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Phylogenetic diversity of bulbil-forming lichenicolous fungi in Cantharellales including a new genus and species

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Abstract. Lichenicolous species are widely distributed in the Basidiomycota, and many are known to produce sclerotia or bulbils with few additional structures to permit taxonomic placement. The Cantharellales include many of these species and we here describe a new species that grows over *Cladonia rangiferina* and forms yellow-orange, initially immersed bulbils similar to *Burgella flavoparmeliae* Diederich & Lawrey, a familiar species in the order. We obtained sequences of nuLSU representing an isolated culture and herbarium specimen of the species, and initial searches in GenBank indicated it was a member of the Cantharellales. We inferred its phylogenetic placement in the order using an existing dataset that included all known lichenicolous species. Our results indicate that it is not closely related to any described lichenicolous species or to any other described bulbilliferous species in the order. Based on these results, we are now establishing a new genus and species, *Neoburgoa freyi*, in the Hydnaceae sensu Hibbett et al. (2014). We also introduce the new name *Adamflakia* for the genus *Bulbilla* as the latter coincides with the technical term 'bulbilla' used in previous descriptions of bulbil-forming species and is therefore not validly published following the ICN (Art. 20.2); *Adamflakia applanata* comb. nov. is proposed.

Keywords. Basidiomycota, Clavulinaceae, Corticiales, fungi, mycoparasitism, phylogenetics, nomenclature, taxonomy.

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Based on the most recent worldwide checklist (Lawrey & Diederich 2016), lichenicolous fungi in the Agaricomycetes are concentrated primarily in the Corticiales, with 4 species in 3 genera, and the cantharelloid clade of Hibbett & Thorn (2001), Binder et al. (2005), Moncalvo et al. (2006), Matheny et al. (2007) and Hibbett et al. (2014), which is made up of Cantharellales, including Ceratobasidiaceae, Tulasnellaceae, Botryobasidiaceae and Hydnaceae, with 7 species in 6 genera. In addition to inhabiting lichens, many of these species are bulbilliferous, producing tightly coiled hyphal masses—bulbils—that form within host lichen tissues and erupt onto the outer surface (Clémençon 2004), or are superficial from the beginning. Their small size and the absence of additional diagnostic structures have hindered identification and phylogenetic placement of these species until recently. Sikaroodi et al. (2001) were the first to use molecular techniques to place bulbil-forming lichenicolous fungi in the Corticiales and Cantharellales, and studies since then (Diederich & Lawrey 2007; Diederich et al. 2014; Lawrey et al. 2007, 2008) suggest that a potentially high diversity of these fungi remains to be discovered.

We recently had the opportunity to study a bulbilliferous species found growing over *Cladonia rangiferina* in Switzerland that resembles *Burgella flavoparmeliae* Diederich & Lawrey; however, a different host ecology, slight differences in color and morphology, different anatomical characters, and more immersed bulbils when young suggested that it was not the same species. We were able to obtain a culture of the fungus and rDNA sequences from both the culture and the specimen. Based on our preliminary searches in GenBank, the specimen represents a species in Cantharellales that is unre-

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lated to any described species in that order. In this paper, we provide results of phylogenetic analyses of the sequences, anatomical/morphological descriptions of the specimen and the isolated culture, and a discussion of the phylogenetic diversity of lichenicolous species in the cantharelloid clade of Agaricomycetes. In addition, due to a nomenclatural error, we are also establishing a new genus name for *Bulbilla*.

Material and Methods

Specimens studied, anatomical methods and isolation of cultures. Fresh specimens of the unknown were collected in Switzerland by one of us (EZ), and samples were sent to other co-authors for study. Herbarium specimens are deposited in G and in the private collection of P. Diederich. Dry herbarium specimens were examined and measured under a binocular microscope Leica MZ 7.5 (magnification up to 50×), and photographed 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, or with a binocular microscope Leica M165C and a Jenoptik ProgResC5 camera, and the free software Combine ZM. Entire or sectioned bulbils were studied in water, 5% KOH, Phloxin B, lactophenol cotton blue or Congo Red, either with or without pressure on the coverslip. Microscopic photographs of bulbils were prepared using a Leica DMLB microscope and a Leica EC3 camera, and Helicon Focus.

A single culture representing the unknown was isolated from herbarium material (*Zimmermann LF1256*) following methods of Lawrey (2002). Bulbils were washed in 70% ethanol, dried on a glass slide and crushed in sterile water. Fragmented tissues were then collected in water and plated onto potato dextrose agar (PDA) or malt extract agar (ME, Difco, Detroit, Michigan, USA). Emergence of hyphae from fragments was observed within three days, and mycelial outgrowths were isolated after two weeks for liquid culture in ME. This was assigned the isolate ID JL596-16. Approximately 2 mg dry mycelial mass was harvested from liquid cultures after two weeks and extracted for DNA analysis.

Molecular data. Genomic DNA was extracted from (1) a sample containing 8 bulbils excised from the specimen and washed in 70% ethanol and (2) 2 mg dry mycelial mass of the culture using the Fast DNA Spin Kit from MP Biomedicals (Santa Ana, CA) according to the manufacturer's protocol. About 10 ng of extracted DNA were subjected to a standard PCR in a 20 μL reaction volume using Taq Gold polymerase (Applied Biosystems, Foster City, CA), also according to manufacturer's protocols, with the objective of amplifying the nuclear large subunit (nuLSU) rDNA. The PCR primers were forward primers ITS5 and/or LR0R and the reverse primers LR7 and/or ITS4 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The products were purified with Ampure magnetic beads (Agencourt Bioscience, Beverly, MA) and the purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). The primers used in sequencing reactions were LR0R, LR3R, LR5, LR7, LR16, ITS4, and ITS5. The sequencing reactions were purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, MO), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run on an ABI3130-xl capillary sequencer (Applied Biosystems). The data collected were analyzed using ABI software, and the sequences were then assembled together with the software Sequencher version 5.0 (Gene Codes, Ann Arbor, MI) for manual corrections in base calling and to make contiguous alignments of overlapping fragments. We obtained sequences from both the culture and the washed bulbils of the specimen.

Phylogenetic analysis. In addition to our newly generated sequences (GenBank accession numbers: Cantharellales sp. culture JL596-16 nuLSU: KX423755; Cantharellales sp. *Zimmermann LF1256* nuLSU: KX423756), we included sequences used in earlier analyses of lichenicolous Cantharellales (Diederich et al. 2014; Lawrey et al. 2007) and those from a broad range of taxa representing recognized orders and families within the cantharelloid clade (Binder et al. 2005; Hibbett et al. 2007, 2014; Matheny et al. 2007; Moncalvo et al. 2006), the "Cantharellaceae" (Dunham et al. 2003; Wilson et al. 2006), and newly published sequences representing *Burgoa* species (Kiyuna et al. 2015). The final data set (all GenBank accession numbers included in **Fig. 1**) contained 73 ingroup terminals, and sequences from *Cerinomyces crustulinus* (Bourdot & Galzin) G.W.Martin, *Tilletiaria anomala* Bandoni & B.N.John and *Platygloea disciformis* (Fr.) Neuhoff used as outgroups.

Figure 1. Best-scoring nuLSU RAxML phylogram of species used in the analysis, showing the placement of *Neoburgoa freyi*. Internal branches in boldface indicate posterior probabilities ≥0.95 and numbers are ML-BS values ≥70. Arrows indicate described bulbilliferous species that are ± lichenicolous.

The newly generated nuLSU sequences were edited in Geneious v.8.1.6 (http://www.geneious.com/) and automatically aligned with MAFFT using the –auto option (Katoh & Toh 2005). The alignments were trimmed and subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn et al. 2010a,b); regions aligned with low confidence (below 0.93) were removed. The final nuLSU data set had an alignment length of 1088 bases, 513 of which were variable. The GUIDANCE score for the nuLSU alignment was 0.9472. Maximum likelihood (ML) searches were done using RAxML 7.2.6 (Stamatakis 2006; Stamatakis et al. 2005) with non-parametric bootstrapping of 1000 replicates under the universal GTRGAMMA model. A Bayesian analysis was also performed for the same data sets using Markov chain Monte Carlo sampling (Larget & Simon 1999) in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Substitution models for each data set were selected in jModelTest 0.1.1 (Posada 2008), which employs PhyML 3.0 (Guindon & Gascuel 2003) to estimate the likelihood of the

data under 24 models of evolution using a fixed topology. The AICc values under each model were compared and the model with the lowest AICc value (GTR+I+G) was selected. Two parallel analyses were then run in MrBayes for 10,000,000 generations, with 4 chains each, sampling every 100 generations. Burn-in trees (initial 25%) were discarded for each run and posterior probabilities (PP) of the nuLSU matrix were determined by calculating a majority-rule consensus tree generated from the post-burnin trees by the MCMCMC runs using the sumt option of MrBayes. RAxML and MrBayes analyses were performed using the CIPRES Web Portal 3.1 (Miller et al. 2010) and the University of Oslo Bioportal (http://www.bioportal.uio.no). The most likely tree was then produced (–lnL=9878.3376). Relationships were considered supported if they had ML-BS values of 70 or greater and Bayesian posterior probabilities (PP) of 0.95 or greater. Phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut 2012).

Results

Phylogenetic placement of new sequences in the Cantharellales. ML and Bayesian analyses of the nuLSU alignment recovered trees with the same topology for the strongly supported branches, so only the ML tree is shown (**Fig. 1**), with the branches having ML bootstrap values $\geq$70 % in bold and with the posterior probabilities of the Bayesian analysis added above the internal branches. The phylogeny consistently resolved most of the recognized clades with good support, including the cantharelloid clade (Binder et al. 2005; Hibbett et al. 2014; Hibbett & Thorn 2001; Matheny et al. 2007; Moncalvo et al. 2006) and the order Cantharellales in the sense of Hibbett et al. (2014), which includes the families Ceratobasidiaceae, Tulasnellaceae, Botryobasidiaceae and Hydnaceae, the latter of which includes what were in the past considered Cantharellaceae, Clavulinaceae and Sistotremataceae.

There are a number of recognized ± lichenicolous and bulbilliferous species in the Hydnaceae, including *Burgella flavoparmeliae*, which resembles the unknown specimen based on morphology. Other species include *Adamflakia applanata* (Diederich, Flakus & Etayo) Diederich & Lawrey (syn. *Bulbilla applanata*; see below), *Burgella lutea* Diederich, Capdet, A.I.Romero & Etayo, *Burgellopsis nivea* Diederich & Lawrey, *Burgoa angulosa* Diederich, Lawrey & Etayo and *Minimedusa pubescens* Diederich, Lawrey & Heylen. Sequences of the unknown were not closely related to any described lichenicolous genera or species in the family.

Our results consistently placed the unknown specimen as a singular species in the Hydnaceae (sensu Hibbett et al. 2014), but not closely related to any recognized genera or phylogroups in the family. Given this result, we believe there is need to establish for now a new genus and species for the unknown.

Taxonomy

Neoburgoa freyi Diederich, Zimmermann & Lawrey, *gen. et sp. nov.* **Figs. 2–3**

MycoBank MB 818611 (genus) and MB 818612 (species).

Characterized by immersed to superficial, yellow to orange, roundish to ellipsoid or irregular bulbils, 150–300(–700) μm diam., internally of roundish to ellipsoid or polyhedral, thick-walled cells, 10-20 μm diam., clamps absent in bulbils, but present in culture.

Type: Switzerland. Kanton Wallis, Oberwald, Grimselpass, westlich vom Totensee (Swiss grid 668′600, 156′800), alt. 2200 m, alpiner Rasen, Windkantenrasen (Elynion), Silikat, on *Cladonia rangiferina*, Sept. 2015, *E. Zimmermann LF1256* (G–holotypus, herb. Diederich–isotypus). Ex-type culture: JL596-16.

Description. Basidiomata and conidiomata unknown. Colonies appearing as dispersed bulbils overgrowing thalli of *Cladonia rangiferina* (**Fig. 2A**). Mycelium not observed. Bulbils (**Fig. 2B–D**) entirely immersed when young, later becoming superficial, pale yellow to orange, without hairs, surface smooth, with no individual cells visible, roundish to ellipsoid or irregular in form, 150–300(–700) μm diam.; bulbils externally without specialized cells, covered by an amorphous layer 3–15 μm thick (**Fig. 2E & F**); bulbils internally composed of more or less roundish to ellipsoid or polyhedral cells (**Fig. 2E & H**) separating rather easily (with pressure on the cover glass), 10–20 μm diam.; cell wall 0.8–1.7 μm thick; clamps not observed; content of cells occasionally yellowish, with yellow oil droplets emerging when observed in lactophenol cotton blue; no crystals visible in polarized light.

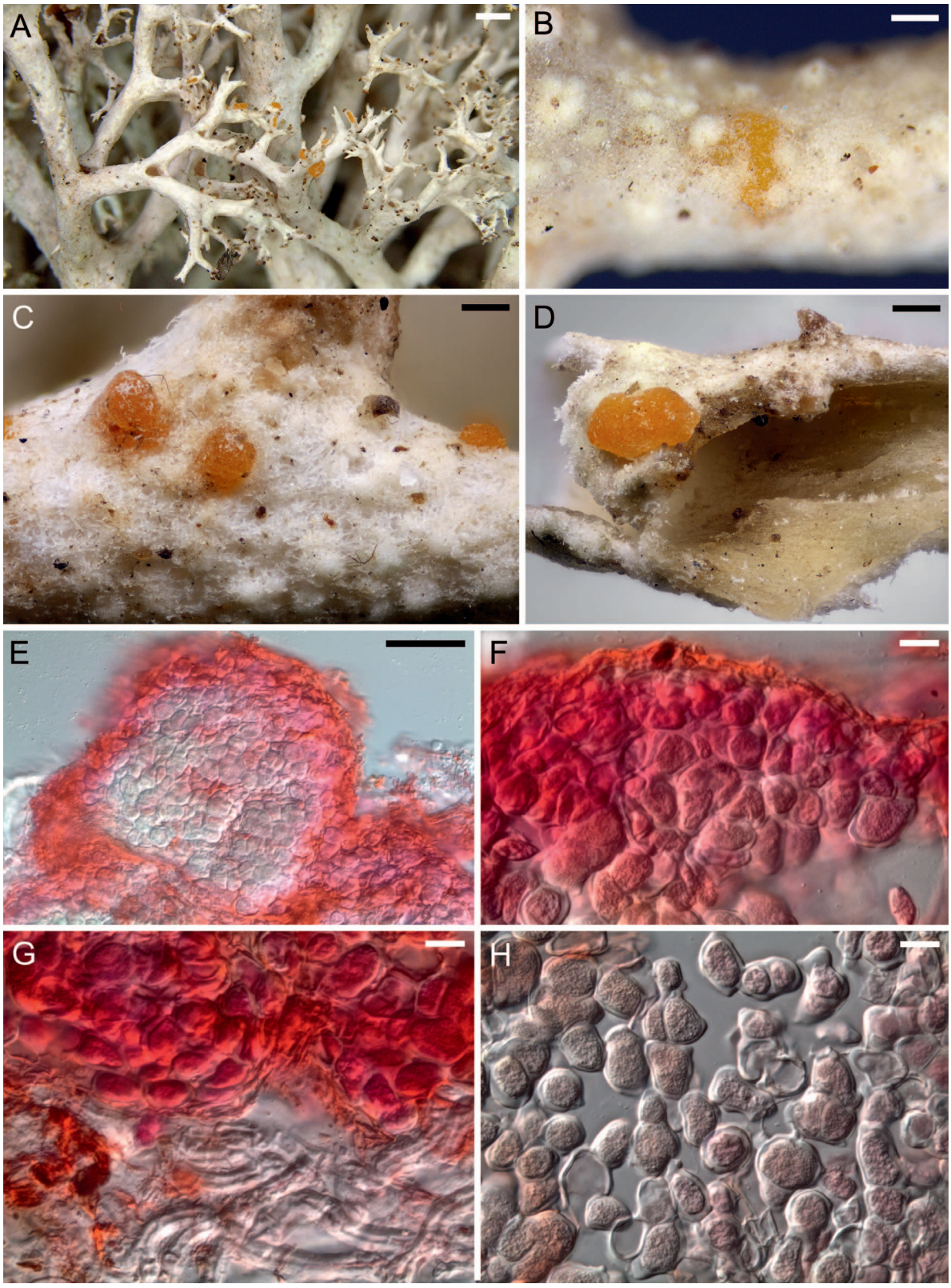

Figure 2. *Neoburgoa freyi* bulbils. **A–D.** Bulbils on thallus of *Cladonia rangiferina*. **E.** Section through bulbil. **F.** Upper part of bulbil covered by a thin amorphous layer. **G.** Lower part of bulbil showing connection with host thallus. **H.** Individual cells inside bulbil after pressure on cover glass. (A–B: *Zimmermann LF1151*; C–H: *Zimmermann LF1256* – holotype, G) Scale bars: A=1 mm, B–D=200 µm, E=50 µm, F–H=10 µm.

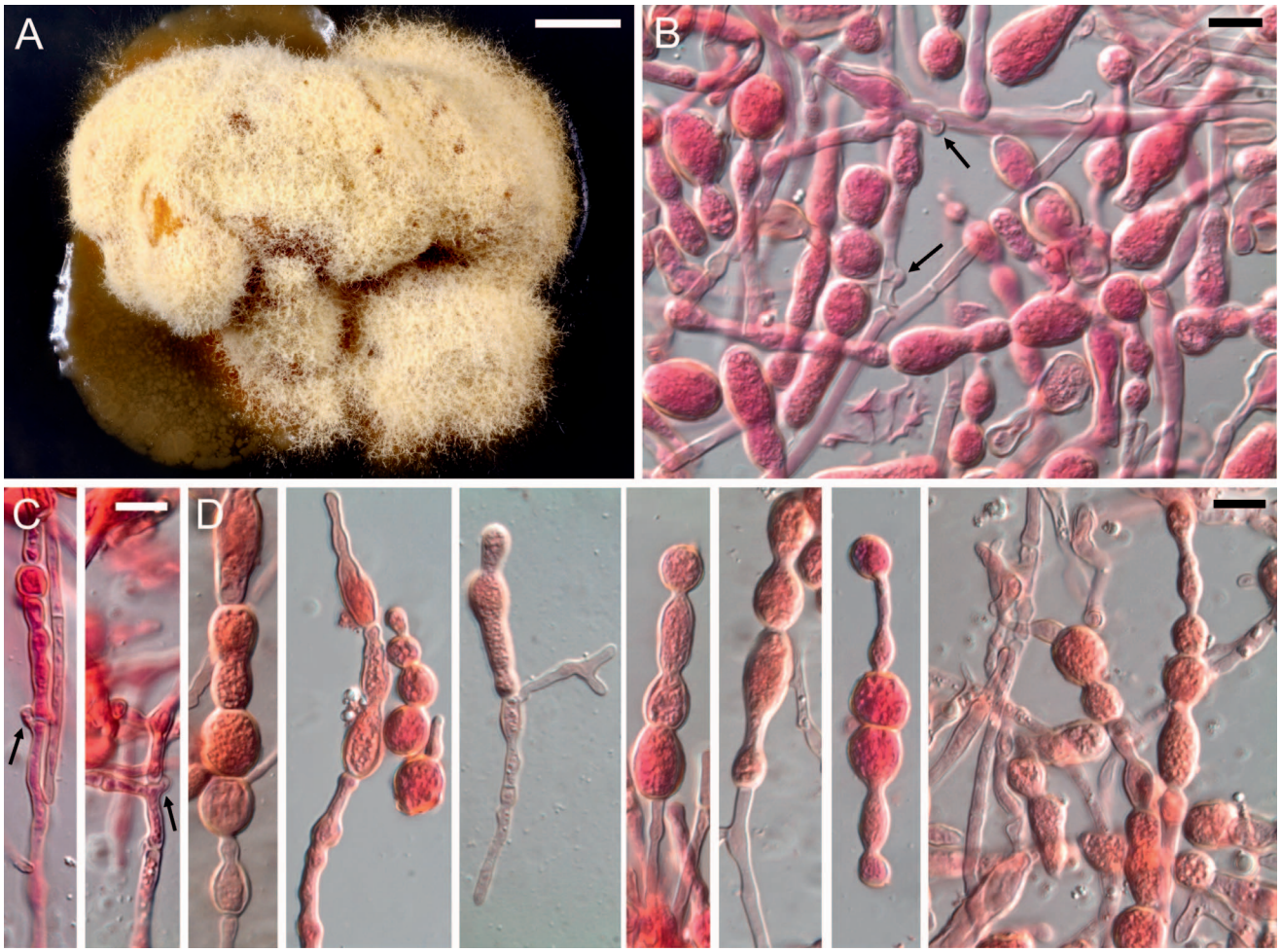

Figure 3. *Neoburgoa freyi* ex-type culture (JL596-16). **A.** Culture on agar plate. **B–D.** Hyphae producing chains of swollen cells; arrows show clamp connections. Scale bars: A=1 mm, B–D=10 µm.

Colonies on agar plates showing yellowish aerial hyphae (**Fig. 3A**). Basidiomata, conidiomata and bulbils not observed. Mycelium 2.5–3.5 µm diam., septa with clamp connections (**Fig. 3B & C**), frequently producing chains of strongly swollen, elongate to roundish or irregularly constricted cells (**Fig. 3B–D**), 6–33 × 6–15 µm.

Distribution and ecology. The new species is known from several alpine localities in Switzerland and is certainly much more widespread in the Alps. It is known only from thalli of *Cladonia rangiferina*. As young bulbils are entirely immersed in the host thallus and soon become superficial, and as they do not visibly damage the host thallus, it is likely that they have evolved together with the host over a long period and that they are confined to this host genus or species. No visible interactions between bulbils and hosts have been observed.

Observations. *Neoburgoa freyi* is mainly characterized by the yellow to orange bulbils that are first immersed and then become superficial on the thallus of *Cladonia rangiferina*. *Burgella* species are similar in color, but bulbils are much smaller and superficial from the beginning: those of *B. flavoparmeliae* are honey-colored, 60–110 µm diam. (Diederich & Lawrey 2007), while those of *B. lutea* are yellow to orange yellow, 50–80 µm diam. (Diederich et al. 2014). *Erythricium aurantiacum* (Lasch) D.Hawksw. & A.Henrici (syn. *Marchandiobasidium aurantiacum* Diederich & Schultheis), a species belonging to the Corticiaceae, is distinguished by orange (carrot red) bulbils (Diederich et al. 2003; Diederich & Lawrey 2007). Microscopically, the new species is distinguished from most lichenicolous, bulbilliferous fungi by the roundish to ellipsoid or polyhedral cells with a particularly thick wall.

Etymology. The new genus strongly resembles *Burgoa* and related genera. The new species is named after the Swiss lichenologist Eduard Frey (1888–1974), an eminent scholar of alpine lichens and founder of the Swiss Association for Bryology and Lichenology.

Additional specimen examined. SWITZERLAND: Graubünden, Wergenstein, Caschgliun, Tguma, Windkantenrasen (Elynion), Silikat, on *Cladonia rangiferina*, Sept. 2015, *E. Zimmermann LF1151* (herb. Zimmermann).

A new name for *Bulbilla* Diederich, Flakus & Etayo

Diederich et al. (2014) published the new genus and species *Bulbilla applanata*, belonging to the "Clavulinaceae" (Cantharellales), lichenicolous over Peltigerales in South America. Unfortunately, the generic name *Bulbilla* is not validly published, following Art. 20.2 (ICN), as it "coincides with a Latin technical term in use in morphology," and the introduction of a new name is therefore necessary.

Adamflakia Diederich & Lawrey nom. nov.

REPLACED SYNONYM: *Bulbilla* Diederich, Flakus & Etayo, in Diederich et al., Lichenologist 46: 340 (2014). TYPE: *Bulbilla applanata* Diederich, Flakus & Etayo.

MYCOBANK MB 818613

Etymology. The new genus is named after Adam Flakus (Krakow), a distinguished explorer of Bolivian lichens, and collector of the type material of *Adamflakia applanata*.

Adamflakia applanata (Diederich, Flakus & Etayo) Diederich & Lawrey comb. nov.

BASIONYM: *Bulbilla applanata* Diederich, Flakus & Etayo, in Diederich et al., Lichenologist 46: 340 (2014).

MYCOBANK MB 818614

Discussion

The phylogeny we recovered for the cantharelloid clade is similar in structure to those obtained by us earlier and by other investigators (Binder et al. 2005; Diederich et al. 2014; Hibbett et al. 2014; Lawrey et al. 2007; Matheny et al. 2007; Moncalvo et al. 2006). The concept of Cantharellales of Hibbett et al. (2014) is supported here; this consists of four families (Ceratobasidiaceae, Tulasnellaceae, Botryobasidiaceae, Hydnaceae) with the Hydnaceae being a diverse assemblage of groups formerly recognized as families (Clavulinaceae, Cantharellaceae and Sistotremataceae) and genera not previously assigned to families. Lichenicolous and bulbilliferous species are found throughout the clade, and the new species appears to be most closely associated with the clade formerly recognized as Clavulinaceae. This clade includes species in *Clavulina*, *Multiclavula*, *Membranomyces*, *Sistotrema* (notably *S. oblongisporum* M.P.Christ. & Hauerslev. and *S. brinkmannii* (Bres.) J.Erikss.), "*Sistotremastrum niveocremeum*" (a misidentified sequence the identity of which is not known; Moncalvo et al. 2006), and the bulbilliferous/lichenicolous genera *Adamflakia*, *Burgella* and *Burgellopsis*. Other species of *Sistotrema* (notably *S. eximum* (H.S.Jacks.) Ryvarden and *S. sernanderi* (Litsch.) Donk) are recovered in an entirely separate clade in the family that includes *Burgoa verzuoliana* Goid. (type of *Burgoa*, which is usually considered the asexual state of *Sistotrema*) and the bulbilliferous and weakly lichenicolous *Burgoa angulosa*. Another well-supported clade in the family is made up of *Minimedusa* species, including the lichenicolous bulbilliferous *M. pubescens*. It is certainly possible at this time to assign *Neoburgoa freyi* to Hydnaceae sensu Hibbett et al. (2014); however, it does not appear to have a close relationship to any presently recognized genus or phylogroup in the family.

The fact that all lichenicolous species (and the lichen-forming *Multiclavula* spp.) in the cantharelloid clade are bulbilliferous raises the possibility that the morphology may have contributed in some way to the evolution of the lichenicolous (and possibly lichen-forming) habit. However, there are many sclerotial and bulbilliferous fungi in the cantharelloid clade, and in many other major clades of Agaricomycetes, and many are not lichenicolous, so bulbil-formation is more likely a characteristic of these fungi generally. It is worth mentioning, however, that the inconspicuous nature of these fungi makes their discovery usually a matter of chance. As the phylogeny of the clade becomes better resolved, the contribution of lichenicolous and bulbilliferous species will be more evident.

The number of lichen-associated fungi in the Basidiomycota has always been low (Lawrey & Diederich 2003); the most recent checklist of lichenicolous species (www.lichenicolous.net; Lawrey & Diederich 2016) shows approximately 80

species in 1800 total species, around 4%. The new species represents the seventh described lichenicolous genus in the cantharelloid clade, some of which include only a single species: *Neoburgoa freyi*, *Adamflakia applanata*, *Burgella flavoparmeliae*, *B. lutea*, *Burgellopsis nivea*, *Burgoa angulosa*, *Ceratobasidium bulbillifaciens* Diederich & Lawrey, and *Minimedusa pubescens* (all except *A. applanata* included in **Fig. 1** and indicated with arrows). This low within-genus diversity combined with the wide distribution of presently described genera across the clade would strongly suggest that the lichenicolous habit arose numerous times recently and independently and represents an important evolutionary theme in the clade. It also suggests that more of these fungi await discovery.

Acknowledgments

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Literature Cited

Binder, M., D. S. Hibbett, K. H. Larsson, E. Larsson, E. Langer & G. Langer. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). Systematics and Biodiversity 3: 113–157.

Clémençon, H. [coll. Emmet, V., Emmet, E.]. 2004. Cytology and Plectology of the Hymenomycetes. Bibliotheca Mycologica 199: 1–488.

Diederich, P. & J. Lawrey. 2007. New lichenicolous, muscicolous, corticolous and lignicolous taxa of *Burgoa* s.l. and *Marchandiomyces* s.l. (anamorphic Basidiomycota), a new genus for *Omphalina foliacea*, and a catalogue and a key to the non-lichenized, bulbilliferous basidiomycetes. Mycological Progress 6: 61–80.

Diederich, P., J. D. Lawrey, M. Capdet, S. Pereira, A. I. Romero, J. Etayo, A. Flakus, M. Sikaroodi & D. Ertz. 2014. New lichen-associated bulbil-forming species of *Cantharellales* (Basidiomycetes). Lichenologist 46: 333–347.

Dunham, S. M., T. E. O'Dell & R. Molina. 2003. Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. Mycological Research 107: 1163–1177.

Guindon, S. & O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.

Hibbett, D. S., R. Bauer, M. Binder, A. J. Giachini, K. Hosaka, A. Justo, U. Kõljalg, E. Larsson, K. H. Larsson, J. D. Lawrey, O. Miettinen, L. Nagy, R. H. Nilsson, M. Weiß & R. G. Thorn. 2014. Agaricomycetes. Pp. 373–429. In: D. J. McLaughlin et al. (eds.), The Mycota, Vol. VII. Systematics and Evolution. Part A. Springer-Verlag, Berlin.

Hibbett, D. S., M. Binder, J. F. Bischoff, M. Blackwell, P. F. Cannon, et al. (67 authors). 2007. A higher-level phylogenetic classification of the *Fungi*. Mycological Research 111: 509–547.

Hibbett, D. S. & R. G. Thorn. 2001. Basidiomycota: Homobasidiomycetes. Pages 121–168. In: D. J. McLaughlin, E. G. McLaughlin & P. A. Lemke (eds.), The Mycota. VIIB. Systematics and Evolution. Springer-Verlag, Berlin.

Huelsenbeck, J. P. & F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Katoh, K. & M. Toh. 2005. MAFFT Version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518.

Kiyuna, K., K.-D. An, R. Kigawa, C. Sano, S. Miura & J. Sugiyama. 2015. "Black particles", the major colonizers on the ceiling stone of the stone chamber interior of the Kitora Tumulus, Japan, are the bulbilliferous basidiomycete fungus *Burgoa anomala*. Myco-Science 56: 293–300.

Larget, B. & D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16: 750–759.

Lawrey, J. D. 2002. Isolation and culture of lichenicolous fungi. Pages 75–84. In: I. Kranner, R. P. Beckett & A. Varma (eds.), Protocols in lichenology: Culturing, biochemistry, physiology and use in biomonitoring. Springer-Verlag, Berlin.

Lawrey J. D., M. Binder, P. Diederich, M. C. Molina, M. Sikaroodi & D. Ertz. 2007. Phylogenetic diversity of lichen-associated homobasidiomycetes. Molecular Phylogenetics and Evolution 44: 778–789.

Lawrey, J. D. & P. Diederich. 2003. Lichenicolous fungi: Interactions, evolution, and biodiversity. The Bryologist 106: 80–120.

Lawrey, J. D. & P. Diederich. 2016. Lichenicolous fungi – worldwide checklist, including isolated cultures and sequences available. URL: http://www.lichenicolous.net [accessed May 10 2016].

Lawrey, J. D., P. Diederich, M. Sikaroodi & P. Gillevet. 2008. Remarkable nutritional diversity in the Corticiales, including a new foliicolous species of *Marchandiomyces* (anamorphic Basidiomycota, Corticiaceae) from Australia. American Journal of Botany 95: 816–823.

Matheny, P. B., Z. Wang, M. Binder, J. M. Curtis, Y. W. Lim, R. H. Nilsson, K. W. Hughes, V. Hofstetter, J. F. Ammirati, C. L. Schoch, E. Langer, G. Langer, D. J. McLaughlin, A. W. Wilson, T. Frøslev, Z.-W. Ge, R. W. Kerrigan, J. C. Slot, Z.-L. Yang, T. J. Baroni, M. Fischer, K. Hosaka, K. Matsuura, M. T. Seidl, J. Vauras, D. S. Hibbett. 2007. Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Molecular Phylogenetics and Evolution 43: 430–451.

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

Moncalvo, J. M., R. H. Nilsson, B. Koster, S. M. Dunham, T. Bernauer, P. B. Matheny, T. M. Porter, S. Margaritescu, M. Weiss, S. Garnica, E. Danell, G. Langer, E. Langer, E. Larsson, K. H. Larsson & R. Vilgalys. 2006. The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. Mycologia 98: 937–948.

Penn, O., E. Privman, G. Landan, D. Graur & T. Pupko. 2010a. An alignment confidence score capturing robustness to guide-tree uncertainty. Molecular Biology and Evolution 27: 1759–1767.

Penn, O., E. Privman, H. Ashkenazy, G. Landan, D. Graur & T. Pupko. 2010b. GUIDANCE: a web server for assessing alignment confidence scores. Nucleic Acids Research 38: W23–W28.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25: 1253–1256.

Rambaut, A. 2012. FigTree v1.4.2, available from: http://tree.bio.ed.ac.uk/software/figtree/

Sikaroodi, M., J. D. Lawrey, D. L. Hawksworth & P. T. DePriest. 2001. Phylogenetic position of selected lichenicolous fungi: *Hobsonia*, *Illosporium* and *Marchandiomyces*. Mycological Research 105: 453–460.

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

Stamatakis, A., T. Ludwig & H. Meier. 2005. RAxML-III: A fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21: 456–463.

Wilson, A. W., M. C. Aime, J. Dierks, G. M. Mueller & T. W. Henkel. 2012. Cantharellaceae of Guyana I: new species, combinations and distribution records of *Craterellus* and a synopsis of known taxa. Mycologia 104: 1466–1477.

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