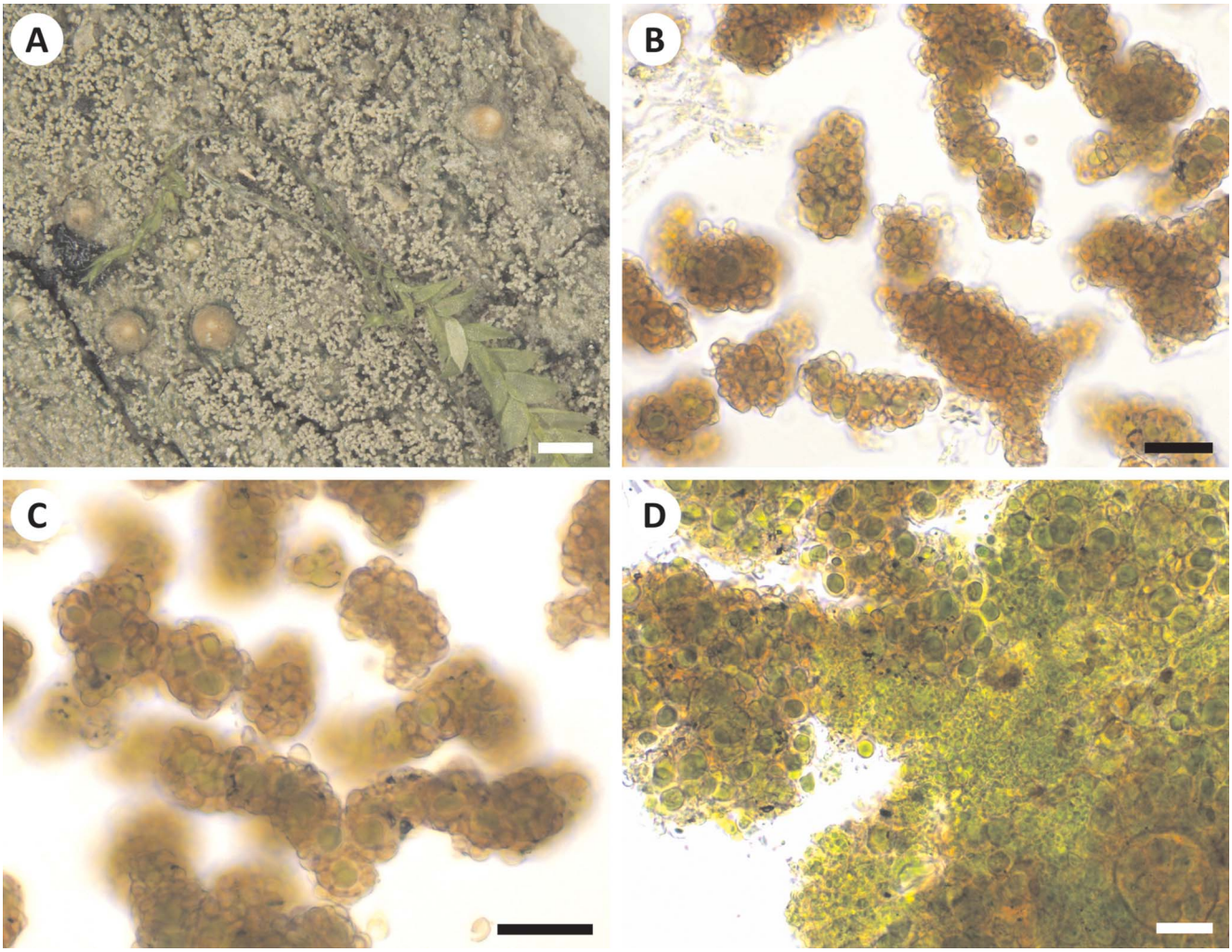

Figure 3. Habitus of *Porina rosei*. A, isidiate thallus with perithecia (PRA-JV22903). B & C, purple pigment in isidia *c.* 20 hours after KOH application (PRA-ZP29299). D, trentepohlioid photobiont (large cells) and a colony of *Coccomyxa* (small cells) from isidia in KOH (PRA-JV22903). Scales: A = 0.5 mm; B & C = 50 µm; D = 20 µm. In colour online.

90 µm) in *C. luteum* s. lat. A comparison of the 10 isidiate taxa of the genus known so far was recently made (Davydov *et al.* 2021). Until now, only *C. isidiatum* (G. Thor & Vězda) Lücking *et al.* has been reported from the temperate to boreal zone of the Northern Hemisphere. It differs in the bluish grey, locally shiny thallus with concolorous isidia and bigger ascospores of 9–14 × 3–4 µm (Thor & Vězda 1984; Davydov *et al.* 2021). *Coenogonium nimisii* is very similar to the New Zealand species *C. fruticulosum* L. Ludw., which differs in several minor anatomical characters (see the diagnosis) and having no observed pycnidia and a wider ecology, including also rotting tussock bases in subalpine grasslands and bryophytes or detritus in montane shrublands (Ludwig 2014).

Enterographa brezhonega Sparrius & Aptroot is occasionally parasitic on *C. nimisii* and rarely on *C. luteum*, and could easily be mistaken as myxomycete fruiting bodies or blobs of *Lepraria*; however, if examined closely the convoluted white lirellae are highly distinctive.

Additional specimens examined. **Great Britain:** *England*: **V.C. 5,** South Somerset, Ley Combe, Hawkcombe, SS885.456, *Quercus* high forest derived from coppice on steep slope, on base-rich streak on downhill side of *Quercus* stub, 2001, *N. Sanderson* 436 (hb. Sanderson; fertile!); **V.C. 11,** South Hampshire, Southampton, New Forest National Park, *c.* 4 km west of Lyndhurst, 50.86893°N, 1.63327°W, 60 m, on mossy bark of *Fagus sylvatica*, 2018, *N. Sanderson, A. Acton, M. Powell* & *J. Vondrák* 19844 (PRA); *ibid.*, *c.* 3 km east of Lyndhurst, 50.86941°N, 1.52586°W, 40 m, on bark of *Quercus*, 2018, *N. Sanderson, A. Acton, M. Powell* & *J. Vondrák* 19912 (PRA); Matley Wood, on flushed side of old *Quercus* trunk, 2018, *M. Powell* 4685 (hb. Powell, hb. Malíček). *Scotland*: **V.C. 98,** Argyll Main, Grampian Mts, Oban, Invercreran, Glen Creran, old-growth oak-dominated forest with wet places, ESE-facing slope, 56°35′48″N, 5°11′23″W, 40–60 m, on bark of *Quercus robur*, 2018, *J. Malíček* 12373, *A. Acton, Z. Palice, M. Powell* & *J. Vondrák* (hb. Malíček); Ardfern, Eilean Mhic Chrion, old-growth hazel-dominated wood on a ridge and slopes below, 56°10′25″N, 5°32′23″W, alt. 50–60 m, on mossy *Fraxinus*, infected by *Enterographa brezhonega*, 2018, *A. Acton, B. Coppins, J. Malíček, Z. Palice* 25138, *M. Powell* & *J. Vondrák* (PRA).—**Ireland: V.C. H2,** North Kerry, Killarney, Meeting of the Waters, on *Quercus*, infected by *Enterographa brezhonega*, 1996, *B. Coppins* 25862 (E).

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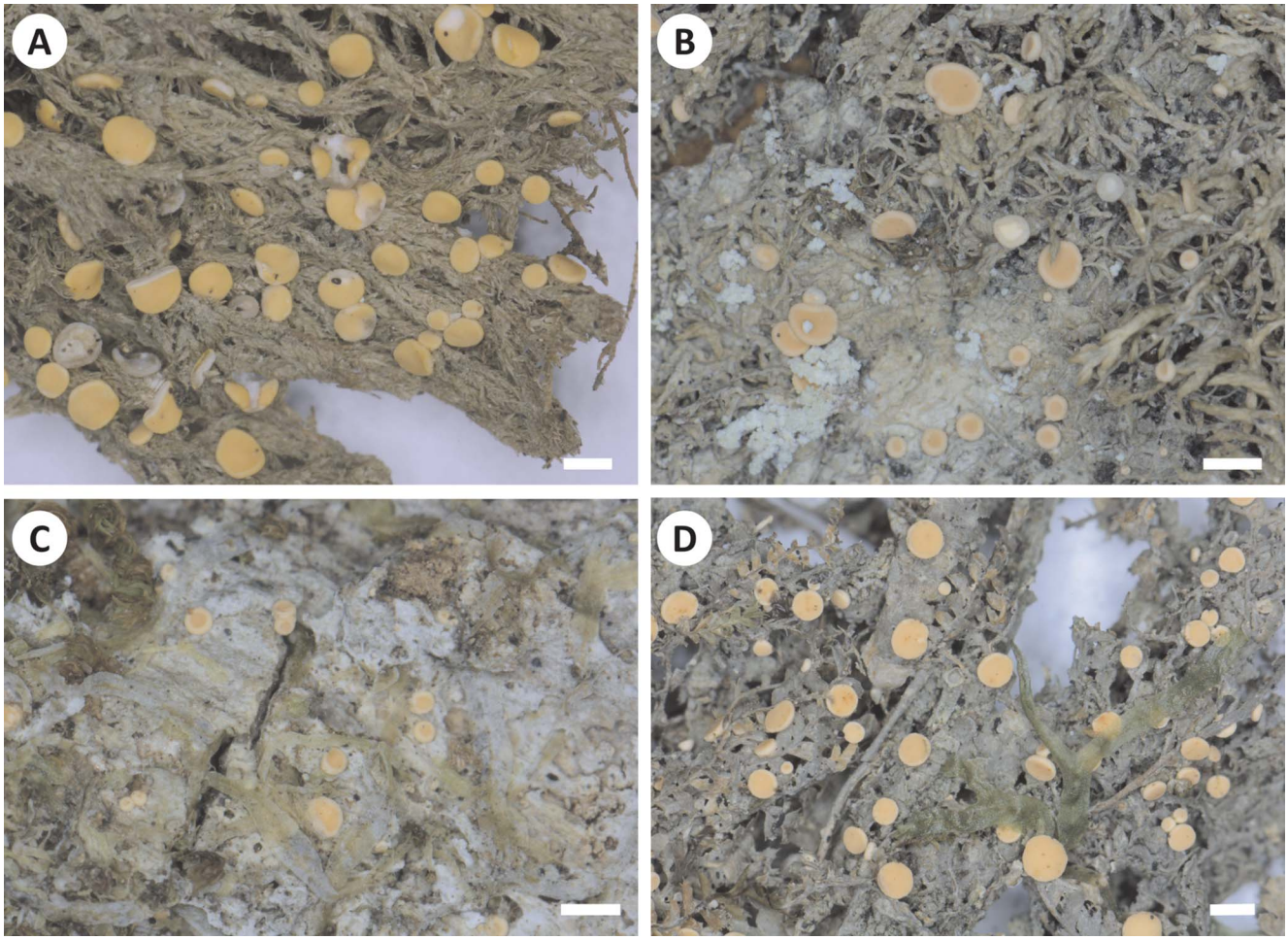

Figure 4. Variability of *Coenogonium luteum* s. lat. (A–C) and *C. luteolum* (D). A, specimen JM 12550. B, JM 10624. C, JM 9141. D, JM 14105. Scales = 1 mm. In colour online.

Specimens of Porina rosei *examined.* **Czech Republic:** *South Bohemian Region*: Šumava, Lenora, Zátoňská hora Nature Reserve, old-growth scree forest on S-facing slope of Mt Zátoňská hora, 48°56′27″N, 13°49′52″E, 900 m, on overhanging siliceous rock, 2018, *J. Malíček* 12126 & *Z. Palice* (hb. Malíček); Šumava, Prachatice, the valley of Blanice, rock outcrop with a fragment of relic pine forest above the right bank of the rivulet, 48°57′47.2″N, 13°55′21.8″E, 760 m, on shaded overhanging granite rock, 2020, *Z. Palice* 29299 (PRA).—**France:** Vercors, Gorges de la Vernaison, en amont de Echevis, amont du lieu-dit Grangeage, 400–450 m, tailis avec buis en bord de rivière, 1986, *E. Sérusiaux* (PRA-V-03285, isotype).—**Great Britain:** *England*. **V.C. 11,** South Hampshire, New Forest, Busketts Wood, Great Stubby Hat, 50.896588°N, 001.564699°W, 35 m, fertile on bark of senescent *Fagus sylvatica* in old-growth *Fagus-Ilex* pasture woodland, 2017, *N. Sanderson* (hb. Sanderson); *ibid*., Coomy Hat, 50.897609°N, 001.558788°W, 30 m, on bark of ancient *Fagus sylvatica* in old-growth *Fagus-Ilex* pasture woodland, 2020, *N. Sanderson* (hb. Sanderson).—**Russia:** *Adygea*: Maykop, Guzeripl, protected area Kavkazskiy zapovednik, 43.98889°N, 40.12507°E, 700 m, on bark of *Carpinus orientalis*, 2016, *J. Vondrák* 15450 (PRA). *Krasnodar Region*: Adler, Khosta, protected area Tiso-samshitovaya roshcha, 43.53116°N, 39.87683°E, 120 m, on twig of *Buxus sempervirens*, 2019, *J. Vondrák* 22903 (PRA); *ibid*., on bark of *Quercus*, 2019, *Z. Palice* 33401, *J. Vondrák* 22938 (PRA).

Coenogonium luteum (Dicks.) Kalb & Lücking

(Fig. 4)

For a comparison of phylogenetic relationships, we included seven specimens of *C. luteum* in the analysis. However, we found this to be a polyphyletic species, represented by at least three lineages in this study (Fig. 2). The lectotype (E00455320, coll. *J. Dickson* 1785) of *C. luteum* comes from the United Kingdom. Sequences cover two distinct clades from Britain, but the specimens differ in the ascospore length: 7.0–9.0(–10.5) versus 9.5–12 µm. We consider the clade represented by three collections (marked s. str.), which contain shorter ascospores, to be identical with the type specimen with ascospores of (6.7–)8–9(–11) µm. The clade represented by the specimen JM10624 corresponds to *C. luteolum*, which is characterized by yellowish apothecia. Kalb & Hafellner (1992) also described differences in the length of ascospores (7–9 µm in *C. luteolum* vs 8–12 µm in *C. luteum*); however, these differences were not observed by us and we regard them as part of intraspecific variability. The remaining two lineages (marked s. lat.) probably represent undescribed or similar tropical

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species (see Rivas Plata *et al.* 2006). The identity of the North American *C. luteum* (*Ryan* 31430), which is strikingly close genetically to *C. nimisii*, is debatable and the possibility that it could be *C. nimisii* cannot be ruled out. The sequenced *C. nimisii* samples come from a geographically limited area (i.e. Great Britain), which may account for the low genetic variability.

Conclusions

In contrast to the high number of described species in the genus *Coenogonium*, the representation of molecular data in the GenBank database is very poor (< 10 species). In this study, we produced 16 mitochondrial SSU and 12 nuclear ITS sequences from five morphologically delimited taxa of *Coenogonium*. Additionally, we produced three mycobiont sequences of mtSSU and three ITS for *Porina rosei*, as well as four ITS and nine *rbcL* sequences of algal partners isolated from both species. The sequences of *C. tavaresianum*, *C. luteolum* and *Porina rosei* are published here for the first time. *Coenogonium tavaresianum* is genetically close to *C. pineti*. *Porina rosei* is published here as new for the Czech Republic.

The newly described species *C. nimisii* forms a well-defined and supported branch within the phylogenetic tree (Fig. 2), but this is in contrast to two other well-known, widely distributed and traditionally defined taxa, *C. luteum* and *C. pineti*. The first mentioned taxon represents at least three distinct species according to molecular data. Similarly, *C. pineti* is genetically variable and consists of at least two distinct but apparently cryptic species. The species complexes of the traditionally circumscribed taxa *C. luteum* and *C. pineti* are deliberately not addressed in detail within this paper as they merit a more comprehensive study.

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Author ORCIDs. Jiří Malíček, 0000-0002-3119-8967; Brian Coppins, 0000-0001-9464-0495; Zdeněk Palice, 0000-0003-4984-8654; Lucie Vančurová, 0000-0002-1370-1020; Jan Vondrák, 0000-0001-7568-6711; Neil Sanderson, 0000-0002-3719-3104.

Competing Interests. The authors declare none.

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