**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.

**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*, *Jianyuniaeae*, *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.

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**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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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 optimizations 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 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 + I 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 & Skaloud 2019) and in the Tremellomycetes (*Biotropolipsis, Heteroanathella, Heterocephalacria, Syzygospora* s. lat. and *Tremellella* 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** |                         |                                |       |        |         |
| *Ballistosporomycyes sasicola* |             | AB021688                        | AF444548 | AF177412 |
| *B. taupoensis*       |                           | -                              | AF444592 | AF177413 |
| *B. xanthus*          |                           | D64118                         | AF444547 | AF177414 |
| *Bensingtonia ciliata* |                           | D38233                         | AF444563 | AF189887 |
| *B. naganoensis*      |                           | -                              | AF444558 | AF189893 |
| **Chionosphaera apobasidialis** |               |                                 |       |        |         |
| *C. cuniculicola*     |                           | KJ708368                       | KJ778640 | KJ708465 |
| *C. coppinsii Melanelixia glabratula* |          | UK, Scotland, Coppins 16400 (Isotype) (K(M)-39188) DNA: AM267 |       |        |         |
| *C. coppinsii*        | Melanelixia glabratula    | Norway, 2017, Westberg & Olsson s. n. (UPS) DNA: AM1045 |       |        |         |
| *C. coppinsii Melanohalea exasperatula* |            | Sweden, Westberg, Ekman & von Hirschheydt s. n. (UPS F-796396) DNA: SAR380 |       |        |         |
| *C. coppinsii*        | Melanohalea exasperatula  | Belgium, 2016, van den Boom 54983 (hb. van den Boom) DNA: AM696 |       |        |         |
| *C. coppinsii*        | Melanohalea exasperatula  | Sweden, Westberg s. n. (S, UPS F-805352) DNA: AM850 |       |        |         |
| *C. coppinsii*        | Melanohalea exasperatula  | Sweden, Westberg s. n. (S, UPS F-805353) DNA: AM852 |       |        |         |
| *C. coppinsii*        | Melanohalea exasperatula  | Switzerland, 2017, Zimmermann 1946 (hb. Zimmermann) DNA: AM1043 |       |        |         |
| *C. coppinsii #*      | Melanohalea exasperatula  | Sweden, Odelvik 11471 (S F-206720) DNA: AM819 |       |        |         |
| *C. coppinsii #*      | Melanohalea exasperatula  | Sweden, Westberg, Ekman & von Hirschheydt s. n. (UPS F-796396) DNA: AM821 |       |        |         |
| *C. coppinsii #*      | Melanohalea exasperatula  | Sweden, Westberg s. n. (S, UPS F-805352) DNA: AM849 |       |        |         |
| *C. coppinsii #*      | Melanohalea exasperatula  | Sweden, Westberg s. n. (S, UPS F-805353) DNA: AM851 |       |        |         |
| *Crittendenia sp.*    | On foliicolous *Bacidina* | Canary Islands, 2007, Diederich 16490 (hb. Diederich) DNA: AM1113 |       |        |         |
| *Crittendenia sp.*    | On foliicolous *Bacidina* | Azores, 2017, van den Boom 56783 (hb. van den Boom) DNA: AM1073 |       |        |         |
| *Crittendenia sp.*    | Hypotrachyna endochlora   | Azores, 2017, Etayo 31093 (hb. Etayo) DNA: AM1130 |       |        |         |
| *Crittendenia sp.*    | Hypotrachyna sp.          | Azores, 2017, Etayo 30945 (hb. Etayo) DNA: AM1131 |       |        |         |
| *Crittendenia sp.*    | Lecidella elaeochroma     | Spain, 2011, Zamora s. n. (hb. Zamora) DNA: AM1132 |       |        |         |
| *Crittendenia sp.*    | Lecidella sp.             | Canada, 2009, Björk 17999 (hb. Björk) DNA: AM530 |       |        |         |
| *Crittendenia sp.*    | Physconia distorta        | Spain, 2010, Zamora s. n. (hb. Zamora) DNA: AM1133 |       |        |         |
| *Cystobasidiopsis lactophila* |             | AB021675                       | AF444545 | AF177411 |
| *C. lophatheri*       |                           | -                              | AB126046 | AB124561 |

(Continued)
| Species name               | Host         | Specimen data | nSSU      | ITS       | nLSU       |
|---------------------------|--------------|---------------|-----------|-----------|------------|
| Jianyunia sakaguchii      |              | A8001746      | AF444626  | AF363646  |            |
| Kondaia 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  |            |
| Ruinaria clavata          |              | -             | AY364839  | AY364839  |            |
| R. rubra                  |              | AB021686      | AF444550  | AF189992  |            |
| Sterigmatomyces elviorae  |              | -             | AF444551  | AF177415  |            |
| S. halophilus             |              | D64119        | AF444556  | AF177416  |            |
| S. hyphoenes              |              | AY665775      | AY789077  | AY634278  |            |
| Stilbum vulgare           |              | AY373387      | GU291281  | -         |            |
| **Atractiellomycetes**    |              |               |           |           |            |
| Atractiella solani        |              | DQ198797      | DQ198781  | AY512831  |            |
| Phleogena faginea*        |              | DQ831022      | MN989991  | DQ831021  |            |
| **Classiculosycetes**     |              |               |           |           |            |
| Classicula fluitans       |              | AY124478      | -         | AY512838  |            |
| **Cryptomycocolacomyces** |              |               |           |           |            |
| Cryptomyocolax abnormis   |              | -             | -         | AY512841  |            |
| **Cystobasidiomycetes**   |              |               |           |           |            |
| Buckleyzyma aurantiaca    |              | KJ708436      | AF444538  | AF189921  |            |
| Cyphobasidium hypogymnicola|            | KU587705      | KU587700  | KU587694  |            |
| Cystobasidium pallidum    |              | AB126651      | AB078492  | AF189962  |            |
| Erythobasidium hasegawa   |              | D12803        | AF444522  | AF189899  |            |
| Naohidea sebacea          |              | KP216515      | DQ911616  | DQ831020  |            |
| Microsporomycetes magnisporus|          | KJ708428      | AB112078  | AB111954  |            |
| Lichenomyces pisutiana    |              | MK491257      | MK491194  | MK491265  |            |
| Sakaguchia dacryoida      |              | D13459        | AF444597  | AF189972  |            |
| Symmetrospora gracilis    |              | KJ708433      | AF444578  | AF189985  |            |
| **Mixiomyces**            |              |               |           |           |            |
| Mixia osmundae            |              | D14163        | DQ831010  | DQ831009  |            |
| **Microbotryomycetes**    |              |               |           |           |            |
| Chrysospora griseoflava   |              | D66884        | AF444557  | AF189986  |            |
| Glaczospora antarctica    |              | D768578       | AF444529  | AF189906  |            |
| Heterogastridium pycnidiaoidum|        | KJ708412      | GU291276  | GU291290  |            |
| Kriegeria eriophori       |              | DQ419918      | AF444602  | KY108178  |            |

(Continued)
Table 1. (Continued)

| Species name                  | Host     | Specimen data            | nSSU   | ITS   | nLSU   |
|-------------------------------|----------|--------------------------|--------|-------|--------|
| Leucosporidium scottii        |          | X53499 AF444495 AF070419 |        |       |        |
| Microbotryum violaceum        |          | KJ708388 KJ778635 KJ708462 |        |       |        |
| Sporabolomyces salmonicolor   |          | AB021697 AY015434 AF070439 |        |       |        |
| **Pucciniomycetes**           |          |                          |        |       |        |
| Endocronartium harknessii     |          | AY665785 DQ206982 AY700193 |        |       |        |
| Platygloea disciformis        |          | DQ234563 DQ234556 AY629314 |        |       |        |
| Puccinia graminis             |          | AY125409 AF468044 AF522177 |        |       |        |
| **Spiculogloeomycetes**       |          |                          |        |       |        |
| Phyllozyma dimennoe           |          | D66881 AB038046 AB644404 |        |       |        |
| P. subbrunneo                 |          | AB021691 AF444549 AF189997 |        |       |        |
| **Tritirachiomycetes**        |          |                          |        |       |        |
| Tritirachium oryze            |          | JF779647 GQ329853 MH870621 |        |       |        |
| **Ustilaginomycotina**        |          |                          |        |       |        |
| Ustilago tritici              |          | DQ846895 DQ846894 DQ94784 |        |       |        |

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 usnecorum s. lat. on Usnea as ‘small fleshy nodules closely appressed to the branches’ (oribculos raro profert, sunt vero ii exigui carnei, ramiis 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 ascomycota for star-shaped conidia, arising from characteristic conidigenous 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
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 mycobion (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 Platylgloeales. 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, 2014a, 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 fulfillment 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 yeast species 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 cataulate 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. 2014b). We chose the Biatoropsis-Unea 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. 2014b, 2016a). 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 lobariaevarum (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 Licheneszyma. 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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Table 2. Primers newly designed for this study to selectively amplify the DNA of Crittendenia.

| Primer name (direction) | Sequence (5′ → 3′) |
|------------------------|--------------------|
| Cc-F1 (forward)        | TTTTTGTAAACGTCGTCG |
| Cc-R1 (reverse)        | CGAAGTAAACTTATGCC |
| ChioLSU 3-3 (reverse)  | GCAAGTCTAACCTCAATCG |

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monophyletic group distinct from Cystobasidium s. str. and outside the Cystobasidiales (Millanes et al. 2016b). 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 thallus.

Almost simultaneously with Millanes et al. (2016b), Spribille and co-workers (2016) discovered numerous yeast lineages from this group and recognized the new order Cypobasidiales 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 Cyphobasidiales 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 cysobasidiomycete 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 cysobasidiomycete 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 Černájová & Škaloud (2019) had characterized a high and widespread diversity of cysobasidiomycete yeasts associated with Cladonia. Interestingly, Černájová & Škaloud (2019) also isolated these yeasts from corticate 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 Cyphobasidiales 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 cysobasidiomycete 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 capitata synnemata-like fruiting bodies. The reniform spores are produced from 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 (2012) later erected the new order Atractiellales (currently in Pucciniomycotina) that included, among others, the new family Chionosphoraceae to accommodate both Chionosphaera and Stilbium.

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. phylacticola (Seifert & Bandoni) R. Kirschner & Oberw. (Seifert et al. 1992; Kirschner et al. 2001). Stilbium 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. phylacticola, 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 Chionosphoraceae 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, 1955–2301). The combined matrix corresponding to dataset 2 contained 995 aligned characters (ITS1, 1–90; 5.8S, 91–241; ITS2, 242–367; nuLSU, 368–995).
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.

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).
Taxonomy

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

MycoBank No.: MB 835604

Diffs 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. *Basidiospores* hyaline, asperate, 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
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 *Felhanera 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.
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* (C. coppinsii) later assigned to *Lecidella elaeochroma* 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. exasperata*. 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* (Zhrbenko & Himelbrant 2002). *Crittendenia coppinsii* should be actively searched for on other species of *Melanohalea* 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 Romsdal: 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°21.5’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 Fruttl, Flaschenwald, 1240 m, 1989, Groner 714 (hb. Groner).

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

MycoBank No.: MB 835607

Basionym: *Chionosphaera lichenica* 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 lichenica* 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 micrococa* by Coppins & Coppins (2005). The specimen growing on *Lecidella elaeochroma* reported by Roberts (1997) as *Chionosphaera coppinsii* and eventually assigned to *C. lichenica* 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 Agaricostibules (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 Parmeliaceae clade of the *Parmeliaceae* (Melanelixia and Melanohalea) (Fig. 3A & B); *C. lichenicola* occurs on a host of the *Parmeliaceae*. 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 seldom collected, it is possible that the true range of hosts is even larger than reported here. Within Pucciniomycotina, *Crittendenia*, *Cyphobasidium* and *Lichenozyina* are the only taxa with a lichenicolous habit that have been described so far, and they are not closely related (Wang *et al.* 2016; Černájová & Škaloud 2019; *Li et al.* 2020). *Lichenozyina*, 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 families *Agaricostilbomycetes* and are also formed in the families *Pucciniomycotina*. Stilboid fruiting bodies are frequent in the families *Agaricostilbomycetes* and are also formed in the families *Pucciniomycotina*. Many additional lineages are still to be discovered in *Pucciniomycotina*. All these taxa have, relative 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 clampsless species in Chionosphaera (i.e. C. apobasidialis and C. cuculinocola), whereas those producing clamp connections (i.e. C. coppinisi 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 pocaliformes (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 Agaricomycotina 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 Pucciniozymomyces (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 coppinisi is a well-delimited species according to our phylogenetic studies and microscopical observations, and apparently grows on Melanohalea 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, 2016a) 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. coppinisi from several completely asymptomatic thalli of Melanohalea exasperatula (Table 1, Fig. 2). The yeast phase of a yet unidentified lichenicolous species of Crittendenia on Lecidella claeochroma 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 Pucciniozymomyces (Srippible et al. 2016; Černajová & Škaloud 2019; Kachalkin et al. 2019; Li et al. 2020).

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