



The family *Pyrenidiaceae* resurrected

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Abstract

Pyrenidium is a lichenicolous genus which was included in the family *Dacampiaceae* (Pleosporales) based on morphological characters. The classification of this genus within *Dacampiaceae* has been controversial due to the lack of sequence data. In this study, the genus *Pyrenidium* is sequenced for the first time using five freshly collected specimens belonging to the generic type and two other species. Although the morphology of *Pyrenidium* is quite similar to other genera of *Dacampiaceae*, phylogenetic analyses from nuLSU and nuSSU sequence data demonstrate that *Pyrenidium* is distantly related to *Dacampiaceae* and it forms a distinct lineage within the Dothideomycetes. Therefore, we resurrect the family *Pyrenidiaceae* to accommodate *Pyrenidium*. Morphological descriptions of the sequenced specimens of *Pyrenidium* are provided and include the description of a new species, *P. borbonicum*.

Key words – 1 new taxon – *Dacampiaceae* – Dothideomycetes – Lichenicolous fungi – Phylogenetic analyses

Introduction

Dacampiaceae Korb. belongs to the order Pleosporales. Members of this family are represented by lichenicolous, lichenized and saprobic fungi which are characterized by blackish, perithecioid ascomata, pseudoparenchymatous exciple and brown ascospores (Hyde et al. 2013, Wijayawardene et al. 2018). *Dacampiaceae* was introduced by Körber (1855) and six genera are currently included: *Aaosphaeria* Aptroot., *Dacampia* A. Massal., *Leptocurthis* Aptroot., *Pseudonitschkia* Coppins & S.Y Kondr., *Pyrenidium* Nyl. and *Weddellomyces* Hawksw. (Hyde et al. 2013, Ertz et al. 2015, Wijayawardene et al. 2018, Doilom et al. 2018).

Pyrenidium was introduced by Nylander (1865), with *P. actinellum* Nyl. as the type species. Species of the genus are all lichenicolous and have perithecioid ascomata that often have blue-green pigment located in upper peridial wall, and ascomata are immersed to erumpent in host thalli with the upper part exposed, or that are prominent but covered by tissue of the host thalli, sometimes causing gall-like malformations of the host (Hawksworth 1982, Eriksson &

Hawksworth 1993, Matzer 1996, Pirogov 2014, Lendemer 2017, Muscavitch et al. 2017, Diederich et al. 2018). The genus has been placed in *Pyrenidiaceae* Zahlbr., however, as *Pyrenidium* shares some similar morphological characters with *Dacampia* (*Dacampiaceae* Körb.), Hawksworth (1980) suggested a close relationship between *Dacampiaceae* and *Pyrenidiaceae* and the latter has been considered as a synonym of *Dacampiaceae* (Lumbsch & Huhndorf 2010, Hyde et al. 2013, Wijayawardene et al. 2014, 2018, Jaklitsch et al. 2016). Crivelli (1983) suggested that *Pyrenidium* is similar to *Dacampia* by having similar ascomatal structures and wall tissues (Hawksworth 1977, Crivelli 1983,). However, these taxonomic assumptions have not been verified based on phylogenetic analyses due to the lack of sequence data.

In this paper, we aim to provide morphological and molecular data for the genus *Pyrenidium* to clarify its phylogenetic placement. A phylogenetic tree including new sequence data from *Pyrenidium* generated by nuLSU and nuSSU is provided. Detailed morphological descriptions and illustrations of materials collected in Belgium, Madagascar and Reunion Island are included with notes.

Materials & Methods

Sample collection, morphological study and isolation

Voucher specimens from Belgium, Madagascar and Reunion Island collected by the second author are deposited in BR (Meise Botanic Garden). They were morphologically studied and photomicrographs were made following the method of Thambugala et al. (2015). Dried herbarium materials were examined using a Motic SMZ 168 dissecting microscope to locate and isolate ascomata. Hand section of the fruiting structures were done and mounted in water, 5% KOH (K), or 1% I₂ Lugol's reagent without (I) or with KOH pre-treatment (K/I) for microscopic studies and photomicrography. The taxa were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Cannon 600D digital camera fitted to the microscope. Measurements were made by Tarosoft (R) Image Frame Work program. Photoplates were prepared by using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA).

DNA extraction, PCR amplification and sequencing

Well-preserved and freshly collected specimens were used for sequencing. Hand sections of the hymenium were used for direct PCR as described in Ertz et al. (2015). The outer wall of perithecia was removed with a sterile razor blade to isolate the hymenium. The material was then added into a tube containing the PCR reaction mixture and amplified directly. Amplification reactions were prepared for 50 µl final volume containing 5 µl 10× DreamTaq Buffer (Thermo Scientific; www.thermoscientific.com/onebio), 1.25 µl of each of the 20 µM primers, 5 µl of 2.5 mg ml⁻¹ bovin serum albumin (Thermo Scientific #B14), 4 µl of 2.5 mM each dNTPs (Thermo Scientific), 1.25 U Dreamtaq DNA polymerase (Thermo Scientific), and tiny fragments of fungal material. DNA amplification was performed by polymerase chain reaction (PCR) using the primer pairs LIC15R (Miadlikowska et al. 2002) and LR6 (Vilgalys & Hester 1990) to amplify the partial ribosomal RNA for the nuclear large subunit (28S, nuLSU), nssu131 (Kauff & Lutzoni 2002) and NS24 (Gargas & Taylor 1992) to amplify the partial ribosomal RNA for the nuclear small subunit (18S, nuSSU). The yield of the amplified products was verified by running the products on 1% agarose gel stained with ethidium bromide. Both strands were sequenced by Macrogen® using the same primers as for the PCR amplifications. Additional primers were used for sequencing LR3 for nuLSU (Vilgalys & Hester 1990), and nssu1088 and nssu1088R for nuSSU (Kauff & Lutzoni 2002). Sequenced fragments were assembled with Sequencher v.5.3 (Gene Codes Corporation, Ann Arbor, Michigan). Sequence data were subjected to MEGABLAST searches to verify their closest relatives and to detect potential contaminations.

Phylogenetic analyses

Phylogenetic analyses were performed for combined nuLSU and nuSSU sequence data. The dataset of this study included 121 strains from GenBank (Table 1). Datasets were aligned by using MAFFT version 7.310 (Kato & Standley 2016: <http://mafft.cbrc.jp/alignment/server/>) and manually aligned wherever necessary in MEGA version 6.0 (Tamura et al. 2013). The sequence datasets were combined using the CIPRES Science Gateway version 3.3. (Miller et al. 2011). RAxML rapid bootstrapping and subsequent ML search used distinct model/data partitions with joint branch length optimization, 1000 rapid bootstrap inferences and thereafter a thorough ML search, all free model parameters were estimated by RAxML, ML estimate of 50 per site rate categories. Likelihood of final tree was evaluated and optimized under GAMMA+P-Invar. Model parameters were estimated up to an accuracy of 0.1000000000 Log Likelihood units. Maximum-parsimony (MP) analyses were carried out using PAUP version 4.0b10 (Swofford 2002). Parsimony bootstrap analyses were performed using the full heuristic search option, random stepwise addition and 1000 replicates with maxtrees set at 5000 (will not be increased).

The model for Bayesian inference analysis was determined by using MrModeltest 2.3 (Nylander 2004) and the GTR+I+G nucleotide substitution model was used for each partition. Posterior probabilities (PP) were performed (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) in MrBayes V. 3.2 (Ronquist et al. 2011). Phylogenetic trees were illustrated in Fig Tree Drawing Tool version 1.4.0 (Rambaut 2012). Bootstrap support values (> ML 60%/MP 60%/BYPP 0.60) are indicated on the branches of the tree (Fig. 1).

Results

Phylogenetic analyses

The combined nuLSU and nuSSU sequence data comprised 121 strains, including strains of *Pyrenidium* (*P. cf. actinellum* Ertz 18063, *P. actinellum* Nyl. *sensu stricto*. Ertz 16557, *P. borbonicum* Ertz 18031, *P. borbonicum* Ertz 18066 and *P. aff. aggregatum* Ertz 20089). *Acolium tigillare* (AFTOL-ID4839) (*Caliciaceae*) in Lecanoromycetes was selected as an outgroup.

Maximum parsimony, maximum likelihood and Bayesian inference resulted in similar topologies. Therefore, only the Maximum parsimony tree is presented, with the support values of the maximum likelihood and the Bayesian inference analyses (Fig. 1). Parsimony analysis comprised a dataset of 1619 total characters, of which 730 characters were constant, 725 parsimony-informative and 164 parsimony-uninformative. The first tree generated among 1000 equally parsimonious trees is selected (Fig. 1; TL = 3724, CI = 0.366, RI = 0.732, RC = 0.268, HI = 0.634).

The phylogenetic tree obtained from Maximum parsimony tree showed that the taxa of *Pyrenidium* form the clade with high bootstrap support outside the family *Dacampiaceae* (Fig. 1). The clade of *Pyrenidium* forms a lineage related to *Natipusillaceae* (Natipusillales), *Microthyriaceae* (Microthyriales) and *Zeloasperisporiaceae* (Zeloasperisporiales). However, the external node does not receive support. The best scoring tree obtained from maximum likelihood analysis received a final value of -20235.541082.

Table 1 Specimens and DNA sequences used in this study, with their respective voucher information. Sequences generated in this study are in blue and ex-type strains are in bold and shown by a T after the strain number.

Species	Culture collection	GenBank accession numbers	
		nuLSU	nuSSU
<i>Acolium tigillare</i>	AFTOL-ID 4839 ^T	JQ301593	JQ301646
<i>Acrocordiopsis patilii</i>	BCC 28166^T	GU479772	GU479736
<i>Acrocordiopsis patilii</i>	BCC 28167	GU479773	GU479737
<i>Aigialus grandis</i>	BCC 20000^T	GU479775	GU479739

Table 1 Continued.

Species	Culture collection	GenBank accession numbers	
		nuLSU	nuSSU
<i>Aigialus mangrovis</i>	BCC 33563	GU479776	GU479741
<i>Aigialus mangrovis</i>	BCC 33564	GU479777	GU479742
<i>Aigialus parvus</i>	BCC 18403	GU479778	GU479744
<i>Alternaria alternata</i>	AFTOL-ID 1610	DQ678082	DQ678031
<i>Alternariaster helianthi</i>	CBS 199.86^T	KC609343	–
<i>Anteaglonium globosum</i>	GKML101N	GQ221875	–
<i>Antennariella placitae</i>	CBS 124785	GQ303299	–
<i>Aquastroma magniostiolata</i>	KT 2485^T	AB807510	AB797220
<i>Aquaticheirospora</i> sp.	RK-2006a	AY736378	AY736377
<i>Aquilomyces patris</i>	CBS 135661^T	KP184041	–
<i>Arthopyrenia salicis</i>	CBS 368.94	AY538339	AY538333
<i>Ascochyta phacae</i>	CBS 184.55	KT389692	–
<i>Ascocylindrica marina</i>	MD6011^T	KT252905	KT252907
<i>Asteromassaria pulchra</i>	CBS 124082	GU301800	GU296137
<i>Bambusicola massarinia</i>	MFLUCC 11–0389^T	JX442037	JX442041
<i>Capnodium salicinum</i>	AFTOL-ID 937^T	DQ678050	–
<i>Clypeoloculus akitaensis</i>	KT 788^T	AB807543	AB797253
<i>Cryptoclypeus oxysporus</i>	KT 2772	LC194345	LC194303
<i>Dacampia engeliana</i>	Hafellner 72868	KT383791	–
<i>Dacampia hookeri</i>	Hafellner73897 (GZU)^T	KT383792	–
<i>Dacampia hookeri</i>	Hafellner74269 (GZU)	KT383793	–
<i>Dacampia hookeri</i>	Hafellner75980 (GZU)	KT383794	–
<i>Dacampia hookeri</i>	Hafellner81840 (GZU)	KT383795	–
<i>Delitschia anisomera</i>	GKM 1205	GU385171	DQ678026
<i>Delitschia chaetomioides</i>	GKM 1283	GU385172	JX254656
<i>Delitschia winteri</i>	AFTOL-ID 1599	DQ678077	DQ018079
<i>Deniquelata barringtoniae</i>	MFLUCC 11-0422^T	JX254655	JX254656
<i>Dictyosporium elegans</i>	NBRC 32502^T	DQ018100	DQ0181079
<i>Didymella exigua</i>	CBS 183.55	EU754155	–
<i>Didymocyrtis</i> cf. <i>epiphyscia</i>	Ertz 17411 (BR)	KT383799	–
<i>Didymocyrtis consimilis</i>	Voucher Gardiennet 12041^T	KT383796	–
<i>Didymosphaeria rubi-ulmifolii</i>	MFLUCC 14-0024	KJ436585	KJ436587
<i>Digitodesmium bambusicola</i>	CBS 110279	DQ018103	–
<i>Dothidotthia aspera</i>	CPC 12933	EU673276	EU673228
<i>Dothidotthia symphoricarpi</i>	CPC 12929^T	EU673273	–
<i>Elsinoe verbenae</i>	CPC 18561	KX887061	JN940562
<i>Extremus antarcticus</i>	CCFEE 5312^T	KF310020	–
<i>Falciformispora lignatilis</i>	BCC 21118^T	GU371827	GU371835
<i>Fuscostagonospora sasae</i>	KT 1467^T	AB807548	AB797258
<i>Gregarithecium curvisporum</i>	KT 922^T	AB807547	AB797257
<i>Halomassarina thalassiae</i>	JK 5262D^T	GU301816	–
<i>Halothia posidoniae</i>	BBH 22481^T	GU479786	GU479752
<i>Hysterium angustum</i>	CBS 236.34	FJ161180	GU397359
<i>Kalmusia brevispora</i>	KT 1466	AB524600	AB524459
<i>Keissleriella cladophila</i>	CBS 104.55	GU205221	GU296155
<i>Kirschsteiniothelia elaterascus</i>	HKUCC7769	AY787934	–
<i>Lentithecium fluviatile</i>	CBS 122367^T	GU301825	GU296158
<i>Lepidosphaeria nicotiae</i>	CBS 559.71^T	DQ384106	DQ384068
<i>Leptosphaeria doliolum</i>	CBS 505.75	GQ387576	GQ387515

Table 1 Continued.

Species	Culture collection	GenBank accession numbers	
		nuLSU	nuSSU
<i>Leptosphaeria maculans</i>	CBS 260.94	JX681096	–
<i>Leptoxyphium cacuminum</i>	MFLUCC10–0049	JN832602	JN832587
<i>Lichenocoonium aeruginosum</i>	JL359–09	HQ174269	HQ174268
<i>Lichenocoonium erodens</i>	JL363–09	HQ174267	HQ174266
<i>Lichenocoonium lecanorae</i>	JL382	HQ174263	HQ174262
<i>Lichenocoonium usneae</i>	JL352–09	HQ174265	HQ174264
<i>Lindgomyces ingoldianus</i>	ATCC 200398^T	AB521736	AB521720
<i>Longipedicellata aptrootii</i>	MFLUCC 10–0297^T	KU238894	KU238895
<i>Lophiostoma macrostomum</i>	KT508^T	AB619010	AB618691
<i>Lophiotrema nucula</i>	CBS 627.86^T	GU301837	GU296167
<i>Lophium mytilinum</i>	AFTOL–ID 1609^T	DQ678081	DQ678030
<i>Macrodiplodiopsis desmazieri</i>	CPC 24971^T	KR873272	–
<i>Magnicamarosporium iriomotense</i>	KT 2822^T	AB807509	AB797219
<i>Massarina ramunculicola</i>	BCC 18404	GQ925853	GQ925838
<i>Massariosphaeria typhicola</i>	CBS 609.86	EF165033	EF165037
<i>Melanomma pulvis-pyrius</i>	CBS 371.75^T	GU301845	FJ201989
<i>Microthyrium microscopicum</i>	CBS 115976^T	GU301846	GU296175
<i>Microthyrium</i> sp.	MFLUCC 15–0213	KT306552	KT306550
<i>Montagnula aloes</i>	CBS 132531	JX069847	–
<i>Multilocularia bambusae</i>	MFLUCC 11–0180^T	KU693438	KU693442
<i>Multiseptospora thailandica</i>	MFLUCC 11–0183^T	KP744490	KP753955
<i>Murilentithecium clematidis</i>	IT1078^T	KM408758	KM408760
<i>Myriangium duriaei</i>	CBS 260.36^T	NG027579	–
<i>Mytilinidion rhenanum</i>	CBS 135.45	FJ161175	–
<i>Natipusilla bellaspora</i>	OTU–0–0212.44	MF331811	
<i>Natipusilla bellaspora</i>	PE91–1a	JX474863	JX474868
<i>Natipusilla bellaspora</i>	PE91–1b	JX474864	JX474869
<i>Natipusilla decorospora</i>	LA236.1A^T	HM196369	–
<i>Natipusilla limonensis</i>	L–AF286–1A	HM196370	HM196377
<i>Natipusilla limonensis</i>	PE3–2a	JX474861	JX474867
<i>Natipusilla limonensis</i>	PE3–2b	JX474862	JX474870
<i>Natipusilla naponensis</i>	LAF217–1A	HM196371	HM196378
<i>Natipusilla naponensis</i>	LAF217–1B	HM196372	HM196379
<i>Neoastrisphaeriella krabiensis</i>	MFLUCC11–0025^T	JN846729	JN846739
<i>Neoastrisphaeriella</i> sp.	MFLUCC 18–0209	MK138829	MK138789
<i>Neoastrisphaeriella sribooniensis</i>	MFLUCC 13–0834	MF588997	MF588987
<i>Neobambusicola strelitziae</i>	CBS 138869^T	KP004495	–
<i>Parabambusicola bambusina</i>	KT 2637^T	AB807538	–
<i>Periconia homothallica</i>	KT 916	AB807565	AB797275
<i>Phaeosphaeria oryzae</i>	CBS 110110^T	GQ387591	GQ387530
<i>Pleomassaria siparia</i>	AFTOL–ID 1600^T	DQ678078	DQ678027
<i>Pleospora herbarum</i> var. <i>herbarum</i>	CBS 191.86^T	JX681120	–
<i>Polyschema terricola</i>	CBS 301.65^T	EF204504	EF204519
<i>Preussia funiculate</i>	CBS 659.74^T	GU301864	GU296187
<i>Pseudoasteromassaria fagi</i>	KT3432^T	LC061590	LC061585
<i>Pseudocoleophoma calamagrostidis</i>	KT3284^T	LC014609	LC014604

Table 1 Continued.

Species	Culture collection	GenBank accession numbers	
		nuLSU	nuSSU
<i>Pseudodictyosporium wauense</i>	NBRC 30078 ^T	DQ018105	DQ018083
<i>Pseudomonodictys tectonae</i>	MFLUCC 12–0552 ^T	KT285573	KT285574
<i>Pseudoxylomyces elegans</i>	KT 2887 ^T	AB807598	AB797308
<i>Pyrenidium actinellum</i> Nyl. <i>sensu stricto.</i>	Ertz 16557	MK713524	–
<i>Pyrenidium</i> aff. <i>aggregatum</i>	Ertz 20089	MK713526	–
<i>Pyrenidium borbonicum</i>	Ertz 18031	MK713527	MK713523
<i>Pyrenidium borbonicum</i>	Ertz 18066	MK713528	MK713522
<i>Pyrenidium</i> cf. <i>actinellum</i>	Ertz 18063	MK713525	MK713521
<i>Repetophragma ontariense</i>	HKUCC 10830	DQ408575	–
<i>Rhytidhysterion opuntiae</i>	GKM1190	GQ221892	–
<i>Rhytidhysterion rufulum</i>	AFTOL–ID 2109	FJ469672	–
<i>Salsuginea ramicola</i>	KT 2597.2 ^T	GU479801	GU479768
<i>Splanchnonema platani</i>	CBS 221.37	JX681100	–
<i>Stagonospora pseudocaricis</i>	CBS 135132	KF251762	–
<i>Sulcatispora acerina</i>	KT2982 ^T	LC014610	LC014605
<i>Tetraplosphaeria sasicola</i>	KT 563 ^T	AB524631	AB524490
<i>Trematosphaeria pertusa</i>	CBS 122368 ^T	FJ201990	FJ201991
<i>Ulospora bilgramii</i>	AFTOL–ID 1598 ^T	DQ678076	DQ678025
<i>Wicklowia aquatic</i>	F76–2 ^T	GU045445	–
<i>Wicklowia aquatica</i>	AF289–1	GU045446	–
<i>Zeloasperisporium wrightiae</i>	MFLUCC 15–0225	KT387737	KT387738
<i>Zopfia rhizophila</i>	CBS 207.26 ^T	DQ384104	DQ384086

Taxonomy

Pyrenidiaceae Zahlbr., in Engler, Syllabus der Pflanzenfamilien, Edn 2 (Berlin): 46 (1898)

Type genus – *Pyrenidium* Nyl.

The *Pyrenidiaceae* was a synonym of *Dacampiaceae*, however based on our phylogenetic results herein we resurrect the family *Pyrenidiaceae*. It includes only the genus *Pyrenidium* (Fig. 1).

Pyrenidium Nyl., Flora, Regensburg 48: 210 (1865)

Type species – *Pyrenidium actinellum* Nyl.

Ascomata perithecioid, densely grouped or dispersed over large areas of the thallus sessile or immersed in the host-thallus, usually black, in longitudinal section subglobose to broadly ovoid or pyriform, often with light greenish-blue tint in ostiolar channel. *Peridium* with isodiametric to elongated cells, brown to dark brown. *Hymenium* hyaline, I–, KI–. *Hamathecial* filaments numerous, persistent, *pseudoparaphyses*, branched and anastomosing and *periphyses* non-branching, apical cells not enlarged. *Asci* 4–8-spored, bitunicate, shortly pedicellate, I–, KI–, ocular chamber not distinct. *Ascospores* dark brown, often paler brown in the spore tips, smooth. Asexual morph: Unknown.

Notes – Lichenicolous, associated with various unrelated hosts occurring on various substrates such as bark, rock, soil, bryophytes or living leaves. The genus *Pyrenidium* has a cosmopolitan distribution (Hawksworth 1980, 1983, Matzer 1996, Navarro–Rosinés & Roux 2007, Knudsen & Kocourková 2010, Aptroot 2014, Doilom et al. 2018).

Pleosporales

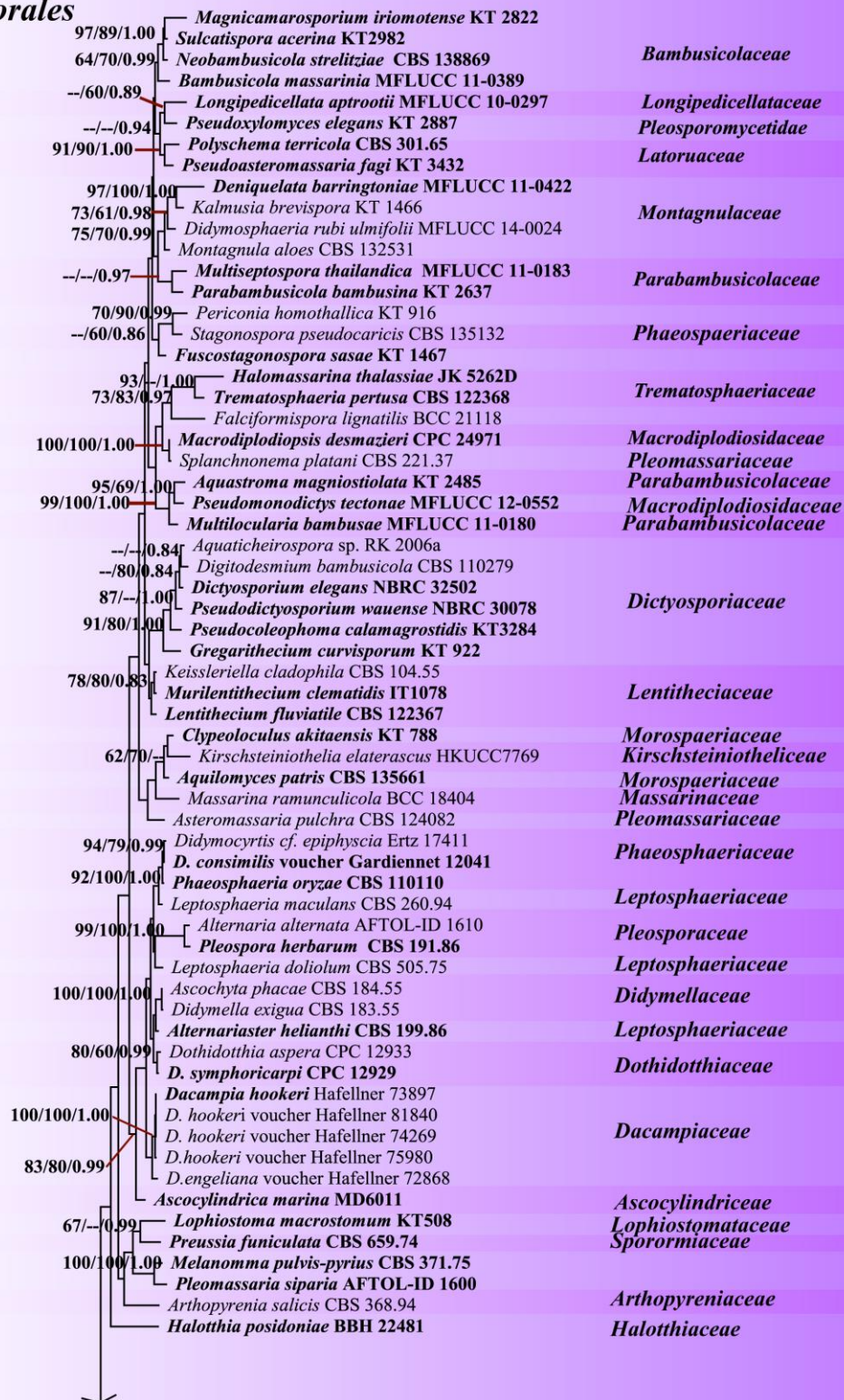


Figure 1– Phylogram generated from Maximum Parsimony tree of combined nuLSU and nuSSU sequence data of representative species in Dothideomycetes. Bootstrap support values (>ML 60%/MP 60%/BYPP 0.60) are given above the branches. Culture accession numbers are mentioned along with the species name. The tree is rooted by *Acolium tigillare* (AFTOL-ID4839) in Lecanoromycetes. Types are in black bold, and the strains of *Pyrenidiaceae* are highlighted in blue bold.

Pleosporales

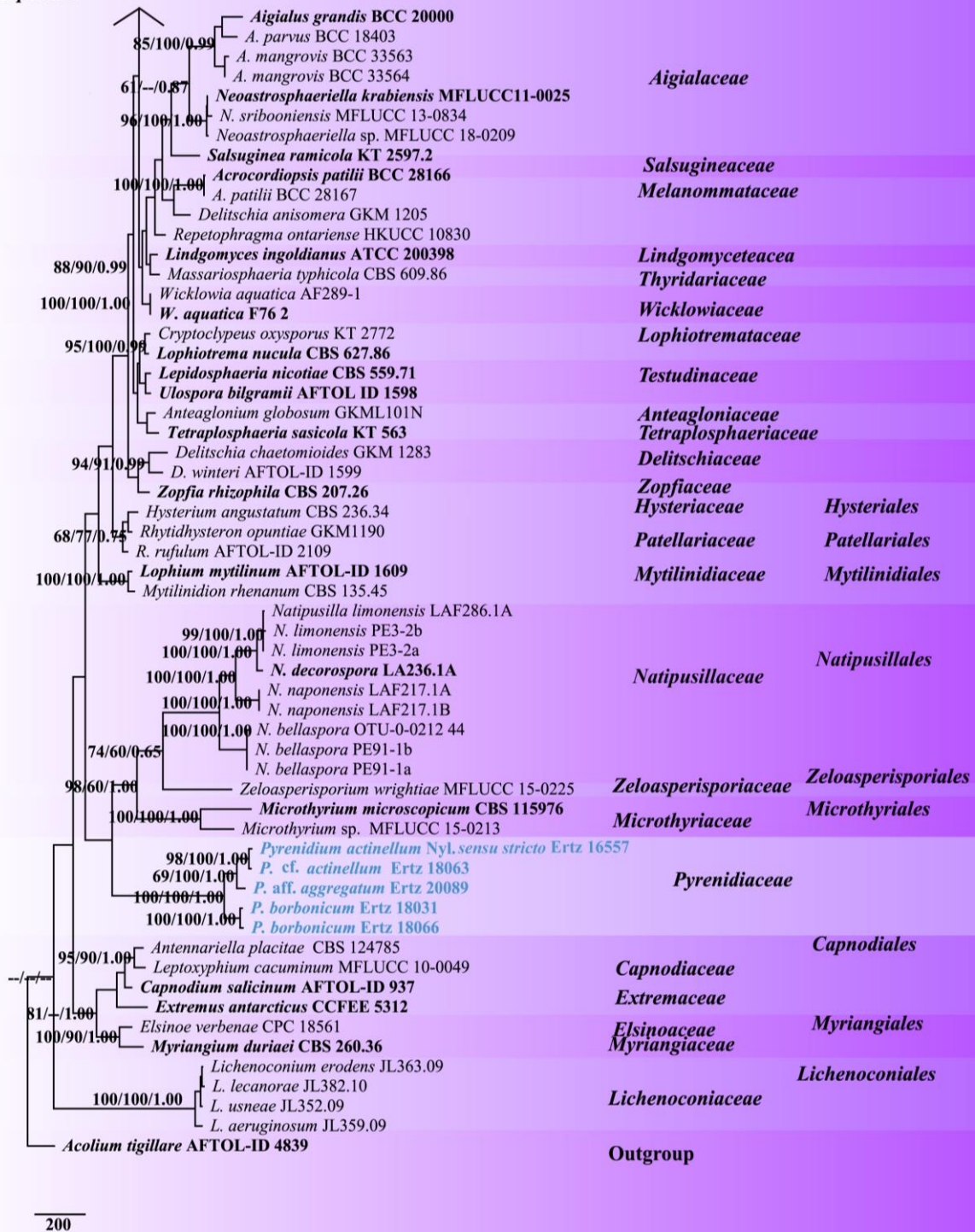


Figure 1 – Continued.

Pyrenidium actinellum Nyl. sensu stricto

Fig. 2

Index Fungorum number: IF403001; Facesoffungi number: FoF04634

Ascomata 175–380 µm high (130–188 µm high, Doilom et al. 2018), 162–350 µm diam. (150–190 µm diam, Doilom et al. 2018), sessile or rarely semi-immersed on host thalli, scattered, solitary or 2–4 contiguous, black, subglobose to pyriform, ostiole central, with pore-like opening; without distinct gall formation or with barely visible malformations of the host thallus. *Peridium* 30–50 µm wide (25–50 µm wide, Doilom et al. 2018), thick-walled, composed of several layers of dark brown

to reddish brown pseudoparenchymatous cells, arranged in *textura angularis* to *textura prismatica*, inner layers comprising hyaline cells. *Hamathecium* composed of dense, filiform, anastomosed, cellular pseudoparaphyses 1.5–2.5 μm wide (1.5–2.5 μm wide, Doilom et al. 2018), constricted at the septa, embedded in a gelatinous matrix. *Asci* 75–80 \times 12–16 μm ($n = 3$) (40–88 \times 11–18 μm , Doilom et al. 2018), 4-spored, subcylindrical, apically rounded with indistinct ocular chamber. *Ascospores* 18–26 \times 7.5–11 μm ($\bar{x} = 23.1 \times 9.5 \mu\text{m}$, $n = 30$) (19–27 \times 7–9 μm , Doilom et al. 2018), overlapping 1–2-seriate, pale brown in immature state, reddish brown to dark brown in mature state, paler in the spore tips, oblong to ellipsoidal, or fusiform, rounded or obtuse at the apex, usually 3-septate, constricted at the septa with the two inner cells much larger than the end cells. Asexual morph: undetermined.

Material examined – BELGIUM, Aywaille, right bank of the Amblève river, Heid des Gattes, 200 m elev. sandstone rocky slope with *Anema*, on the lichen *Scytinium plicatile*, 2011, Ertz 16557 (BR).

Notes – *Pyrenidium actinellum* was introduced by Nylander (1865), and was described from *Scytinium teretiusculum* (Wallr.) Otálora et al. *sensu lato* (= *Leptogium teretiusculum* (Wallr.) Arnold). The type specimen was described and illustrated in more details by Doilom et al. (2018). *Pyrenidium actinellum* is characterized by perithecioid ascomata with a bluish green hue (not or barely visible in our specimen 16557, and in the type according to the figure in Doilom et al. (2018), 4-spored, subcylindrical asci and brown to dark brown, paler at the ends, ellipsoidal, 3-septate ascospores (Hawksworth 1983, Navarro-Rosinés & Roux 2007, Knudsen & Kocourková 2010).

The type specimen of *P. actinellum* and our sequenced specimen 16557 grow on the cyanolichen genus *Scytinium* (*S. teretiusculum* and *S. plicatile* respectively). The latter specimen differs from the type specimen by larger perithecia, which are semi-immersed on the host thalli. *Pyrenidium actinellum* has been reported from a wide range of lichen hosts, but our molecular analyses suggest that more species are involved in this complex, of which one is described here as new. Sequencing of specimens of *P. actinellum sensu lato* from other hosts lichen genera will likely reveal a higher cryptic diversity in the genus.

***Pyrenidium cf. actinellum* Nyl.**

Fig. 3

Index Fungorum number: IF403001; Facesoffungi number: FoF04634

Ascomata 168–205 μm high (130–188 μm high, Doilom et al. 2018) \times 117–179 μm diam. (150–190 μm diam, Doilom et al. 2018), ($\bar{x} = 187 \times 146 \mu\text{m}$, $n = 5$), numerous, evenly dispersed over large areas of the thallus, immersed in the host-thallus, only the upper, blackish part of the ascomata visible, ascomata in longitudinal section subglobose to broadly ovoid, with light greenish-blue tint in ostiolar channel. *Peridium* 22–36 μm wide, composed of 5–9 layers of *textura prismatica*, with isodiametric to elongated cells, brown to dark brown. *Hymenium* not interspersed. *Hamathecial* filaments numerous, persistent, composed of pseudoparaphyses, 1–2 μm diam., richly branched and anastomosing, developing between the asci, and paraphyses 1–2 μm wide (1.5–2.5 μm wide, Doilom et al. 2018), with light greenish-blue tint in ostiole region, apical cell not enlarged. *Asci* 51–115 \times 12–18 μm ($\bar{x} = 84 \times 16 \mu\text{m}$, $n = 20$), (40–88 \times 11–18 μm , Doilom et al. 2018), 4-spored, narrowly ellipsoid, ocular chamber not distinct. *Ascospores* 20–25 \times 6–11 μm ($\bar{x} = 23 \times 9 \mu\text{m}$, $n = 20$), (19–27 \times 7–9 μm , Doilom et al. 2018), biseriate, partially overlapping, dark brown, K⁺ turning black, often paler brown in the spore tips, narrowly ellipsoid, 3-septate, sometimes slightly curved toward the ends, constricted at the septa, without a gelatinous sheath. Asexual morph: undetermined.

Material examined – FRANCE, Reunion; Cilaos, trail to the Col du Taibit, just before the pass, ca 2,010m, 21°06'47"S, 55°26'03"E, rocky slope under *Erica* gr arborea on the edge of a path, on *Solorina simensis* (*Peltigeraceae*) 2012, Ertz 18063 (BR).

Notes – see under *Pyrenidium actinellum sensu stricto*. Our specimen likely represents *P. actinellum* because of a similar morphology and our phylogenetic results (Fig. 1). However, as the

host lichen genus is different, we refrain to include it into *P. actinellum sensu stricto* until the host range will be studied.

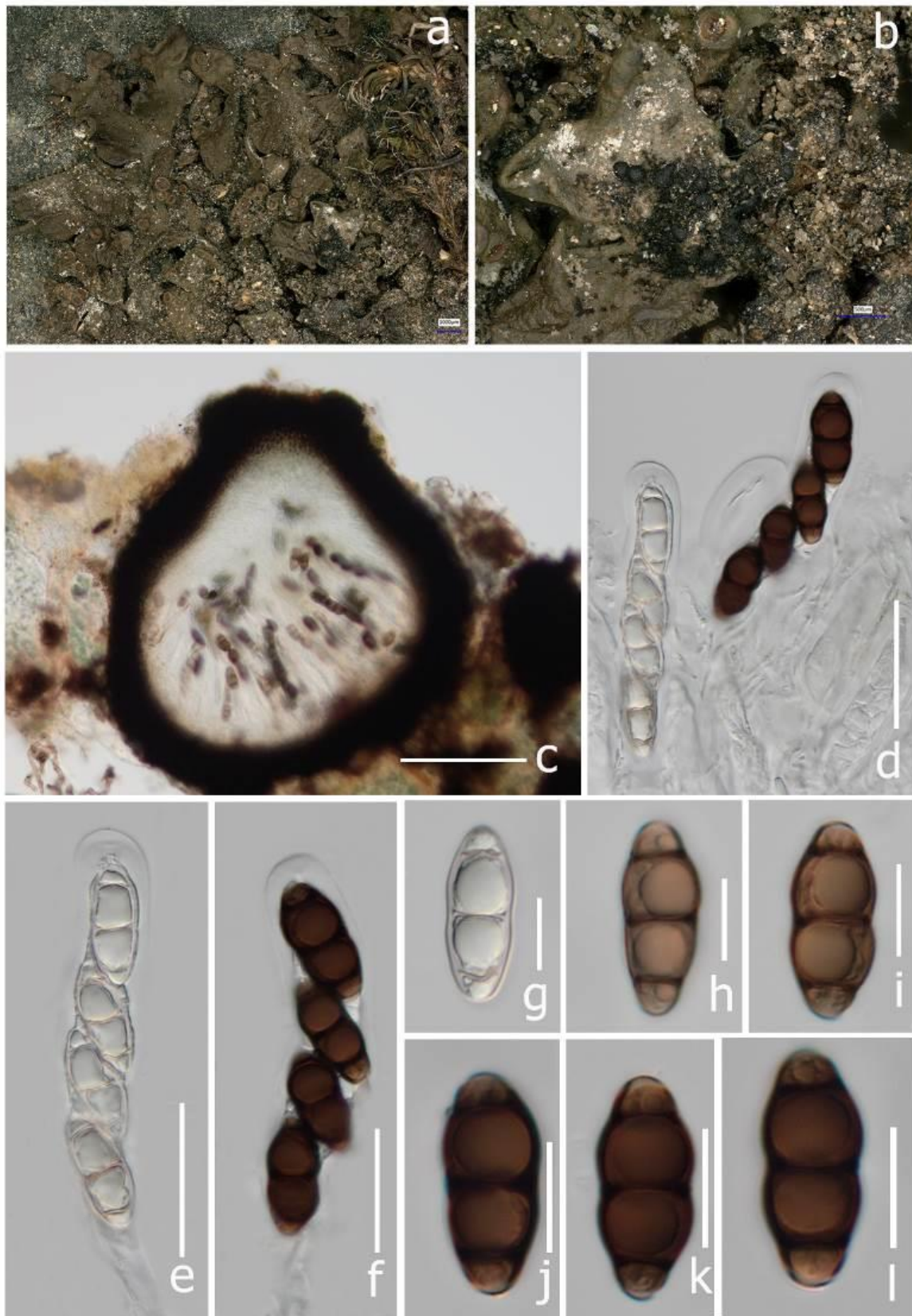


Figure 2 – *Pyrenidium actinellum* Nyl *sensu stricto*. (Ertz 16557) a, b Appearance of ascomata on host surface. c Vertical section of host with perithecioid ascomata visible. d Hymenium with asci. e, f Asci. g–l Ascospores. Scale bars: c = 50 µm, d = 10 µm, e, f = 10 µm, g–l = 5 µm.



Figure 3 – *Pyrenidium* cf. *actinellum* (Ertz 18063) a–c Appearance of ascomata on host surface. d Vertical section of host with perithecioid ascomata visible. e Vertical section of perithecioid ascoma. f Ostiolar channel. g Peridium. h Hymenium with asci in water. i Hymenium with asci in KOH. j Pseudoparaphyses. k–m Asci. n–s Ascospores. Scale bars: d–e = 50 μ m, f = 50 μ m, g = 10 μ m, h = 20 μ m, i = 20 μ m, j = 20 μ m, k–m = 20 μ m, n–s = 5 μ m.

Pyrenidium aff. aggregatum K. Knudsen & Kocourk

Fig. 4

Index Fungorum number: IF518068; Facesoffungi number: FoF06298

Ascomata 45–106 × 33–79 μm (\bar{x} = 73 × 53 μm, n = 10), solitary or crowded, immersed in gall-like malformations of the host thallus, blackish part arises from host thallus, 2–15 ascomata in one gall, galls strongly convex, of the same colour of the thallus, 0.4–1.3 mm, ascomata in longitudinal section subglobose to broadly ovoid, with light greenish-blue tint in ostiolar channel. *Peridium* 9–12 μm wide, composed of 3–9 layers of *textura prismatica*, with isodiametric to elongated cells, brown to dark brown. *Hymenium* not inspersed. *Hamathecial filaments* numerous, composed of pseudoparaphyses, 1–3 μm diam., richly branched and anastomosing, developing between asci, and paraphyses 15–20 × 3–4 μm (\bar{x} = 18 × 3 μm, n = 10), with light greenish-blue tint in ostiole region, apical cell not enlarged. *Asci* 48–87 × 11–18 μm (\bar{x} = 71 × 14 μm, n = 20), 8-spored, narrowly ellipsoid, ocular chamber not distinct. *Ascospores* 18.8–22.2 × 7–8.5 μm (\bar{x} = 21 × 7 μm, n = 20), biseriate, partially overlapping, dark brown, K⁺ turning black, often paler brown in the spore tips, narrowly ellipsoid, 3-septate, often slightly curved, sometimes slightly curved toward the ends, constricted at the septa, smooth, without a gelatinous sheath. Asexual morph: undetermined.

Material examined – REPUBLIC OF MADAGASCAR, Province. Diego Suarez, Antsiranana, W of Sambava, Marojejy National Park, along the trail from Camp Simpona to summit, 1853 m, 14°26'41"S, 49°44'13"E, rather dense humid montane forest grading into dense sclerophyllous upper montane forests, on twig of shrub, on *Parmeliaceae*, 20 October 2014, Ertz 20089 (BR).

Notes – *Pyrenidium aggregatum* was described from USA by Knudsen & Kocourkova (2010). Our specimen fits well *P. aggregatum* which is characterized by distinct convex galls, with completely immersed perithecia, 8-spored and rather small ascospores. The holotype of *P. aggregatum* was reported from *Phaeophyscia rubropulchra* (*Physciaceae*), while our collection is reported from *Parmeliaceae*. Thus, we prefer to use “aff.” for our specimen because of the different lichen family of the host and the distant geographic locality (Madagascar).

Pyrenidium borbonicum Huanraluek, Ertz & K.D. Hyde, sp. nov

Figs 5, 6

Index Fungorum number: IF556643; Facesoffungi number: FoF06297

Etymology – named after Bourbon Island, the old name for Reunion Island.

Holotype – Reunion Island; Cilaos, trail to the Col du Taibit, Plateau de la Fraise, ca 1,700 m, 21°06'45"S, 55°26'35"E, mossy rocky slope, on *Sticta*, 12 December 2012, D. Ertz 18066 (BR).

Ascomata in groups immersed in the host-thallus, only the upper, blackish part of the ascomata visible, 94–221 × 77–166 μm (\bar{x} = 172 × 129 μm, n = 10) (Ertz 18031), 125–165 × 117–184 μm (\bar{x} = 151 × 145 μm, n = 4) (Ertz 18066), subglobose to broadly ovoid in longitudinal section, with hyaline paraphyses in ostiolar channel. *Peridium* 18–29 μm wide (Ertz 18031) 12–15 μm wide (Ertz 18066), composed of 3–7 layers (Ertz 18031), 4–6 layers (Ertz 18066) of *textura prismatica*, with isodiametric to elongated cells, brown to dark brown. *Hymenium* not inspersed. *Hamathecial filaments* numerous, composed of pseudoparaphyses 2–3 μm diam, richly branched and anastomosing, present between the asci, and paraphyses 18–20 × 2–4 μm (Ertz 18031), 11–24 × 1–2 μm (Ertz 18066) in ostiole region, apical cell not enlarged. *Asci* 47–96 × 10–16 μm (\bar{x} = 71 × 13 μm, n = 20) (Ertz 18031), 64–98 × 11–17 μm (\bar{x} = 80 × 15, n = 20) (Ertz 18066), 8-spored, narrowly ellipsoid, ocular chamber not distinct. *Ascospores* 19–23 × 5–8 μm (\bar{x} = 21 × 6 μm, n = 20) (Ertz 18031), 22–27 × 4–8 μm (\bar{x} = 25 × 6 μm, n = 20) (Ertz 18066) biseriate, partially overlapping, dark brown, K⁺ turning black, often pale brown in the spore tips, narrowly ellipsoid, 3-septate, sometimes slightly curved toward the ends, constricted at the septa, without a gelatinous sheath. Asexual morph: undetermined.

Additional material examined – FRANCE, Reunion Island, Cilaos, forest of Grand Matarum, trail to the Piton des Neiges, ca 1,150 m, 21°07'13"S, 55°29'03"E, on rock, on *Sticta*, 11 December 2012, Ertz 18031 (BR).

Notes – *Pyrenidium borbonicum* differs from *P. aggregatum* by the absence of strongly convex gall formation (Knudsen & Kocourkova 2010). It differs from *P. actinellum* by 8-spored asci and narrower ascospores. Phylogenetically, *P. borbonicum* forms a distinct lineage with high bootstrap supports (MP 100%/ML 100%/ BYPP 1.00, Fig. 1).

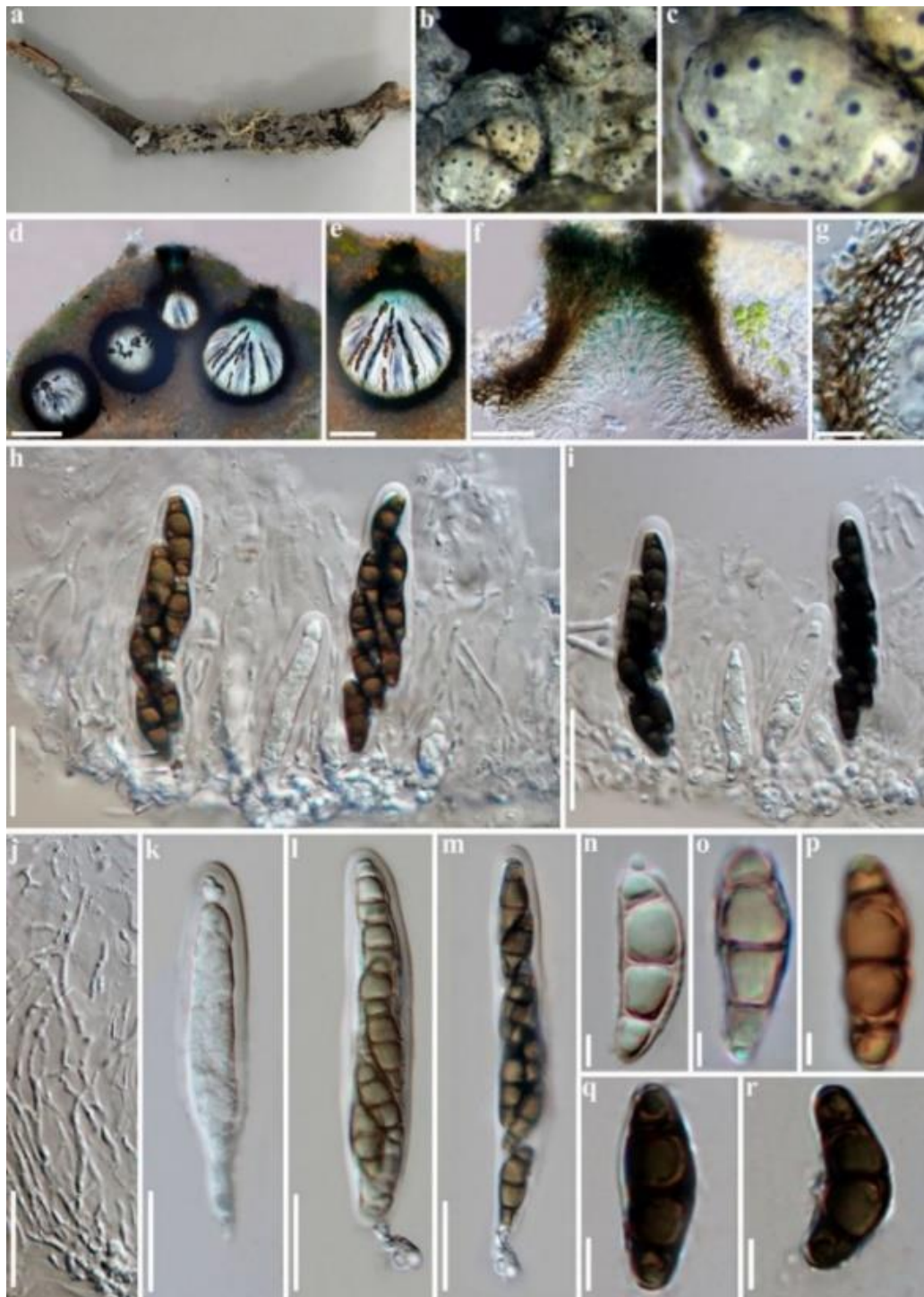


Figure 4 – *Pyrenidium* aff. *aggregatum* (Ertz 20089) a–c Appearance of ascomata on host surface. d Vertical section of a gall with four perithecioid ascomata visible. e Vertical section of one perithecioid ascoma. f Ostiolum. g Peridium. h Hymenium with asci in water. i Hymenium in KOH and brownish ascospores that turned black. j Pseudoparaphyses, k–m Asci. n–r Ascospores. Scale bars: d–e = 50 μ m, f = 20 μ m, g = 10 μ m, h = 20 μ m, i = 20 μ m, j = 20 μ m, k–m = 20 μ m, n–r = 5 μ m.

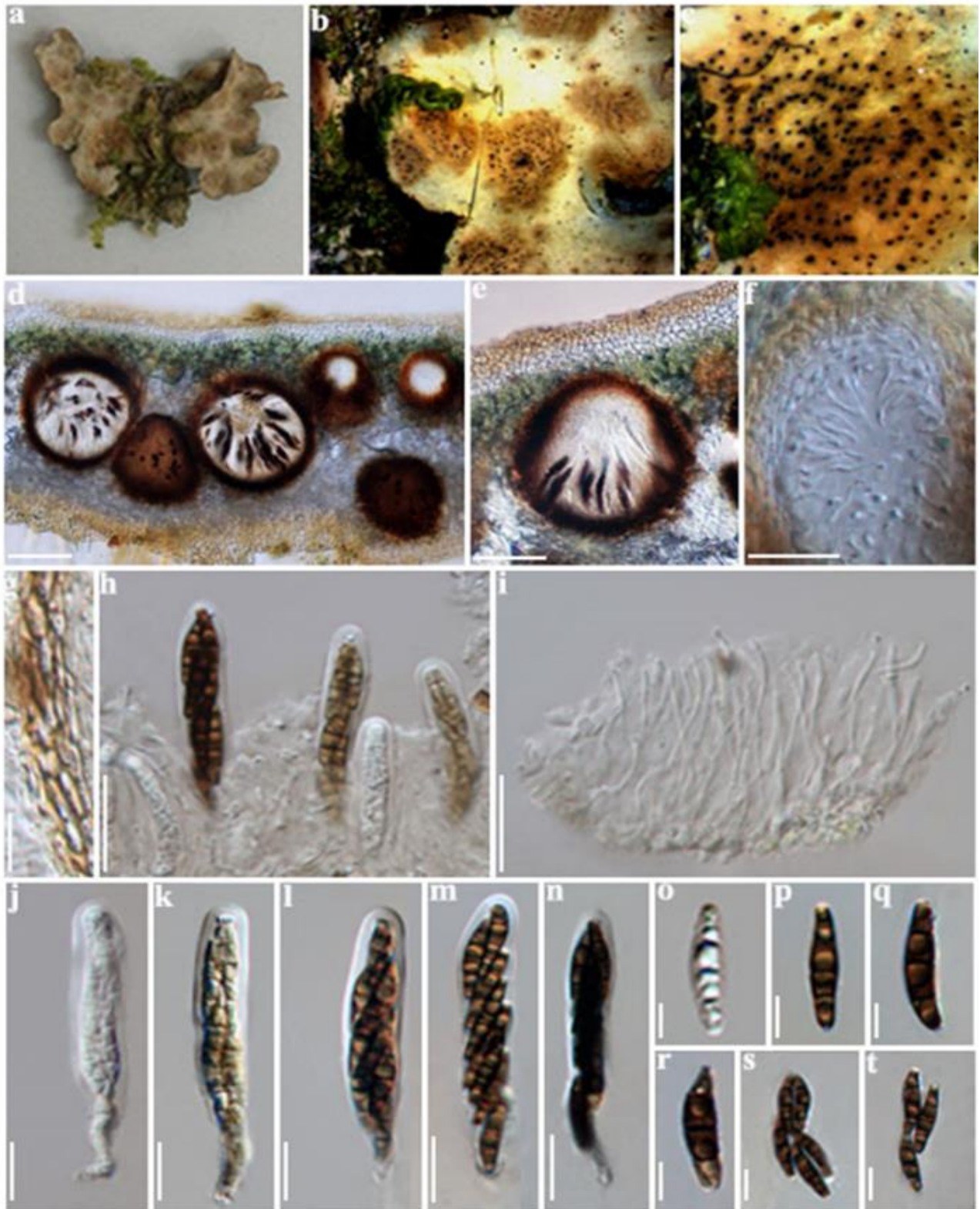


Figure 5 – *Pyrenidium borbonicum* (Ertz 18066 – holotype) a–c Appearance of ascomata on host surface. d Vertical section of host with perithecioid ascomata visible. e Vertical section of perithecioid ascoma. f Ostiolar channel. g Peridium. h Hymenium with asci. i Pseudoparaphyses, j–m Asci in water. n Asci in KOH showing the ascospores with the brownish pigment that turned black. o–t Ascospores. Scale bars: d–e = 100 μ m, f = 50 μ m, g = 10 μ m, h = 20 μ m, i = 20 μ m, j–n = 20 μ m, o–t = 10 μ m.

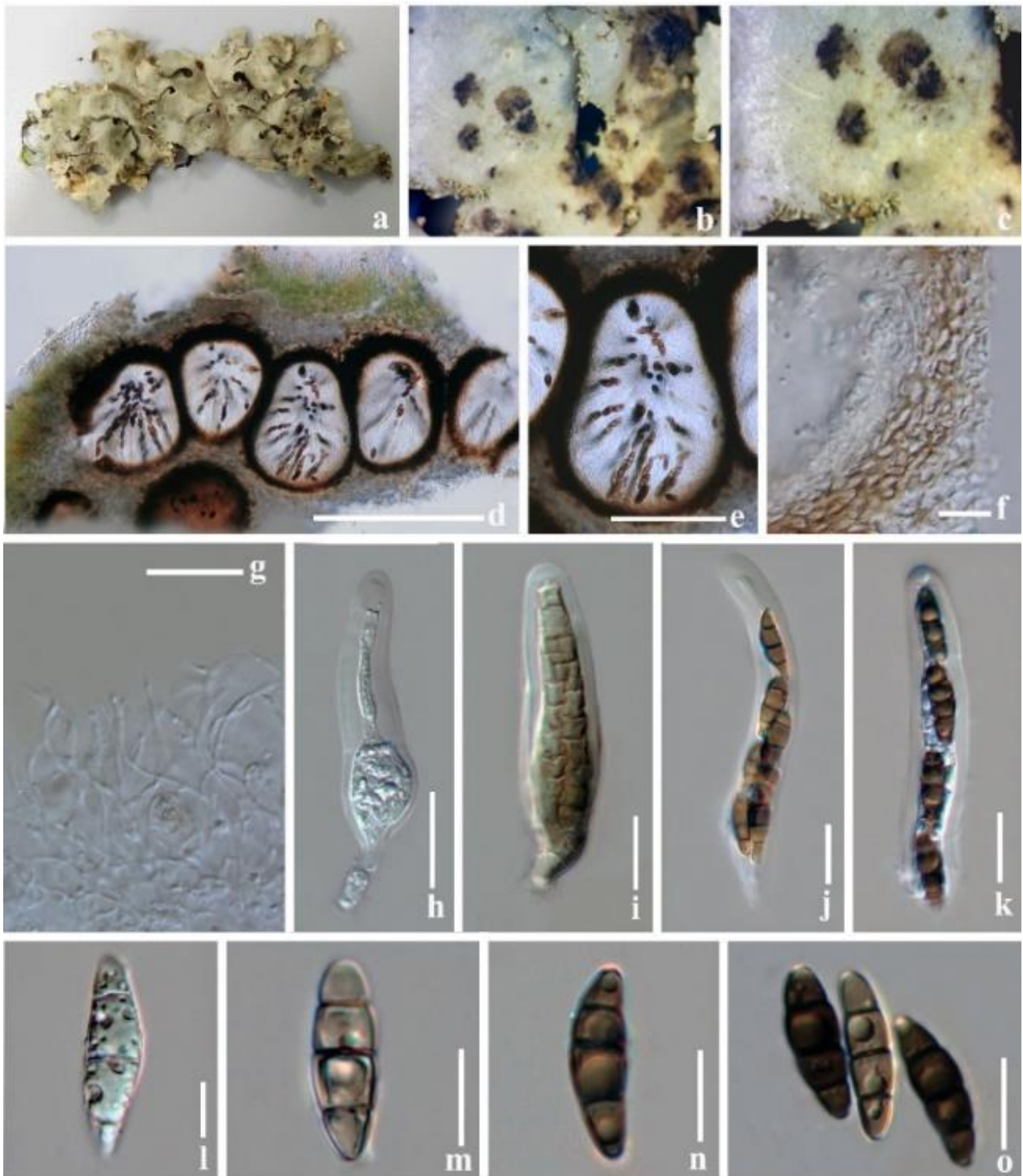


Figure 6 – *Pyrenidium borbonicum* (Ertz 18031) a–c Appearance of ascomata on host surface. d, e Vertical section of perithecioid ascomata. f Peridium. g Pseudoparaphyses. h–k Asci in water. l–o Ascospores. Scale bars: d, e = 50 μ m, f = 20 μ m, g–k = 20 μ m, l–o = 10 μ m.

Discussion

The family *Dacampiaceae* was shown to be polyphyletic by Ertz et al. (2015), with several genera (viz. *Clypeococcum*, *Munkovalsaria* and *Polycoccum*) transferred to other families and orders. *Dacampiaceae* was placed in the Pleosporales based on DNA sequence data of its type genus (*Dacampia*). Since then, the monophyly of *Dacampiaceae* as currently accepted has never been tested, and there is a lack of molecular data for its six other currently accepted genera. Our phylogenetic analyses using new nuLSU and nuSSU sequence data indicate that *Pyrenidium* does

not belong to the *Dacampiaceae* (Fig. 1). Despite these phylogenetic analyses, *Dacampiaceae* still appears to be a heterogeneous assemblage of genera (e.g. unbranched vs. branched pseudoparaphyses, slit-like vs. punctiform ostiole). Therefore, it is likely that molecular studies of the other genera currently included in *Dacampiaceae* can lead to further dismantling of the family.

Pyrenidium has affinities with genera of *Dacampiaceae* by having a lichenicolous habit, perithecioid ascomata, a similar ascus type, and brown ascospores, but differs notably by different type of ascospores (Table 3). The closest relatives of *Pyrenidium* are *Natipusillaceae* (Natipusillales), *Microthyriaceae* (Microthyriales) and *Zeloasperisporiaceae* (Zeloasperisporiales) but these are not well supported (Fig. 1). A more robust phylogeny is needed to confirm this relationship using markers that are more informative than the nuLSU and nuSSU loci.

The family *Natipusillaceae* was described for saprobic fungi living on submerged wood in fresh water habitats of the neo-tropical environment (Raja et al. 2012, Hyde et al. 2013). Species of *Natipusilla* differs from *Pyrenidium* notably by hyaline to light brown ascomata, globose, subglobose or obclavate asci, fusiform to cylindrical, hyaline (but becoming brown with age) ascospores sometimes having gelatinous appendages and a very different life habit being saprobic on submerged wood (Ferrer et al. 2011, Raja et al. 2012, Hyde et al. 2013). *Microthyriaceae* are reported as epiphytes on dried leaves of plants. In addition to the substrate, species of *Microthyriaceae* differ from *Pyrenidium* by thyriothecioid ascomata with hyaline, rarely brown, usually 1-septate ascospores (Müller & von Arx 1962, Ellis 1976, Ramaley 1999, von Arx & Müller 1975, Barr 1987, Hawksworth et al. 1995, Lumbsch & Huhndorf 2010, Wu et al. 2010, 2011, b, Hyde et al. 2013). *Zeloasperisporiaceae* are reported as saprobes on dead and living leaves (Wu et al. 2011, Hongsanan et al. 2015). This family can be distinguished from *Pyrenidium* by thyriothecioid, superficial ascomata, globose to ovoid or clavate, apedicellate asci and 1-septate, hyaline, asymmetric ascospores (Jayasiri et al. 2018). Based on our molecular results and the morphology, the family *Pyrenidiaceae* is resurrected from its synonymy with *Dacampiaceae*.

Pyrenidium actinellum has been recorded from a wide range of lichen host genera (e.g. Hawksworth 1983) but the species is considered to be heterogeneous. In our phylogenetic analyses, specimen '16557' from Belgium grows on the same lichen host genus as the type, i.e. *Scytinium plicatile*. It also shares morphological characters with the lectotype of *P. actinellum*, such as the 4-spored asci (Table 2). Therefore, we consider specimen 16557 to represent *P. actinellum sensu stricto*.

Most of the 2000 obligately lichenicolous taxa known worldwide are assumed to be restricted to a single host genus (Diederich et al. 2018). In our phylogenetic tree, specimen '18063' from Reunion groups with *P. actinellum sensu stricto* (strain Ertz 16557). The vouchers also have 4-spored asci and probably belong to the same species. Interestingly, specimen '18063' has a different host genus (= *Solorina*), suggesting that *P. actinellum* can grow on different host lichen genera (*Scytinium* and *Solorina*). However, further studies should test if *P. actinellum sensu stricto* is also able to grow on lichens having green algae as photobiont (cyanobacteria in *Scytinium* and *Solorina*). This species is also reported from different lichen genera having trebouxoid photobionts such as *Caloplaca*, *Phaeophyscia*, *Teloschistes* and *Trapeliopsis* (e.g. Navarro-Rosinés & Roux 2007). The closest relative of these two specimens of *P. actinellum* is *P. aff. aggregatum* (specimen 20089) from Madagascar. It differs from *P. actinellum sensu stricto* notably by the formation of strongly convex galls, more immersed perithecia, 8-spored asci and a lichen host having trebouxoid photobiont (Table 2). The new species *P. borbonicum* from Reunion is more similar to *P. aff. aggregatum* by its 8-spored asci and size of ascospores, but it is phylogenetically distinct from *P. cf. actinellum* and *P. aff. aggregatum* (Fig. 1). A hidden diversity might be expected in this genus as revealed by the discovery of the new species that is genetically clearly deviant from *P. actinellum*, a name that perhaps has been misused for the identification of *Pyrenidium* specimens from various host genera.

Table 2 Comparison of *Pyrenidium* species. Species in this study are in bold. *Pyrenidium ucrainicum* is probably a synonym of *Didymocyrtis ramalinae* according to Ertz et al. (2015).

<i>Pyrenidium</i> species	<i>Perithecioid</i> ascomata size (µm)	Asci size (µm)	Ascospores size (µm)	Number of ascospores per asci	Host Lichen	References
<i>Pyrenidium actinellum</i> (holotype, BM)	130–188 × 150–190	40–88 × 11–18	19–27 × 7–9, 3-septate	4-spored	<i>Scytinium teretiusculum</i>	Doilom et al. (2018)
<i>Pyrenidium</i> aff. <i>aggregatum</i> (Ertz 20089)	45–106 × 33–79	48–87 × 11–18	19–22 × 7–9, 3-septate	8-spored	<i>Parmeliaceae</i>	This study
<i>Pyrenidium actinellum</i> Nyl. sensu stricto. (Ertz 16557)	175–380 × 162–350	75–80 × 12–16	18–26 × 8–11, 3-septate	4-spored	<i>Scytinium plicatile</i>	This study
<i>Pyrenidium aggregatum</i>	150–250	85–95 × 17–20	15–21 × 6.5–8.5, 3-septate	8-spored	<i>Phaeophyscia</i> ssp. and <i>Physcia atrostriata</i>	Knudsen & Kocourkova (2010)
<i>Pyrenidium borbonicum</i> (Ertz 18031)	94–221 × 77–166	47–96 × 10–16	19–23 × 5–8, 3-septate	8-spored	Sticta	This study
<i>Pyrenidium borbonicum</i> (Ertz 18066)	125–165 × 117–184	64–98 × 11–17	22–27 × 4–8, 3-septate	8-spored	Sticta	This study
<i>Pyrenidium</i> cf. <i>actinellum</i> (Ertz 18063)	168–205 × 117–179	51–115 × 12–18	20–25 × 6–11, 3-septate	4-spored	<i>Solorina simensis</i>	This study
<i>Pyrenidium coccineum</i>	200–300	–	17–21 × 7.5–8.5, 3-septate	8-spored	<i>Siphula decumbens</i>	Aptroot (2014)
<i>Pyrenidium cryptotheciae</i>	100–150 × 80–150	55–80 × 10–14	9–13 × 4–6, 3-septate	8-spored	<i>Cryptothecia candida</i>	Matzer (1996)
<i>Pyrenidium hetairizans</i>	75–150	–	24–26 × 6–9, 3-septate	6–8-spored	<i>Verrucaria hydrela</i>	Hawksworth (1986)
<i>Pyrenidium hypotrachynae</i>	63–90 × 85–110	50–70 × 8.5–11	22.5–28 × 5–11, 3-septate	4-spored	<i>Hypotrachyna coorgiana</i>	Joshi et al. (2018)
<i>Pyrenidium octosporum</i>	–	65 × 28	20–26 × 7–10, 3-septate	8-spored	an unidentified lichen	Looman (1963)
<i>Pyrenidium santessonii</i>	50–100	50–60 × 10–13	13–15 × 4–5, 3-septate	8-spored	<i>Bacidia psychotriae</i> and <i>Bapalmuia</i> sp.	Lücking (1998)
<i>Pyrenidium sporopodium</i>	110–150 × 100–120	45–65 × 9–11	12–18 × 5–6, (2)–3-septate	4-spored	<i>Sporopodium</i> cf. <i>leprieurii</i> var. <i>citrinum</i>	Matzer (1996)
<i>Pyrenidium ucrainicum</i>	180–300 × 150–250	80–85 × (7.5–)8–10	12–22 × 4.5–7, 3(–4)-septate	8-spored	<i>Ramalina</i> sp.	Kondratyuk et al. (2014)
<i>Pyrenidium zamiae</i>	100–170 × 100–150	48–70 × 10–14	15–22 × 4–6, 3-septate	6–8-spored	<i>Porina epiphylla</i> , <i>P. ludica</i>	Matzer (1996)

Table 3 Morphological characters of *Dacampiaceae* and *Pyrenidiaceae*.

Family	Fruiting body Character	Peridium	Asci character	Ascospores character	References
<i>Dacampiaceae</i>	<i>Ascomata perithecioid</i> , blackish with a central ostiole. Includes lichenicolous, lichenized and saprobic fungi.	<i>Textura angularis</i> with thick-walled, reddish to dark brown cells.	Bitunicate, subcylindrical, apically thickened ocular chamber, 8-spored.	1–2-seriate, pale brown to dark brown, in mature state, wall not distinctly paler in the spore tips, muriform, ellipsoid to fusiform, often constricted at the septa, smooth, without visible gelatinous sheath.	Lutzoni et al. 2004, Zhang et al. 2009, Hyde et al. 2013, Ertz et al. 2015.
<i>Pyrenidiaceae</i>	<i>Ascomata perithecioid</i> , blackish with a central ostiole. Some species with light greenish-blue tint in ostiole. Includes lichenicolous.	<i>Textura angularis</i> to <i>Textura prismatica</i> with thick-walled, composed of several layers of dark brown to reddish brown.	Bitunicate, narrowly ellipsoid, shortly pedicellate, 4–8-spored.	1–2-seriate, reddish brown to dark brown in mature state, paler in , the spore tips, oblong to ellipsoidal, or fusiform usually 3-septate, constricted at the septa, smooth-walled, with the two inner cells often much larger than the end cells, without visible gelatinous sheath.	This study

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