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New lichen-associated bulbil-forming species of *Cantharellales* (Basidiomycetes)

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Abstract: Two new genera and four new species of bulbil-forming basidiomycetes are described. Phylogenetic analyses of nuLSU and ITS sequences place them in *Cantharellales*. A facultative lichenicolous species with yellow to orange-yellow bulbils from South America groups with the type of *Burgella* and is consequently described as *B. lutea*. The new species and genus *Burgellopsis nivea* is introduced for material from Scotland with white bulbils overgrowing saxicolous lichens. An obligate lichenicolous species with particularly large, applanate bulbils developing over *Peltigerales* in South America could not be placed accurately using ITS sequences and is described as the new species and genus *Bulbilla applanata*. A European species with brown, facultatively lichenicolous bulbils grouped with *Ceratobasidium* and *Thanatephorus* species and is described as the new *Ceratobasidium bulbillifaciens*.

Key words: Europe, lichenicolous fungi, sclerotia, South America

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Introduction

Lichen-associated homobasidiomycetes are known to occur in at least five major clades, the *Agaricales* (*Arrhenia*, *Gamundia*), *Atheliales* (*Athelia*), *Boletales* (*Leucogyrophana*), *Corticiales* (*Laetisaria*, *Marchandiobasidium* and *Marchandiomyces*) and *Cantharellales* (*Burgella*, *Burgoa* and *Minimedusa*) (Lawrey *et al*. 2007; Barrasa & Rico 2010; Lawrey & Diederich 2013). Many of these species form bulbils, minuscule sclerotia-like structures that are always sterile, with no known basidiomata or conidiomata. Lichenized basidiomycetes species are also known from many of these same lineages, the *Agaricales* (*Acantholichen*, *Cyphellostereum*, *Dictyonema*, *Lichenomphalia*), *Atheliales* (*Athelia*), *Corticiales* (*Marchandiomphalina*), *Cantharellales* (*Multiclavula*) and *Lepidostromataceae* (unknown order) (*Lepidostroma*) (Jülich 1978; Lawrey *et al*. 2007, 2009; Ertz *et al*. 2008), and in two lineages, the *Cantharellales* and *Agaricales*, lichenized species produce lichenized bulbils (*Multiclavula* and *Lichenomphalia*). In both the *Cantharellales* and *Corticiales*, lichenicolous species are also closely related to lichenized species, which led Lawrey *et al*. (2007) to propose the hypothesis that

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This paper is dedicated to our friend Brian Coppins on the occasion of his 65th birthday, in recognition of his longstanding interest in lichens and lichenicolous fungi, including bulbil-forming fungi.

lichenized species may have evolved from lichenicolous ancestors, and that the production of bulbils may promote the evolution of the lichenized habit. This and other interesting hypotheses cannot be tested fully until more is learned about the phylogenetic relationships of lichenized and lichen-associated basidiomycetes. A review of all known bulbil-forming species was provided by Diederich & Lawrey (2007), with the description of several new taxa within the *Cantharellales* and *Corticiales*. One of those species, belonging to the *Ceratobasidiaceae*, was not formally described in that paper, as the authors preferred to wait for more material and additional sequences to confirm the placement in that family. The aim of this paper is to formally describe this species in *Ceratobasidium* and to describe several additional, at least facultatively lichen-associated, species in the *Cantharellales*.

Material and Methods

Morphological study

Specimens from the herbaria B, BAFC, BR, E, KRAM, M, LPB, STU and UPS, and from the private collections of R. Cezanne & M. Eichler, P. Diederich, A. Flakus, J. Etayo, Z. Palice and P. van den Boom were examined. 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 and a Nikon BD Plan 10 microscope objective, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Entire unsectioned bulbils, more rarely sections of bulbils (*Bulbilla applanata*), were studied on material mounted in water, 5% KOH, lactophenol cotton blue (LCB), Phloxine B or Congo Red, either without or with pressure on the coverslip. Microscopic photographs of bulbils in LCB were prepared using a Leica DMLB microscope and a Leica EC3 camera, and using Helicon Focus.

Isolation of fungal cultures

Cultures of *Burgella flavoparmeliae* (*Flakus* 23513), *Burgellopsis nivea* (*Coppins* 21845) and *Ceratobasidium bulbillifaciens* (*Eichler-Cezanne* 8193, 8067) were isolated from freshly collected material. Isolation techniques are discussed in Lawrey (2002). Bulbils were removed from lichens using sterile pin tools and placed in 70% ethanol for *c.* 30 s, after which time they were placed on culture media. All fungi grew on either malt/yeast agar (MYA), potato dextrose agar (PDA) or Sabouraud's dextrose agar (SDA). Mycelial outgrowths were subcultured monthly. After 2 weeks, *c.* 2 μg dry mycelial mass was harvested from liquid cultures and extracted for DNA analysis.

Molecular techniques

Cultures of *Burgellopsis nivea* (*Coppins* 21845) and the *Ceratobasidium bulbillifaciens* specimens from Germany (*Eichler-Cezanne* 8193, 8067) were sequenced at George Mason University using standard fluorescent sequencing methods. Genomic DNA was extracted from isolated fungal tissue using the Bio 101 Fast DNA Spin Kit (Qbiogen, Illkirch, France) according to the manufacturer's protocol, with slight modifications. Approximately 10 ng of extracted DNA was subjected to either a standard PCR in a 25 μl reaction volume using Taq Gold polymerase (Applied Biosystems Inc., Foster City, California, USA) or a Bio-X-Act Long Mix (Bioline USA, Inc., Taunton, Massachusetts, USA), according to the manufacturer's protocol. Different methods of PCR were used depending on the size of the target fragment. After visualizing the PCR products on a 1% agarose gel with ethidium bromide and confirming the size, the products were purified with Agencourt Ampure magnetic beads solution (Beckman Coulter, Inc., Indianapolis, IN, USA). The purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems Inc.). The sequencing reactions were purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, Missouri, USA), dried in Automatic Environmental Speed Vac System (Savant Instruments, Inc., Holbrook, NY), denatured in HiDi Formamide (Applied Biosystems Inc.), and run on an ABI3130-xl capillary sequencer (Applied Biosystems Inc.). The data collected were analyzed using Sequencing Analysis v5.4 software (Applied Biosystems Inc.). In the region of 500–700 bases were obtained for each primer used. These sequences were analyzed with Sequencher v4.7 (Gene Codes Corporation, Ann Arbor, Michigan, USA) software for manual base calling and to make contiguous alignments of overlapping fragments.

Sequence data were obtained from the nuclear rDNA internal transcribed spacer 1, 5.8S and internal transcribed spacer 2 (collectively referred to as ITS), and *c.* 1700 bases of the 5′ end of the nuclear large subunit rDNA (nuLSU) using the 5.8SR, LR0R, LR3R, LR8R, LR5, LR7, LR16, and ITS2, ITS3, ITS4, ITS5 primers available from the Duke University Mycolab web site (http://www.biology.duke.edu/fungi/mycolab/primers.htm).

The culture of *Burgella flavoparmeliae* (*Flakus* 23513) was sequenced in the National Botanic Garden of Belgium following the methods used by Ertz *et al.* (2011), but with primer LIC15R only to obtain a single strand sequence of *c.* 800 bases.

The method of direct PCR as explained in Lawrey *et al.* (2007: 780) was performed on bulbils of *Burgella lutea* (*Etayo* 27623), using primers LROR and LR7 for amplification of nuLSU, and primers ITS1 and ITS4 for amplification of ITS. In addition, primers LR3R and LR3 were used for the sequencing of nuLSU.

TABLE 1. *Specimens and newly acquired cultures and sequences featured in the study*

Species	Country	Specimen	Substratum	Isolate	Genbank Accession No. ITS	Genbank Accession No. nuLSU
Bulbilla applanata (holotype)	Bolivia	*Flakus* 16422	*Pseudocyphellaria*	—	KC336078	—
B. applanata	Bolivia	*Flakus* 16424	*Lobariella crenulata*	—	KC336079	—
Burgella flavoparmeliae (holotype)	USA, Oklahoma	*Buck* 38682	*Flavoparmelia baltimorensis*	ATCC MYA-2157	—	DQ915469
B. flavoparmeliae	Bolivia	*Flakus* 23513	*Parmotrema*	—	—	KC336074
B. lutea (holotype)	Bolivia	*Etayo* 27623	Corticolous lichens	—	KC336076	KC336075
Burgellopsis nivea (holotype)	Great Britain, Scotland	*Coppins* 21845	Crustose saxicolous lichen	ATCC MYA-4209	—	KC336077
Ceratobasidium bulbillifaciens	Germany	*Eichler-Cezanne* 8193	Bark of *Acer platanoides*	CBS 129339	—	KC336073
C. bulbillifaciens	Germany	*Wirth* 32360	Bark of *Sambucus*	ATCC 208870	—	DQ915470
C. bulbillifaciens	Germany	*Eichler-Cezanne* 8067	Bark of *Fraxinus*	CBS 132236	KC336072	KC336071

Multitag pyrosequencing (MTPS)

We employed the multitag pyrosequencing (MTPS) process to characterize the fungal components from the two Bolivian specimens (*Flakus* 16422, 16424) that did not produce a clean sequence with standard Sanger sequencing methods. Specifically, we used a set of fusion primers that contain 454 emulsion PCR linkers (Roche Diagnostics corp., Indianapolis, IN, USA) and a unique 8-base barcode on the forward primer to recognize each sample after sequencing. Each sample was amplified with a uniquely barcoded set of forward ITS1F primers and a reverse ITS2 primer. Both ITS1F and ITS2 primers are universal fungal primers and the reverse primer was FAM (6-carboxyfluorescein) labelled to fingerprint the products and quantify them before emulsion PCR for pyrosequencing (Sikaroodi & Gillevet 2012). The PCR products were pooled based on quantification of individual Fam-labelled samples using Genemapper software v4.1 (Applied Biosystems Inc.). They were then quantified as a pool on a DTX880 Multimode Fluorescene detector (Beckman Coulter, Indianapolis, IN, USA), subjected to emulsion PCR and pyrosequenced using a GS Junior sequencing instrument (Roche Diagnostics corp., Indianapolis, IN, USA). Original Roche protocols were used for emulsion PCR and the pyrosequencing process, with slight modifications for optimization (Sikaroodi & Gillevet 2012). Sequence data from each pooled sample were sorted into bins using custom PERL scripts based on the unique barcodes, and the *Bulbilla* sequences, which were distinct from those of the host lichens, were extracted and used in phylogenetic analyses.

Phylogenetic analyses

The nuLSU dataset consisted of sequences obtained from all specimens except the two Bolivian specimens of *Bulbilla applanata* (*Flakus* 16422, 16424), sequences from a nuLSU alignment of sclerotial and bulbil-forming lichenicolous members of the *Cantharellales* published previously (Lawrey *et al.* 2007), and those downloaded from GenBank. GenBank sequences were obtained following BLAST searches using as queries the new sequences of *Burgella*-like specimens and excluding sequences of uncultured/environmental samples. After retrieval, duplicated sequences were identified in BIOEDIT 7.1.5 (Hall 2007) and deleted. The final nuLSU dataset contained 62 ingroup terminals and sequences from *Basidiodendron caesiocinereum*, *Exidia glandulosa*, *Pseudohydnum gelatinosum*, *Tilletiaria anomala* and *Platygloea disciformis* were used as an outgroup (GenBank accession numbers in Table 1 for new sequences and Fig. 1 for published sequences).

ITS sequences from *Burgella lutea*, the two Bolivian specimens of *Bulbilla applanata* obtained by 454 pyrosequencing, and two bulbil-forming specimens of *Ceratobasidium bulbillifaciens* from Germany were added to those from GenBank, representing most families of the *Cantharellales*. BLAST searches were carried out using ITS of *Ceratobasidium bulbillifaciens* and the *Burgella*-like specimens as query sequences, and excluding sequences of uncultured/environmental samples. In a preliminary alignment of the ITS sequences representing identified members of the *Ceratobasidiaceae*, it was discovered that most were duplicates, and so all but one of these were deleted from the final dataset. ITS sequences of *Cantharellus*

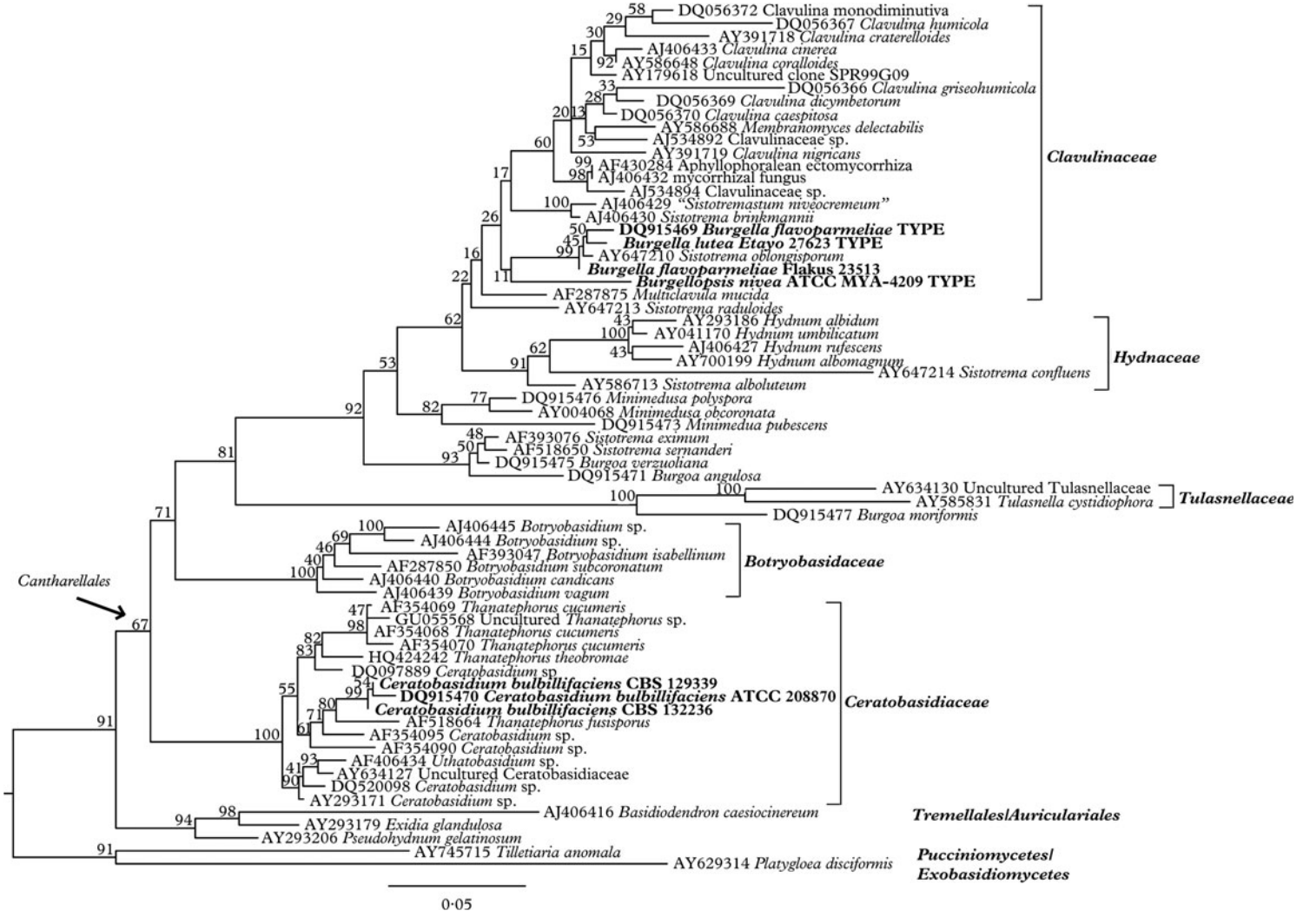

FIG. 1. Best-scoring maximum-likelihood tree of the nuLSU alignment of selected *Cantharellales* including new bulbil-forming *Burgella*, *Burgellopsis* and *Ceratobasidium* species. Scale indicates expected substitutions per site, and numbers at nodes are bootstrap values based on 500 replicates. GenBank accession numbers for sequences obtained in this study (in bold) are provided in Table 1.

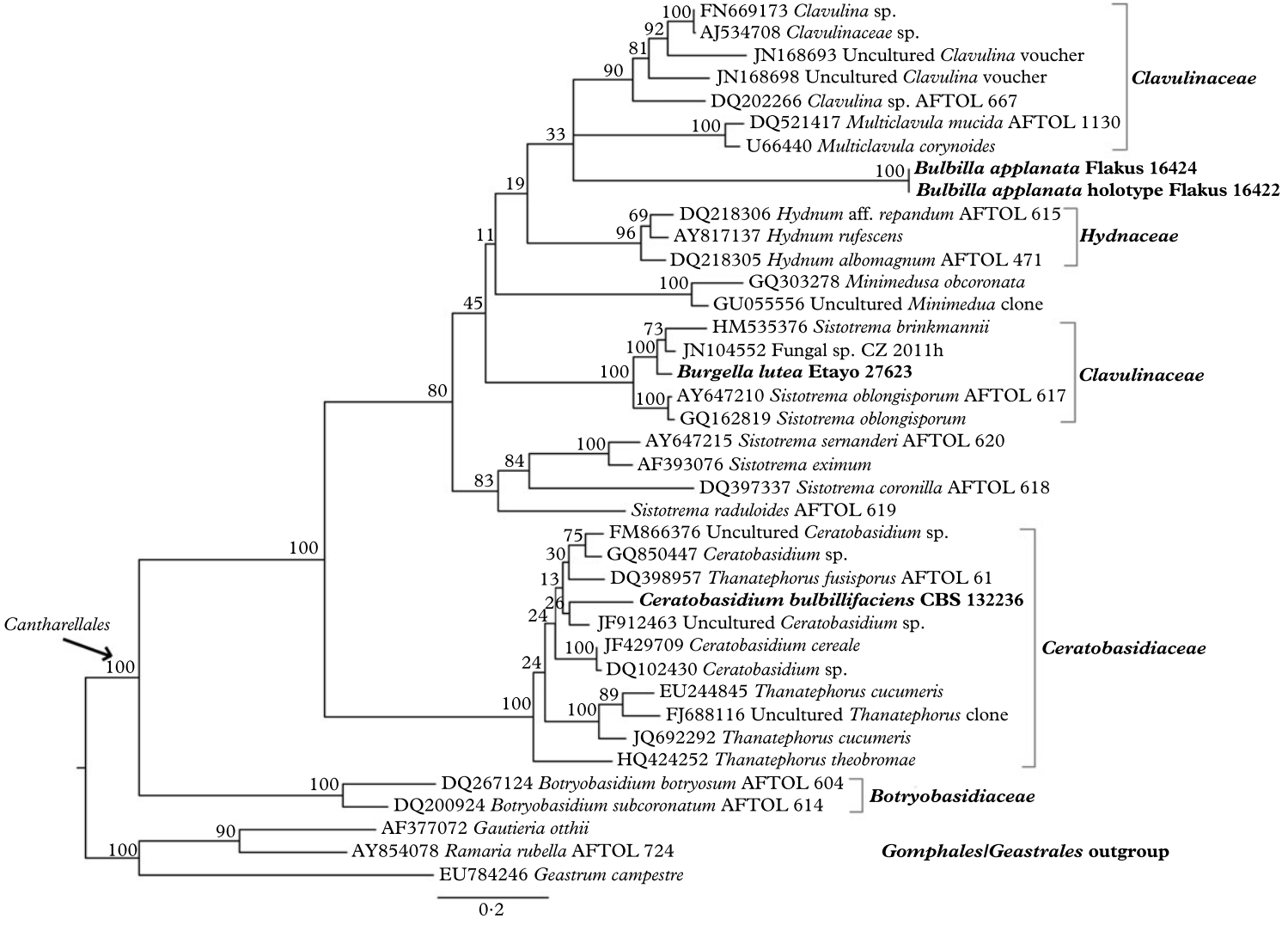

FIG. 2. Best-scoring maximum-likelihood tree of the ITS alignment of selected *Cantharellales* including new bulbil-forming *Bulbilla*, *Burgella* and *Ceratobasidium* species. Scale indicates expected substitutions per site, and numbers at nodes are bootstrap values based on 500 replicates. GenBank accession numbers for sequences obtained in this study (in bold) are provided in Table 1.

spp. and members of the *Tulasnellaceae* proved to be unusually difficult to align and were dropped from the dataset. BLAST searches of *Bulbilla applanata* sequences obtained by 454 pyrosequencing (Bolivian specimens *Flakus* 16422, 16424) indicated they were members of the *Cantharellales* but not similar to *Burgella* species in our dataset, so the closest ITS sequences from BLAST searches were added to the dataset, the final version of which contained 36 ingroup terminals, with sequences of *Gauthierea otthii*, *Ramaria rubella* and *Geastrum campestre* used as an outgroup (GenBank accession numbers in Table 1 for new sequences and Fig. 2 for published sequences).

Sequences were arranged into multiple sequence alignments for each gene using BIOEDIT 7.1.5 (Hall 2007) and automatically aligned with MAFFT 6.850b using the –auto option (Katoh & Toh 2005, 2010). The individual nuLSU and ITS alignments were subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn *et al.* 2010*a*, *b*) and introns and regions aligned with low confidence, particularly in the ITS, were removed. This resulted in an alignment length of 985 for the nuLSU and 1130 for the ITS. The two datasets contained sequences representing different species and specimens and were therefore analyzed separately. The alignments were subjected to maximum likelihood (ML) searches using RAxML 7.2.6 (Stamatakis *et al.* 2005; Stamatakis 2006), with parametric bootstrapping using 500 replicates under the GTRGAMMA model.

Results

Phylogenetic analyses

The best-scoring nuLSU ML tree indicated a monophyletic but weakly-supported *Cantharellales* with bulbil-forming species represented in two clades (Fig. 1). Sequences representing the new species *Ceratobasidium bulbillifaciens* were recovered in a strongly supported clade that includes *Thanatephorus fusisporus* as its closest relative and two unidentified *Ceratobasidium* species. The new *Burgella lutea* was recovered in a strongly-supported clade containing two separate sequences of *Burgella flavoparmeliae*, the type from the USA and another from Bolivia, and a sequence of *Sistotrema oblongisporum*. This clade was sister to another new species, *Burgellopsis nivea*, but without support, and the entire *Burgella* + *Burgellopsis* clade was sister to another, consisting of *Sistotrema brinkmannii* and a sequence labelled "*Sistotremastum niveocremeum*" (probably misidentified, but correct identification unknown, see Moncalvo *et al.* 2006) and a much larger, weakly supported assemblage representing the *Clavulinaceae*. The nuLSU alignment contained relatively few ambiguous regions (overall GUIDANCE alignment score = 0·9656), and support for the backbone of the tree is moderately strong, but since our alignment was designed to emphasize mainly the groups known to harbour our specimens, support for other groups tended to be weaker.

The best-scoring ITS ML tree (Fig. 2) indicated a well-supported *Cantharellales* with bulbil-forming specimens observed in the same clades as in the nuLSU tree, but with weaker support. A single sequence representing *Ceratobasidium bulbillifaciens* (*Eichler-Cezanne* 8067) was recovered in the *Ceratobasidiaceae*, but another sequence (*Eichler-Cezanne* 8193) proved to be too short to be reliably placed phylogenetically. The new species *Burgella lutea* was recovered in a weakly supported clade containing members of *Sistotrema* s. lat., and the new genus and species *Bulbilla applanata* (represented by ITS only) was recovered in a poorly-supported polytomy containing members of the *Clavulinaceae*, *Multiclavula* spp. and *Hydnum* spp. The highly length-variable ITS1 and ITS2 regions may be partly responsible for this uncertainty (overall GUIDANCE alignment score = 0·7056), but these same groups were also not well resolved in the nuLSU tree.

Discussion

Phylogenetic analysis of nuLSU sequences (Fig. 1) places a Bolivian specimen developing on *Parmotrema* (*Flakus* 23513), morphologically similar to the lichenicolous *Burgella flavoparmeliae* (described from *Flavoparmelia*), as sister to the type of *B. flavoparmeliae*, suggesting that both specimens are either conspecific or represent two closely related species. These two sequences, together with *Sistotrema oblongisporum* and the new *Burgella lutea*, form a strongly supported clade. Bulbils of both species of *Burgella* are ochraceous or yellow-coloured and in microscopical preparations, orange lipid droplets emerge from cells, especially when examined in LCB, and these might be responsible for the bulbil colour. Basidiomata of *Sistotrema oblongisporum*,

on the contrary, are greyish white, without any yellowish tinge. Moncalvo *et al.* (2006) have shown that *Sistotrema* is a polyphyletic complex of morphologically similar wood saprophytes that need further study. The type species, *S. confluens*, is more closely related to *Hydnum* than to most other *Sistotrema* species, and therefore major nomenclatural changes will be needed in this group. Although our phylogenetic results suggest that *S. oblongisporum* belongs to *Burgella*, we do not formally combine the species in that genus, leaving such a decision for future studies focusing on the entire genus *Sistotrema*.

The new *Burgellopsis nivea* is sister to the *Burgella* clade, but with low support. Bulbils are pure white and no yellowish lipid drops are visible under microscopical examination, unlike in species of *Burgella*. Consequently, a new genus, *Burgellopsis*, is described for this species.

From a rather common lichenicolous, bulbil-forming species developing over *Peltigerales* in South America that we describe as the new *Bulbilla applanata*, no cultures and no nuLSU sequences could be obtained. Instead, short ITS sequences obtained from two specimens by 454 pyrosequencing, and a phylogenetic analysis of these (Fig. 2), clearly places them within *Cantharellales*, but not close to any other species. A more accurate phylogenetic placement will require additional genes to be sequenced. As the species is morphologically distinct from all other bulbil-forming genera of *Cantharellales*, the new species is therefore included in a new genus *Bulbilla*.

An undescribed bulbil-forming species clustering with *Ceratobasidium* was studied by Lawrey *et al.* (2007). Our new phylogenetic analyses, including sequences from three specimens (Figs 1 & 2), confirm that the species, here newly described as *Ceratobasidium bulbillifaciens*, belongs to the *Ceratobasidium-Thanatephorus* complex, which initially included *Ceratobasidium* D. P. Rogers, *Thanatephorus* Donk and *Uthatobasidium* Donk. These three genera were primarily differentiated on ecological characters, the latter two being morphologically indistinguishable, leading Knudsen & Hansen (1996) to unite them, with *Uthatobasidium* becoming a synonym of *Thanatephorus*. Morphological characters to separate the genus *Thanatephorus* (including *Uthatobasidium*) from *Ceratobasidium*, and also to distinguish individual species, are mainly based on basidiomata, which are unfortunately unknown in the new species. Roberts (1999) revised the species of *Ceratobasidium* and *Thanatephorus* having *Rhizoctonia* anamorphs and presented modern descriptions of many species. He furthermore explained that the generic type of *Ceratobasidium*, *C. calosporum* D. P. Rogers, is morphologically not close to the other species of the genus and possibly not even related to them, but no sequences of this taxon are available yet. In a recent phylogenetic analysis using ITS sequences, Veldre (2011, fig. 2) showed that the genus *Thanatephorus* represents a single, well-defined clade, whilst *Ceratobasidium* species are spread over several clades. Our nuLSU tree (Fig. 1) recovers the *Thanatephorus*-clade, with the generic type *T. cucumeris* and *T. theobromae*, which is sister to a clade comprising the new *C. bulbillifaciens*, two unidentified *Ceratobasidium* species and *Thanatephorus fusisporus*, and these two clades together are sister to another clade including several unidentified sequences labelled *Ceratobasidium* and *Uthatobasidium*. Our ITS tree (Fig. 2) presents similar results. Although one of the *Thanatephorus* species (*T. fusisporus*) does not group with the generic type *T. cucumeris*, our results nevertheless suggest that an inclusion of the new species in *Ceratobasidium* is the best choice.

Following Veldre (2011), species of the *Ceratobasidium-Thanatephorus* complex "spend most of their life in morphologically simple asexual stages during which they can only be macroscopically observed as irregular sclerotia less than a centimetre in size". Sclerotia were mentioned from several species studied by Roberts (1999), but these are all much bigger than the minuscule bulbils of the new species (up to 0·2 mm diam.). Sclerotia of *C. anceps* (Bres. & Syd.) H. S. Jacks. are much larger, up to 5 mm diam., cream at first then dark brown; *C. bicorne* J. Erikss. & Ryvarden develops sclerotia in culture, up to 0·9 mm diam., 'fulvous to umber' aggregating into larger clusters; sclerotia of *C. cornigerum* (Bourdot) D. P. Rogers are pale to brown,

0·5–3·0 mm diam.; sclerotia of *C. setariae* (Sawada) Oniki *et al.* are 0·5–2·0 mm diam., composed of subglobose hyphal compartments 13–30 µm diam.; *Thanatephorus cucumeris* (A. B. Frank) Donk produces sclerotia up to 8 mm diam.; and *T. ochraceus* (Massee) P. Roberts produces loose, white sclerotia in culture (Roberts 1999).

Taxonomy

Bulbilla applanata Diederich, Flakus & Etayo gen. et sp. nov.

MycoBank Nos.: MB807650 (genus), MB807651 (species)

Characterized by relatively large [200–400(–500) µm diam.], lichenicolous, beige, greyish yellow to greyish orange, translucent, basally constricted, applanate bulbils, internally of adherent polyhedral cells, without clamps.

Type: Bolivia, Dept. La Paz, Prov. Nor Yungas, Coroico village, Yungas montane forest, 16°11′10″S, 67°43′16″W, alt. 1550 m, on epiphytic *Pseudocyphellaria*, 6 June 2010, *A. Flakus* 16422 & *P. Rodrigues F.* (KRAM—holotype; LPB, hb. Diederich—isotypes).

(Figs 3A–C, 4A & B)

Basidiomata and conidiomata unknown. *Colonies* appearing as dispersed bulbils overgrowing lichen thalli or more rarely apothecia. *Mycelium* not observed. *Bulbils* slightly to distinctly immersed in the host thallus, usually leaving conspicuous scars in the thallus when removed, pale beige, greyish yellow or greyish orange, translucent, and colour consequently varying with the colour of the host thallus, not or faintly shiny, without hairs, roundish to shortly ellipsoid, 200–400(–500) µm diam., sometimes confluent and up to 700 µm, flattened, *c.* 200 µm tall, basally strongly constricted; *bulbils externally* without specialized cells, cells in surface view polyhedral, smooth, mainly 5–12 µm diam.; *bulbils internally* composed of strongly adherent, more or less roundish to ellipsoid or polyhedral cells separating only with difficulty, 4–11(–14) µm diam., clamps not observed; agglomerations of small crystals visible in polarized light present on the bulbil surface in some specimens examined.

Distribution and ecology. This species is known from several South American countries (Bolivia, Chile and Ecuador), where it parasitizes thalli, more rarely apothecia, of large, cyanobacterial macrolichens belonging to the *Peltigerales* (*Lobariella*, *Peltigera*, *Pseudocyphellaria*, *Sticta*). Infected thalli often, but not always, change colour (e.g., in the type collection, the brown thallus of *Pseudocyphellaria* turns dark green, surrounded by a blackish necrotic line), suggesting that the new species may be a virulent parasite on some hosts. This reaction on the host thallus, the observation that bulbils are slightly immersed in the thallus and often (but not always) leave holes when removed, and the host choice suggest a long evolutionary history between the new *Bulbilla* species and the *Peltigerales* hosts, and let us speculate that the species is strictly lichenicolous. Thin sections through bulbils reveal at their base a zone mainly composed of *Bulbilla* cells, intermixed with photobiont cells of the host (Fig. 4A–D), suggesting that some kind of interaction takes place between bulbils and their hosts.

Observations. This species differs from all other known lichenicolous, bulbil-forming basidiomycetes by the particularly large bulbils, typically reaching 400 µm in diam. Bulbils are slightly translucent, show some variability in colour, and are often (but not always) applanate. We hesitated to describe this species, as no cultures could be obtained. However, small ITS sequences obtained by 454 pyrosequencing clearly included the species within the *Cantharellales*. Our phylogenetic analysis (Fig. 2) did not allow us to recognize the closest relatives of the new species, but clearly excluded it from the genus *Burgella*. Although no ITS sequences of *Burgoa* species are available, the closest relatives of the generic type *Burgoa verzuoliana*, viz. *Sistotrema eximum* and *S. sernanderi* (see Fig. 1), are only distantly related to the new species (Fig. 2). Similarly, no ITS sequences of *Minimedusa* are available, but these species strongly differ morphologically from the new species (Diederich & Lawrey 2007). Consequently, a new genus *Bulbilla* is described to accommodate this characteristic species.

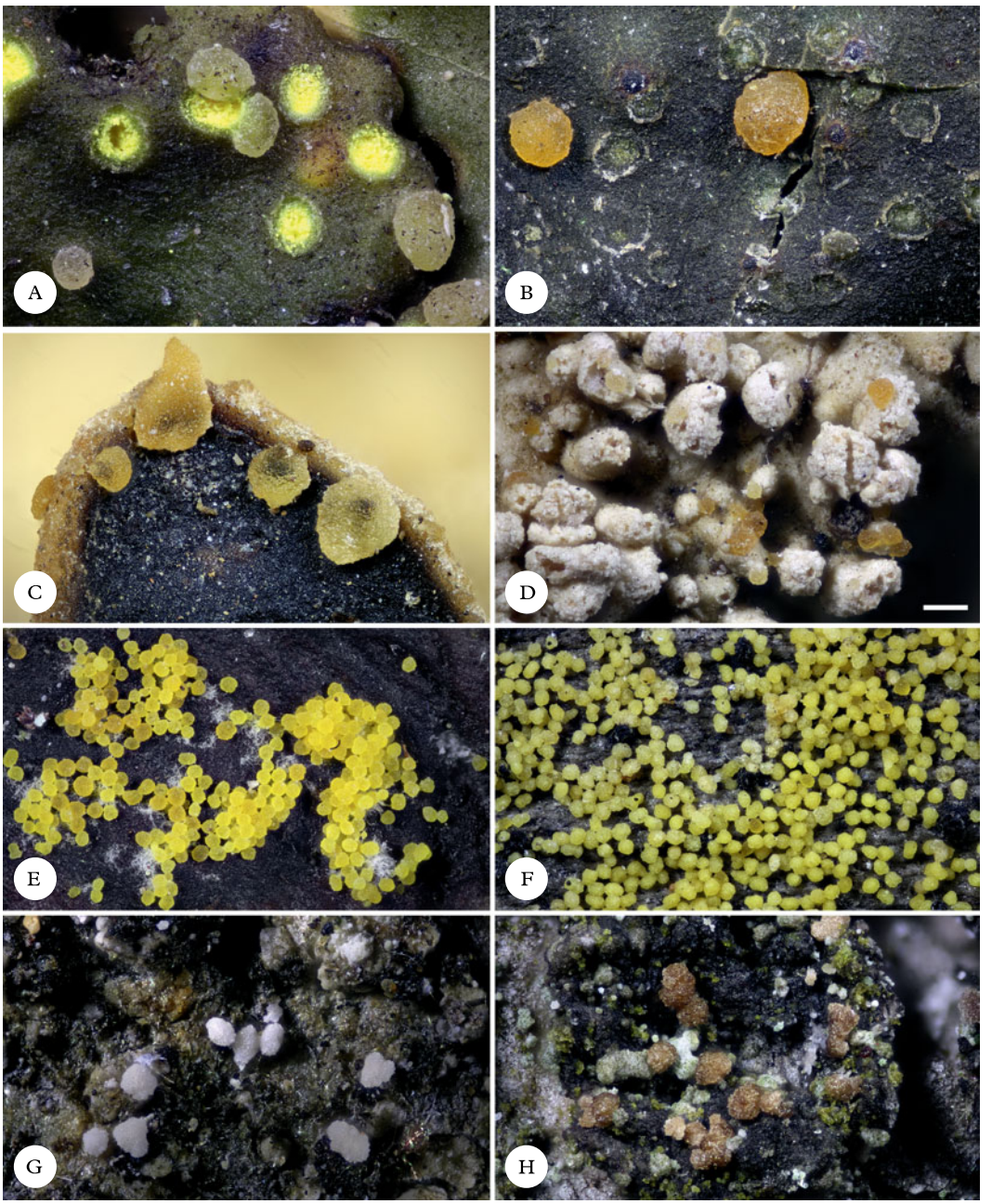

FIG. 3. A, *Bulbilla applanata* on the thallus of *Pseudocyphellaria* (holotype); B, *B. applanata* on the thallus of *Sticta*, leaving distinct scars when removed (*Etayo* 26437); C, *B. applanata* on apothecia of *Pseudocyphellaria faveolata* (*Etayo* 23562); D, *Burgella flavoparmeliae* on the thallus of *Flavoparmelia baltimorensis* (holotype); E, *Burgella lutea* on the thallus of *Sticta* (holotype); F, *B. lutea* on *Butia yatay* (*Capdet & Romero* 51690); G, *Burgellopsis nivea* over sterile, saxicolous lichen (holotype); H, *Ceratobasidium bulbillifaciens* on dying lichenized crust and young *Physcia tenella* (holotype). Scale (the same for all photographs) = 200 μm.

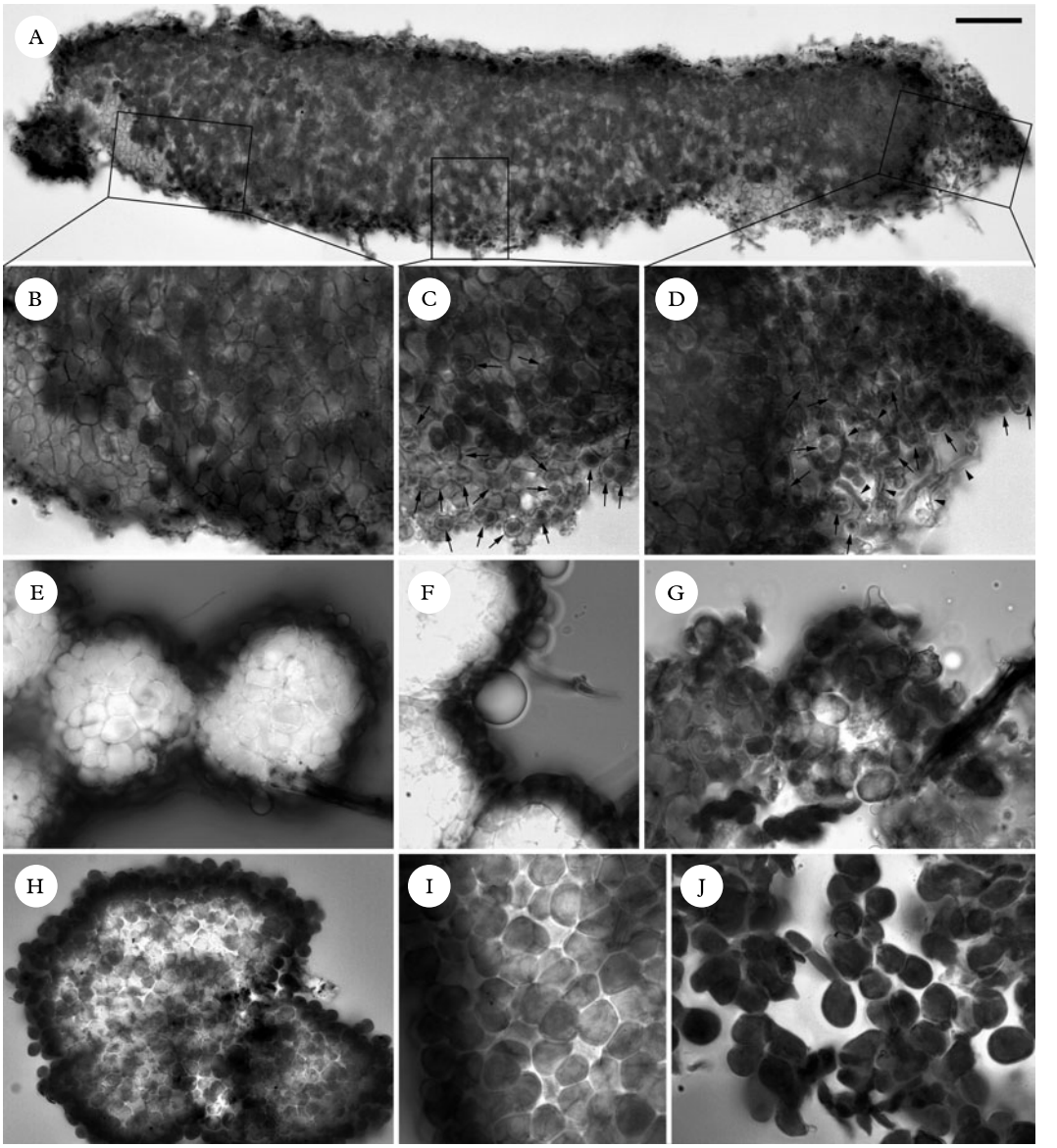

FIG. 4. A–D, *Bulbilla applanata* (holotype) on *Pseudocyphellaria*; A, section through flattened bulbil in LCB; B–D, details of A at a higher magnification; B, lower part of bulbil not in contact with host, showing polyhedral cells; C, lower part of bulbil in contact with host thallus, showing a mixture of polyhedral cells of bulbil and photobiont cells of host (arrows); D, lower part of bulbil in loose contact with host, showing polyhedral cells of bulbil, photobiont cells (arrows) and host hyphae (arrow heads). E–G, *Burgella lutea* (holotype); E, bulbils in LCB (only outer parts of bulbils stained), showing ellipsoid cells; F, the same at a higher magnification, showing emerging oil drops and (indistinctly) hyphae with clamps; G, the same bulbils after pressure on cover glass, slightly separating inner, ellipsoid cells. H–J, *Burgellopsis nivea* (holotype); H, bulbils in LCB, showing subspherical to ellipsoid cells; I, bulbil (surface view) at a higher magnification; J, bulbil after pressure on cover glass, separating inner cells. Scales (the same for all photographs): A, E & H = 50 μm; B–D, F, G, I & J = 20 μm.

Additional specimens examined. **Bolivia:** Same locality as type, on epiphytic *Lobariella crenulata*, *Flakus* 16424 & *Rodriguez F.* (LPB, hb. Flakus).—**Chile:** *Región de Los Lagos*: Valdivia, P. N. Vicente Pérez Rosales, subida al volcán Osorno, bosque de grandes *Nothofagus dombeyi*, 41°07′59″S, 72°32′18″W, alt. 920–980 m, on *Nothofagus*, on *Pseudocyphellaria faveolata*, 2006, *Etayo* 23562 (hb. Etayo).—**Ecuador:** *Prov. Loja*: Parque Nacional Podocarpus, on *Peltigera*, 1999, *Palice* 3166 (hb. Palice, hb. Etayo 23468). *Prov. Tungurahua*: entre Pondoa y Tungurahua, bosque nublado, alt. 2400–3800 m, on *Peltigera*, 1999, *Etayo* 25914 & *Palice* (hb. Etayo); Río Verde (*c.* 13 km E of Baños), descent waterfalls at the confluence of Río Pastaza and Río Verde (Pailón del Diablo), 2°24′03″S, 78°17′43″W, on *Sticta*, 2003, *Etayo* & *Palice* 8564 (hb. Palice, hb. Etayo). *Prov. Zamora-Chinchipe*: N.R. of Estación Científica San Francisco, S of road Loja-Zamora, primary montane forest on steep slope, *c.* 40 km from Loja, 3°58′S, 79°04″W, 1945 m, on *Hyeronima asperifolia*, on *Lobariella crenulata*, 2004, *Sipman* 52521 (B, hb. Etayo).

Burgella flavoparmeliae Diederich & Lawrey

Mycol. Progress **6:** 64 (2007).

(Fig. 3D)

This species was originally described from three specimens from the USA on *Flavoparmelia baltimorensis* (Diederich & Lawrey 2007). Here we report morphologically similar populations from South America growing on another host genus, *Parmotrema*. Sequences from bulbils on both hosts group together in our phylogeny (Fig. 1), but they form two rather long branches on a short, unsupported branch, leaving some doubt as to whether they are really conspecific. Due to the similar morphology, we provisionally include the material on *Parmotrema* in *Burgella flavoparmeliae*, awaiting additional populations on both hosts being sequenced. It is interesting to note that *Flavoparmelia* belongs to the *Parmotrema* clade in a recent molecular phylogeny of parmelioid genera (Crespo *et al.* 2010).

Specimens examined. **Bolivia:** *Dept. La Paz*: Prov. Franz Tamayo, between Apolo and Mapiri villages, 13 km from Apolo, 14°50′51″S, 68°21′38″W, 1530 m, savannah with scattered trees and shrubs, along stream, on *Parmotrema* sp., 2011, *Flakus* 23037 & *Kukwa* (BR, KRAM, LPB, hb. Flakus). *Dept. Santa Cruz*: Prov. Cordillera, between Tucavaca and Roboré, 18°36′11″S, 59°53′06″W, 320 m, transition between Chaqueño and Chiquitano forests, on thallus of *Parmotrema* sp., 2011, *Flakus* 23513 & *Kukwa* (KRAM, LPB, BR). *Dept. Tarija*: Prov. O'Connor, Lomas de Soledad, road between Entre Ríos and Chiquiacá, 21°39′38″S, 64°07′31″W, 1670 m, Tucumano-Boliviano altimontano forest, on *Parmotrema* sp., 2012, *Etayo* 28110, *Flakus* & *Kukwa* (LPB, hb. Etayo).—**Ecuador:** *Imbabura*: Otavalo, Reserva bosque nublado INTAG, La Delicia, bosque nublado, 2700 m, con *Gunnera* y helechos arborescentes, on *Parmotrema*, 2003, *Etayo* 25640 & *Palice* (hb. Etayo).

Burgella lutea Diederich, Capdet, A. I. Romero & Etayo sp. nov.

MycoBank No.: MB807652

Characterized by superficial, yellow to orange-yellow, roundish bulbils 50–80 µm diam., internally of adherent polyhedral cells, clamps in mycelium present, in bulbils unknown.

Type: Bolivia, Dept. La Paz, Prov. Franz Tamayo, near Apolo village, small valley amongst meadows, 14°50′15″S, 68°26′58″W, alt. 1430 m, pre-Andean Amazon forest, on corticolous lichens, 17 May 2011, *J. Etayo* 27623, *A. Flakus*, *M. Kukwa* & *U. Schiefelbein* (LPB—holotype; hb. Etayo, hb. Diederich—isotypes).

(Figs 3E–F, 4C–G)

Basidiomata and *conidiomata* unknown. *Colonies* appearing as dispersed or agglomerated bulbils overgrowing lichen thalli, ascomycete perithecia or rotten woody parts of palms. *Mycelium* superficial, 3·0–3·5 µm thick, with clamps. *Bulbils* superficial, yellow to orange-yellow (Kornerup & Wanscher 1984: 3A5–8, 4A6–8; colours based on dry herbarium material), without hairs, surface at a high magnification (×50) rather rough, with individual convex cells slightly distinguishable, slightly shiny, roundish to shortly ellipsoid, 50–80 µm diam.; *bulbils externally* without specialized cells, cells in surface view polyhedral, smooth, mainly 7–15 µm diam.; *bulbils internally* composed of strongly adherent, more or less roundish to ellipsoid or polyhedral cells, 7–15 µm diam., clamps not observed; large, orange-coloured oil drops are present in these cells and emerge from them when examined in lactophenol cotton blue; no crystals visible in polarized light.

Distribution and ecology. This species is known from Argentina and Bolivia. In the type collection (Bolivia), it develops over contiguous corticolous thalli of *Sticta weigelii*, *Heterodermia* sp., cf. *Pertusaria* sp. (sterile, without soralia or isidia), or directly on the supporting bark, where bulbils probably spread from the nearest infected lichen thalli.

Infected portions of *Sticta weigelii* are darker brown, partly with a superficial mycelium, but it is not known if these necrotic areas are caused by the fungus, or if bulbils preferably colonize dying parts of the host. On *Heterodermia* also, only brown dying parts of the host are covered by bulbils. The cf. *Pertusaria* thallus is not visibly damaged. No scars are left on the host thalli. In a second Bolivian locality, the species grows over *Hypotrachyna*. The Argentinian material is not associated with lichens: bulbils densely cover the rotten fallen foliar rachis and spathe of *Butia yatay* and also the ascomycete *Cannonia australis* developing on the spathe. In all these populations, bulbils are only loosely attached to the substrata, and they are easily removed using a dissecting needle. No visible interactions between bulbils and hosts could be detected. As the new species has repeatedly been observed overgrowing lichen thalli, but also ascomata of a pyrenomycete and rotten parts of plants, it may be considered as facultatively lichenicolous or fungicolous.

Observations. The new species is well characterized by the abundant, yellow to orange-yellow bulbils, 50–80 μm diam., covering the substratum. Phylogenetically, it is very close to the generic type *Burgella flavoparmeliae*, with which it shares most morphological and anatomical characters, including the orange oil drops present in the cells. *Burgella flavoparmeliae* differs in the slightly larger (60–110 μm diam.), honey-coloured bulbils that appear to be strictly lichenicolous over parmelioid lichens, on the thallus of which they leave distinct scar-like holes when removed. In the type collection of *B. lutea*, a second, unidentified bulbil-forming species is present, but is readily distinguished by the pale, almost translucent bulbils developing over the bark. Bulbils in three-year old herbarium specimens of the non-lichenicolous Argentinian material are more matt and light yellow than those of the holotype (Fig. 3E & F), but the original colour of fresh material was deep yellow to orange-yellow; otherwise they are almost identical, morphologically and anatomically. Unfortunately, no sequences could be obtained from those populations.

Additional specimens examined. **Argentina:** *Prov. Entre Ríos*: Dept. Colón, Parque Nacional El Palmar, Sendero Yatay, 31°53′24″S, 58°14′35″W, on spathe of *Butia yatay* and on *Cannonia australis*, 2009, *Capdet* & *Romero* 51690 (BAFC 51690; hb. Diederich); *ibid*., El Mollar, 31°51′45″S, 58°12′44″W, on foliar rachis of *Butia yatay*, 2009, *Capdet* & *Romero* 51689 (BAFC 51689; hb. Diederich).—**Bolivia:** *Dept. La Paz*: Prov. Nor Yungas, P. N. y A. N. M. I. Cotapata, between Tunkini and Chairo villages, above Tunkini, near Biological Station, 16°11′S, 67°52′W, 1300–1600 m, Yungas montane forest, on *Hypotrachyna*, 2011, *Etayo* 27717, *Flakus*, *Kuwka*, *Plata* & *Schiefelbein* (LPB, hb. Etayo).

Burgellopsis nivea Diederich & Lawrey gen. et sp. nov.

MycoBank Nos.: MB807653 (genus), MB807654 (species)

Characterized by superficial, white, roundish to irregular bulbils 100–220 μm diam., internally of indistinctly catenate, roundish to polyhedral cells, clamps absent.

Type: Great Britain, Scotland, VC 82, East Lothian, Lammermuir Hills, Lamb Burn, on small stone in scree, over sterile, sorediate, crustose lichen, 36(NT)604·630, alt. 350 m, 6 April 2006, *A. M.* & *B. J. Coppins* 21845 (E—holotype). Ex-type culture: ATCC MYA-4209.

(Figs 3G, 4H–J)

Basidiomata and *conidiomata* unknown. *Colonies* appearing as dispersed bulbils overgrowing lichen thalli. *Mycelium* not observed. *Bulbils* superficial, white, without hairs, surface at a high magnification (×50) rather rough, with individual convex cells slightly distinguishable, slightly shiny, roundish to shortly ellipsoid or rarely irregular in form, 100–220 μm diam.; *bulbils externally* without specialized cells, cells in surface view polyhedral, smooth, mainly 8–15 μm diam.; *bulbils internally* composed of indistinctly catenate, more or less roundish to ellipsoid or polyhedral cells, 8–15 μm diam., clamps not observed; no oil droplets emerging from cells when observed in lactophenol cotton blue; no crystals visible in polarized light. *Colonies on agar plates* showing white aerial hyphae; *bulbils* not observed.

Distribution and ecology. As the new species is known only from the type specimen from Scotland, little is known about its biology and its trophic stages. Most bulbils develop over the thallus of a sterile, sorediate saxicolous lichen, whilst some are found between thalline squamules. They are rather loosely

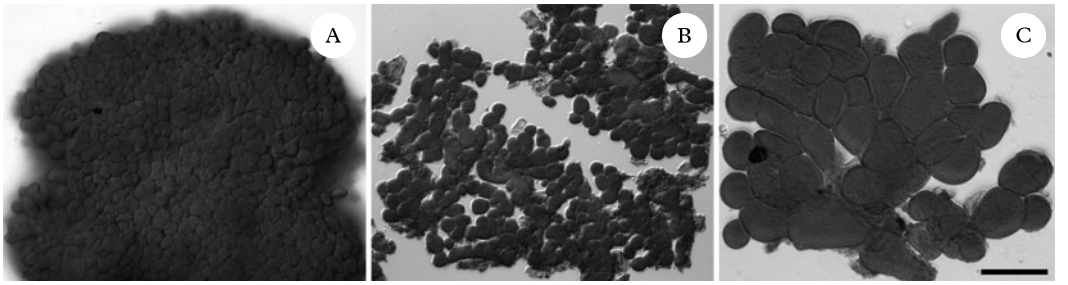

FIG. 5. *Ceratobasidium bulbillifaciens* (holotype); A, bulbil in a KOH-Congo Red-Phloxine mixture, showing subspherical outer cells; B, the same, after pressure on cover glass, showing catenate, ellipsoid cells; C, the same, at a higher magnification. Scales (the same for all photographs): A & B = 50 μm; C = 20 μm.

attached to the substratum and can easily be removed using a dissecting needle. No visible interactions between bulbils and hosts could be detected. Therefore, the species might be considered as facultatively lichenicolous, without causing any visible damage to the host.

Observations. In our phylogenetic analysis (Fig. 1), this species is sister to the clade containing *Burgella*, but with low support. It differs from the generic type *Burgella flavoparmeliae* and also from *B. lutea* by the absence of any yellowish or ochraceous pigments, the absence of yellow oil drops emerging from cells in microscopical preparations, and by the distinctly larger bulbils (60–110 μm in *B. flavoparmeliae* and 50–80 μm in *B. lutea*). As the new species is genetically rather distant from *Burgella* (long, unsupported branch in LSU analysis, Fig. 1), and shows clear morphological differences to the two *Burgella* species, it cannot be included in that genus. *Burgoa angulosa* Diederich *et al.*, a species also producing white bulbils, differs by the more irregular, often angulose bulbils, and anatomically by the distinctly catenate arrangement of cells and the presence of clamps. Two of the three known *Minimedusa* species differ morphologically by the conical outer cells of the bulbils. Both *Burgoa* and *Minimedusa* species are only distantly related to the new species in our phylogenetic analysis (Fig. 1). The new genus *Bulbilla* strongly differs morphologically from the new *Burgellopsis nivea* (bulbils larger, differently coloured, translucent); furthermore, the closest relatives of *B. nivea* in our analysis of LSU sequences (Fig. 1) are *Burgella* species, and the generic type *Burgella lutea* does not group with *Bulbilla applanata* in our analysis of ITS sequences (Fig. 2). Consequently, a new genus, *Burgellopsis*, has to be described to accommodate the new species *B. nivea*.

Ceratobasidium bulbillifaciens Diederich & Lawrey sp. nov.

MycoBank No.: MB807655

Characterized by superficial, greyish yellow to dark brown, matt, roundish bulbils 100–200 μm diam., internally of branched chains of subspherical to elongate cells, clamps absent.

Type: Germany, Hessen, Gießen, Parkplatz bei Sporthalle im Süden von Heuchelheim, alt. 155 m, on *Acer platanoides*, on unidentified, dying lichenized crust, also on young thalli of *Physcia tenella*, 6 September 2010, *R. Cezanne* & *M. Eichler* 8193 (BR—holotype; hb. Diederich—isotype). Ex-type culture: CBS 129339.

(Figs 3H, 5; figs 3d and 4c in Diederich & Lawrey 2007)

Basidiomata and *conidiomata* unknown. *Colonies* appearing as dispersed bulbils growing over or between corticolous lichen thalli. *Mycelium* not observed. *Bulbils* superficial, dispersed, roundish to shortly ellipsoid, greyish yellow when young (Kornerup & Wanscher 1984: 4B3–5B4), brown when mature (6D6–7E8), dark brown when old (7F6) (colour ±unchanged in old herbarium specimens), matt, not translucent, without hairs, 100–200 μm diam.; *bulbils externally* without specialized cells; *bulbils internally* composed of

branched chains of subspherical to elongate, hyaline cells, 7–23 × 6–10 µm, septa without clamps, crystals absent in polarized light. *Colonies on agar plates* showing white aerial hyphae; *bulbils* not observed; *hyphae* hyaline, septate, straight, rarely branched or anastomosed, 3·5–4·5 µm thick; *septa* without clamp connections.

Distribution and ecology. This species is widespread in Europe and is known from Belgium, France, Germany, Luxembourg, the Netherlands and Sweden. Bulbils develop over or between lichen thalli, frequently on unidentified, lichenized crusts, not visibly damaging the lichen thalli, usually in *Xanthorion* communities. Most specimens have been collected on the bark of various trees, but one, from the Netherlands, was growing over concrete. Owing to the inconspicuous colour of the small bulbils, the species is difficult to recognize in the field. Therefore, it is surely much more common than the specimens examined might suggest. In all these populations, bulbils are only loosely attached to the substratum, and they are easily removed using a dissecting needle. No visible interactions between bulbils and hosts could be detected. As the new species has repeatedly been observed overgrowing lichen thalli, it may be considered as facultatively lichenicolous.

Observations. Lawrey *et al*. (2007) included sequences of one specimen of this species in a phylogenetic analysis of bulbil-forming fungi and found it belongs to the subclade of the *Cantharellales* containing *Ceratobasidium* and *Thanatephorus* (i.e. the *Ceratobasidiaceae*). Diederich & Lawrey (2007) provided a description and illustrations of the species, without naming it. As more specimens became available in the meantime, and especially as sequences of two additional specimens could be obtained, we decided to formally describe the species here.

The species is characterized by small, brown, dispersed bulbils, less than 200 µm diam., internally composed of branched chains of subspherical to elongate, hyaline cells, and by the absence of clamps. Bulbils of *Bulbilla*, *Burgella*, *Burgellopsis*, *Burgoa* and *Minimedusa* species are internally composed of polyhedral, subspherical or elongate cells, rarely of branched chains of cells (*Burgoa moriformis*), and clamps are always present in these species. The species should not be confused with sclerotia of *Athelia arachnoidea*, which are typically much larger, over 500 µm diam., and often associated with a superficial white mycelium or thin, resupinate basidiomata.

Additional specimens examined (if not otherwise indicated, on unidentified, lichenized crusts). **Belgium:** Meise, National Plantentuin, on ×*Chitalpa tashkentensis*, xi 2011, *Van den Broeck* s. n. (BR).—**France:** *Pyrénées-Orientales*: Targassonne, Chaos de Targassonne, on *Populus*, on *Phaeophyscia orbicularis*, 1985, *Diederich* 6561 (hb. Diederich).—**Germany:** *Baden*: Oberrhein, Kleinkems, Gewann Kroatenschanze, on *Sambucus*, 1998, *Wirth* 32360 (STU-Wirth; specimen lost?; culture ATCC 208870). *Hessen*: Allmendfeld, Freifläche vor dem Bürgerhaus (TK 6217-1), on *Acer platanoides*, 2012, *Cezanne & Eichler* 8680 (hb. Diederich). *Bayern*: Spessart, Südrand von Röllbach (TK 6221-1), on *Fraxinus*, 2010, *Cezanne & Eichler* 8067 (hb. Cezanne-Eichler, hb. Diederich; culture CBS 132236).—**Luxembourg:** W of Schengen, Grouf, on *Populus*, over *Candelariella reflexa*, *C. xanthostigma* and *Phaeophyscia orbicularis* and on bark, 1987, *Diederich* 8442 (hb. Diederich); *ibid*., 1997, *Diederich* 13475 (hb. Diederich).—**The Netherlands:** *Friesland*: Schiermonnikoog, W side of the village, on *Ulmus*, 1996, *Aptroot* 40082 (M–6826). *Noord-Brabant*: SW of Greveschutven, fish-nursery, concrete of diver, 1997, *van den Boom* 19742 (hb. van den Boom); SW of Leende, S of Molenheide, on *Sambucus*, on *X. parietina*, 2006, *van den Boom* 37312 (hb. van den Boom).—**Sweden:** *Jämtland*: Brunflo par., Torvalla, on *Salix caprea*, 1948, *Santesson* 48.502 (d) (UPS).

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