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Standard Paper

Crittendenia gen. nov., a new lichenicolous lineage in the Agaricostilbomycetes (Pucciniomycotina), and a review of the biology, phylogeny and classification of lichenicolous heterobasidiomycetes

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Abstract

The lichenicolous 'heterobasidiomycetes' belong in the Tremellomycetes (Agaricomycotina) and in the Pucciniomycotina. In this paper, we provide an introduction and review of these lichenicolous taxa, focusing on recent studies and novelties of their classification, phylogeny and evolution. Lichen-inhabiting fungi in the Pucciniomycotina are represented by only a small number of species included in the genera *Chionosphaera*, *Cyphobasidium* and *Lichenozyma*. The phylogenetic position of the lichenicolous representatives of *Chionosphaera* has, however, never been investigated by molecular methods. Phylogenetic analyses using the nuclear SSU, ITS, and LSU ribosomal DNA markers reveal that the lichenicolous members of *Chionosphaera* form a monophyletic group in the Pucciniomycotina, distinct from *Chionosphaera* and outside the *Chionosphaeraceae*. The new genus *Crittendenia* is described to accommodate these lichen-inhabiting species. *Crittendenia* is characterized by minute synnemata-like basidiomata, the presence of clamp connections and aseptate tubular basidia from which 4–7 spores discharge passively, often in groups. *Crittendenia*, *Cyphobasidium* and *Lichenozyma* are the only lichenicolous lineages known so far in the Pucciniomycotina, whereas *Chionosphaera* does not include any lichenicolous taxa.

Key words: basidiomycetes, lichens, parasites, taxonomy, yeasts

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Introduction

'Heterobasidiomycetes' is an old, taxonomically redundant name for a heterogeneous assemblage of distantly related basidiomycete groups, which share the characteristics of having predominantly septate basidia and gelatinous basidiomata. Several of these groups contain lichen-inhabiting fungi and have lately attracted much attention among lichenologists. Despite this, some of these groups have remained surprisingly poorly studied and here we will introduce a study of the lichenicolous Pucciniomycotina, focusing on the representatives presently classified in *Chionosphaera*. We will also review the recent rapid development in our understanding of the evolution and natural relationships of heterobasidiomycete groups, including the long overdue integration of the classifications of yeasts and filamentous taxa, and we do this with a particular focus on lichenicolous representatives.

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Material and Methods

Taxon sampling

We used two different datasets for our phylogenetic analyses. The first one (dataset 1) included a representative sampling in the Pucciniomycotina (i.e. representative taxa of the 10 classes currently assigned to the group, and three lichenicolous *Chionosphaera* samples). In this dataset, a species in the Ustilaginomycotina (*Ustilago tritici*) was used as outgroup. Based on preliminary analyses of the first dataset, we used a second dataset focusing only on the Agaricostilbomycetes (dataset 2). This second sampling included representatives of the five accepted families in the Agaricostilbomycetes (i.e. *Agaricostilbaceae*, *Chionosphaeraceae*, *Jianyuniaceae*, *Kondoaceae* and *Ruineniaceae*: Wang *et al.* 2016; Li *et al.* 2020). Here we included a larger number of lichenicolous *Chionosphaera* samples, to test the monophyly of the lineage including lichenicolous species and to focus on the systematic position and affinities of the lichenicolous taxa within the Agaricostilbomycetes. *Chionosphaera* is represented by *C. apobasidialis*, *C. cuniculicola* and a number of lichenicolous specimens assigned to *Chionosphaera*, including an isotype of *C. coppinsii*. *Phyllozyma dimmenae* was used as outgroup based on Wang *et al.* (2016). Species names, voucher information, and GenBank Accession numbers are given in Table 1.

Morphological studies

Herbarium specimens are deposited in K, S, TRH and UPS, and in the private collections of C. Björk, P. Diederich, J. Etayo, P. van den Boom, U. Groner, J.C. Zamora, and E. Zimmermann. Macroscopic images were captured using a Canon 40D camera with a Nikon BD Plan 10 microscope objective, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Microscopic structures were studied on material mounted in water, 5% KOH, a mixture of phloxine B and 5% KOH, Congo red and Melzer's reagent using a Leica DMLB microscope fitted with DIC optics. Images were captured with a Leica EC3 camera and Helicon Focus.

DNA extraction, amplification and sequencing

DNA from lichenicolous specimens was extracted from either recently collected or dried herbarium material. *Chionosphaera* fruiting bodies were carefully sectioned and separated from the lichen thallus with a scalpel and tweezers, in order to minimize the lichen material in the DNA extraction. Approximately three to ten basidiomata were selected from each specimen for DNA extraction. Total DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Venlo, the Netherlands), according to the manufacturer's instructions. We used three molecular markers for dataset 1: the small subunit (nuSSU), the internal transcribed spacer (ITS) and the large subunit (nuLSU) of the nuclear ribosomal DNA. For dataset 2, we used ITS and nuLSU only, to avoid introducing too much missing data since we did not obtain nuSSU sequences of the lichenicolous taxa from the present study.

We designed specific primers to selectively amplify the DNA of the new genus, avoiding that of other basidiomycetes and of the lichenized host. Suitable priming sites were identified by aligning available sequences of representatives of *Chionosphaera* s. lat. against sequences of other lichenicolous basidiomycetes and lichenized hosts (*Lecanorales*, Ascomycota), selecting conserved fragments that differed markedly between them.

General fungal primers, viz. ITS1F (Gardes & Bruns 1993), ITS4 (White *et al.* 1990) LR0R (Rehner & Samuels 1994), LR3 (Vilgalys & Hester 1990), and newly designed primers (Table 2) were combined to amplify the ITS and a fragment of *c.* 600 bp of the nuLSU in the nuclear ribosomal DNA. Asymptomatic thalli of known hosts of *C. coppinsii* (*Melanohalea* spp.) were PCR screened for the presence of *Chionosphaera*. PCR amplifications were performed using Illustra™ Hot Start PCR beads (GE Healthcare Life Sciences, Pittsburg, California, USA), according to the manufacturer's instructions. PCR amplifications using the primer pair LR0R/LR3 were performed following Rehner & Samuels (1994). For the primer pairs ITS1F/ChioLSU 3-3 and ITS1F/Cc-R1, we used initial denaturing at 95 °C for 3 min, four cycles of 95 °C for 40 s, 53 °C for 40 s and 72 °C for 90 s, four cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 90 s, and finally 32 cycles of 95 °C for 30 s, 47 °C for 30 s and 72 °C for 90 s, with a final extension at 72 °C for 420 s. For the primer pair Cc-F1/ITS4, we used initial denaturing at 95 °C for 3 min, four cycles of 95 °C for 40 s, 52 °C for 40 s and 72 °C for 90 s, four cycles of 95 °C for 30 s, 49 °C for 30 s and 72 °C for 90 s, and finally 32 cycles of 95 °C for 30 s, 46 °C for 30 s and 72 °C for 90 s, with a final extension at 72 °C for 420 s.

Before sequencing, the PCR products were purified with Exo-sap-IT™ (USB Corporation, Cleveland, Ohio, USA). The purified samples were either run on an automated sequencer (ABI Prism 377) located in the Molecular Systematic Laboratory at the Swedish Museum of Natural History, or sequenced by Macrogen (Madrid, Spain).

Multiple alignment and phylogenetic analyses

For phylogenetic analyses, sequences were aligned using MAFFT version 7 (Katoh *et al.* 2019) with the Q-INS-i algorithm. The alignments were trimmed to exclude ambiguously aligned regions using GBlocks (Castresana 2000), following the relaxed conditions described by Talavera & Castresana (2007). Individual marker datasets were analyzed individually by maximum likelihood bootstrapping to assess for conflicts. No incongruence was found, and data were further analyzed as two- or three-marker concatenations. Phylogenetic relationships were reconstructed using maximum likelihood (ML) and Bayesian approaches. We considered five independent partitions, nuSSU, ITS1, 5.8S, ITS2 and nuLSU, for dataset 1, and four independent partitions, ITS1, 5.8S, ITS2 and nuLSU, for dataset 2. Maximum likelihood analyses were carried out in RAxMLGUI 1.5 (Silvestro & Michalak 2012), a graphical front-end for RAxML (Stamatakis 2014). The GTRGAMMA model of nucleotide substitution was applied to all partitions because of constraints of the software RAxML. We performed a thorough ML search with a total of 100 runs and assessed node support by thorough bootstrap using 1000 bootstrap pseudoreplicates. Bayesian analyses were performed by Markov chain Monte Carlo (MCMC) sampling as implemented in the software MrBayes 3.2.6 (Ronquist *et al.* 2012). We selected substitution models for each of the regions using the corrected Akaike information criterion (AICc) as implemented in jModelTest 2 (Darriba *et al.* 2012), using full likelihood optimization and six discrete gamma categories. For dataset 1, the SYM + I + Γ was selected for the nuclear SSU rDNA, the HKY + Γ was selected for the ITS1, the SYM + I + Γ for the 5.8S, the HKY + Γ for the ITS2, and the GTR + Γ for the nuclear LSU rDNA. For dataset 2, the JC model was selected for the ITS1, the K80 + I for the 5.8S, the K80 + Γ for the ITS2, and the GTR + I + Γ for the nuclear LSU rDNA. The combined analyses treated the different regions as separate partitions with topology linked across partitions but separate model parameter values and proportional rates across partitions. For each combined dataset, three parallel runs were performed, each with five chains, four of which were incrementally heated with a temperature of 0.15. The analyses were diagnosed for convergence every 100 000 generations and were set to halt automatically when the average standard deviation of splits across runs in the last half of the analysis descended below 0.01. Every 100th tree was saved. The first 50% of each run was discarded as burn-in.

Results

Biology, phylogeny and classification of lichenicolous heterobasidiomycetes

Lichenicolous heterobasidiomycetes are now known to be very common, many are widespread, and the species are usually host-specific. This includes genera in the Pucciniomycotina (*Chionosphaera*, *Cyphobasidium* and *Lichenozyma*: Diederich *et al.* 2018; Černajová & Škaloud 2019) and in the Tremellomycetes (*Biatoropsis*, *Heteroacanthella*, *Heterocephalacria*, *Syzygospora* s. lat. and *Tremella* s. lat.; Diederich *et al.* 2018). Other than lichenicolous fungi, Pucciniomycotina and Tremellomycetes mainly

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Table 1. Sequences included in this study, either newly produced (in bold) or retrieved from GenBank. Host, specimen data and DNA extraction code are given for newly sequenced samples. An asterisk indicates one case where the ITS sequence comes from a different culture than that of nuSSU and nuLSU. Hash symbols '#' indicate sequences of *Crittendenia coppinsii* obtained either from asymptomatic lichen specimens, or from the asymptomatic areas of lichen specimens with *C. coppinsii*.

Species name	Host	Specimen data	nSSU	ITS	nLSU
Pucciniomycotina					
Agaricostilbomycetes					
Ballistosporomyces sasicola			AB021688	AF444548	AF177412
B. taupoensis			-	AF444592	AF177413
B. xanthus			D64118	AF444547	AF177414
Bensingtonia ciliata			D38233	AF444563	AF189887
B. naganoensis			-	AF444558	AF189893
Chionosphaera apobasidialis			U77662	AF444599	AF177407
C. cuniculicola			KJ708368	KJ778640	KJ708465
Crittendenia coppinsii	*Melanelixia glabratula*	UK, Scotland, *Coppins* 16400 (Isotype) (K(M)-39188) DNA: AM267	-	**MT520689**	**MT482329**
C. coppinsii	*Melanelixia glabratula*	Norway, 2017, *Westberg* & *Olsson* s. n. (UPS) DNA: AM1045	-	**MT520690**	-
C. coppinsii	*Melanohalea exasperatula*	Sweden, *Westberg, Ekman* & *von Hirschheydt* s. n. (UPS F-796396) DNA: SAR380	-	**MT520691**	**MT482330**
C. coppinsii	*Melanohalea exasperatula*	Belgium, 2016, *van den Boom* 54983 (hb. van den Boom) DNA: AM696	-	**MT520692**	**MT482331**
C. coppinsii	*Melanohalea exasperatula*	Sweden, *Westberg* s. n. (S, UPS F-805352) DNA: AM850	-	**MT520693**	-
C. coppinsii	*Melanohalea exasperatula*	Sweden, *Westberg* s. n. (S, UPS F-805353) DNA: AM852	-	**MT520694**	-
C. coppinsii	*Melanohalea exasperatula*	Switzerland, 2017, *Zimmermann* 1946 (hb. Zimmermann) DNA: AM1043	-	**MT520695**	-
C. coppinsii #	*Melanohalea exasperatula*	Sweden, *Odelvik* 11471 (S F-206720) DNA: AM819	-	**MT520696**	-
C. coppinsii #	*Melanohalea exasperatula*	Sweden, *Westberg, Ekman* & *von Hirschheydt* s. n. (UPS F-796396) DNA: AM821	-	**MT520697**	-
C. coppinsii #	*Melanohalea exasperatula*	Sweden, *Westberg* s. n. (S, UPS F-805352) DNA: AM849	-	**MT520698**	-
C. coppinsii #	*Melanohalea exasperatula*	Sweden, *Westberg* s. n. (S, UPS F-805353) DNA: AM851	-	**MT520699**	-
Crittendenia sp.	On foliicolous *Bacidina*	Canary Islands, 2007, *Diederich* 16490 (hb. Diederich) DNA: AM113	-	**MT520700**	**MT482332**
Crittendenia sp.	On foliicolous *Bacidina*	Azores, 2017, *van den Boom* 56783 (hb. van den Boom) DNA: AM1073	-	**MT520701**	**MT482333**
Crittendenia sp.	*Hypotrachyna endochlora*	Azores, 2017, *Etayo* 31093 (hb. Etayo) DNA: AM1130	-	**MT520702**	**MT482334**
Crittendenia sp.	*Hypotrachyna* sp.	Azores, 2017, *Etayo* 30945 (hb. Etayo) DNA: AM1131	-	**MT520703**	**MT482335**
Crittendenia sp.	*Lecidella elaeochroma*	Spain, 2011, *Zamora* s. n. (hb. Zamora) DNA: AM1132	-	**MT520704**	**MT482336**
Crittendenia sp.	*Lecidella* sp.	Canada, 2009, *Björk* 17999 (hb. Björk) DNA: AM530	-	**MT520705**	**MT482337**
Crittendenia sp.	*Physconia distorta*	Spain, 2010, *Zamora* s. n. (hb. Zamora) DNA: AM1133	-	**MT520706**	**MT482338**
Cystobasidiopsis lactophila			AB021675	AF444545	AF177411
C. lophatheri			-	AB126046	AB124561

(*Continued*)

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Table 1. (*Continued*)

Species name	Host	Specimen data	nSSU	ITS	nLSU
Jianyunia sakaguchii			AB001746	AF444626	AF363646
Kondoa malvinella			D13776	AF444498	AF189903
K. miscanthi			-	AF444516	AF189891
K. subrosea			D38238	AF444565	AF189895
Kurtzmanomyces nectairei			D64122	AF444494	AF177409
K. tardus			-	AF444566	AF177410
Mycogloea nipponica			-	KJ778629	KJ708456
Pseudobensingtonia ingoldii			D38234	AF444519	AF189888
P. musae			-	AF444569	AF189892
Ruinenia clavata			-	AY364839	AY364839
R. rubra			AB021686	AF444550	AF189992
Sterigmatomyces elviae			-	AF444551	AF177415
S. halophilus			D64119	AF444556	AF177416
S. hyphaenes			AY665775	AY789077	AY634278
Stilbum vulgare			AY373387	GU291281	-
Atractiellomycetes					
Atractiella solani			DQ198797	DQ198781	AY512831
*Phleogena faginea**			DQ831022	MN989991	DQ831021
Classiculomycetes					
Classicula fluitans			AY124478	-	AY512838
Cryptomycocolacomycetes					
Cryptomycocolax abnormis			-	-	AY512841
Cystobasidiomycetes					
Buckleyzyma aurantiaca			KJ708436	AF444538	AF189921
Cyphobasidium hypogymniicola			KU587705	KU587700	KU587694
Cystobasidium pallidum			AB126651	AB078492	AF189962
Erythrobasidium hasegawae			D12803	AF444522	AF189899
Naohidea sebacea			KP216515	DQ911616	DQ831020
Microsporomyces magnisporus			KJ708428	AB112078	AB111954
Lichenozyma pisutiana			MK491257	MK491194	MK491265
Sakaguchia dacryoidea			D13459	AF444597	AF189972
Symmetrospora gracilis			KJ708433	AF444578	AF189985
Mixiomycetes					
Mixia osmundae			D14163	DQ831010	DQ831009
Microbotryomycetes					
Chrysozyma griseoflava			D66884	AF444557	AF189986
Glaciozyma antarctica			DQ785788	AF444529	AF189906
Heterogastridium pycnidioideum			KJ708412	GU291276	GU291290
Kriegeria eriophori			DQ419918	AF444602	KY108178

(*Continued*)

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Table 1. (*Continued*)

Species name	Host	Specimen data	nSSU	ITS	nLSU
Leucosporidium scottii			X53499	AF444495	AF070419
Microbotryum violaceum			KJ708388	KJ778635	KJ708462
Sporobolomyces salmonicolor			AB021697	AY015434	AF070439
Pucciniomycetes					
Endocronartium harknessii			AY665785	DQ206982	AY700193
Platygloea disciformis			DQ234563	DQ234556	AY629314
Puccinia graminis			AY125409	AF468044	AF522177
Spiculogloeomycetes					
Phyllozyma dimennae			D66881	AB038046	AB644404
P. subbrunnea			AB021691	AF444549	AF189997
Tritirachiomycetes					
Tritirachium oryzae			JF779647	GQ329853	MH870621
Ustilaginomycotina					
Ustilago tritici			DQ846895	DQ846894	DQ094784

comprise representatives with a variety of nutritional habits, including fungal and animal parasitism, plant parasitism and saprotrophy. Their diversity and evolution are still very poorly known and they may show potential co-evolutionary patterns with their hosts where host-specialization may be an important driver of speciation (Antonovics *et al.* 2002; Refrégier *et al.* 2008; Millanes *et al.* 2014*b*; Aime *et al.* 2018).

The diversity of lichen-inhabiting heterobasidiomycetes is clearly very large but it took a surprisingly long time before the common, gall-like structures occurring on lichens were recognized as basidiomycetes. The earliest reference to these structures was by Dillenius (1742), who referred to the deformations by *Biatoropsis usnearum* s. lat. on *Usnea* as 'small fleshy nodules closely appressed to the branches' (*orbiculos raro profert, sunt vero ii exigui carnei, ramis absque limbo arcte adnati*). A number of later authors, including Acharius (1795; and other references cited by Diederich & Christiansen 1994), mentioned gall-like structures on *Usnea* using different terminologies and interpretations, but it was not until Räsänen (1934) that they were connected to the presence of another fungus. When first described as an independent organism, *Biatoropsis* was believed to be an ascomycete (Räsänen 1934). Most lichenicolous heterobasidiomycetes were thus overlooked, if not completely neglected, until the studies by Diederich (1986, 1996) and Diederich & Christiansen (1994). The first published observation of a lichenicolous *Tremella* species was by Coppins & James (1979) on *Violella fucata*, even if this fungus remained formally unpublished until Diederich (1986) published it as *Tremella lichenicola*. The monograph by Diederich (1996) was effectively the start of a more thorough study of these fungi, including not only taxonomic descriptions of 41 new species (of the 54 species treated) but also the first discussions of putative relationships and a first serious attempt to classify them. Since Diederich's monograph, the number of newly discovered lichenicolous heterobasidiomycetes has continuously increased, currently reaching 74 species, and this number will certainly keep increasing in the future (Diederich *et al.* 2018, 2019; Diederich & Ertz 2020). Before the first molecular studies, classifications including lichenicolous heterobasidiomycetes were based on a small number of morphological characters only and were often difficult to interpret. Diederich (1996) had already pointed out that the taxonomic assignment of some taxa was only tentative. This was particularly the case in the genera *Biatoropsis*, *Chionosphaera*, *Cystobasidium* and *Syzygospora*, where the classification was principally based on basidium morphology and was still uncertain at that time (Diederich 1996). The same is true for *Heteroacanthella ellipsospora*, which is so far the only described lichenicolous species with acanthoid basidia (Zamora *et al.* 2014).

Diederich (1996) studied not only sexual stages of heterobasidiomycetes, but also numerous conidia-forming species. He coined the term asteroconidia for star-shaped conidia, arising from characteristic conidiogenous cells that have been observed in both *Filobasidiales* (*Heterocephalacria*) and *Tremellales* (*Tremella* s. lat.) but, intriguingly, never in non-lichenicolous species. Diederich also observed and illustrated spores that multiplied through unicellular budding (Diederich 1996; figs 17 and 111), in effect the first observations of yeast-stages in lichenicolous representatives. Prillinger *et al.* (1997) shortly after described five species of tremellalean yeasts isolated from epiphytic lichens. Zamora *et al.* (2016) also mentioned and illustrated the germination of spores by budding in species of lichenicolous *Tremella*. Many, perhaps most, heterobasidiomycete groups are dimorphic, switching between a unicellular haploid yeast phase and a filamentous phase, frequently including dikaryotic hyphae and a spore-producing hymenium, in their life cycle. These phases often occur on different hosts or substrata. The switch between phases at least sometimes activates pathogenicity, although frequently the yeast stages form large masses of cells that do not harm the host in any visible way (Lin 2009; Oberwinkler 2017). Recently, it was shown that two groups of lichenicolous heterobasidiomycetes, *Cyphobasidium* spp. (Pucciniomycotina) and *Tremella lethariae* (*Tremellales*, Agaricomycotina), were able to complete their whole life cycle within the lichen thallus (Spribille *et al.* 2016; Tuovinen *et al.* 2019), since yeasts, mycelium and hymenia are produced within

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Table 2. Primers newly designed for this study to selectively amplify the DNA of *Crittendenia*.

Primer name (direction)	Sequence (5′→3′)
Cc-F1 (forward)	TTTTTGTTAAACACTCGTGAC
Cc-R1 (reverse)	CGAAGATAAACTTATGCTGGCC
ChioLSU 3-3 (reverse)	GCAAGTCTAACTTCAATCGT

the same lichen thallus. The life cycle of lichenicolous taxa has been studied less than non-lichenicolous heterobasidiomycetes, as lichen-inhabiting fungi are usually very difficult to grow in culture.

For many years, the lack of cultures created problems in assessing the classification of lichen-inhabiting heterobasidiomycetes, since key characters for the classification, such as the anatomy of the septal pores, required material in culture to be studied (Bandoni 1984; Weiss *et al.* 2004). Molecular phylogenetics, however, has increased our understanding of these fascinating fungi considerably in recent decades. Tremellalean fungi that accidentally amplified instead of the expected lichen fungus were frequent sources of error and confusion in early PCR-based lichen studies, as noted, for example, by Ekman (1999). *Tremellales* have been later widely reported in metabarcoding studies of the lichen mycobiome (Fernández-Mendoza *et al.* 2017; Banchi *et al.* 2018). Millanes *et al.* (2011) introduced a set of nuclear rDNA primers designed to specifically amplify tremellalean basidiomycetes and thus the lichen host could usually be excluded from amplification experiments. Millanes *et al.* (2011) were the first to include lichenicolous *Tremella* s. lat. species in a larger phylogeny of Tremellomycetes. This study showed convincingly that the lichenicolous *Tremella* species form several unrelated groups within *Tremellales*, none of which is closely related to *T. mesenterica*, the type species of *Tremella*. *Biatoropsis* formed a group together with some lichenicolous *Tremella* species, despite the morphologically very different basidia that *Biatoropsis* species possess which initially caused Diederich & Christiansen (1994) to suggest that it should be classified within *Platygloeales*. Utilizing these primers and the dataset produced by Millanes *et al.* (2011), a number of morphologically distinct new *Tremella* species were later described (e.g. Millanes *et al.* 2012, 2014*a*, 2015; Diederich *et al.* 2014; Ariyawansa *et al.* 2015; Zamora *et al.* 2016, 2017, 2018).

For a long time, the classification of filamentous and yeast-like heterobasidiomycetes unfortunately developed in parallel, as different groups of researchers traditionally focused on studies of either macrofungi or yeasts (Swann & Taylor 1995; Chen 1998; Fell *et al.* 1999). This also hampered the development of a modern classification of heterobasidiomycete groups and the fulfilment of the 'One fungus, one name' principle, since some species had separate anamorph and teleomorph names for yeast and filamentous stages, respectively. In many cases the connection between these stages is also unknown, and often the sexual, filamentous stage seems to have been lost or reduced in frequency. Despite the previous attempts of integrated phylogenetic studies (Fell *et al.* 2000; Scorzetti *et al.* 2002; Sampaio 2004; Inácio *et al.* 2005; Matheny *et al.* 2007; Boekhout *et al.* 2011; Wuczkowski *et al.* 2011), it was only recently that a larger group effort resulted in the first integrated phylogenetic classification of yeasts and filamentous groups in the Tremellomycetes (Liu *et al.* 2016). This study is a very good introduction to Tremellomycetes phylogeny (including the lichenicolous lifestyle) and could serve as a baseline for future studies focusing on the evolution of mycoparasitism in the group. It further highlights the polyphyly of *Tremella* and other genera, and discusses the widespread occurrence of morphologically defined but polyphyletic yeast groups within the Tremellomycetes. In the most recent phylogenetic and taxonomic studies (Liu *et al.* 2015, 2016; Li *et al.* 2020), five orders, 17 families and 55 genera were accepted. The works by Liu *et al.* (2015, 2016) and Li *et al.* (2020) represent a good framework on which to incorporate information and taxa, but many taxonomic issues remain unresolved, particularly regarding lichenicolous species.

Although the systematics and classification in the Tremellomycetes is far from stable, several molecular phylogenetic studies of lichenicolous representatives have contributed substantially to the recent progress. They have also provided valuable information on other aspects of the evolution of these fungi. An example is the evolution of the basidium morphology. Although this character was traditionally considered to be of great taxonomic importance in the group, it has now repeatedly been shown to be misleading when trying to circumscribe higher taxa. However, the morphological characteristics of the basidium are still useful to circumscribe taxa in the *Tremellales* at lower taxonomic levels. Millanes *et al.* (2011) studied the evolution of the 'basidium habit' (i.e. single basidia or basidia forming chains) and of the septation patterns in individual basidia. They found that the single basidium and the longitudinally septate basidium were the more ancestral character states, whereas catenulate basidia and other septation patterns in individual basidia were derived character states. It was also interesting to note that different basidium-related characters had evolved following different evolutionary models, for example, either a punctuated model (basidium habit) or a model of gradual evolution (basidium septation). This, together with frequent and independent transformations of the basidium in the phylogeny of the group, again indicated different evolutionary mechanisms involved in the variety of basidia observed, and pointed to a limited usefulness of basidium-related traits as taxonomic characters to characterize higher taxa in the Tremellomycetes (Millanes *et al.* 2011). More recently, thorough phylogenetic studies of basidiomycetes have enabled dating the origin of both the Tremellomycetes (estimated mean value: 303 MA) and *Tremellales* (estimated mean value: 156 MA) (Floudas *et al.* 2012; He *et al.* 2019).

Our phylogenies allowed us to investigate the possible influence of a joint evolutionary story between hosts and parasites in the diversification of tremellalean species, particularly in lichenicolous species (Millanes *et al.* 2014*b*). We chose the *Biatoropsis-Usnea* system to explore early stages of speciation and to investigate mechanisms generating diversity and found that co-speciation was not a main evolutionary event in this system. Instead, divergence resulting from host specialization seemed to occur frequently through host-switch speciation (Millanes *et al.* 2014*b*, 2016*a*). Similarly, our earlier population haplotype and coalescent-based studies on lichenicolous *Tremellales* in Macaronesia revealed that host species and not geography influenced the genetic structure of *Tremella lobariacearum* (Werth *et al.* 2013).

In addition to the Tremellomycetes, the Pucciniomycotina is the other larger group of fungi including lichenicolous heterobasidiomycetes. It mainly comprises plant pathogens in the *Pucciniales*, and the rest of the group is remarkably ecologically and biologically diverse. Lichen-inhabiting species are represented only in the genera *Chionosphaera*, *Cyphobasidium* and *Lichenozyma*. *Cyphobasidium* was recently described as a result of our own phylogenetic studies that showed that the lichenicolous members of *Cystobasidium* (*C. hypogymniicola* and *C. usneicola*) formed a

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monophyletic group distinct from *Cystobasidium* s. str. and outside the *Cystobasidiales* (Millanes *et al.* 2016*b*). *Cyphobasidium* is characterized by having distinctive basidia that arise from a thick-walled structure, the probasidium, and by its lichenicolous occurrence on species of *Hypogymnia* and *Usnea*. It induces conspicuous gall-like structures, containing basidia, on the host lichen thalli.

Almost simultaneously with Millanes *et al.* (2016*b*), Spribille and co-workers (2016) discovered numerous yeast lineages from this group and recognized the new order *Cyphobasidiales* to accommodate them, although the systematics of this order is still unsettled (Kachalkin *et al.* 2019). Spribille *et al.* (2016) found that the relative abundance of *Cyphobasidium* yeasts in the cortex of *Bryoria tortuosa* was correlated with the production of vulpinic acid in this lichen. The variable production of vulpinic acid had led in the past to the treatment of *B. tortuosa* and *B. fremontii* as distinct species, until molecular studies suggested they were conspecific (Velmala *et al.* 2009). Spribille *et al.* (2016) and Spribille (2018) also suggested that cystobasidiomycete yeasts constituted a third component of the lichen symbiosis, particularly in the lichen family *Parmeliaceae*, and that they could play a role in the formation of the lichen cortex. This hypothesis, however, still remains to be tested. In a large review, Oberwinkler (2017) stated that '… these mycoparasites are dimorphic … having a haploid yeast phase as initial stage … it is a common phenomenon of yeasts that they propagate mitotically to produce yeast colonies …' and concluded that the basidiomycete yeasts in lichen thalli are not a third component of the lichen symbiosis but the typical yeast-formed vegetative propagules so frequently produced by other mycoparasites. In a recent study, Lendemer *et al.* (2019) used metagenomic data to investigate the presence of *Cyphobasidium* across a much wider range of lichens, and observed that neither *Cyphobasidium* nor other cystobasidiomycete yeasts were commonly found outside lichens of the *Parmeliaceae*. Smith *et al.* (2020) also found little evidence of a widespread presence of *Cyphobasidium* yeasts in macrolichens, although Černajová & Škaloud (2019) had characterized a high and widespread diversity of cystobasidiomycete yeasts associated with *Cladonia*. Interestingly, Černajová & Škaloud (2019) also isolated these yeasts from ecorticate species and, in addition, were able to produce cultures of these yeasts from the medulla of some *Cladonia* specimens. This indicates that the yeasts are not exclusively limited to growing in the cortex. However, some species could still be part of a superficial biofilm, as suggested by Spribille (2018) and Spribille *et al.* (2020). Regarding host-specialization, Mark *et al.* (2020) recently investigated the specificity of *Cyphobasidium* yeasts, found in six common lichen species of *Lecanoraceae*, *Parmeliaceae* and *Physciaceae*, towards the lichen mycobionts, and they could not confirm a strong specialization of the yeasts, compared to that observed in the photobiont. But irrespective of their potential role or abundance, the discovery of a vast diversity of lichenicolous cystobasidiomycete yeasts associated with lichens in the *Parmeliaceae* (Spribille *et al.* 2016) raised new challenges for the taxonomic characterization of this diversity, and it is likely that *Cyphobasidium* and its possible role in lichens will continue to be discussed for some time.

In contrast with the recent activities focusing on *Cyphobasidium*, other lichenicolous taxa in the Pucciniomycotina remain comparatively poorly studied. One of the most intriguing and still poorly understood genera in the Pucciniomycotina is *Chionosphaera,* and we will focus the second part of this manuscript on some recent and novel results on its phylogeny and classification.

Chionosphaera was described by Cox (1976) based on a single species (*C. apobasidialis*) which is dimorphic and heterothallic, and forms characteristic white capitate synnemata-like fruiting bodies. The reniform spores are produced on holobasidia and the spores germinate by budding (i.e. have a distinct yeast phase). In nature, *C. apobasidialis* presumably grows associated with *Cladosporium herbarum* s. lat. (Dothideomycetes, Ascomycota). Cox (1976) included *Chionosphaera* in the *Filobasidiales* (Agaricomycotina) because of the basidium morphology that closely resembles that of *Filobasidium floriforme*. Oberwinkler & Bandoni (1982) later erected the new order *Atractiellales* (currently in Pucciniomycotina) that included, among others, the new family *Chionosphaeraceae* to accommodate both *Chionosphaera* and *Stilbum*.

Since the description of *Chionosphaera apobasidialis*, four additional species of *Chionosphaera* have been described: *C. lichenicola* Alstrup *et al.* (Alstrup 1993), *C. coppinsii* P. Roberts (Roberts 1997), *C. cuniculicola* Kirschner *et al.* (Kirschner *et al.* 2001) and *C. phylaciicola* (Seifert & Bandoni) R. Kirschner & Oberw. (Seifert *et al.* 1992; Kirschner *et al.* 2001). *Stilbum erythrinae* Hansf. was further tentatively combined into *Chionosphaera* because its spores are thin-walled and the expected two-celled basidia with denticulate sterigmata were not observed (Kirschner & Chen 2008). The genus currently includes six species, of which only *C. coppinsii* and *C. lichenicola* have a lichenicolous habit. Both *C. coppinsii* and *C. lichenicola* have clamp connections, just as *C. erythrinae* and *C. phylaciicola*, whereas *C. apobasidialis* and *C. cuniculicola* lack clamps. This, together with the differences in ecology, suggests that the genus is heterogeneous.

Based on molecular data, Bauer *et al.* (2006) and Wang *et al.* (2015, 2016) placed *Chionosphaeraceae* in the *Agaricostilbales* (Agaricostilbomycetes, Pucciniomycotina). To date, only *Chionosphaera apobasidialis* and *C. cuniculicola* have been included in molecular studies (Sampaio *et al.* 1999; Kirschner *et al.* 2001; Bauer *et al.* 2006; Wang *et al.* 2015, 2016) and the phylogenetic position of other species currently assigned to *Chionosphaera*, particularly of those with a lichenicolous habit, is uncertain. Although the life cycle of some *Chionosphaera* species (viz. *C. apobasidialis* and *C. cuniculicola*) has been studied in detail using cultured material, the yeast phase of the lichenicolous species (although mentioned and illustrated already by Roberts (1997)) is very poorly understood. The hypothesis that these species can also complete their life cycle within the lichen thallus, as shown in other lichenicolous heterobasidiomycetes, remains untested.

Here we present recent results on the classification, diversity and biology of lichenicolous taxa currently included in *Chionosphaera*. Using molecular data, we investigate 1) the phylogenetic position of the lichenicolous *Chionosphaera*, 2) the diversity of lichenicolous species comprised in the genus, and 3) the hypothetical occurrence of *Chionosphaera* in asymptomatic thalli of lichenized hosts.

Phylogeny and systematics of lichenicolous Chionosphaera

Molecular results. We generated 28 new sequences (18 ITS and 10 nuLSU rDNA) that were aligned together with sequences already available in GenBank (Table 1). The combined matrix corresponding to dataset 1 contained 2501 aligned characters (nuLSU, 1–1645; ITS1, 1646–1692; 5.8S, 1693–1844; ITS2, 1845–1954; nuLSU, 1955–2501). The combined matrix corresponding to dataset 2 contained 995 aligned characters (ITS1, 1–90; 5.8S, 91–241; ITS2, 242–367; nuLSU, 368–995).

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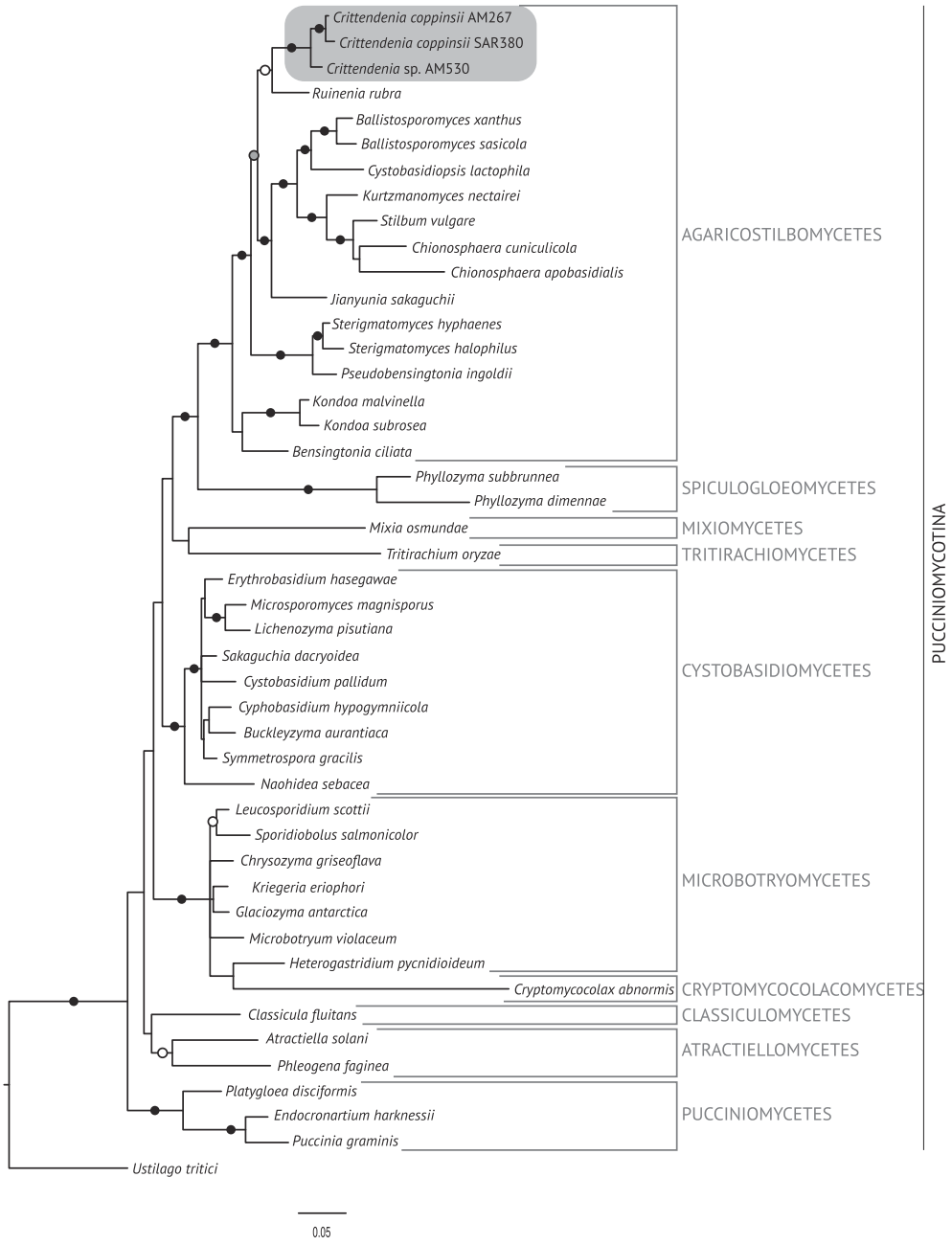

Fig. 1. Fifty percent majority-rule Bayesian consensus tree from the combined analysis including nuSSU, ITS and nuLSU, and representing the Pucciniomycotina. Black dots indicate branches supported by both Bayesian and ML analyses. White dots indicate branches supported only by Bayesian analysis. Branch lengths are scaled to the expected number of substitutions per site. *Crittendenia* representatives are enclosed in a grey box. Classes currently included in the Pucciniomycotina are indicated in the right margin.

The best trees obtained from the ML analyses had lnLikelihood values of −5226.293179 for dataset 1 and −8467.292203 for dataset 2. The Bayesian analyses halted after 2 000 000 generations in analyses of dataset 1, and after 400 000 generations in analyses of dataset 2, when the average standard deviation of split frequencies across runs was < 0.01, indicating that the three runs had converged (< 0.01). In all analyses, Potential Scale Reduction Factor (PSRF) values for all model parameters as well as all branch lengths were close to 1. A majority-rule consensus tree was constructed from the 30 000 trees (dataset 1) or 6000 trees (dataset 2) of the stationary tree sample. There was no incongruence between the ML and Bayesian trees in any of the two analyzed datasets. Therefore, only the 50% majority-rule consensus tree from the Bayesian analyses corresponding to datasets 1 and 2 are shown in Figs 1 and 2, respectively, with information on ML bootstrap values added. Our analyses revealed a distinct group including all lichenicolous specimens previously assigned to *Chionosphaera*, placed outside *Chionosphaera* s. str. (Figs 1 & 2). The new genus *Crittendenia* is consequently described to accommodate these lichen-inhabiting taxa. *Crittendenia* sequences were also obtained from totally asymptomatic thalli of *Melanohalea exasperatula*, possibly representing an asexual yeast phase of this fungus (Table 1).

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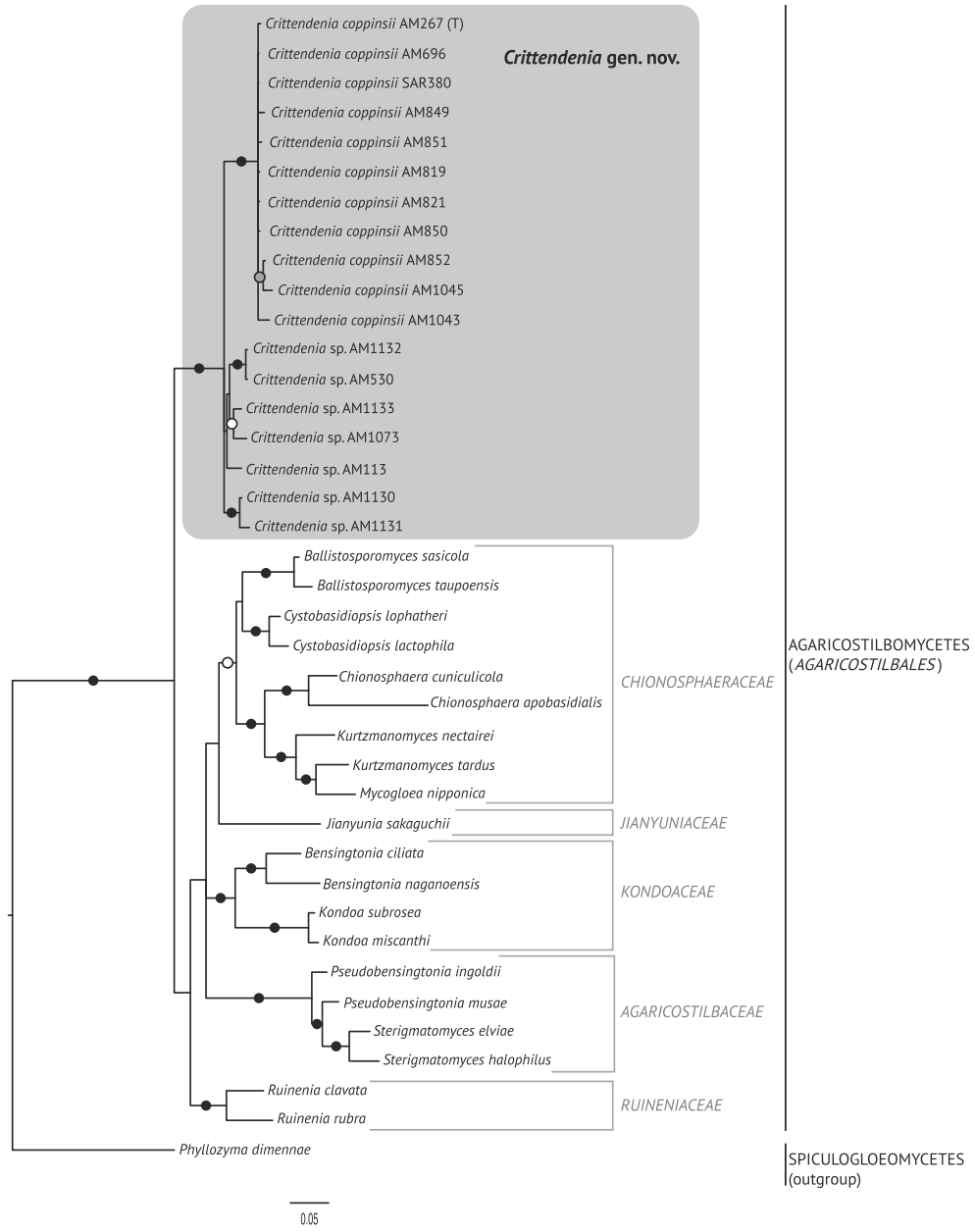

Fig. 2. Fifty percent majority rule Bayesian consensus tree from the combined analysis including ITS and nuLSU, and representing the Agaricostilbomycetes. Black dots indicate branches supported by both Bayesian and ML analyses. White and grey dots indicate branches supported only by Bayesian or ML analyses, respectively. Branch lengths are scaled to the expected number of substitutions per site. *Crittendenia* representatives are enclosed in a grey box and the type is indicated with '(T)'. Suprageneric taxa are indicated in the right margin.

Taxonomy

Crittendenia Diederich, Millanes, M. Westb., Etayo, J.C. Zamora & Wedin gen. nov.

MycoBank No.: MB 835604

Differs from *Chionosphaera* by the presence of basidial clamps.

Type species: *Crittendenia coppinsii* (P. Roberts) Diederich, M. Westb., Millanes & Wedin.

(Fig. 3)

Basidiomata developing on lichens, stipitate-capitate, synnemata-like, fleshy waxy, pale, slightly translucent; capitulum slightly to strongly differentiated and enlarged. Stipe composed of parallel, rarely branched hyphae with few septa; hyphidia and haustorial branches unknown. *Basidia* apical, tubular, aseptate, thin-walled, with basal clamps, when immature apically rounded, when mature with 4–7 apical, short, inconspicuous sterigmata, collapsing after spore detachment. *Basidiospores* hyaline, aseptate, ellipsoid to fusiform, with a small, often indistinct, basal apiculus, not forcibly discharged, often liberating together as a cluster of 4–7 spores. Basidiospores probably capable of germination by budding.

Asexual conidial stage unknown.

Etymology. We are very happy to dedicate the new genus to our friend and colleague Peter Crittenden to endorse the importance of his lichenological career. This is not only to recognize his

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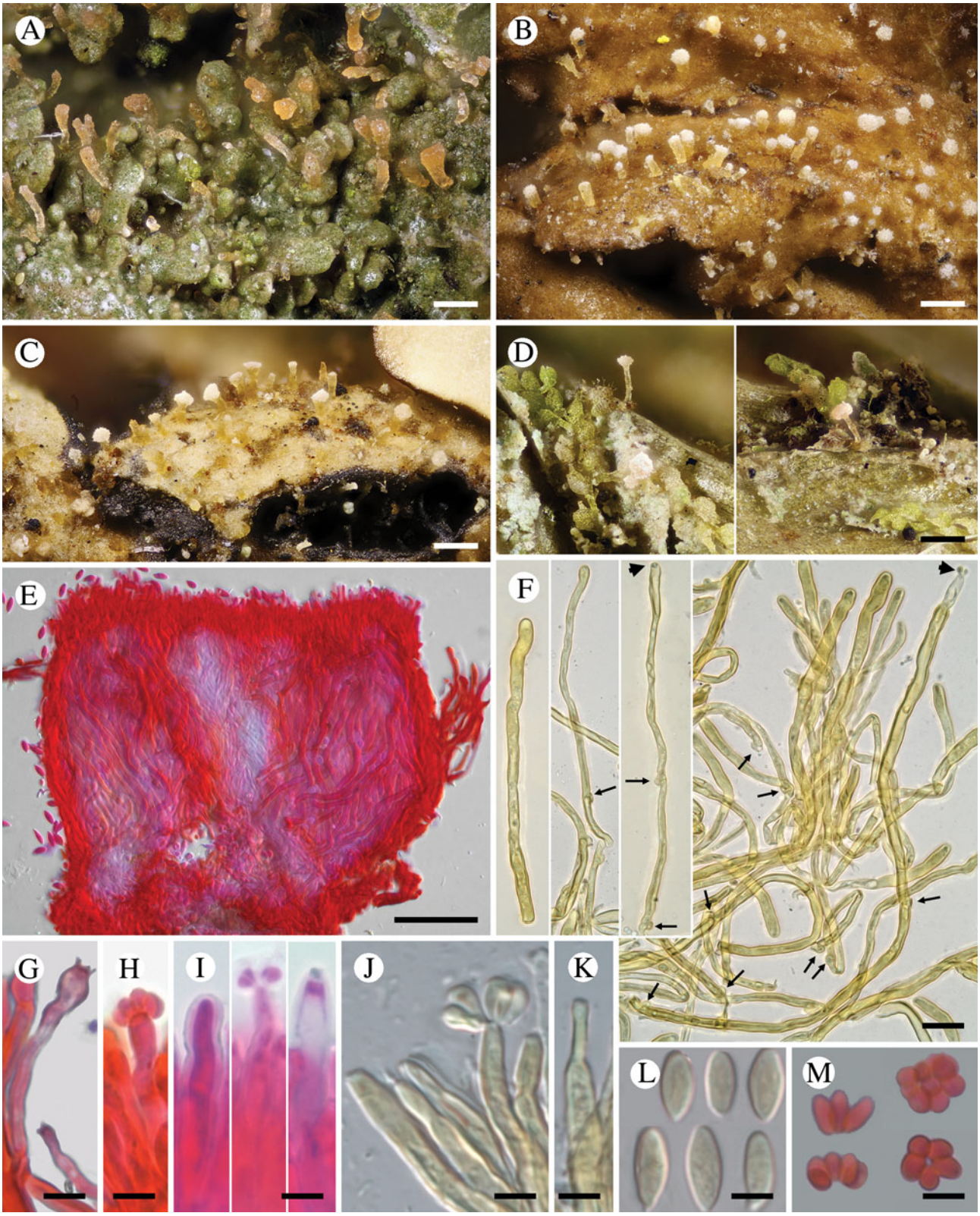

Fig. 3. Diversity of *Crittendenia* species. A, immature basidiomata on *Melanohalea exasperatula*. B, mature basidiomata on *Melanelixia glabratula*. C, basidiomata on *Hypotrachyna laevigata*. D, slender basidiomata on *Fellhanera bouteillei*. E, entire basidioma (in Congo red). F, basidia with basal clamps (arrows), some with inconspicuous sterigmata and/or young basidiospores (arrow heads) (in Melzer's reagent). G & H, basidia with sterigmata and basidiospores (in Congo red). I, basidium development: immature basidium (left), mature basidium with basidiospores (middle), old basidium (right) (in phloxine B). J, mature basidium with basidiospores (in Melzer's reagent). K, cystidium-like structure of unknown function (in Melzer's reagent). L, basidiospores (in Melzer). M, clusters of basidiospores separated from basidia. A, B, E, F, I–L, *Crittendenia coppinsii* (A & F, *van den Boom* 54983; B, E, I–L, *Groner* 714); C, *Crittendenia* sp. (*Diederich* 4913); D, *Crittendenia* sp. (*van den Boom* 56901); G, H & M, *Crittendenia* sp. on *Bacidia* (*Kalb* 26946). Scales: A–D = 200 μm; E = 50 μm; F = 10 μm; G–M = 5 μm.

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impressive and highly valuable work as a lichen symbiosis researcher, but above all to acknowledge his outstanding contributions as Senior Editor of *The Lichenologist* over the past 20 years.

Ecology. Lichenicolous, associated with a large variety of lichens belonging to different phylogenetic lineages.

Crittendenia coppinsii (P. Roberts) Diederich, M. Westb., Millanes & Wedin comb. nov.

MycoBank No.: MB 835606

Basionym: *Chionosphaera coppinsii* P. Roberts, *Mycotaxon* **63**, 195 (1997); type: Scotland, Wester Ross, Torridon, Inveralligan, wood & gorge of Abhainn Alligin, on *Melanelixia glabratula*, 21 vi 1994, *B. J. Coppins* 16400 & *A. M. O'Dare* (E—holotype, non vid.; K 39188—isotype!).

A detailed description of this species is provided by Roberts (1997). *Crittendenia coppinsii* was originally described from the UK (Scotland) and the type grows on *Melanelixia glabratula* (Roberts 1997). A second specimen provisionally reported by P. Roberts (1997) and growing on *Lecidella elaeochroma* was later assigned to *C. lichenicola* by Kirschner *et al.* (2001). Coppins *et al.* (2009) also found *C. coppinsii* on a new host, *Melanelixia subaurifera*. Here we report *Crittendenia coppinsii* as new to Belgium, Norway, Sweden and Switzerland, based on specimens examined by us that grow on *Melanelixia glabratula*, *Melanohalea exasperata* and *M. exasperatula*. A record of '*Chionosphaera* cf. *apobasidialis*' from Russia on *Melanohalea olivacea* is further accepted as belonging to *C. coppinsii* because the description fully agrees with *C. coppinsii* s. str. and the host is a species of *Melanohalea* (Zhurbenko & Himelbrant 2002). *Crittendenia coppinsii* should be actively searched for on other species of *Melanelixia* and *Melanohalea*.

Additional specimens examined. **Belgium:** *Liège*: Eupen, between road N68 and N620, 50°35′N, 6°2.5′E, 420 m, 2016, *van den Boom* 54983 (hb. van den Boom).—**Norway:** *Møre og Romsal*: Halsa, S-facing slope by Halsafjorden, Kalsetlia, 100 m, 2000, *Holien* 8105 (TRH); Rama, the Romsdalen Valley, S of Trollveggen Camping by the River Rauma (WP54), 6 vi 2017, *Westberg* & *Olsson* s. n. (UPS).—**Sweden:** *Uppland*: Vänge, Fiby Urskog Nature Reserve, southernmost part near entrance, 59° 52.9′N, 17°21.15′E, 7 iv 2016, *Westberg* (S, UPS F-805352); *ibid.*, 8 iv 2016, *Westberg, Ekman* & *von Hirschheydt* (UPS F-796396); *ibid.*, NE of the nature reserve, 59°53.4′N, 17°21.6′E, 7 iv 2017, *Westberg* (S, UPS F-805353).—**Switzerland:** *Bern*: Lenk, Zelg, Simmenfälle, 1030 m, 2017, *Zimmermann* 1946 (hb. Zimmermann). *Schwyz*: Muotathal, E Fruttli, Flaschenwald, 1240 m, 1989, *Groner* 714 (hb. Groner).

Crittendenia lichenicola (Alstrup, B. Sutton & Tønsberg) Diederich, Millanes & Wedin comb. nov.

MycoBank No.: MB 835607

Basionym: *Chionosphaera lichenicola* Alstrup *et al.*, *Graphis Scripta* **5**, 97 (1993); type: Norway, Hordaland, Fjell, Lokøy, the peninsula S of Storafjellet, alt. 10 m, on *Sorbus aucuparia*, on *Micarea prasina*, 27 viii 1989, *Tønsberg* 12000 (BG—holotype!; C, IMI—isotypes!).

Descriptions of this species are provided by Alstrup (1993), Diederich (1996) and Kirschner *et al.* (2001). *Crittendenia lichenicola* differs from *C. coppinsii* in the much narrower and more delicate basidiomata, shorter basidia, smaller basidiospores, and host selection. *Crittendenia lichenicola* was originally described from Norway, growing on *Micarea prasina* (Alstrup 1993), and has never been re-collected on this host. However, a second specimen has been published from Scotland on *Micarea micrococca* by Coppins & Coppins (2005). The specimen growing on *Lecidella elaeochroma* reported by Roberts (1997) as *Chionosphaera coppinsii* and eventually assigned to *C. lichenicola* by Kirschner *et al.* (2001) is here tentatively excluded from *C. lichenicola*, awaiting a taxonomic revision of the genus.

Discussion on Crittendenia *gen. nov.*

Crittendenia is a distinct and mainly lichen-inhabiting lineage in the *Agaricostilbales* (Pucciniomycotina), different from *Chionosphaera*. *Crittendenia* includes two known species that grow on lichen hosts of the *Lecanorales*: *C. coppinsii* is apparently confined to hosts in two closely related genera in the *Parmelia*-clade of the *Parmeliaceae* (*Melanelixia* and *Melanohalea*) (Fig. 3A & B); *C. lichenicola* occurs on a host of the *Pilocarpaceae*. Many additional specimens growing on a large variety of hosts in the families *Lecanoraceae*, *Lobariaceae*, *Parmeliaceae*, *Physciaceae*, *Ramalinaceae* and *Teloschistaceae*, some of which have been included in our phylogenetic analysis (Figs 2, 3C & D), await a morphological, taxonomic revision and are not considered further here. In a small number of cases, the interaction with a lichen host is difficult to ascertain. Since species in *Crittendenia* are difficult to observe in the field owing to the extremely small size of the basidiomata and the pale coloration, some of them are even difficult to detect under a binocular microscope and therefore relatively seldom collected, it is possible that the true range of hosts is even larger than reported here. Within Pucciniomycotina, *Crittendenia*, *Cyphobasidium* and *Lichenozyma* are the only taxa with a lichenicolous habit that have been described so far, and they are not closely related (Wang *et al.* 2016; Černajová & Škaloud 2019; Li *et al.* 2020). *Lichenozyma*, isolated from *Cladonia*, is known only from its yeast stage, and Li *et al.* (2020) suggested that it is a synonym of *Microsporomyces*. The latter genus would then be the only known lineage in Pucciniomycotina including both lichen-inhabiting taxa and species isolated from plant substrata. It is probable that more lichenicolous lineages are still to be discovered in Pucciniomycotina.

In addition to *Chionosphaera* and *Crittendenia,* representatives with synnematous fruiting bodies are common in Pucciniomycotina. Stilboid fruiting bodies are frequent in the Agaricostilbomycetes and are also formed in *Atractiella* and *Phleogena* (*Atractiellales*, Pucciniomycotina). All these taxa have, however, transversely septate basidia (Oberwinkler & Bandoni 1982; Aime *et al.* 2014), and none is phylogenetically closely related to *Crittendenia* (Fig. 1). Microscopically, the basidium of *Filobasidium* (*Filobasidiales*, Tremellomycetes) could suggest affinities to *Crittendenia* but *Filobasidium* is also phylogenetically unrelated to the new genus. Our results support existing evidence indicating that the morphology of both fruiting bodies and basidia has limited value in characterizing natural higher taxonomic groups in Basidiomycota.

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Other species morphologically resembling *Chionosphaera* and *Crittendenia*, and still requiring study, are the non-lichenicolous species *Chionosphaera erythrinae* and *C. phylaciicola* (Seifert *et al.* 1992; Kirschner *et al.* 2001; Kirschner & Chen 2008). Our results suggest retaining the clampless species in *Chionosphaera* (i.e. *C. apobasidialis* and *C. cuniculicola*), whereas those producing clamp connections (i.e. *C. coppinsii* and *C. lichenicola*) are transferred to the new genus *Crittendenia* (Fig. 3F). *Chionosphaera erythrinae* and *C. phylaciicola* also have clamp connections, which suggests a possible connection with the new genus *Crittendenia*. *Chionosphaera erythrinae* is known only from the type specimen that consists of two fruiting structures associated with a *Cladosporium*-like hyphomycete on leaves of *Erythrina tomentosa*. *Chionosphaera phylaciicola* was described from South America as *Fibulostilbum phylaciicola*, growing on stromata of the ascomycete *Phylacia poculiformis* (Seifert *et al.* 1992). It was later transferred to *Chionosphaera* by Kirschner *et al.* (2001) as they considered the slight morphological differences with *Chionosphaera apobasidialis*, and the different fungal association, insufficient to segregate it from *Chionosphaera*. We unfortunately could not obtain any specimens of these two taxa for our study. Based on the host selection, the two species may be more closely related to *Chionosphaera* or perhaps represent different lineages, but molecular investigations will be needed to elucidate their systematic position.

The family assignment of *Crittendenia* within the Agaricostilbomycetes is also uncertain, until additional molecular markers other than the nuclear ribosomal DNA can be sequenced and utilized in more robust phylogenies of the group. Our analyses of the Pucciniomycotina (Fig. 1) suggest an affinity with the family *Ruineniaceae*. However, only the ITS and the nuLSU of the ribosomal DNA have been amplified for *Crittendenia* specimens. Therefore, the family allocation of the new genus should wait until a larger dataset with more markers is available for this taxon.

Our results show that the species delimitation in the new genus will need further investigation. *Crittendenia coppinsii* is a well-delimited species according to our phylogenetic studies and microscopical observations, and apparently grows on *Melanelixia* and *Melanohalea* only, two closely related genera in the *Parmeliaceae* (Blanco *et al.* 2004; Arup & Sandler Berlin 2011; Divakar *et al.* 2017). In contrast, herbarium specimens currently assigned to *C. lichenicola* probably represent a heterogeneous assemblage of several species, some of which are possibly host-specific. Host selection is an important factor that characterizes monophyletic groups in other mycoparasitic basidiomycetes (Millanes *et al.* 2015, 2016*a*) and this might also be the case in *Crittendenia*.

Some questions still remain regarding the life cycle of *Crittendenia*, among them whether there is an asexual yeast phase. In addition to the observed basidiomata, we have amplified and sequenced *C. coppinsii* from several completely asymptomatic thalli of *Melanohalea exasperatula* (Table 1, Fig. 2). The yeast phase of a yet unidentified lichenicolous specimen of *Crittendenia* on *Lecidella elaeochroma* was illustrated by Roberts (1997); this leads us to suspect that the sequences obtained from asymptomatic thalli correspond to yeast phases of *Crittendenia*, although we cannot fully exclude the presence of mycelia which, however, seem difficult to observe by light microscopy. If a yeast stage is indeed present, *Crittendenia* would expand the increasing quantity of previously overlooked yeast-stage diversity, recently reported from the Pucciniomycotina (Spribille *et al.* 2016; Černajová & Škaloud 2019; Kachalkin *et al.* 2019; Li *et al.* 2020).

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