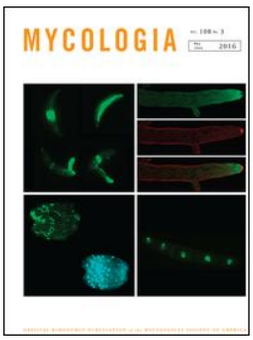

Mycologia

ISSN: 0027-5514 (Print) 1557-2536 (Online) Journal homepage: http://www.tandfonline.com/loi/umyc20

Phylogenetic assessment of Chromocyphellaceae (Agaricineae, Basidiomycota) and a new lamellate species of Chromocyphella

Gabriel Moreno, Maria Prieto, Fernando Esteve-Raventós & Ibai Olariaga

To cite this article: Gabriel Moreno, Maria Prieto, Fernando Esteve-Raventós & Ibai Olariaga (2017): Phylogenetic assessment of Chromocyphellaceae (Agaricineae, Basidiomycota) and a new lamellate species of Chromocyphella, Mycologia

To link to this article: http://dx.doi.org/10.1080/00275514.2017.1377586

MYCOLOGIA
https://doi.org/10.1080/00275514.2017.1377586

Taylor & Francis
Taylor & Francis Group

Phylogenetic assessment of Chromocyphellaceae (Agaricineae, Basidiomycota) and a new lamellate species of *Chromocyphella*

Gabriel Moreno [a], Maria Prieto [b], Fernando Esteve-Raventós [a], and Ibai Olariaga [a]

[a]Departamento de Ciencias de la Vida (Botánica), Facultad de Biología, Universidad de Alcalá, E-28805 Alcalá de Henares, Madrid, Spain; [b]Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos, C/Tulipán s/n, 28933 Móstoles, Madrid, Spain

ABSTRACT

Cyphelloid fungi represent a polyphyletic assemblage of reduced agarics, including the brown-spored family Chromocyphellaceae. In order to elucidate the phylogenetic position of Chromocyphellaceae, newly generated sequences of *Chromocyphella* were included in a multigene alignment of the Agaricineae and phylogenetically analyzed. The current analyses show that the *Chromocyphella muscicola* specimen used to phylogenetically place Chromocyphellaceae in its original description was misidentified and that the Chromocyphellaceae nests in the Hymenogastraceae, *Chromocyphella* being sister to *Flammula*. *Chromocyphella* is emended, including now a new species with lamellate and stipitate basidiomata, *C. lamellata*. The name *Cymbella crouanii*, type species of *Chromocyphella*, is lecto- and epitypified. Our analyses support a new origin of cyphelloid fungi. The shift to a cyphelloid basidioma from an agaric ancestor is suggested to have occurred in two evolutionary steps in *Chromocyphella*, an initial reduction in basidioma size and a subsequent loss of lamellae and stipe.

ARTICLE HISTORY
Received 9 March 2017
Accepted 18 August 2017

KEYWORDS
Agaricales; Cyphellaceae; cyphelloid; Laurisilva; *Phaeosolenia*

INTRODUCTION

Agaricales is the largest order of mushroom-forming fungi, with ca. 13 500 described species (Kirk et al. 2008), within which the Agaricineae is a group characterized mostly by species with thick-walled pigmented basidiospores (Garnica et al. 2007). An agaricoid basidioma type is largely predominant in the Agaricales, but derived clavarioid, cyphelloid, and gasteroid representatives exist. Main lineages of Agaricales have been defined by molecular phylogenies (Moncalvo et al. 2002; Matheny et al. 2006, 2014; Binder et al. 2010), but the backbone of the Agaricales has not received support until phylogenomic data have been implemented (Dentinger et al. 2016). Nevertheless, numerous generic and family delimitation issues remain unaddressed, also in cyphelloid fungi.

Cyphelloid fungi are a poorly known group of Agaricales, characterized by sessile, cup-shaped to tubular, minute (typically not exceeding 2 mm) basidiomata with a smooth hymenophore (Donk 1966). This group has been estimated to comprise 120 species in ca. 40 genera, but actual species diversity is probably considerably higher (Bodensteiner et al. 2004). Cyphelloid fungi represent a polyphyletic assemblage of simplified agarics (Donk 1959; Bodensteiner et al. 2004) and constitute an excellent example of parallel evolution (Bodensteiner et al. 2004; Petersen et al. 2010). Cyphelloid forms are the result of 10–12 independent origins according to Bodensteiner et al. (2004) and are classified in six families (Cyphellaceae, Inocybaceae, Marasmiaceae, Niaceae, Schizophyllaceae, and Tricholomataceae; according to MycoBank, viewed on 7 March 2017). Nevertheless, the phylogenetic position of most cyphelloid lineages has been inferred with not fully resolved 28S nuc rDNA (28S) phylogenies (Bodensteiner et al. 2004; Petersen et al. 2010), and modern family-level classification of cyphelloid fungi is based on these (Knudsen and Vesterholt 2012). Hence, the phylogenetic position of some cyphelloid lineages needs to be further explored in the light of more robust phylogenies.

Chromocyphellaceae was erected to accommodate *Phaeosolenia* Speg. and *Chromocyphella* De Toni & Levi and was characterized as containing cyphelloid fungi with smooth or verrucose spores with a germ pore or with some callus formation (Petersen et al. 2010). *Chromocyphella* is characterized by mostly subglobose, ornamented basidiospores furnished with a plage and associated with mosses (Senn-Irlet 2008), whereas *Phaeosolenia* has smooth spores and a subiculum of brown hyphae (Petersen et al. 2010).

CONTACT Ibai Olariaga ibai.olariaga@ehu.eus

Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/umyc.
Supplemental data for this article can be accessed on the publisher's Web site.

Both genera formed a supported clade in the 28S analyses performed by Petersen et al. (2010), but its position among the Agaricineae could not be resolved. The type species of *Chromocyphella, C. muscicola* (Fr.: Fr.) Donk, was represented in the latter data set by a single sequence.

During a foray in the Canary Islands, a tiny dark-spored agaric was encountered on bark of *Erica arborea* L. The material showed clear microscopic similarities to the cyphelloid genus *Chromocyphella* but had stipitate and lamellate basidiomata, unlike all species placed in *Chromocyphella*. Molecular data from two specimens of *Chromocyphella muscicola* collected by us confirmed the closeness between the Canarian fungus and *C. muscicola*, but these sequences differed significantly from the sequence of *C. muscicola* employed to infer the phylogenetic position of Chromocyphellaceae by Petersen et al. (2010).

Under this scenario, the aim of this study was to reassess the phylogenetic position of the family Chromocyphellaceae and to determine the correct generic placement of the Canarian fungus employing a six-locus phylogeny including protein-coding genes. Furthermore, using *Chromocyphella* as a case study, we will study the processes driving the evolution of cyphelloid fungi from reduction of agaricoid ancestors.

MATERIALS AND METHODS

Morphological study.—Microscopic characters were observed in 10% KOH, Congo red in ammonia, and Melzer's reagent. Basidiospore characterization and statistics follow Heinemann and Rammeloo (1985). Twenty mature spores were measured from hymenial mounts, and on the basis of those measurements, mean length (L), width (l), and Q coefficient (L/l) were calculated, with their respective standard deviations (δ). Values higher or lower than L ± 2δ, l ± 2δ, or L/l ± 2δ are quoted between parentheses. Microphotographs were obtained with a Nikon (model Eclipse 80i; Nikon, Spain) microscope with an incorporated system of automatic digital photography (Nikon DS-5 M). Scanning electronic microscopy (SEM) photographs were made with a Zeiss DSM-950 microscope (Zeiss, Spain) using critical point technique (Moreno and Castillo 2013). The material studied or cited is deposited in AH, ARAN, C, and FH herbaria (Thiers 2012).

Molecular study and phylogenetic analyses.—DNA was extracted from dried material of *Chromocyphella lamellata* and from fresh material stored in sodium dodecyl sulfate (SDS) extraction buffer (*C. muscicola*). The extraction method followed Hansen et al. (1999). Material was ground with a plastic pestle after adding a few sand particles in an Eppendorf tube. We sequenced these regions: the nuc ITS1-5.8S-ITS2 region, using the primers ITS1–ITS4 (White et al. 1990); the nuc 28S rDNA region (D1–D2), using the LR0R-LR5 and LR3R-LR7 primers (Vilgalys and Hester 1990); the nuc 18S rDNA region using primers NS1–NS8 (White et al. 1990); the *RPB2* region between primers fRPB2-5F and bRPB2-7R; the *RPB1* region between gRPB1A-for and fRPB1C-rev (Stiller and Hall 1997; Matheny et al. 2002); and the partial *TEF1* using primers 983F and 2218R (Rehner and Buckley 2005). Polymerase chain reaction (PCR) products were purified with ExoSAP-IT (USB, Cleveland, Ohio, USA). Sequencing was performed by Macrogen Inc. (Amsterdam, The Netherlands). Sequences were edited and assembled with Sequencher 4.1.4 (Gene Codes Corporation Ann Arbor, Michigan, USA) and subsequently submitted as BLAST queries to verify their identity and check their similarities to sequences in GenBank.

To explore the phylogenetic affinities of *Chromocyphella*, available nucleotide sequences of genera nested in the Agaricineae were assembled. This taxon sampling was largely based on Matheny et al. (2006, 2014). We constructed alignments of the six gene regions studied (SUPPLEMENTAL TABLE 1). Each protein coding region was translated to amino acids in AliView (Larsson 2014) to determine intron positions and to refine the alignment. All introns except for the conserved region of intron 2 of *RPB1* (Matheny et al. 2002) could not be aligned and were excluded. In addition, we obtained sequences of the markers mentioned above from the genomes of *Agaricus bisporus* (H97), *Agrocybe pediades* (Hildén et al. 2014), *Crepidotus variabilis* and *Cortinarius glaucopus* (AT 2004 176), *Galerina marginata* (Riley et al. 2014), *Hebeloma cylindrosporum* (Dore et al. 2015), *Hypholoma sublateritium* (Kohler et al. 2015), *Laccaria bicolor* (Martin et al. 2008), and *Pholiota connisans*, through the JGI Web site (http://genome.jgi.doe.gov). To test the position of Chromocyphellaceae sensu Petersen et al. (2010), sequences of *Phaeosolenia densa* (AY571018) and *C. muscicola* (FJ603306) were employed. Sequences of *Infundibulicybe gibba* were used as an outgroup.

Gene congruence was evaluated manually by comparing supported clades among single-gene genealogies (Mason-Gamer and Kellogg 1996). Each locus was subjected to a maximum likelihood (ML) analysis using the "RAxML HPC2 on XSEDE" tool (Stamatakis 2006) via CIPRES Science Gateway (Miller et al. 2010), employing mixed models of evolution and starting from a random

tree. A GTR-GAMMA model with four rate categories was selected. For branch confidence, 1000 ML bootstrap replicates were conducted using rapid bootstrapping. A supported clade for one marker was considered to be in conflict when contradicted with significant support by another (bootstrap support >70%). As no conflict was detected, all the markers were concatenated into a single alignment with seven partitions: 28S, 18S, 5.8S, *RPB2, RPB1, RPB1* intron 2, and *TEF1*. Based on preliminary analyses, the third codon position was excluded for *RPB2, RPB1*, and *TEF1* regions. Since preliminary analyses showed that addition of sequences of *Phaeosolenia densa* (AY571018) and *C. muscicola* (FJ603306) caused severe changes in the topology and support of the trees, probably due to missing data. We excluded those sequences in the first matrix (Agaricoid matrix). This data set was subjected to ML and Bayesian analyses. The ML analysis was performed as explained above.

The Bayesian analysis was carried out in MrBayes 3.2.1. (Ronquist et al. 2012; MB), with two parallel runs of eight Metropolis-coupled Markov chain Monte Carlo (MCMCMC) chains for 30 million generations, starting from a random tree, and sampling one tree every 100th generation. The substitution models were sampled across the GTR space by the MCMCMC analysis (Ronquist et al. 2012). To check whether the chains had converged, to determine whether the mixing was adequate, and to choose an appropriate burn-in, log-likelihood values were plotted against the time generation with Tracer 1.6 (Rambaut et al. 2014). Stationarity was assumed when average standard deviation of split frequencies fell below 0.01. A burn-in sample of 300 000 trees was discarded. To assess branch confidence, a 50% majority rule consensus tree was computed with the remaining 300 002 trees using the SUMT command of MrBayes. Bayesian posterior probability (PP) values ≥0.95 were considered to be significant.

To test the position of Chromocyphellaceae sensu Petersen et al. (2010), we included sequences of *Phaeosolenia densa* (AY571018) and *C. muscicola* (FJ603306) in a second matrix (*Phaeosolenia* matrix) that was subjected to a Bayesian analysis as explained above. To avoid major changes in the topology of the trees, nodes supported in the Bayesian analysis of the Agaricoid data set were constrained with the "partial" option of MrBayes, allowing these sequences to fall into or out of the constrained clades.

RESULTS

***Chromocyphella* nests in the Hymenogastraceae.**— Both data sets contained 5129 aligned characters (1287 28S, 1665 18S, 158 5.8S, 697 *RPB2*, 426, *RPB1*, 278 *RPB1* intron 2, and 618 *TEF1*) after excluding ambiguous regions. The Agaricoid analysis reached an average standard deviation of split frequencies of 0.01 after 2 490 000 generations. The 50% majority rule consensus of the *Phaeosolenia* analysis is presented in FIG. 1, including Bayesian PPs of both analyses.

Sequences of *C. muscicola* generated in this study (ARAN-Fungi 3210 and ARAN-Fungi 3324) are encompassed in a strongly supported clade with the Canarian fungus described here as *C. lamellata* (FIG. 1). This clade is sister to *Flammula alnicola*, and both clades nest in the Hymenogastraceae. Clades corresponding to the Strophariaceae s. str., Tubariaceae, and Inocybaceae-Crepidotaceae, as circumscribed by Matheny et al. (2014), are supported in the Agaricoid analysis. Clades corresponding to Hymenogastraceae and Strophariaceae s. str. form a major supported clade in the Agaricoid analysis (PP = 0.99). *Gymnopilus sapineus* does not nest in the Hymenogastraceae but hold an unresolved position within the Agaricineae.

As for the *Phaeosolenia* analysis, sequences of *Phaeosolenia* and *Choromocyphella muscicola* generated by Petersen et al. (2010) fall in the Tubariaceae, but without support (FIG. 1).

TAXONOMY

Chromocyphella De Toni & Levi, Naturalist 155:1588. 1888.

≡ *Cymbella* Pat. in Doassans & Patouillard, Revue Mycol (Toulouse) 8(29):27. 1886 (nom. illeg. Art. 53.1; later homonym of *Cymbella* C. Agardh, Consp Crit Diat 1:1. 1830).

≡ *Phaeocarpus* Pat., Hyménomyc Eur:154. 1887 (nom. illeg. Art. 53.1; later homonym of *Phaeocarpus* Mart. & Zucc., Flora 7(1), Beil 4:136. 1824).

≡ *Phaeocyphella* Pat., Essai Tax Hyménomyc:57. 1901. (nom. illeg., Art. 52.1; superfluous name).

Type species: Cymbella crouanii Pat. & Doass. (= *Chromocyphella muscicola* Fr.: Fr.) Donk).

Chromocyphella muscicola (Fr.: Fr.) Donk, Persoonia 1:95. 1959.

≡ *Cyphella muscicola* Fr.: Fr., Syst Mycol 2:202. 1822.

≡ *Calyptella muscicola* (Fr.: Fr.) Quél., Enchir Fung:217. 1886.

≡ *Arrhenia muscicola* (Fr.: Fr.) Quél., Fl Mycol France:33. 1888.

≡ *Chaetocypha muscicola* (Fr.: Fr.) Kuntze, Revis Gen Pl 2:847. 1891.

≡ *Phaeocyphella muscicola* (Fr.: Fr.) Rea, Br Basidiomyc:704. 1922.

Typification: No type designated. No specimen in the E. Fries herbarium (UPS).

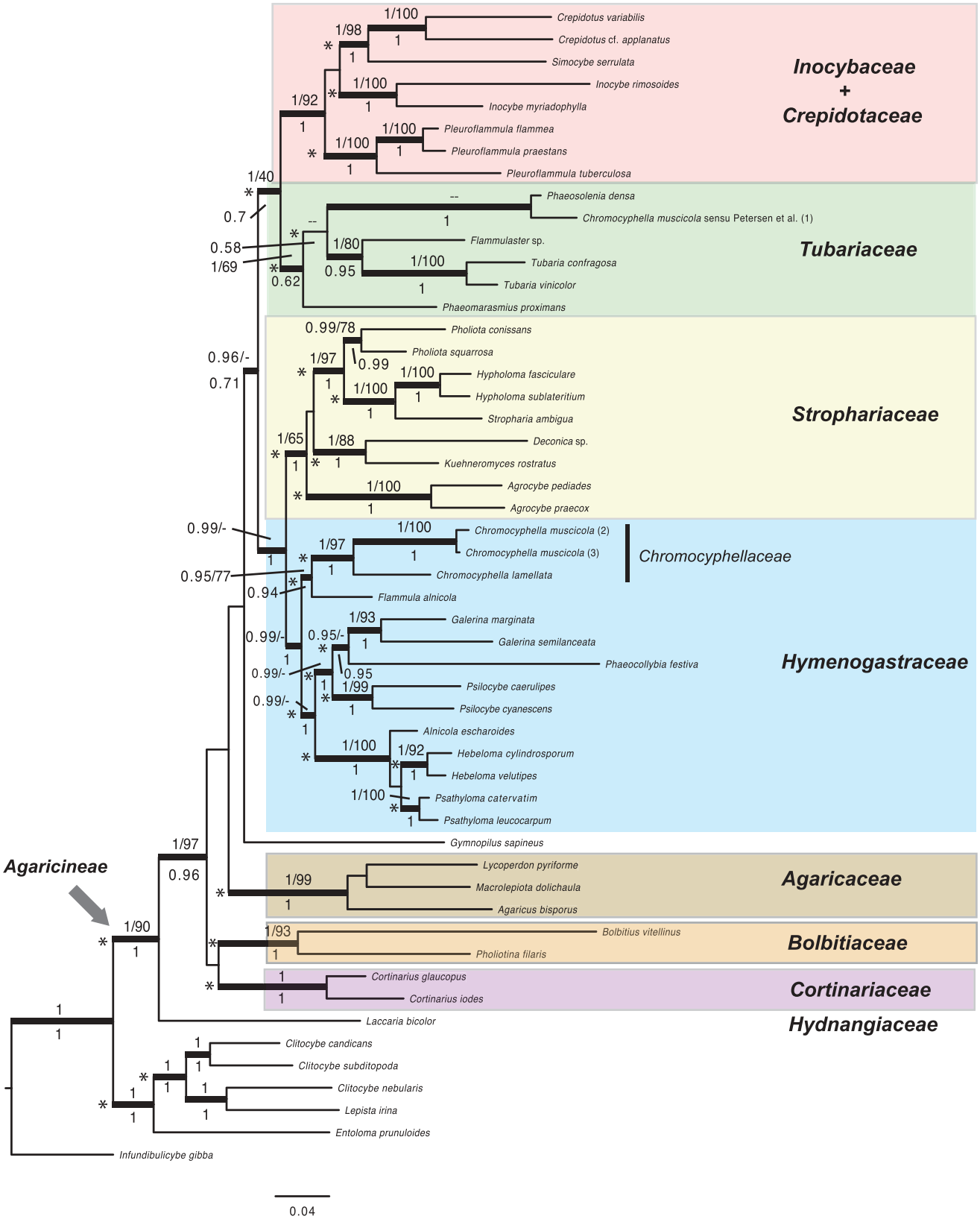

Figure 1. Bayesian inference 50% majority rule consensus phylogram of the Agaricineae from 28S-18S-5.8S-*RPB2*-*RPB1*-*RPB1* intron 2-*TEF1* sequence data (*Phaeosolenia* matrix). Sequences in bold were generated in this study. Constrained nodes are marked with an asterisk. Bayesian posterior probabilities (PP) ≥95%/maximum likelihood bootstrap values of the analyses of the Agaricoid matrix are showed above nodes, whereas Bayesian posterior probabilities of the constrained analysis (*Phaeosolenia* matrix) are shown under nodes. Black thickened branches denote a high support in at least one of the analyses of the Agaricoid matrix.

≡ *Cyphella abieticola* P. Crouan & H. Crouan, Fl Finistère:61. 1867.

non *Cyphella abieticola* P. Karst., Fung Fenn Exs no. 718. 1868. (nom. illeg., Art. 53.1, later homonym of *Cyphella abieticola* P. Crouan & H. Crouan).

Typification: No type designated. No type specimen is known.

≡ *Cymbella crouanii* Pat. in Doassans & Patouillard, Revue Mycol (Toulouse) 8(29):27. 1886.

≡ *Phaeocarpus crouanii* (Pat.) Pat., Hyménomyc Eur:154. 1887. "crouani."

≡ *Cyphella crouanii* (Pat.) Sacc., Syll Fung 6:672. 1888. "crouani."

≡ *Chaetocypha crouanii* (Pat.) Kuntze, Revis Gen Pl 2:847. 1891.

≡ *Phaeocyphella crouanii* (Pat.) Pat., Essai Tax Hyménomyc:58 1901. "crouani."

≡ *Calyptella crouanii* (Pat.) Bigeard & H. Guill., Fl Champ Sup France 2:481. 1913. "crouani."

Typification: FRANCE. FONTENAY: Bois de Vincennes, Eaux Bonnes, tronc de tilleul dans la mousse, Oct 1885, FH-Patouillard, barcode 00458664 (**lectotype** designated here; MBT 375519). SPAIN. BASQUE COUNTRY: Gipuzkoa, Aia, Iturraran, on bark of pollard *Quercus robur* tree, 12 Mar 2016, *J.M. Lekuona, P.M. Pasaban, J. Martín, J.I. López, J.L. Albizu & I. Olariaga, ARAN-Fungi 3324* (**epitype** designated here; MBT 375522) (FIG. 2).

***Nomenclatural notes on* Cymbella crouanii.**—There is not full agreement about the place of valid publication of *Cymbella crouanii*, which is here ascribed to Doassans and Patouillard (1886). These authors provided a description only for *Cymbella* Pat., but *C. crouanii*

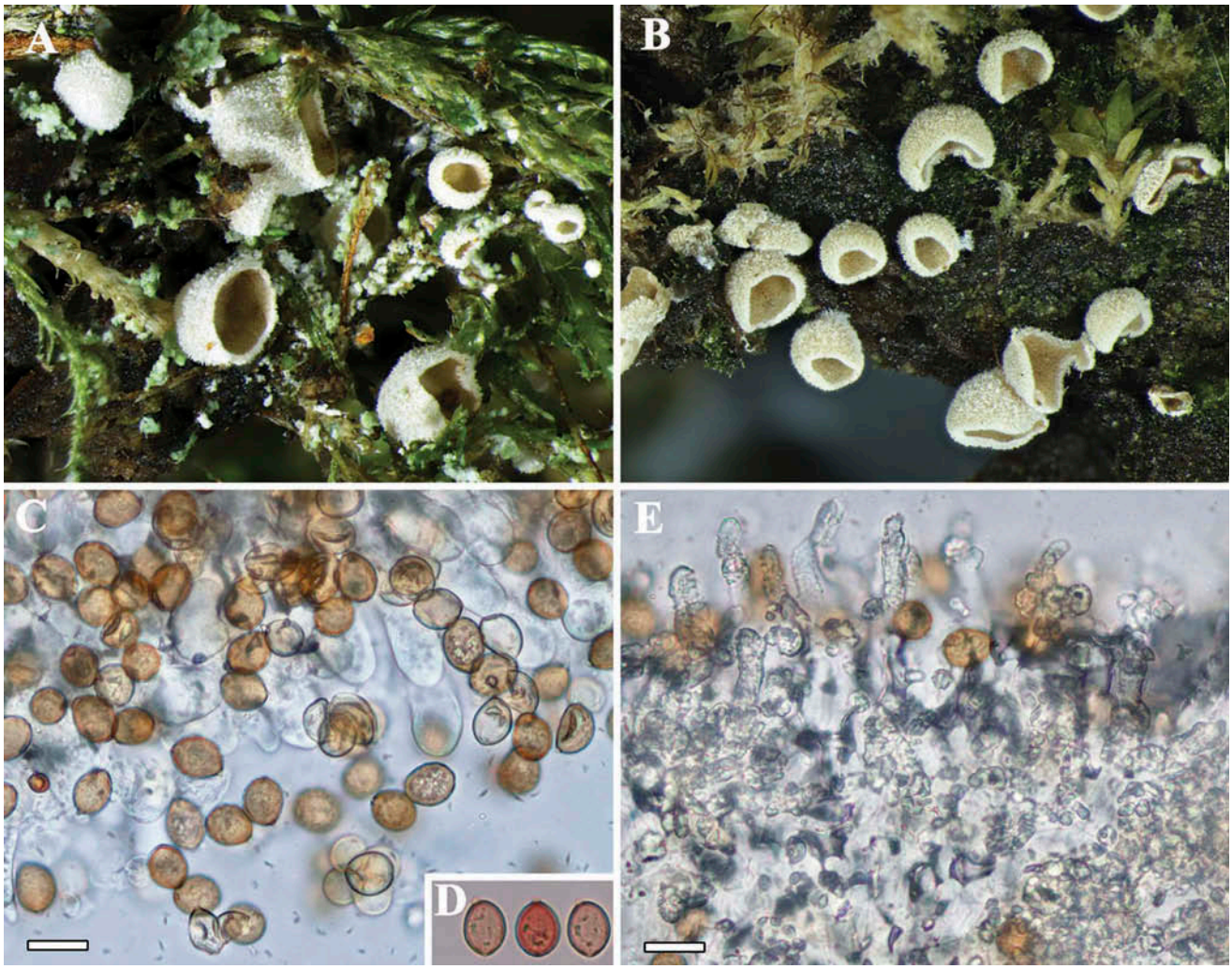

Figure 2. *Chromocyphella muscicola*. A. Basidiomata of *C. muscicola* (ARAN-Fungi 3210). B. Basidiomata of *C. muscicola* (ARAN-Fungi 3324; epitype of *Cymbella crouanii*). C. Basidiospores in water. D. Basidiospores in Congo Red showing an apical germ pore. E. Pileipellis in water. Bars = 10 µm.

achieved valid publication simultaneously under Art. 38.5 (descriptio generico-specifica). The description of *C. crouanii* by Doassans and Patouillard (1886), published on 1 January according to the front page of fascicle 29, precedes the description in Patouillard (1886), published between January and May 1886 according to Stafleu and Cowan (1983). Furthermore, the facts that Patouillard (1886) did not add "sp. nov." after the heading of *C. crouanii* and that he noted "Revue Mycol. Janvier" indicate that the description by (Patouillard 1886) was published later than the one by Doassans and Patouillard (1886).

Doassans and Patouillard (1886) described *C. crouanii* in an account of fungi collected in the French Béarn. The only specimen of *Cymbella crouanii* in the Patouillard herbarium (FH) was collected in the forest of Vincennes, near Paris, and thus not in the Béarn. Nevertheless, this specimen represents original material through the reference to "Tab. Analyt. nº 467" made in the protologue, where "bois de Vincennes" was cited by Patouillard (1886). This specimen is here selected as the lectotype of *Cymbella crouanii*, and it represents *C. muscicola* according to the enclosed notes by S. A. Redhead. The lecto- and epitype designated here constitute the type of *Chromocyphella* and of the whole family Chromocyphellaceae.

Chromocyphella lamellata G. Moreno & Olariaga, sp. nov. FIG. 3

MycoBank MB820345; internal transcribed spacer (ITS) barcode: GenBank MF623832

Typification: SPAIN. Canary Islands: Tenerife: Monte Verde, on bark of *Erica arborea* L., covered with pleurocarpous bryophytes, 4 Jan 2004, *G. Moreno, Gl. Moreno, J. Ayllón, J.L. Vallejo, A. García, A. Vallejo & J. Vallejo* (**holotype** AH 45802; **isotype**, AH 45948).

Etymology: From Latin *lamella*-, which means "gill," referring to its lamellate hymenophore.

Diagnosis: Characterized by having crepidotoid basidiomata, finely ornamented spores and abundant pileocystidia; multigenic phylogenetic analyses reveal that it is closely allied to *Chromocyphella muscicola*, type species of *Chromocyphella*, from which it markedly differs in the presence of lamellae and a well-developed stipe.

Basidiomata growing isolated or gregariously, crepidotoid. Pileus 1.5–3.5(–4) mm diam, subdimidiate to circular, convex and becoming plane-convex, pale brownish, brown ochraceous to brown (color exposed beneath the furfuraceous covering) (Mu 7.5YR 7/4-8; Mu 7.5YR 6/6-8), apparently not hygrophanous, not striate at margin; surface dry, densely furfuraceous to velvety-pubescent under strong magnification; margin straight to slightly incurved. Lamellae well developed, very few (L = 5–7), lamellulae present (l = 1–2), adnexed-decurrent, arcuate to straight, ochre brown to reddish brown, spaced; edge paler, pruinose. Stipe 0.5–1.2 (–1.5) × 0.1–0.25 mm, lateral, small but well developed, cylindrical, pubescent, concolorous with the lamellae. Veil absent. Odor and taste not remarkable.

Basidiospores (7–)7.5–*8.9*–10.5(–11) × 6.5–*7.4*–8 (–8.5) µm, Q_m = 1–*1.2*–1.4 (n = 40), broadly ovoid to subglobose, yellowish brown to brown, finely verrucose or apparently rugose to rugulose under light microscope, sometimes with short ridges, dextrinoid, with a vague plage visible in SEM; apicula distinct, 0.5–1.2 µm long. Basidia clavate to cylindrical, sometimes constricted in the middle, hyaline, 4-spored, 20–28 × 6–8 µm. Lamella edge homogeneous and sterile, covered with cylindrical and capitate cheilocystidia, 30–60 × 3–4 µm, 5–6.5 µm broad at the (sub)capitate apex. Pleurocystidia absent. Lamellar trama interwoven to subregular, constituted by 3–5 µm wide cylindrical to branched intricate hyphae, clamped, hyaline to very pale brown, with a finely encrusting to parietal pigment. Pileipellis consisting of a non-gelatinized cutis, formed by cylindrical, septate, 4–6 µm diam hyphae, with yellowish brown incrusting pigment. Pileocystidia abundant, similar to cheilocystidia, (sub)capitate showing a globose, simple to branched apex. Caulocystidia very abundant, similar to the cheilocystidia. Clamp connections present in all septa.

Notes: Chromocyphella lamellata is a unique agaric characterized by its crepidotoid basidiomata furnished with a short lateral stipe, verruculose ovoid to subglobose spores, and the presence of abundant capitate cheilo- and pileocystidia. Its habitat seems also distinctive, but it is still uncertain whether this fungus is strictly bark-associated or bryophilous. Some basidiomata grew directly on bark, whereas others were attached to moss caulidia on bark of *Erica arborea* (Ericaceae) in a laurisilva forest in the Canary Islands, within the Macaronesian biogeographical region.

Our efforts to find an earlier name for *Chromocyphella lamellata* among crepidotoid genera with ornamented spores were unsuccessful. The fungus described here shows affinities with *Pyrrhoglossum* Singer, which, as suggested by Singer (1944), is closely related to *Gymnopilus* P. Karst. (see Rees et al. 2002). BLAST searches using the ITS sequence (AF501569) of the type species *P. pyrrhum* (Berk. & M.A. Curtis) Singer retrieve *Gymnopilus* sequences as closest matches, *Gymnopilus* being distantly related to *Chromocyphella* in our phylogeny (FIG. 1). We did

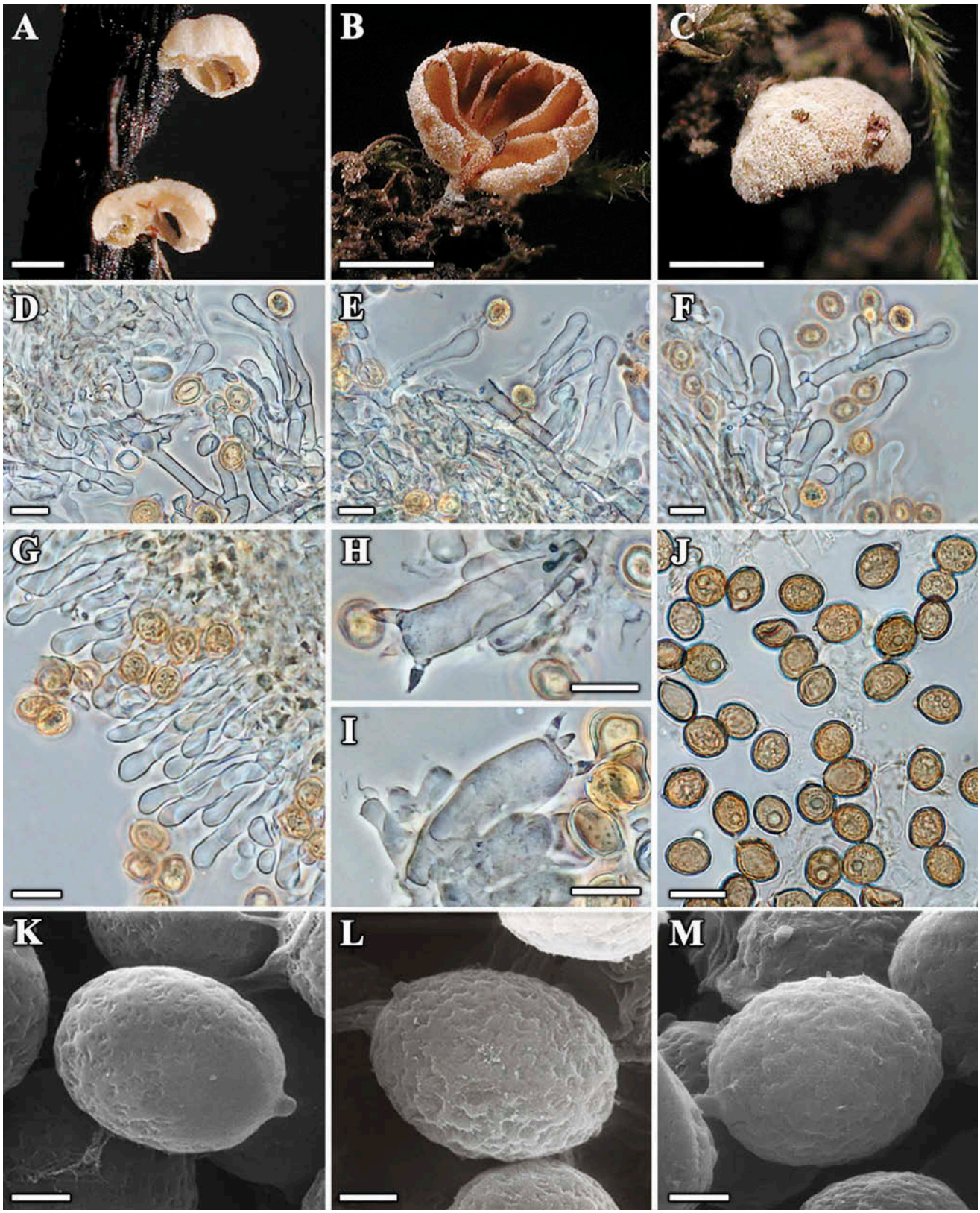

Figure 3. *Chromocyphella lamellata* (AH 45802, holotype). A. Basidiomata growing on *Erica arborea* bark. B. Basidioma showing a rudimentary stipe and well-developed gills. C. Basidioma showing a felty-silky upper surface. D. Cheilocystidia. E. Cheilocystidia. F. Cheilocystidia. G. Homomerous lamellar edge. H. Basidium. I. Basidium. J. Basidiospores viewed in a light microscope. K. Basidiospore in SEM. L. Basidiospore in SEM. m. Basidiospore in SEM. Bars: A–C = 1 mm; D–J = 10 µm; K–M = 2 µm.

not find any taxon assigned to *Pyrrhoglossum* that conforms to *C. lamellata*, nor does our material show the predominant yellow-brown pigments characteristic in *Pyrrhoglossum* (Horak 1989). *Crepidotus* (Fr.) Staude also includes a few species with a lateral or reduced stipe (Hesler and Smith 1965), and many of its species produce ornamented non-dextrinoid spores. Our phylogenetic results show also that *Crepidotus* is distantly related to *C. lamellata* (FIG. 1). The genus *Pleuroflammula* Singer comprises crepidotoid to pleurotoid species as well, but they are characterized by having smooth spores and the presence of a usually well-developed veil (Horak 1978) and hence different from *Chromocyphella*. The cyphelloid habit and lamellar hymenophore in *C. lamellata* are similar to *Phaeomarasmius siquieri* Salom & Esteve-Rav., another small lignicolous agaric found recently on bark of *Juniperus phoenicea* in Formentera (Balearic Islands, Spain). Nevertheless, *P. siquieri* has non-dextrinoid smooth spores, as well as sinuose-elongate and encrusted cystidia, and is better placed in the family Tubariaceae, perhaps close to *Phaeosolenia* (Salom and Esteve-Raventós 2011). Species of *Phaeosolenia* fruit from a well-developed subiculum or woolly stroma (Sulzbacher et al. 2009).

DISCUSSION

***Placement of Chromocyphellaceae and characterization of* Chromocyphella.**—*Chromocyphella* is emended here to encompass also a species with lamellate and stipitate basidiomata, *C. lamellata*. *Chromocyphella muscicola* and *C. lamellata* share a bark-associated or bryophilous habitat, reduced basidiomata, a pale pileal surface composed of pileocystidioid hairs, and subglobose dextrinoid spores with a vague bare suprahilar plage. *Chromocyphella muscicola* grows often on bleached pleurocarpous mosses and is thus believed to be a moss parasite (Senn-Irlet 2008; Clarke 2014). Although *C. lamellata* did not directly grow on bleached mosses, the possibility of its being a bryosymbiont is not excluded. Of interest, *C. muscicola* has lost a stipe and gills and consequently lacks cheilocystidia, as Petersen et al. (2010) interpreted. The ITS sequences of *C. muscicola* and *C. lamellata* differ in 67 positions (substitutions, deletions, and insertions), and both are in long branches in our analyses (FIG. 1). This considerable divergence suggests *C. muscicola* and *C. lamellata* not to be phylogenetically extremely close. Nevertheless, considering their morphological similarities, emending *Chromocyphella* to encompass a lamellate species is considered a suitable and practical option here. The genus *Flammula* (Fr.: Fr.) P. Kumm. is the closest genus to *Chromocyphella* in our phylogeny. *Flammula* possesses basidiospores with a germ pore, a pileipellis of an ixocutis, lacks pileocystidia, and has a veil (Jacobsson 2008). The two species currently accommodated in *Flammula*, *F. alnicola* (Fr.: Fr.) P. Kumm., and *F. pinicola* (Jacobsson) Noordel. are saprotrophic, which is the predominant trophic strategy in the Agaricineae.

The position of Chromocyphellaceae is inferred here from multiple sequences obtained from two specimens collected by us. Both specimens belong unequivocally to *C. muscicola* due to their cyphelloid basidiomata and brown subglobose and ornamented spores showing a vague suprahilar plage, as described in the literature (Donk 1959; Senn-Irlet 2008). These sequences generated by us nest in the Hymenosgastraceae (FIG. 1), whereas the position of the *C. muscicola* (FJ603306) sequence generated by Petersen et al. (2010) is not supported within the Agaricineae. This disagreement questions the identification of the specimen of *C. muscicola* sequenced by Petersen et al. (2010). A revision of this specimen (C-F-62443) showed that it represents a species of *Phaeosolenia* (H. Knudsen, pers. comm.).

Our multigene phylogeny shows that Chromocyphellaceae is a later synonym of Hymenogastraceae, since *Chromocyphella* nests in the latter family. Chromocyphellaceae could only be recognized to accommodate *Flammula* (Fr.: Fr.) P. Kumm. and *Chromocyphella* in a separate family. Nevertheless, finding morphological and ecological traits to discriminate Chromocyphellaceae and Hymenogastraceae proves difficult, and both families are accordingly merged here. The position of *Phaeosolenia* remains unresolved in our phylogeny. Although *Phaeosolenia* nested within Tubariaceae without support in our constrained analysis (*Phaeosolenia* matrix, FIG. 1), Tubariaceae cannot be excluded as its correct placement, as suggested by Matheny et al. (2007).

This study uncovers a new and interesting evolutionary origin of cyphelloid fungi. The sister group of *Chromocyphella* is a genus producing large agaricoid basidiomata, the genus *Flammula*. The common ancestor of both was very likely a large fleshy agaric, which is the synapomorphic and predominant basidioma type in the Agaricineae. From a large agaricoid ancestor, the shift to a cyphelloid basidioma seems to have occurred in two steps: (i) first a reduction in size and (ii) a subsequent loss of lamellae and stipe. In this particular case, the adoption of a bryosymbiont trophic strategy might be the evolutionary force driving basidioma reduction in *Chromocyphella*.

ACKNOWLEDGMENTS

We wish to express our gratitude to Mr. A. Priego and Mr. J.A. Pérez of the Electron Microscopy Service of the University of Alcalá de Henares for their invaluable help with the SEM. We also thank Luis Monje of the Department of Drawing and Scientific Photography at the Alcalá University for his help in the digital preparation of the photographs, and we are grateful to Dr. J. Rejos, curator of the AH herbarium for his assistance with the specimens examined in the present study. We also would like to acknowledge Henning Knudsen for examining specimen C-F-62443 of *Chromocyphella muscicola* and for his collaborative attitude. Genevieve E. Tocci (née Lewis-Gentry) is thanked for sending us valuable information on the *Cymbella crouanii* specimen in the Patouillard herbarium (FH).

ORCID

Gabriel Moreno http://orcid.org/0000-0002-9303-7181
Maria Prieto http://orcid.org/0002-1692-9821
Fernando Esteve-Raventós http://orcid.org/0000-0001-8265-5067
Ibai Olariaga http://orcid.org/0000-0002-0334-7750

LITERATURE CITED

Binder M, Larsson KH, Matheny PB, Hibbett DS. 2010. Amylocorticiales ord. nov. and Jaapiales ord. nov.: early-diverging clades of Agaricomycetidae dominated by corticioid forms. Mycologia 102:865–880.

Bodensteiner P, Binder M, Moncalvo J-M, Agerer R, Hibbett DS. 2004. Phylogenetic relationships of cyphelloid homobasidiomycetes. Molecular Phylogenetics and Evolution 33:501–515.

Clarke D. 2014. Notes on the bryophilous fungus *Chromocyphella muscicola* (Fr.) Donk in Cumbria. Lakeland Naturalist 2:24–28.

Dentinger BTM, Gaya E, O'Brien H, Suz LM, Lachlan R, Díaz-Valderrama JR, Koch RA, Aime MC. 2016. Tales from the crypt: genome mining from fungarium specimens improves resolution of the mushroom tree of life. Biological Journal of the Linnean Society 117:11–32.

Doassans E, Patouillard NT. 1886. Champignons de Béarn (2e liste). Revue Mycologique (Toulouse) 8(29):25–28.

Donk MA. 1959. Notes on "Cyphellaceae". Persoonia 1:25–110.

Donk MA. 1966. A reassessment of the Cyphellaceae. Acta Botanica Neerlandica 15:95–101.

Dore J, Perraud M, Dieryckx C, Kohler A, Morin E, Henrissat B, Lindquist E, Zimmermann SD, Girard V, Kuo A, Grigoriev IV, Martin F, Marmeisse R, Gay G. 2015. Comparative genomics, proteomics and transcriptomics give new insight into the exoproteome of the basidiomycete Hebeloma cylindrosporum and its involvement in ectomycorrhizal symbiosis. New Phytologist 208:1169–1187.

Garnica S, Weiss M, Walther G, Oberwinkler F. 2007. Reconstructing the evolution of agarics from nuclear gene sequences and basidiospore ultrastructure. Mycological Research 111:1019–1029.

Hansen K, Pfister DH, Hibbett DS. 1999. Phylogenetic relationships among species of *Phillipsia* inferred from molecular and morphological data. Mycologia 91:299–314.

Heinemann P, Rammeloo J. 1985. De la mesure des spores et de son expression. Agarica 6:366–380.

Hesler LR, Smith AA. 1965. The North American species of *Crepidotus*. New York and London: Hafner Publishing Company. 126 p.

Hildén K, Mäkelä MR, Steffen KT, Hofrichter M, Hatakka A, Archer DB, Lundell TK. 2014. Biochemical and molecular characterization of an atypical manganese peroxidase of the litter-decomposing fungus *Agrocybe praecox*. Fungal Genetics and Biology 72:131–136.

Horak E. 1978. *Pleuroflammula*. Persoonia 9:439–451.

Horak E. 1989. New and additional data concerning *Pyrrhoglossum* and eccentric or laterally stipitate taxa of *Gymnopilus* (Agaricales). Opera Botanica. 100:115–129.

Jacobsson S. 2008. *Flammula*. In: Knudsen H, Vesterholt J, eds. Funga Nordica. Copenhaghen, Denmark: Nordsvamp. p. 785–787.

Kirk P, Cannon PF, Minter DW, Stalpers JA. 2008. The dictionary of fungi. 10th ed. Wallingford, UK: CAB International. 784 p.

Knudsen H, Vesterholt J. 2012. Funga Nordica. 2nd revised and augmented edition. 2 volumes. Copenhagen, Denmark: Nordsvamp. 1083 p.

Kohler A, Kuo A, Nagy LG, Morin E, Barry KW, Buscot F, Canbäck B, Choi C, Cichocki N, Clum A, Colpaert J, Copeland A, Costa MD, Doré J, Floudas D, Gay G, Girlanda M, Henrissat B, Herrmann S, Hess J, Högberg N, Johansson T, Khouja H-R, LaButti K, Lahrmann U, Levasseur A, Lindquist EA, Lipzen A, Marmeisse R, Martino E, Murat C, Ngan CY, Nehls U, Plett JM, Pringle A, Ohm RA, Perotto S, Peter M, Riley R, Rineau F, Ruytinx J, Salamov A, Shah F, Sun H, Tarkka M, Tritt A, Veneault-Fourrey C, Zuccaro A, Mycorrhizal Genomics Initiative Consortium, Tunlid A, Grigoriev IV, Hibbett DS, Martin F. 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nature Genetics 47:410–415.

Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30:3276–3278.

Mason-Gamer RJ, Kellogg EA. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Graminaeae). Systematics and Biology 45:524–545.

Martin F, Aerts A, Ahr D, Brun A, Danchin EGJ, Duchaussoy F, Gibon J, Kohler A, Lindquist E, Pereda V, Salamov A, Shapiro HJ, Wuyts J, Blaudez D, Bu M, Brokstein P, Canb B, Cohen D, Courty PE, Coutinho PM, Delaruelle C, Detter JC, Deveau A, DiFazio S, Duplessis S, Fraissinet-Tachet L, Lucic E, Frey-Klett P, Fourrey C, Feussner I, Gay G, Grimwood J, Hoegger PJ, Jain P, Kilaru S, Labb J, Y. C. Lin5, Legu V, Le Tacon F, Marmeisse R, Melayah D, Montanini B, Muratet M, Nehls U, Niculita-Hirzel H, Oudot-Le Secq MP, Peter M, Quesneville H, Rajashekar B, Reich M, Rouhier N, Schmutz J, Yin T, Chalot M, Henrissat B, Lucas S, Van de Peer Y, Podila GK, Polle A, Pukkila PJ, Richardson PM, Rouz P, Sanders IR, Stajich JE, Tunlid A, Tuskan G, Grigoriev IV 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452:88–92.

Matheny PB, Hofstetter V, Aime MC, Moncalvo JM, Ge Z-W, Yang Z-L, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98:982–995.

Matheny PB, Liu JY, Ammirati J, Hall BD. 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). American Journal of Botany 89:688–698.

Matheny PB, Moreu P-A, Vizzini A, Harrower E, De Haan A, Contu M, Curti M. 2014. *Crassisporium* and *Romagnesiella*: two new genera of dark-spored Agaricales. Systematics and Biodiversity 13:28–41.

Matheny PB, Vellinga EC, Bougher N, Ceska O, Moreau P-A, Neves MA, Ammirati J 2007. Taxonomy of displaced species of *Tubaria*. Mycologia 99:569–585.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, 14 November 2010, p. 1–8.

Moncalvo J-M, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémençon H, Miller OK Jr. 2002. One hundred and seventeen clades of euagarics. Molecular Phylogenetics and Evolution 23:357–400.

Moreno G, Castillo A. 2013. A comparative study of the types of three species of Myxomycetes: *Trichia crateriformis, T. fallax* var. *olivacea* and *T. fernbankensis*. Boletín de la Sociedad Micológica de Madrid 37:85–98.

Patouillard NT. 1886. Tabulae analyticae fungorum. Fasc. 5. Poligny, France: Librairie C. Klincksieck.

Petersen G, Knudsen H, Seberg O. 2010. Alignment, clade robustness and fungal phylogenetics—Crepidotaceae and sister families revisited. Cladistics 26:62–71.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v. 1.6. [cited 2016 Jul 30]. Available from: http://beast.bio.ed.ac.uk/Tracer

Rees BJ, Zuccarello GC, Orlovich DA. 2002. Relationships between Australian and Northern hemisphere *Gymnopilus* species II. A preliminary phylogeny of species of *Gymnopilus* and related genera based on internal transcribed spacer (ITS) region of ribosomal DNA. Mycotaxon 84:93–110.

Rehner S, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98.

Riley R, Salamov AA, Brown DW, Nagy LG, Floudas D, Held BW, Levasseur A, Lombard V, Morin E, Otillar R, Lindquist EA, Sun H, LaButti KM, Schmutz J, Jabbour D, Luo H, Baker SE, Pisabarro AG, Walton JD, Blanchette RA, Henrissat B, Martin F, Cullen D, Hibbett DS, Grigoriev IV. 2014. Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. Proceedings of the National Academy of Sciences of the United States of America 111:9923–9928.

Ronquist F, Teslenko M, Mark P van der, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

Salom JC, Esteve-Raventós F. 2011. *Phaeomarasmius siquierii* (Agaricoid clade, Tubariaceae), a new Mediterranean resupinate species found in Formentera (Balearic Islands, Spain). Micologia e Vegetazione Mediterranea 26:29–36.

Senn-Irlet B. 2008. *Chromocyphella*. In: Knudsen H, Vesterholt J, eds. Funga Nordica. Copenhaghen, Denmark: Nordsvamp. p. 861–863.

Singer R. 1944. New genera of fungi. Mycologia 36:358–368.

Stafleu FA, Cowan RS. 1983. Taxonomic literature 4. P-Sak. Utrecht, the Netherlands: Bohn, Scheltema & Holkema. 1214 p.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690.

Stiller JW, Hall BD. 1997. The origin of red algae: implications for plastid evolution. Proceedings of the National Academy of Sciences of the United States of America 94:4520–4525.

Sulzbacher MA, Coelho G, Cortez VG. 2009. Studies on cyphelloid fungi in Southern Brazil. The genus Phaeosolenia Speg. Boletín de la Sociedad Micológica de Madrid 33:91–96.

Thiers B. 2012 (continuously updated). Index herbariorum. A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. [cited 2013 Feb 26]. Available from: http://sweetgum.nybg. org/ih/

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172:4238–4246.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Inns MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego, California: Academic Press. p. 315–322.