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# *Basidioascus* and *Geminibasidium*: a new lineage of heat-resistant and xerotolerant basidiomycetes

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***Abstract:*** Using a heat-treatment method, two genera of heat-resistant and xerotolerant basidiomycetes were isolated from soil samples. These two genera, *Basidioascus* and *Geminibasidium* gen. nov., are morphologically similar and phylogenetically related. The genus *Basidioascus* originally was described as an ascomycete, but the structures originally interpreted as single-spored asci appear to represent basidiospores. Morphologically both genera are characterized by the lack of a fruiting body, conspicuously granular and deciduous basidia with a unique basal lateral projection and apparently double-walled basidiospores. The basidia, rather than the basidiospores, are forcibly discharged in *Basidioascus* species but not in *Geminibasidium* species. In *Geminibasidium* species a putative basidium arises from a primary cell. These are novel forms of basidia ontogenesis previously unseen in basidiomycetes. The rDNA (SSU + 5.8S + LSU) Bayesian phylogenetic analysis suggests that these fungi are distantly related to *Wallemia*, another xerotolerant basidiomycete genus commonly found in indoor air dust, dried foods and natural hypersaline environments. Given the physiological similarity and phylogenetic relationships, *Basidioascus* and *Geminibasidium* are classified in a new order, Geminibasidiales, and are taxonomically assigned to the class Wallemiomycetes. Based on morphological observations and molecular phylogeny of the internal transcribed spacer (ITS), two species of *Basidioascus* (*B. undulatus*, *B. magus* sp. nov.) and two species of *Geminibasidium* (*G. donsium* sp. nov., *G. hirsutum* sp. nov.) are described. A key to these species is provided using micromorphological and cultural characters.

***Key words:*** Basidiomycota, deciduous basidium, Geminibasidiaceae, Geminibasidiales, fungi in blueberry jam, soil fungi, Wallemiomycetes

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## Introduction

The genus *Basidioascus* was described first by Matsushima (2001) as an ascomycete. The only species, *B. undulatus,* based on one strain, was isolated from the tropical rainforest soil on CMA (cornmeal agar) in Cape Tribulation National Park, Queensland, Australia. Unfortunately the method of isolation was not mentioned. Matsushima (2003) interpreted the fertile structures as single-spored asci, and although he did not mention it in the text he likely interpreted the basal lateral projections as croziers. His strain of *B. undulatus* also produced a *Geotrichum*-like anamorph in culture. Given these observations, Matsushima concluded that the fungus was an ascomycete, a classification currently followed by the Dictionary of Fungi, MycoBank and Index Fungorum. Although the Latin description and microphotographs are precise enough to allow morphological identification of *B. undulatus*, DNA sequences and phylogenetic analysis were lacking.

During surveys for heat-resistant fungi in different kinds of soil, we isolated a variety of ascomycetes and basidiomycetes from samples collected from provinces in Canada and from Crater Lake National Park, USA. By definition, heat-resistant fungi can survive exposure to 75 C for 30 min (Samson et al. 2000). Among these fungi, we isolated *Basidioascus*-like strains, including several of an apparently undescribed genus related to *Basidioascus*, which we describe below as *Geminibasidium*.

In this study we characterized *Basidioascus* and *Geminibasidium* morphologically, physiologically and phylogenetically. We reinterpreted the structures of *B. undulatus* as those of a basidiomycete rather than as an ascomycete. Basidia and basidiospore ontogeny and colony morphology were detailed, and cardinal growth temperatures established. We also determined the xerotolerance of some strains with glycerol or sucrose-amended media and halotolerance using media supplemented with sodium chloride. Finally we sequenced the ribosomal operon (SSU, ITS and LSU) of *Basidioascus* and *Geminibasidium* and performed a phylogenetic analysis to determine their phylogenetic position in kingdom Fungi and in support of the morphological species concept.

## MATERIALS AND METHODS

*Culture isolation and preservation.*—Cultures were isolated following the method outlined in Seifert et al. (2004). Briefly, soil samples were suspended in a 0.1% (w/v) proteose peptone solution and exposed to 75 C for 30 min in a water bath. A 1 mL aliquot of the sample was mixed with molten chloramphenicol (40 mg/L) potato dextrose agar (PDA, Difco) and dispensed in 9 cm polystyrene Petri dishes. Petri dishes were sealed and incubated upright at room temperature (approximately 22 C) under ambient light. Cultures were deposited in the Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada (DAOM/CCFC) and at the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS) (TABLE I).

*Morphological studies.*—Observations were made from cultures on cornmeal agar (CMA, Acumedia) mounted in 85% lactic acid. Digital photographs were taken with an Evolution MP digital microscope camera on an Olympus BX50 compound microscope, with ImagePro 6.0 software (Media Cybernetics, Bethesda, Maryland). Measurements were carried out with ImagePro 6.0 with means and standard errors calculated in Microsoft Excel.

To assess colony morphology and sizes, strains were grown on 2% malt extract agar (MEA, Difco), dichloran 18% glycerol agar (DG18, Oxoid) (Samson et al. 2010) and malt yeast 40% sucrose agar (M40Y) (http://www.cbs.knaw.nl/service/food_media.aspx). The cultures were incubated upright at room temperature. Colony diameter was measured after 1 wk. Photographs of the colonies were taken after 2 wk with an iPhone 4S camera. To check for arthroconidial anamorphs, type strains (*B. undulatus* DAOM 241956, *B. magus* DAOM 241948, *G. donsium* DAOM 241966, *G. hirsutum* DAOM 241969) were grown in 2% malt broth at room temperature 1 wk on a rotary shaker, then a 20 µL aliquot of the liquid culture was observed microscopically. To test for forcibly discharged basidia or basidiospores, type strains (listed above) were inoculated on CMA in Petri dishes with glass slides taped to the lid. After 5 d incubation in the upright position, the slides were checked under the microscope for structures shot from the colony onto the lid. SEM photomicrograph was prepared by Susan Carbyn, Paula Allan-Wojtas and Milos Kalab with standard techniques.

*Physiological studies.*—To test xerotolerance and halotolerance, the type strains (listed above) were grown on malt yeast agar (MYA) as a basal medium (Wheeler et al. 1988), with increasing amounts of controlling solutes (NaCl, glycerol, sucrose) to achieve water activities of 1.00–0.77. Amounts of solutes were estimated from previous studies (P. Zalar pers comm, A. Patriarcia pers comm, see SUPPLEMENTARY TABLE III). MYA first was prepared without agar, and after addition of solutes to MYA the pH was adjusted to 7.0 with NaOH or HCl. Then agar was added and the media were sterilized 20 min. Strains were inoculated on the 6 cm agar polystyrene Petri dishes in three replicates for each treatment. Petri dishes were incubated upright at room temperature 28 d. Photographs were taken with an iPhone 4S camera every 7 d. Images were imported into the program ImageJ (Schneider et al. 2012) where the colony diameter was measured in proportion to the known diameter of the Petri dish. Growth diameters were averaged and data were compiled in Microsoft Excel.

To determine the cardinal temperatures, type strains (listed above) were inoculated on MYA and M40Y in triplicate. The strains were incubated at 5 C, 10 C, 15 C, 20 C, 25 C, 30 C, 35 C, 37 C and 40 C for 1 wk. Photographs were taken with an iPhone 4S camera. Images were imported into ImageJ (Schneider et al. 2012) for diameter measurement as described above.

*DNA extraction, PCR, sequencing.*—The UltraClean Microbial DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, California) was used for DNA extraction following the manufacturer's instructions. To amplify the ITS region, the primer combination ITS5 and ITS4 or ITS1 and ITS4 (White et al. 1990) were used. Primers ITS1, ITS4 and ITS5 were used as sequencing primers with primers ITS2 and ITS3 as internal sequencing primers if required. The SSU region was amplified with primers NS1 and ITS2. Primers NS1, NS2, NS3, NS4, NS5 and NS8 (White et al. 1990) were used as sequencing primers for the SSU region. The LSU region was amplified with primers LR0R and LR8, with LR0R, LR3R, LR6, LR7R, LR7 and LR8 used as sequencing primers (Vilgalys and Hester 1990).

To amplify DNA, a PCR master mix consisting of 0.1 mM dNTPs, 0.08 µM forward primer, 0.08 µM reverse primer, 1× Titanium Taq buffer (Clontech, Mountain View, California), 0.5× Titanium Taq enzyme (Clontech, Mountain View, California) and 1.00 µL DNA template mixed in sterile water totaling 10 µL per reaction was prepared. The following PCR profile was used for SSU, LSU and ITS amplifications: 95 C for 3 min (initial denaturation), then 35 cycles at 95 C for 1 min (denaturation), 56 C for 45 s (annealing), 72 C for 1.5 min (extension), followed by 72 C for 10 min (final extension). PCR products were checked by agarose gel electrophoresis and sequenced with Big Dye Terminator (Applied Biosystems, Foster City, California).

*Phylogenetic analysis.*—Sequences were assembled and edited with SeqMan II 8.0 (DNA Star Inc., Madison, Wyoming). BLAST analyses were performed with SSU, LSU and ITS sequences to verify their homology with other fungal SSU, LSU and ITS sequences. All sequences were deposited in GenBank (TABLE I). Sequences of each gene were aligned with MAFFT (Katoh et al. 2005). These alignments were trimmed with BioEdit (Hall 1999). Alignments were deposited in TreeBASE (www.treebase.org/treebase/), study accession number S13396.

All molecular phylogenies were determined by Bayesian inference. To select the most appropriate model of sequence evolution, MrModeltest 2.2.6 (Nylander 2004) was applied on each gene (ITS, SSU, LSU, 5.8S) and the model was chosen according to the Akaike information criterion (AIC). The GTR + I + G model was selected for SSU and LSU, SYM + I + G was chosen for 5.8S and GTR + G model was adopted for ITS. Before Bayesian analysis, the SSU, 5.8S and LSU matrices were concatenated with SeaView (Gouy et al. 2010). Bayesian inference was

performed with MrBayes 3.2 (Ronquist and Huelsenbeck 2003). Two independent Markov chain Monte Carlo (MCMC) runs were performed simultaneously. Each MCMC ran for $5.0 \times 10^6$ generations for the rDNA analysis and $2.0 \times 10^6$ generations for the ITS analysis, sampling every 500 generations. The first 25% of trees were discarded as burn-in, the remaining trees (15 002 for rDNA and 6002 for ITS) were kept and combined into one tree with 50% majority rule consensus. Consensus trees in Newick format were imported into TreeView X (Page 1996) and exported as SVG vector graphics for figure assembly in Adobe Illustrator CS5 and Adobe Photoshop CS5.

## RESULTS

*Isolation.*—*Basidioascus* strains were isolated from soils collected at Crater Lake National Park, Oregon, USA, and from soils collected from Ontario, British Columbia, Alberta, Nova Scotia and Manitoba in Canada. We isolated nine strains of *B. undulatus*, nine strains of *B. magus* and three strains of *Basidioascus* sp. that we are not naming. *Geminibasidium* species were isolated from soils collected in British Columbia and Nova Scotia, Canada. We isolated three strains of *G. donsium* and two strains of *G. hirsutum*. These fungi were found in soil from forests, grasslands and blueberry fields, many of which had been exposed to deliberate or accidental burning (TABLE I).

*Colony size and morphology.*—*Basidioascus undulatus* grew faster than *B. magus*, and *G. hirsutum* grew faster than *G. donsium*. Generally colonies of *Basidioascus* species are white and those of *Geminibasidium* species are pink. On MEA, *B. undulatus* colonies are uniform whereas those of *B. magus* appear more stellate. The convex rosette growth of *B. undulatus* differentiates it from the flat, sulcate pattern of *B. magus* on DG18. It was difficult to differentiate *B. undulatus* and *B. magus* on M40Y because both have a flat-white appearance. On MEA, *G. donsium* colonies are pink and *G. hirsutum* colonies are beige. On M40Y and DG18 it was difficult to distinguish *G. donsium* and *G. hirsutum* because both produce pink colonies. There are many slight variations in colony appearance among our strains.

*Development of basidia and basidiospores.*—In *Basidioascus* species a small probasidium develops laterally or terminally on somatic hyphae and matures to a larger clavate basidium. Basidia are deciduous and forcibly discharge at maturity. These basidia produce symmetrical basidiospores attached by tubular sterigmata resembling those of some gasteromycetes basidia and basidiospores. The basidiospores of *B. undulatus* and *B. magus* are subglobose, brown and have a thick, wavy wall. Generally *B. undulatus* produces larger basidia and basidiospores compared to *B. magus*.

In *Geminibasidium* species a basidium-bearing cell (primary cell) develops on terminal and lateral branches of somatic hyphae. The primary cell is attached to hyphae by a thin tubular connector (FIGS. 3P, 5M). Over time a basidium develops on the apical portion of the primary cell. The globose and brown basidiospores in *G. donsium* are slightly verrucose, whereas they are hirsute in *G. hirsutum*. We saw no evidence that either the basidia or basidiospores of *Geminibasidium* species were forcibly discharged.

Both *Basidioascus* and *Geminibasidium* species have conspicuously granular basidia with a basal lateral projection. The basal lateral projection refers to a small protrusion that is found near the base of the basidium in *Basidioascus* species and near the base of the basidium-bearing cell (primary cell) in *Geminibasidium* species. These basal lateral projections sometimes resemble clamp connections (FIGS. 1B, 2D), but they are not attached to the subtending cell; they collapse as basidia mature in *Basidioascus* species but not in *Geminibasidium* species.

*Arthroconidia.*—Only *B. undulatus* produces an arthroconidial, *Geotrichum*-like anamorph. Arthroconidia are produced both in liquid culture and on agar. In liquid culture arthroconidia are predominant perhaps are analogous to a yeast form of *B. undulatus*. When *B. undulatus* arthroconidia were inoculated on MEA and incubated at room temperature, the fungus reverted to hyphal growth. Type strains of *B. magus*, *G. donsium* and *G. hirsutum* did not produce arthroconidia in liquid cultures and on agar.

*Phylogenetic analysis.*—To determine the relationships of *Basidioascus* and *Geminibasidium* in relation to other basidiomycetes, a Bayesian phylogenetic analysis with SSU, 5.8S and LSU genes combined was performed with selected sequences from the AFTOL (Assembling the Fungal Tree of Life) project and other sequences from GenBank (FIG. 6). GenBank accession numbers are provided (SUPPLEMENTARY TABLE I). The results show that *Basidioascus* and *Geminibasidium* are related sister groups with a posterior probability of 0.93. They reside at the base of Agaricomycotina and are related to *Wallemia* on a long branch supported by a posterior probability of 1.00.

A Bayesian phylogenetic analysis of the ITS was performed to delimit species in support of our morphological observations (FIG. 7). Full ITS sequences from *Basidioascus* and *Geminibasidium* are roughly 600 bp long. BLAST queries with our ITS

TABLE 1. Strains, source and sequences

| Taxon | Culture | | GenBank accession no. | | | Country | State or Province | Region | Substrate and plants |
|---|---|---|---|---|---|---|---|---|---|
| | DAOM[a] | CBS[b] | ITS | LSU | SSU | | | | |
| *Basidioascus undulatus* | 241956[c] | 133763 | JX242863 | JX242883 | JX242889 | Canada | MB | Between Thompson and Nelson House | Soil from the site of a forest fire; jack pine and trembling aspen with understory of black spruce |
| | 241958 | 133764 | JX242859 | — | — | Canada | BC | Quamichan Lake | Soil from a stand of black cottonwood (*Populus trichocarpa*) |
| | 241959 | 133765 | JX242875 | — | — | Canada | BC | Shesta Lake, Prince George | Soil from a stand of spruce |
| | 241961 | 133766 | JX242862 | JX242884 | JX242890 | Canada | BC | Helliwell Park, Hornby Island | Soil from a stand of old-growth Douglas fir |
| | 241962 | 133767 | JX242864 | — | — | Canada | AB | AAFC Experimental Farm, Fort Vermillion | Soil from a field of barley stubble |
| | 241963 | 133768 | JX242865 | — | — | Canada | BC | Rolla Research Farm, Dawson Creek | Soil from a fallow field |
| | 241964 | 133769 | JX242866 | — | — | Canada | AB | AAFC Research Farm, Beaverlodge | Soil from a mature pine/spruce stand |
| | 242122 | 133770 | JX242876 | — | — | Canada | AB | Stavely | Soil from rough fescue grasslands |
| | 229809 | 133771 | JX242882 | — | — | Canada | ON | Matawatchan Township, Renfrew County, Cooper Hill Road | Soil from mixed woods |
| *Basidioascus magus* | 241948[d] | 133772 | JX242869 | JX242885 | JX242891 | USA | OR | Wizard Island, Crater Lake National Park | Forest soil near *Goodyera oblongifolia* |
| | 241949 | 133773 | JX242868 | — | — | USA | OR | Wizard Island, Crater Lake National Park | Forest soil near *Vaccinium* |
| | 241950 | 133774 | JX242870 | — | — | USA | OR | Wizard Island, Crater Lake National Park | Forest soil near *Goodyera oblongifolia* |
| | 241951 | 133775 | JX242871 | — | — | USA | OR | Wizard Island, Crater Lake National Park | Forest soil near *Ceanothus velatinus* |
| | 241952 | 133776 | JX242872 | — | — | USA | OR | Wizard Island, Crater Lake National Park | Forest soil near *Vaccinium* |
| | 241953 | 133777 | JX242873 | — | — | USA | OR | Wizard Island, Crater Lake National Park | Forest soil near *Ceanothus velatinus* |
| | 242123 | 133778 | JX242874 | — | — | USA | OR | Wizard Island, Crater Lake National Park | Forest soil near *Ceanothus velatinus* |
| | 241954 | 133779 | JX242857 | — | — | Canada | ON | Madawaska Highlands, Morrow Cr. Trail, Centennial Lake | Soil from a stand of red and white pine |
| | 241955 | 133780 | JX242861 | — | — | Canada | BC | Helliwell Park, Hornby Island | Soil from a stand of *Arbutus menziesii* with some Douglas fir |

TABLE I. Continued

| Taxon | Culture | | GenBank accession no. | | | Country | State or Province | Region | Substrate and plants |
|---|---|---|---|---|---|---|---|---|---|
| | DAOM[a] | CBS[b] | ITS | LSU | SSU | | | | |
| *Basidioascus* sp. | 241957 | 133781 | JX242858 | — | — | Canada | ON | Big Island, Centennial Lake | Soil from a stand of oak interspersed with white pine |
| | 241960 | 133782 | JX242860 | — | — | Canada | BC | Rocky Point near Metchosin, west of Victoria, Vancouver Island | Soil from an old-growth Garry oak and Douglas fir forest |
| | 241965 | 133783 | JX242867 | — | — | Canada | MB | Between Thompson and Nelson House | Soil from the site of a forest fire in July 2003. Sampled underneath burnt moss and areas where organic material was completely burnt off |
| *Geminibasidium donsium* | 241966[d] | 133784 | JX242877 | JX242886 | JX242892 | Canada | NS | Sable River | Soil from a commercial field of lowbush blueberries |
| | 241967 | 133785 | JX242878 | JX242887 | JX242893 | Canada | NS | 5 km from Grafton Lake, Kejimkujik National Park | Soil from a stand of white pine (*Pinus strobus*) |
| | 242124 | 133786 | JX242881 | — | — | Canada | NS | Colquist Road, Shelburne County, Nova Scotia | Soil from a stand of jack pine (*Pinus banksiana*) |
| *Geminibasidium hirsutum* | 241969[d] | 133787 | JX242880 | JX242888 | JX242894 | Canada | BC | Shingle Bolt Trail, Capilano Gorge, base of Grouse Mountain, North Vancouver | Soil from coniferous forest of mainly Douglas fir with understory of *Vaccinium parvifolium* |
| | 241968 | 133788 | JX242879 | — | — | Canada | BC | Shingle Bolt Trail, Capilano Gorge, base of Grouse Mountain, North Vancouver | Soil from coniferous forest of mainly Douglas fir with understory of *Vaccinium parvifolium* |

[a] DAOM = Canadian Collection of Fungal Cultures in Ottawa, Canada. All type strains were also deposited as dried cultures.
[b] CBS = Centraalbureau voor Schimmelcultures in Utrecht, the Netherlands.
[c] Epitype.
[d] Holotype.

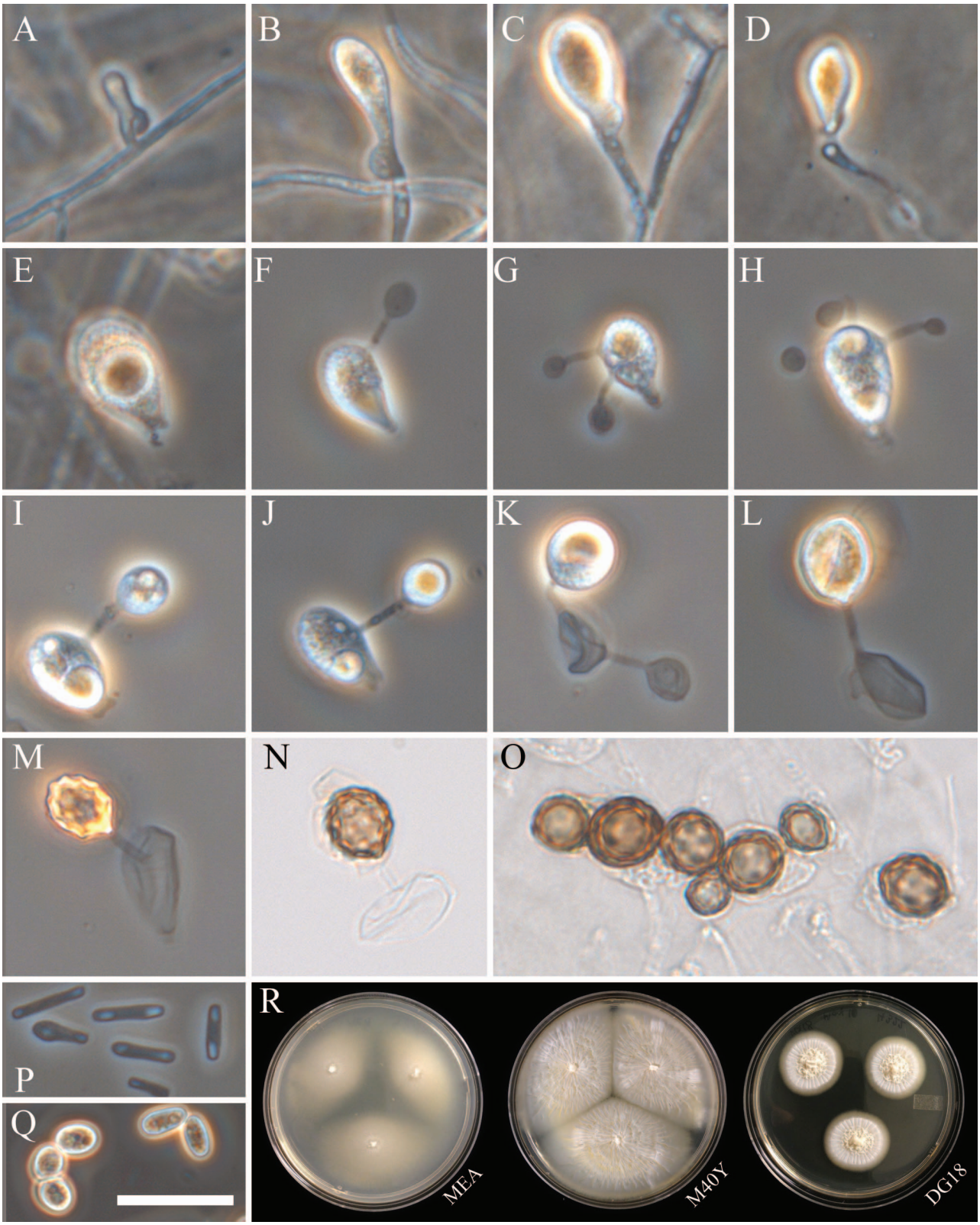


FIG. 1. *Basidioascus undulatus* DAOM 241956 ontogenesis and colonies. A–C. Granular basidia with a basal lateral projection develop on hyphae and over time mature into a clavate basidium. D–E. The basidia then become deciduous and are detached from hyphae. F–J. One or more than one basidiospore, attached by sterigmata, develop on the basidia. K. More than one immature basidiospore can develop on a basidium, but only one basidiospore matures and the other basidiospores are

sequences detected several unidentified but related soil environmental sequences, and we included these in our ITS analysis. Further information on these environmental sequences is provided (SUPPLEMENTARY TABLE II). Representatives from Cystofilobasidiales and Wallemiales were chosen to root the tree because they are the most closely related known groups according to our rDNA phylogenetic analysis. The ITS phylogeny shows that both *Basidioascus* and *Geminibasidium* are monophyletic. The posterior probability at the node grouping strains of *B. magus* is 1.00, *B. undulatus* is 0.98, *G. donsium* is 1.00 and *G. hirsutum* is 0.99. The ITS tree shows a polytomous phylogenetic delimitation between *B. undulatus* and *B. magus* and a dichotomous phylogenetic delimitation between *G. donsium* and *G. hirsutum*.

The strains identified as *Basidioascus* sp. (DAOM 241960, DAOM 241965, DAOM 241957) did not group with *B. undulatus* and *B. magus* type strains phylogenetically. Two environmental sequences from GenBank (sequences FN397319, EU626069) form a strongly supported clade of 1.00. The environmental sequences that are phylogenetically near *Geminibasidium* make up at least four phylogenetic clades composed of various GenBank sequences from Italy, USA, Canada, Japan and Guyana. The sequence named Kikoku was from a fungal contaminant isolated in Japan from blueberry jam (Y. Kikoku pers comm) and groups with *G. donsium*.

*Physiology.*—*Basidioascus* and *Geminibasidium* species are able to grow on low water activity media such as DG18 and M40Y. As such, they are xerotolerant like *Wallemia* species (their closest known relative). To test the xerotolerance of *Basidioascus* and *Geminibasidium* species, neutral pH MYA was mixed with varying amounts of glycerol or sucrose (FIG. 8). The fastest colony growth occurred within the first week. Over the range of water activities tested, *Basidioascus* and *Geminibasidium* species grew faster in $a_w = 1.00$ in the first week. However, from the second to the fourth week, they grew better under lower water activities adjusted by glycerol and sucrose. For example, the peak growth diameter for *B. undulatus*, *B. magus*, *G. donsium* and *G. hirsutum* in a glycerol gradient occurred at $a_w = 0.98$ after 4 wk. The peak growth in the sucrose gradient occurred at $a_w = 0.95$ for *B. undulatus* and *B. magus*, $a_w = 0.90$ for *G. donsium* and $a_w = 0.98$ for *G. hirsutum*.

To test the halotolerance of *Basidioascus* and *Geminibasidium* species, neutral pH MYA was mixed with varying amounts of NaCl (FIG. 8). *Basidioascus undulatus* can tolerate the most salt (up to 2.3 M or 13.5% [w/v]), whereas *B. magus* could not tolerate any salt. Both *G. donsium* and *G. hirsutum* tolerate a low amount of salt (up to 0.6 M or 3.5% [w/v]).

To test the cardinal temperatures, type cultures of *Basidioascus* and *Geminibasidium* species were grown on MYA and M40Y (FIG. 9). Generally *Basidioascus* and *Geminibasidium* are mesophilic. The optimum growth temperature on MYA medium for *B. undulatus* is 25–30 C; *B. magus* is 25 C; *G. donsium* and *G. hirsutum* is 30 C. The optimum under M40Y for *B. undulatus*, *B. magus* and *G. donsium* is 30 C, while it is 37 C in *G. hirsutum*. Of interest, *B. undulatus*, *G. donsium* and *G. hirsutum* all are able to grow at 37 C to some extent on MYA and M40Y.

## TAXONOMY

**Wallemiomycetes** P. Zalar, G.S. de Hoog and H.-J. Schroers, emend H.D.T. Nguyen, N.L. Nickerson and Seifert

MycoBank MB501496

*Description:* Class of xerotolerant and halotolerant basidiomycetes. Basidiomata are absent; basidiospores are produced by some genera. Arthroconidial or basauxic anamorphs are produced in some species. This class is the earliest diverging lineage of Agaricomycotina based on analyses of combined SSU, 5.8S and LSU ribosomal RNA, as well as amino acid analysis of 71 protein-coding genes.

*Type order:* Wallemiales**.**

*Notes:* This description was emended by the addition of information on teleomorphs and more precise information on the phylogenetic position of Wallemiomycetes based on genome sequences.

**Geminibasidiales** H.D.T. Nguyen, N.L. Nickerson and Seifert, ord. nov.

MycoBank MB801330

*Description:* Basidiomata are absent. Basidia are produced singly or in clusters, perhaps representing incipient or vestigial fruiting bodies, arise singly from somatic hyphae or from swollen basidium bearing cells (primary cells), and have granular cellular contents. A basal lateral projection occurs either on the basidium or the swollen primary cell supporting

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aborted. K–N. Basidia lose their cytoplasm and sometimes collapse as basidiospores mature. O. Clusters of basidiospores often are seen in 1 mo old cultures. P. Slender arthroconidia occur in cultures on agar. Q. Oval arthroconidia are prevalent in liquid culture. R. Colonies of *B. undulatus* on MEA, M40Y and DG18 after 2 wk incubation at room temperature. Bars: A–Q = 20 μm.

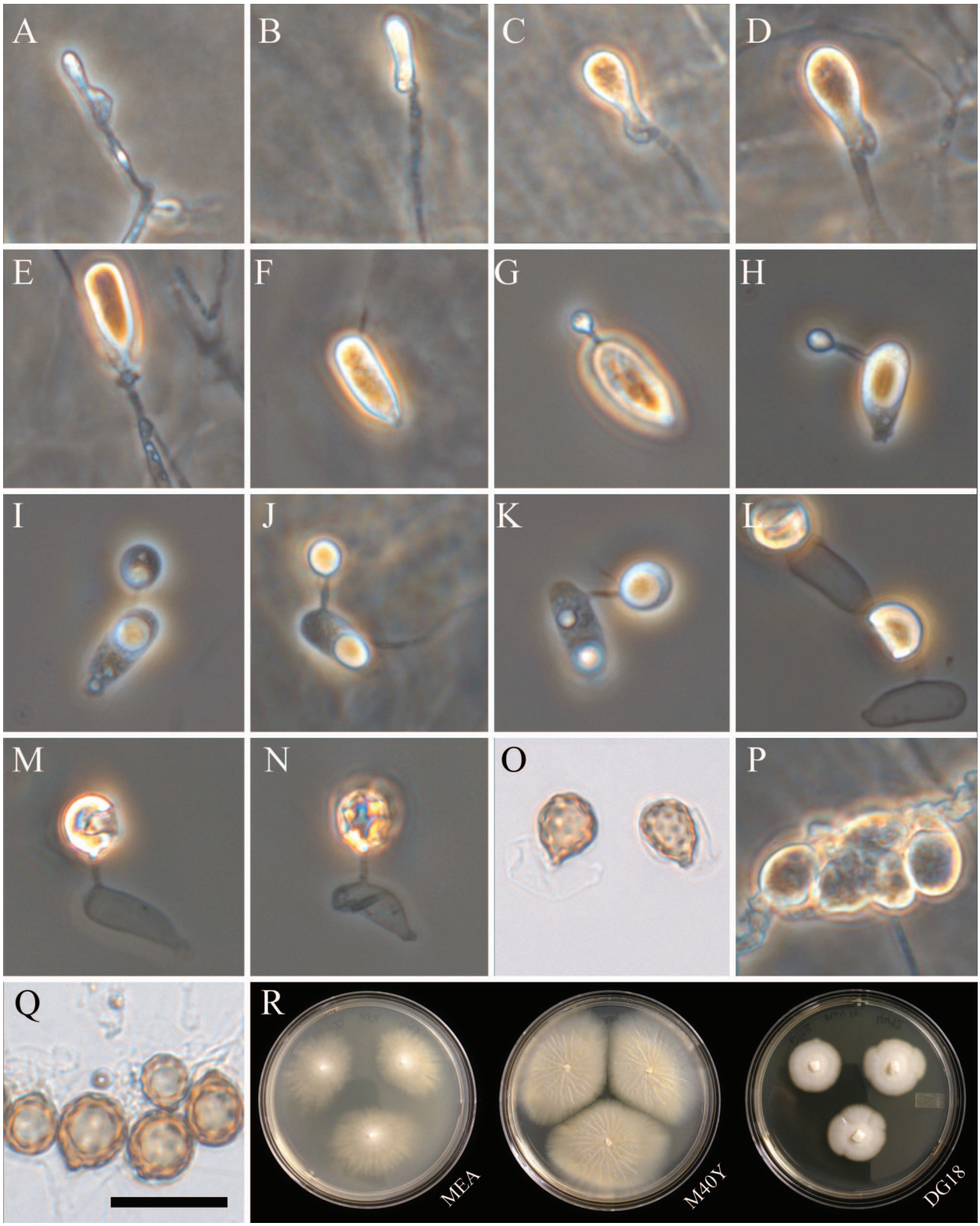


FIG. 2. *Basidioascus magus* DAOM 241948 ontogenesis and colonies. A–E. Granular basidia with basal lateral projection develop on hyphae and over time mature into obovate or cylindrical basidia. E–G. The basidia then become deciduous and are detached from hyphae. G–O. A single basidiospore arises from the basidium on a sterigma. K–O. Basidia lose their cytoplasm and sometimes collapse as the developing basidiospore matures. P–Q. A cluster of basidia gives rise to a cluster of basidiospores over time. R. Colonies of *B. magus* on MEA, M40Y and DG18 after 2 wk incubation at room temperature. Bars: A–Q = 20 μm.

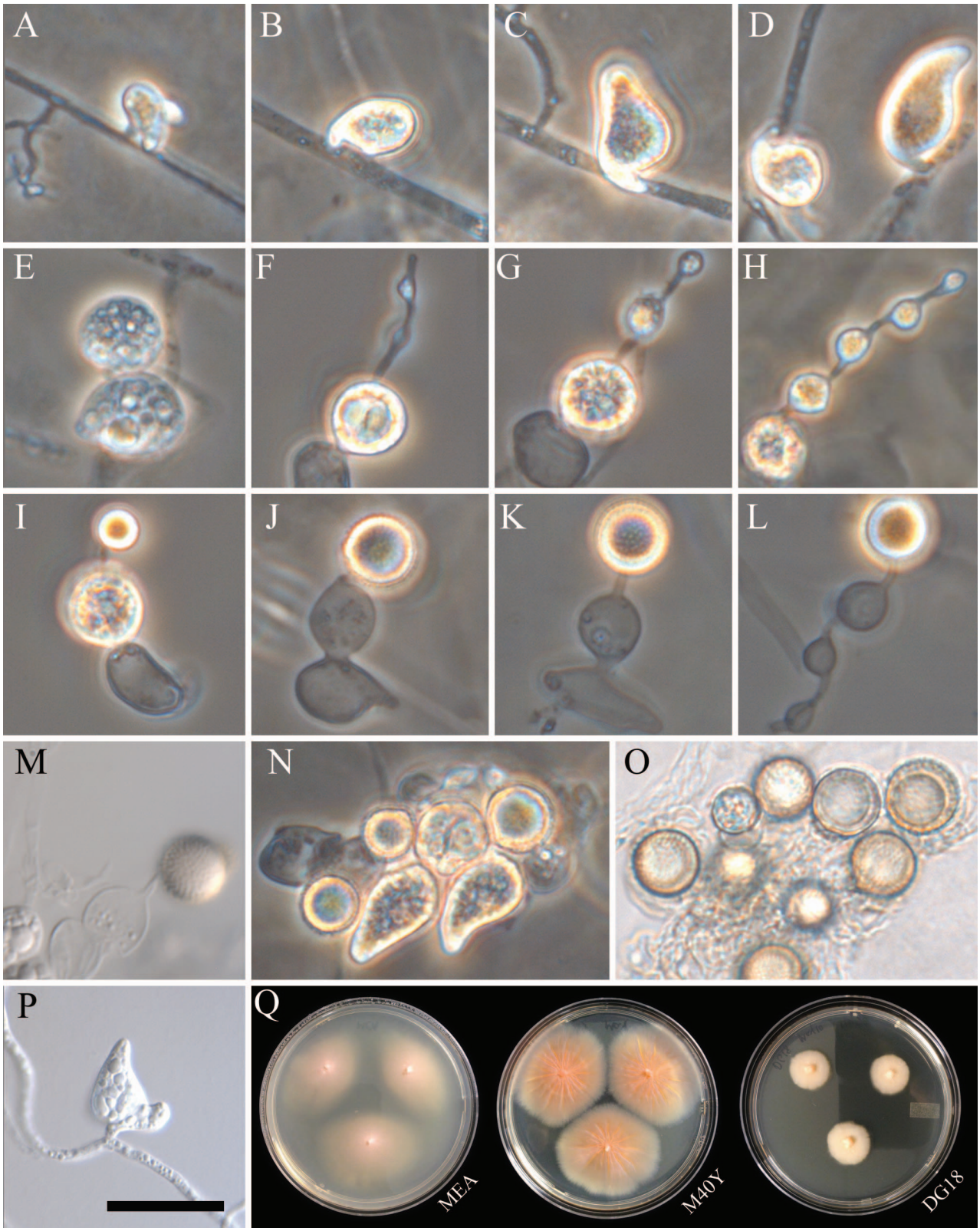


FIG. 3. *Geminibasidium donsium* DAOM 241966 ontogenesis and colonies. A–D. Granular basidium bearing cells (primary cells), with a basal lateral projection, develop on hyphae and over time become swollen and irregular. E, F. Basidia arise from the apex of the primary cells. G–L. Basidia produce a chain of swollen cells with sterigma-like connectors with the terminal swollen cell becoming a mature basidiospore. M. Mature basidiospores are brown and have slightly verrucose walls. N–O. Basidiospores in clusters can be found in 1 mo old cultures. P. The basidium is attached to hyphae by a thin connector. Q. Colonies of *G. donsium* on MEA, M40Y and DG18 after 2 wk incubation at room temperature. Bars: A–P = 20 µm.

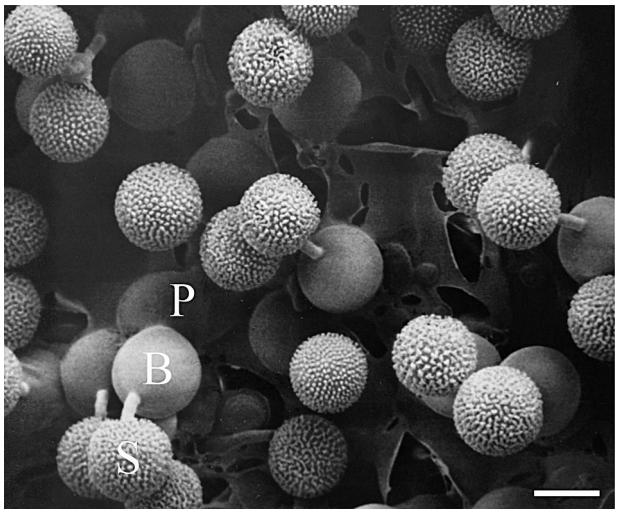


FIG. 4. *Geminibasidium donsium* DAOM 241966. S = basidiospore. B = basidium. P = primary cell. Bar = 10 μm.

the basidium. The basidia, or the basidia attached to the swollen primary cell, are deciduous or forcibly discharged before basidiospores begin to develop. One sterigma, or sometimes more than one sterigma, arises randomly over the apical two-thirds of the surface of the detached basidium. Only one mature basidiospore develops per basidium. Basidiospores are symmetrical on sterigma, not forcibly discharged, initially hyaline, dark brown when mature, with an apparent double wall. Basidia lose their cytoplasm and sometimes collapse as basidiospores mature. Arthroconidial anamorphs are sometimes produced. Species of the Geminibasidiales are heat-resistant and xerotolerant. Analyses of combined SSU, 5.8S and LSU ribosomal RNA suggest a sister relationship to the Wallemiales and a basal phylogenetic position in the Agaricomycotina.

*Type family:* Geminibasidiaceae.

**Geminibasidiaceae** H.D.T. Nguyen, N.L. Nickerson and Seifert, fam. nov.
MycoBank MB801331

*Description:* Conforming to the diagnosis of the order Geminibasidiales given above.

*Type genus: Geminibasidium.*

**Basidioascus** Matsush., Matsushima Mycological Memoirs 10:98 (2003)[2001], emend H.D.T. Nguyen, N.L. Nickerson and Seifert
MycoBank MB28558

*Description:* Basidia are produced singly or in clusters, arise singly from somatic hyphae, and have granular cellular contents and a basal lateral projection. The basidia are forcibly discharged before basidiospores begin to develop. One sterigma, and in one species more than one sterigma, arise randomly over the apical two-thirds of the surface of the detached basidium. Only one mature basidiospore develops per basidium. Basidiospores are symmetrical on sterigma, not forcibly discharged, initially hyaline, dark brown when mature with an apparent double wall. Basidia lose their cytoplasm and sometimes collapse as basidiospores mature. Arthroconidial anamorphs are produced in one species. Species of *Basidioascus* are heat resistant and xerotolerant. Analyses of SSU, 5.8S and LSU ribosomal RNA suggest a sister relationship to *Geminibasidium.*

*Etymology: Basidioascus* = basidia behaving like asci.

*Type species: Basidioascus undulatus.*

*Notes:* The genus *Basidioascus* first was described by Matsushima (2001) as an ascomycete. However, it was ineffectively published on CD-ROM only. To legitimize this name, the information on the CD-ROM was published in hard copy in 2003 and distributed to the biosystematics libraries of Agriculture and Agri-Food Canada, Ottawa, and CABI Bioscience, Egham, UK (S. Redhead pers comm). This description was emended by the morphological reinterpretation of *Basidioascus* as a basidiomycete rather than an ascomycete, information on physiological characters and the addition of phylogenetic data on its relationship with *Geminibasidium.*

**Basidioascus undulatus** Matsush., Matsushima Mycol Mem 10:98 (2003)[2001], FIG. 1
MycoBank MB374219

*Etymology: undulatus* = basidiospores with wavy wall.

*Description:* Colonies grow to a diameter of 2.6–3.4 cm (mean ± SE = 3.0 ± 0.1 cm) on MEA, 2.3–3.3 cm (mean ± SE = 2.9 ± 0.1 cm) on M40Y and 1.2–1.7 cm (mean ± SE = 1.4 ± 0.1 cm) on DG18 after 1 wk at room temperature. The full life cycle can be observed on CMA. The surface of the colony on CMA is glandular because basidia form basidiospores on the surface of the agar. After 2 wk on MEA colonies are planar, uniform, white-beige with diffuse margins; on M40Y colonies are flat and sulcate/velvet, white with diffuse margins; on DG18 colonies are convex and form a rosette pattern in the center, white, with diffuse margins.

Probasidia are at first clavate on terminal and lateral branches of hyphae. Over time, probasidia expand and mature into obovate basidia. Mature basidia are 12.5–17.5 × 8.0–11.0 μm (mean ± SE = 15.1 ± 0.6 × 9.6 ± 0.3 μm), deciduous, forcibly discharged, with cell contents conspicuously granular, often with a basal lateral projection that eventually collapses. Mature basidia are visible as early as 3–4 d.

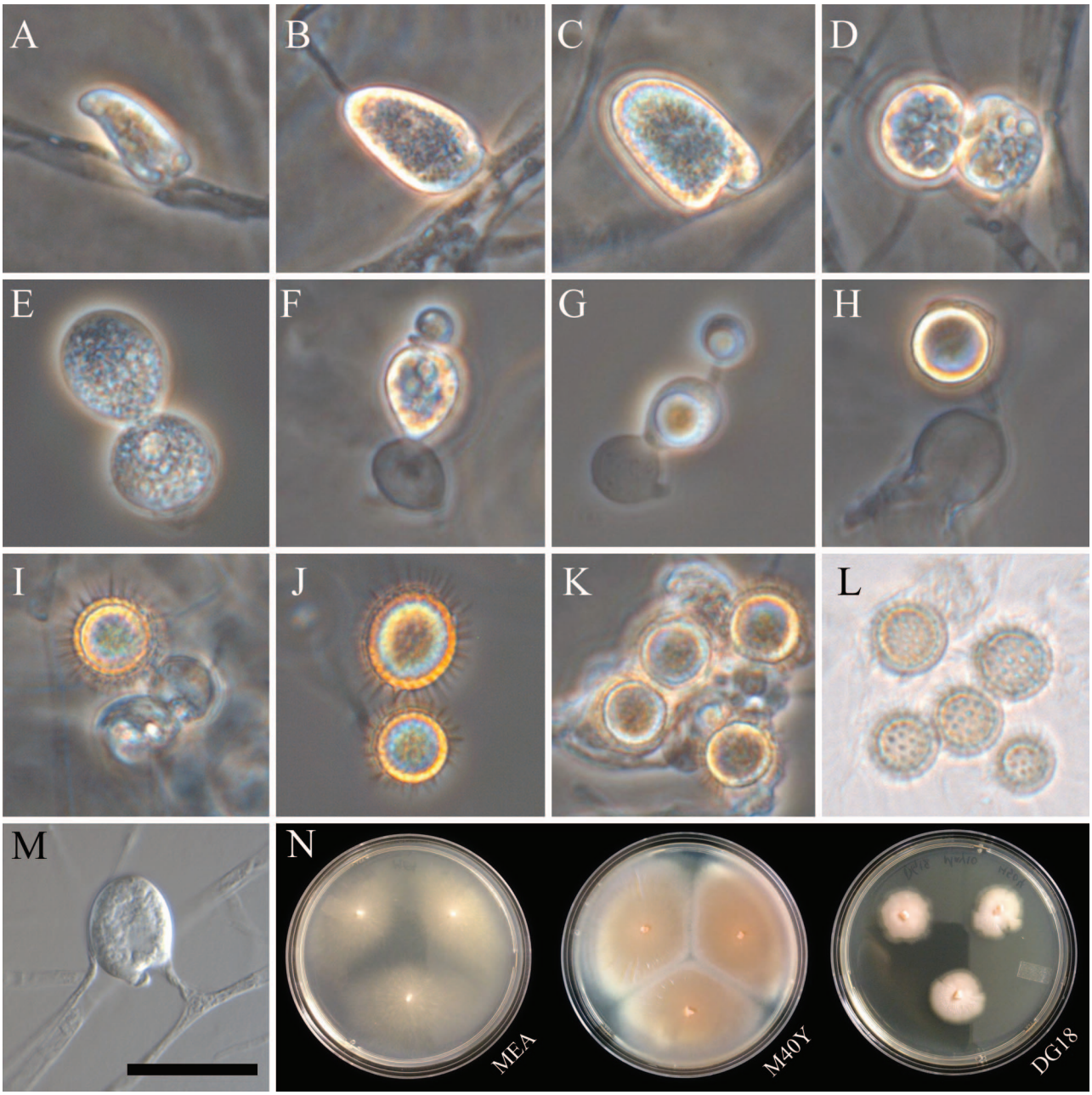


FIG. 5. *Geminibasidium hirsutum* DAOM 241969 ontogenesis and colonies. A–C. Granular basidium bearing cell (primary cells), with a basal lateral projection, develop on hyphae and over time become swollen and irregular. D–I. Basidia arise from the apex of primary cells and produce a basidiospore on a sterigma. H–J. Basidiospores are at first smooth-walled and become hirsute over time. K–L. Basidiospores in clusters can be found in 1 mo old cultures. M. The basidium is attached to hyphae by thin connector. N. Colonies of *G. donsium* on MEA, M40Y and DG18 after 2 wk incubation at room temperature. Bars: A–M = 20 μm.

More than one basidiospore can develop on the basidia, but only one basidiospore develops to maturity and the other basidiospores are aborted. Basidia lose their cytoplasm and sometimes collapse as basidiospores mature. Basidiospores are subglobose, hyaline brown to brown, have thick, wavy, verrucose walls, 9.5–12.5 × 8.0–10.0 μm (mean ± SE = 11.1 ± 0.3 × 8.7 ± 0.2 μm). Basidiospores can be observed after 7 d. In one month old cultures, clusters of basidiospores encased in a gelatinous matrix (presumed to be remnants of basidia) can be observed.

On 1 mo old cultures on agar, arthroconidia, 6.0–12.0 × 1.5–3.0 μm (mean ± SE = 8.0 ± 0.4 × 2.1 ± 0.1 μm) usually attached in chains, are produced

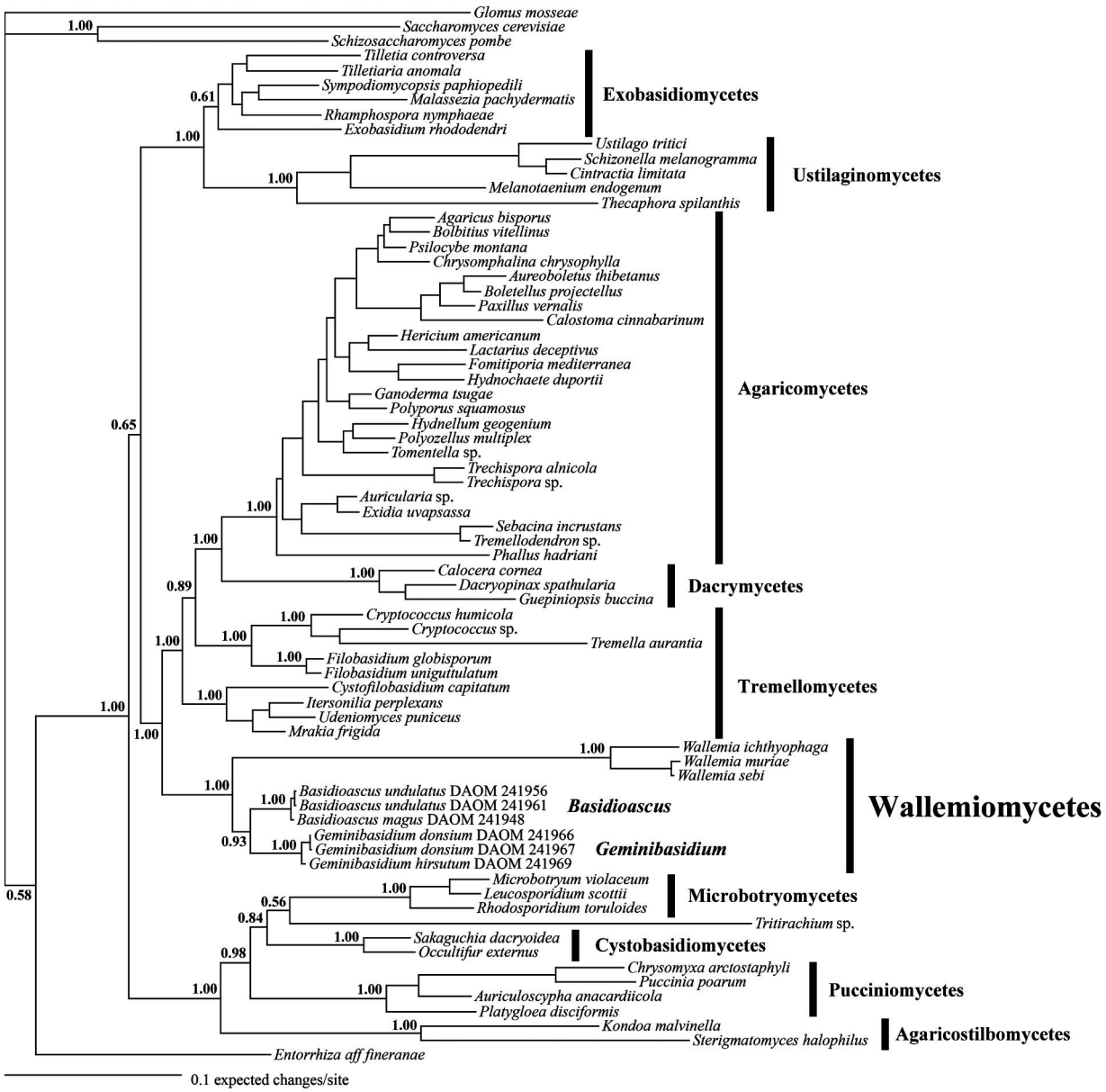


FIG. 6. Ribosomal phylogeny of *Basidioascus* and *Geminibasidium*. The relationship of *Basidioascus* and *Geminibasidium* to the other basidiomycetes is shown in a Bayesian phylogenetic partition analysis with the combined SSU, 5.8S and LSU genes. Posterior probabilities are displayed at nodes of the tree.

above the surface of the agar. In shaking malt extract broth culture, oval arthroconidia, 4.5–8.0 × 3.0–7.0 μm (mean ± SE = 6.8 ± 0.2 × 4.4 ± 0.2 μm) are produced in chains.

*Epitype* (designated here)*: B. undulatus* DAOM 241956; dried culture on CMA (Oct 2012) of an isolate from soil, Manitoba, Canada; living culture deposited at Canadian Collection of Fungal Cultures in Ottawa, Canada, as DAOM 241956.

*Strains examined:* DAOM 241956 (epitype), 241958, 241959, 241961, 241962, 241963, 241964, 242122, 229809 (TABLE I).

*Holotype: B. undulatus* MFC-21004; isolated from soil at Cape Tribulation National Park, Australia (Matsushima 2003).

*Notes:* The production of arthroconidia, presence of abortive basidiospores and ability to grow in MYA with sodium chloride concentrations up to 2.3 M or 13.5% (w/v) are diagnostic characteristics of *B. undulatus*. Despite repeated attempts to confirm the existence of a holotype for this species, we received no response from T. Matsushima but are aware that much of his collection was destroyed in the great Hanshin earthquake of 1995, albeit before the

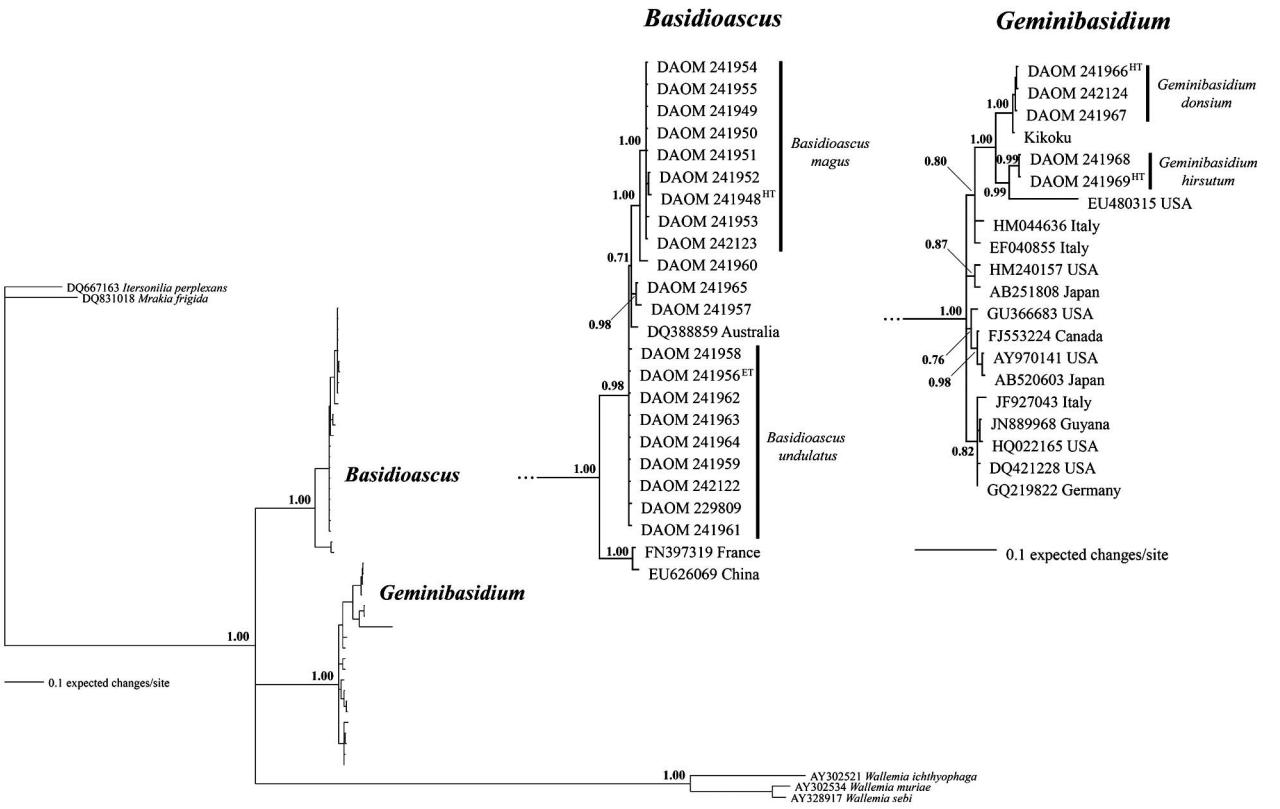


FIG. 7. Phylogenetic species delimitation of *Basidioascus* and *Geminibasidium* species using ITS. A Bayesian phylogenetic analysis was performed and posterior probabilities are displayed at nodes of the tree. Environmental ITS sequences from GenBank also were included. Sequences marked with HT denote the holotype strain, whereas ET indicates the epitype strain. The sequence named Kikoku came from a fungus isolated in Japan from blueberry jam (Y. Kikoku pers comm).

publication of this species (Yamaguchi et al. 2012). Thus the holotype of *B. undulatus* (MFC-21004) was not obtained for this study and probably no longer exists; however, until this can be confirmed we think it would be inappropriate to designate Matsushima's published illustrations as lectotype, and assign our epitypification to the holotype. We initially identified our strains as *B. undulatus* because they produced arthroconidia. Strains of *B. magus* (described below) do not produce arthroconidia and therefore they cannot represent *B. undulatus* sensu Matsushima. Clamp connections are present on some septa of *B. undulatus*, according to Matsushima (2003); we have not observed them.

**Basidioascus magus** H.D.T. Nguyen, N.L. Nickerson and Seifert, sp. nov. FIG. 2
MycoBank MB801332

*Etymology: magus* = wizard. The name refers to Wizard Island at Crater Lake National Park where the type strain was isolated.

*Diagnosis:* Colonies are white on M40Y and DG18. Sporulation occurs on CMA. Granular basidia are 9.5–14.5 × 6.5–10.5 µm, often found with a basal lateral projection, obovate or cylindrical when mature, and produce one mature basidiospore. Basidiospores are brown, 8.0–11.0 × 6.5–8.5 µm, and have wavy, verrucose and double wall. Arthroconidia are not produced.

*Description:* Colonies grow to a diameter of 1.4–2.5 cm (mean ± SE = 1.9 ± 0.1 cm) on MEA, 1.8–2.9 cm (mean ± SE = 2.3 ± 0.1 cm) on M40Y and 0.7–1.5 cm (mean ± SE = 1.1 ± 0.1 cm) on DG18 after 1 wk room temperature. The full life cycle can be observed on CMA. The surface of the colony on CMA is glandular because basidia form basidiospores on the surface of the agar. After 2 wk on MEA colonies are planar, stellate, white-beige, with diffuse margins; on M40Y colonies are flat, uniform, white with diffuse margins; on DG18 colonies are flat and sulcate, white with diffuse margins.

Probasidia are at first clavate on terminal and lateral branches of hyphae. Over time, probasidia expand and mature into obovate or cylindrical basidia. Mature basidia are 9.5–14.5 × 6.5–10.5 µm (mean ± SE = 12.2 ± 0.7 × 7.9 ± 0.5 µm), deciduous, forcibly discharged, with cell contents conspicuously granular, often with a basal lateral

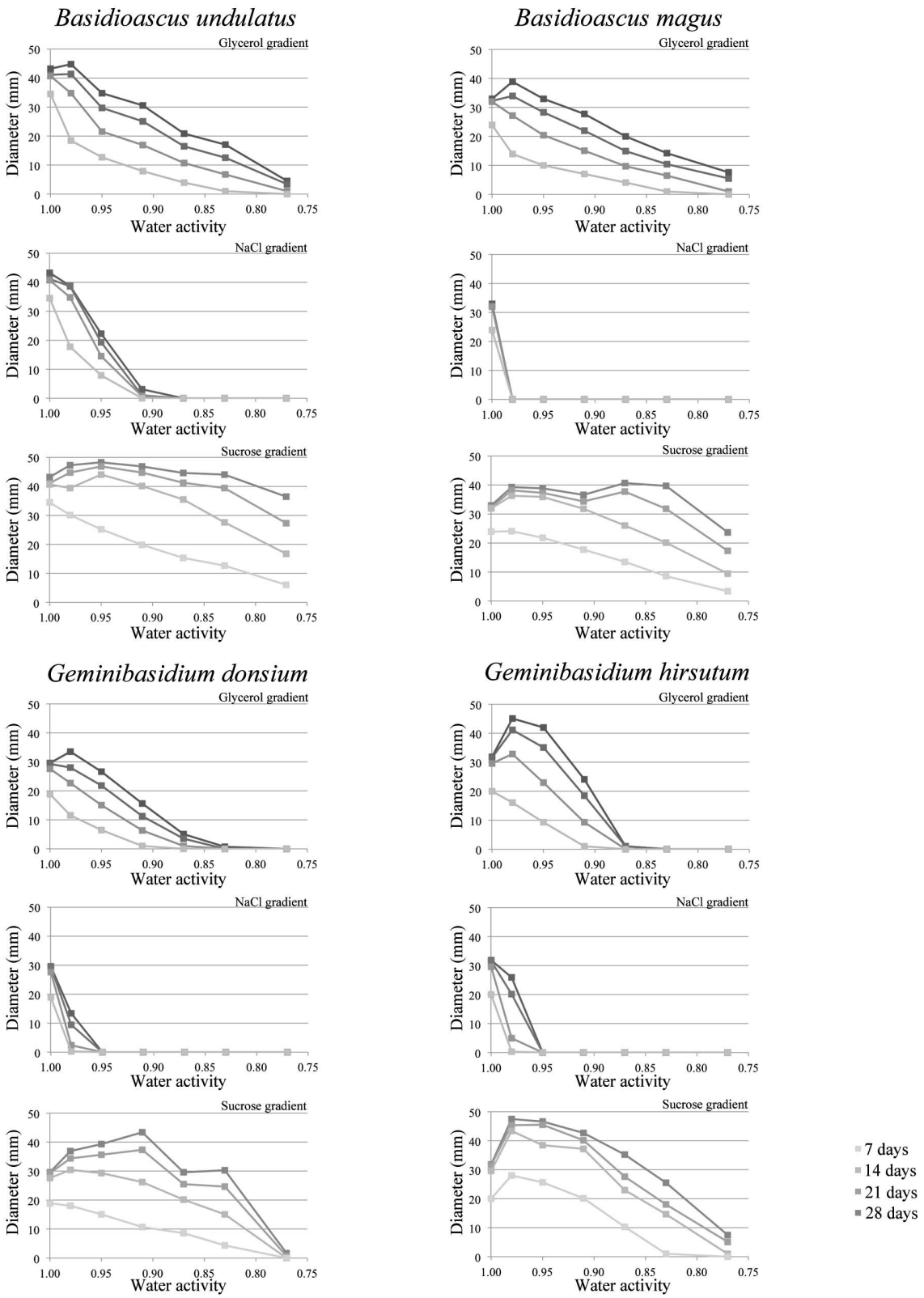


FIG. 8. Growth of *Basidioascus* and *Geminibasidium* type strains in water activities 1.00–0.77 after 7–28 d. Water activity in the media was lowered by addition of glycerol, sodium chloride and sucrose.

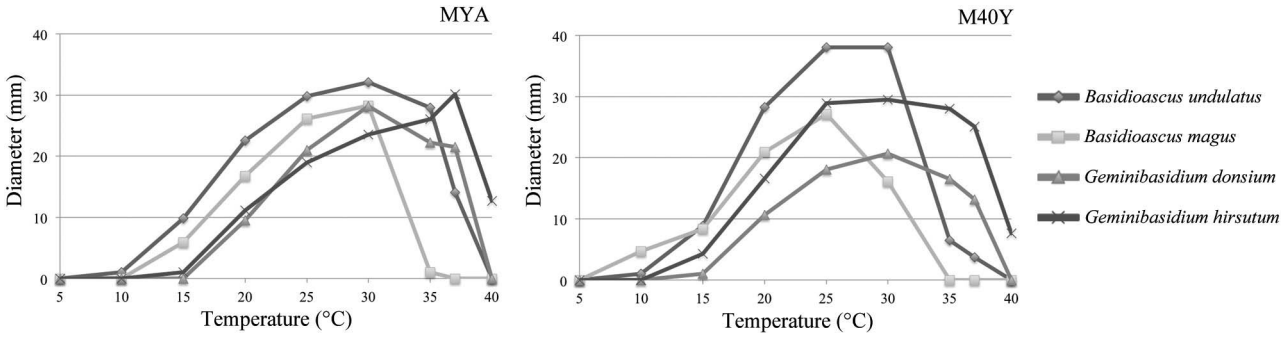


FIG. 9. Average growth of *Basidioascus* and *Geminibasidium* at 5–40 C after 7 d in MYA and M40Y media.

projection that eventually collapses. Mature basidia are visible after 5 d.

One basidiospore on a sterigma grows from the detached basidium. Basidia lose their cytoplasm and sometimes collapse as basidiospores mature. Basidiospores are subglobose, hyaline brown to brown, and have a thick, wavy, verrucose walls, 8.0–11.0 × 6.5–8.5 µm (mean ± SE = 9.6 ± 0.4 × 7.6 ± 0.2 µm). Basidiospores can be observed after 7 d. In 1 mo old cultures, clusters of basidiospores encased in a gelatinous matrix (presumed to be remnants of basidia) can be observed.

Arthroconidia are not produced by any of the *B. magus* strains observed.

*Holotype: B. magus* DAOM 241948; dried culture on CMA (Oct 2012) of an isolate from soil on Wizard Island at Crater Lake National Park, Oregon, United States. (42°56′N 122°08′W, 1900 m).

*Strains examined:* DAOM 241948 (ex-type), 241949, 241950, 241951, 241952, 241953, 242123, 241954, 241955 (TABLE I).

*Notes:* The characters that most readily differentiate *B. magus* and *B. undulatus* are the absence of arthroconidia, the absence of abortive basidiospores, and its limited ability to grow in the presence of sodium chloride.

**Geminibasidium** H.D.T. Nguyen, N.L. Nickerson and Seifert, gen. nov.
MycoBank MB801333

*Description:* Basidia are produced singly or in clusters, arise from swollen basidium bearing cells (primary cells) and have granular cellular contents. A basal lateral projection occurs on the swollen primary cell supporting the basidium and does not collapse during maturation. The basidia attached to the primary cells are deciduous before basidiospores begin to develop. One sterigma, or in one species a series of swollen cells attached by sterigma-like connectors, arise randomly over the apical two-thirds of the surface of the putative basidium. Only one mature basidiospore develops per basidium. Basidiospores are symmetrical on sterigma, not forcibly discharged, initially hyaline, brown when mature with an apparent double wall. Basidia lose their cytoplasm and sometimes collapse as basidiospores mature. Species of *Geminibasidium* are heat tolerant and xerotolerant. Analyses of SSU, 5.8S and LSU ribosomal RNA suggest a sister relationship to *Basidioascus*.

*Etymology: Geminibasidium* = twin basidium. The name refers to the basidium bearing cell (primary cell) and its attached basidium.

*Type species: Geminibasidium donsium.*

**Geminibasidium donsium** H.D.T. Nguyen, N.L. Nickerson and Seifert, sp. nov. FIGS. 3, 4
MycoBank MB801334

*Etymology: donsium* was named after Don Brisson, a good friend of the first author.

*Diagnosis:* Colonies are pink on M40Y and DG18. Sporulation occurs on CMA. Basidium bearing cells (primary cells) are 14.5–17.0 × 11.0–12.0 µm, have granular cell contents, a basal lateral projection, and are irregularly shaped when mature. During maturation, a probasidium, 10.5–14.5 × 9.5–12.5 µm, arises on the apex of the primary cells. The probasidium forms a series of swollen cells with sterigmata-like connectors with the terminal swollen cell becoming a basidiospore. Mature basidiospores are globose, hyaline or brown, 11.0–12.0 µm diam, slightly verrucose and double-walled.

*Description:* Colonies grow to a diameter of 2.2–2.4 cm (mean ± SE = 2.2 ± 0.1 cm) on MEA, 1.5–2.0 cm (mean ± SE = 1.8 ± 0.2 cm) on M40Y and 0.9–1.0 cm (mean ± SE = 0.9 ± 0 cm) on DG18 after 1 wk at room temperature. The full life cycle can be observed on CMA. The surface of the colony on CMA is glandular because basidia form basidiospores on the surface of the agar. After 2 wk on MEA colonies are planar, uniform, pink with diffuse margins; on M40Y colonies are flat, plicate, pink with diffuse margins; on DG18 colonies are flat, pink with diffuse or discrete margins.

Basidium-bearing cells (primary cells) at first are irregularly shaped on terminal and lateral branches of hyphae. Over time they expand and mature into swollen and irregularly shaped primary cells attached to hyphae by a thin connector. Mature primary cells are 14.5–17.0 × 11.0–12.0 µm (mean ± SE = 16.3 ± 0.8 × 11.6 ± 0.3 µm), have granular cell contents and a basal lateral projection. Primary cells are visible after 4 d.

After about 5 d a probasidium, 10.5–14.5 × 9.5–12.5 µm (mean ± SE = 12.3 ± 0.4 × 11.0 ± 0.3 µm), arises on the apex of the primary cells. The probasidium produces a series of swollen cells with sterigmata-like connectors with the terminal swollen cell becoming a basidiospore. These swollen cells appear granular at first, but they eventually become transparent after the terminal swollen cell matures into a single basidiospore. The basidium collapses as the basidiospore matures. One-wk old basidiospores are usually hyaline, and older basidiospores are usually brown. Basidiospores are globose, slightly verrucose, 11.0–12.0 µm (mean ± SE = 11.3 ± 0.3 µm) diam, and possess a thick double wall. Basidiospores are visible after 7 d. In 1 mo old cultures, clusters of basidiospores encased in a gelatinous matrix (presumed to be remnants of basidia) can be observed.

*Holotype: G. donsium* DAOM 241966; dried culture on CMA (Oct 2012) of an isolate from soil of commercial field of low bush blueberries in Sable River, Nova Scotia, Canada.

*Strains examined:* DAOM 241966 (ex-type), 241967, 242124 (TABLE I).

*Notes:* The characters that differentiate *G. donsium* from *G. hirsutum* are the production of a series of swollen cells with sterigma-like connectors on the basidium and the slightly verrucose appearance of mature basidiospores.

**Geminibasidium hirsutum** H.D.T. Nguyen, N.L. Nickerson and Seifert, sp. nov. FIG. 5

MycoBank MB801335

*Etymology: hirsutum* = hairy. The name refers to the hirsute basidiospores.

*Diagnosis:* Colonies are pink on M40Y and DG18. Sporulation occurs on CMA. Basidium-bearing cells (primary cells) are 14.0–20.5 × 11.5–14.0 µm, have granular cell contents, a basal lateral projection and are irregularly shaped when mature. During maturation, a probasidium, 13.5–23.5 × 11.0–17.5 µm, arises on the apex of the primary cells. One mature basidiospore on sterigma arises from the basidium. Basidiospores are globose, hyaline or brown, hirsute, 10.5–13.5 µm diam and double-walled.

*Description:* Colonies grow to a diameter of 2.6–2.7 cm (mean ± SE = 2.7 ± 0.1 cm) on MEA, 2.5–2.7 cm (mean ± SE = 2.6 ± 0.1 cm) on M40Y and 0.8–1.5 cm (mean ± SE = 1.2 ± 0.3 cm) on DG18 after 1 wk at room temperature. The full life cycle can be observed on CMA. The surface of the colony on CMA is glandular because basidia form basidiospores on the surface of the agar. After 2 wk on MEA, colonies are flat, uniform, white-beige or light pink-beige with diffuse margins; on M40Y, colonies are flat, smooth, pink with diffuse margins; on DG18, colonies are flat, pink with diffuse or discrete margins.

Basidium-bearing cells (primary cells) at first are irregularly shaped on terminal and lateral branches of hyphae. Over time they expand and mature into swollen and irregularly shaped primary cells, attached to hyphae by a thin connector. Mature primary cells are 14.0–20.5 × 11.5–14.0 µm (mean ± SE = 17.2 ± 3.4 × 12.9 ± 1.3 µm), have granular cell contents and a basal lateral projection. Primary cells are visible after 4 d.

After about 5 d, a probasidium, 13.5–23.5 × 11.0–17.5 µm (mean ± SE = 18.2 ± 1.4 × 14.5 ± 0.9 µm), arises on the apex of the primary cells. The probasidium produces a smooth immature basidiospore on a sterigma. The basidium collapses as the smooth basidiospore matures into a hirsute basidiospore. Basidiospores are globose (rarely subglobose), hirsute, 10.5–13.5 µm (mean ± SE = 12.0 ± 1.5 µm) diam, and possessing a thick double wall. Basidiospores are visible after 7 d. In 1 mo old cultures, clusters of basidiospores encased in a gelatinous matrix (presumed to be remnants of basidia) can be observed.

*Holotype: G. hirsutum* DAOM 241969; dried culture on CMA (Oct 2012) of an isolate from soil of a coniferous forest of mainly *Pseudotsuga* sp. with understory of *Vaccinium parvifolium* in Shingle Bolt Trail, Capilano Gorge, base of Grouse Mountain, North Vancouver, British Columbia, Canada. (49°22′N, 123°05′W, 310 m).

*Strains examined:* DAOM 241969 (ex-type), 241968 (TABLE I).

*Notes:* The characters that differentiate *G. hirsutum* and *G. donsium* are the presence of hirsute basidiospores and absence of the chain of swollen cells produced by the basidium.

### KEY TO THE SPECIES OF *BASIDIOASCUS* AND *GEMINIBASIDIUM*

1. Basidia forcibly discharged; sessile or on a cylindrical stalk, but lacking a basal, swollen primary cell . . . . . 2 (*Basidioascus*)
1. Basidia arising from a swollen and irregularly shaped primary cell . . . . . 3 (*Geminibasidium*)
2. Abortive basidiospores on some basidia, arthroconidia produced . . . . . *B. undulatus*
2. Only one basidiospore on each basidium, no arthroconidia produced . . . . . *B. magus*

3. Basidiospores with slightly verrucose walls when mature . . . . . *G. donsium*
3. Basidiospores with hirsute walls when mature . . . . . *G. hirsutum*

## DISCUSSION

During surveys for heat-resistant fungi in soil, we isolated *Basidioascus* and *Geminibasidium* species from soil using a pasteurization-like heat treatment. After analyzing these strains morphologically, phylogenetically and physiologically, our data support the description of two species of *Basidioascus* (*B. undulatus*, *B. magus* sp. nov.) and a novel genus, *Geminibasidium*, along with two novel species (*G. donsium*, *G. hirsutum*).

*Geminibasidium* and *Basidioascus* share similar ecology, physiology and morphology but also have several differences. *Basidioascus* species form white colonies, whereas *Geminibasidium* species form pink colonies. In *Geminibasidium* species, the basidium arises on a primary cell, whereas in *Basidioascus* species the basidium matures directly on somatic hyphae. Basidiospores in *Geminibasidium* species are globose, whereas they are subglobose in *Basidioascus* species. To our surprise, the basidia of *Basidioascus* species are forcibly discharged, a unique behavior that to our knowledge does not occur elsewhere in the Basidiomycota, including the sister genus *Geminibasidium*. Given these morphological observations (FIGS. 1–5) and our molecular phylogeny (FIGS. 6, 7), we conclude that *Basidioascus* and *Geminibasidium* are distinct genera.

The topology of our rDNA phylogenetic reconstruction reflects the currently accepted taxonomic view of the basidiomycetes (McLaughlin et al. 2009). In this analysis, *Basidioascus* and *Geminibasidium* were found to be sister groups in Wallemiomycetes (FIG. 6). Our results also show that *Wallemia* is the sister taxon of *Basidioascus* and *Geminibasidium*. The taxonomic position of *Wallemia* was uncertain (Matheny et al. 2006) until Padamsee et al. (2012) found it is unambiguously fixed at the base of the Agaricomycotina with high certainty by phylogenetic analysis with an amino acid dataset from 71 protein-coding genes.

The phylogenetic placement of *Basidioascus* and *Geminibasidium* in the Basidiomycota lead us to interpret their characters in the context of basidiomycete morphology. The structures referred to in this paper as basidia and basidiospores are putatively identified as such. Our preliminary experiments with DAPI staining to study nuclear behavior were unsuccessful, probably suggesting such stains do not effectively penetrate the cell walls of these fungi. We plan detailed follow-up studies of the karyology and ultrastructure of these fungi. Basidia in *Basidioascus* species resemble those of gasteroid Agaricomycetes, such as *Secotium agaricoides* and *Lycoperdon candidum* (Coker and Couch 1974). In common with the gasteroid Agaricomycetes, the basidiospores of *Basidioascus* basidia are not actively discharged. The gasteroid morphological character is polyphyletic in the Agaricomycetes (Binder and Bresinsky 2002). Although similar, basidia from many gasteroid fungi are more elongated than those of *Basidioascus* species, perhaps reflecting the evolution of the gasteroid fungi from the typically elongated clavate basidia of the Agaricales. Our rDNA data (FIG. 6) suggest that *Basidioascus* species are only distantly related to the Agaricomycetes. In *Geminibasidium* species, basidium-bearing cells (primary cells) give rise to basidia. This peculiar basidium arrangement does not resemble that of any other known fungi. As an aside, we note the striking similarity of the simple basidial structures of *Basidioascus* and *Geminibasidium* with the sporangia and subsporangial swellings of the chytrid *Phlyctochytrium aureliae* as illustrated by Letcher et al. (2011).

The basidia in *Basidioascus* and *Geminibasidium* species have a basal lateral projection. The origin and function of this structure are unknown. As basidia mature, the basal lateral projection in *Basidioascus* species collapses but not that of *Geminibasidium* species. Basidia with collapsed basal lateral projections were found on the Petri dish lids above colonies of *Basidioascus* species. However, basidia were not found on the Petri dish lids above *Geminibasidium* species. We speculate that the collapse of this projection is involved in the forcible discharge of the basidia in *Basidioascus* species.

Both *Geminibasidium* and *Basidioascus* have double-walled basidiospores that themselves are not actively discharged. The double-walled basidiospores, which can be hirsute or verrucose, resemble those of gasteroid species of *Lycoperdon* and *Scleroderma* (Coker and Couch 1974) and also those of basidiomycetes with actively discharged basidiospores such as species of *Ganoderma* (Hong and Jung 2004). In older cultures, double-walled basidiospores often cluster together. Perhaps these clusters are immature or degenerated basidiomes, a cultural expression of what might be a more developed structure in nature. We speculate that the double wall adaptation and clustering helps in resisting heat. The outer wall of the spore may provide an additional protective layer. Clustering also decreases exposed surface area, and spores in the center of the cluster could be further shielded from heat. The basidiospores in *G. hirsutum* are hirsute, and this adaptation may help sticking to

an animal or insect vector for spore dispersal or could be an additional way for spores to cluster for increased protection.

The majority of basidiomycetes in the Agaricales, Pucciniales and Tremellales have basidia that produce four meiotic basidiospores. Some basidiomycetes produce other numbers (e.g. the tuning fork basidium in *Dacrymyces* with two basidiospores and *Sistotrema* species with eight). Each basidium in *Basidioascus* and *Geminibasidium* species produces only one mature basidiospore. The adaptive benefits of this are uncertain. Perhaps CMA is not the ideal medium to observe ontogenesis because *B. undulatus* produces abortive basidiospores and *G. donsium* can produce a series of swollen cells, which may be interpreted as immature basidiospores, before the terminal mature basidiospore. Therefore, the production of one basidiospore may be an artifact of growth in culture.

*Basidioascus* and *Geminibasidium* species share other common characters with *Wallemia*. They commonly are xerotolerant, have similar optimal growth temperature, are found in soil and lack a basidiocarp. *Wallemia* is mostly isolated from indoor air dust, dried and salty foods but also can be isolated from soil (Zalar et al. 2005, Zajc et al. 2011). However, they differ in ontogenesis, morphology and heat resistance. In *Wallemia* conidia develop basipetally (Padamsee et al. 2012) in a unique pattern known as basauxic conidiogenesis (Zalar et al. 2005). Furthermore, *Wallemia* is not heat resistant (Vytrasová et al. 2002).

Given all these unusual characters, we suggest the placement of *Basidioascus* and *Geminibasidium* in a novel family, Geminibasidiaceae, within a novel order, Geminibasidiales. As well, we propose that Geminibasidiales tentatively be placed under the class Wallemiomycetes. Class-rank assignment based on the septal pore ultrastructure would provide conclusive evidence for this higher taxonomic classification.

*Ecology and physiology.—Basidioascus* and *Geminibasidium* species were isolated from soil from forests, grasslands and blueberry fields (TABLE I), suggesting that they are common and have been overlooked. These soils sometimes were accidentally or intentionally burned. Few published studies deal with basidiomycetes recovered in heat-treated or burned soils (Bollen 1969, 1974; Izzo et al. 2006). The only basidiomycetes reported to be recovered after heat treatment of soils were *Tephrocybe* sp. (Bollen 1974), *Coprinus fimetarius* (Bollen 1969) and *Rhizopogon olivaceotinctus* (Izzo et al. 2006). Our study adds *Basidioascus* and *Geminibasidium* species to the list of heat-resistant basidiomycetes.

Our ITS analysis shows that there remain undescribed genera or species related to *Basidioascus* and *Geminibasidium* (FIG. 7). Location data in the environmental sequences suggest that these fungi are widely distributed in soil around the world. Additional soil sampling likely will help isolate these environmental taxa now that a method of isolation for these fungi is established. The environmental sequences FN397319 from France and EU626069 from China form an additional well supported clade and may represent a yet to be described genus or species of *Basidioascus*. The environmental sequences near *Geminibasidium* species form four additional clades with moderate node support that could represent additional undescribed species of *Geminibasidium*. Many of the studies (see citations in SUPPLEMENTARY TABLE II) that generated these environmental sequences focused on ectomycorrhizal communities. One environmental ITS sequence was reported to be from ''ectomycorrhized root tips'' in the GenBank (HM044636 otherwise unpubl) record. This ITS sequence is closely related to *Geminibasidium* strains found in our study (FIG. 7). If this information is accurate, *Geminibasidium* or a *Geminibasidium*-like fungus may be ectomycorrhizal. Future in vitro studies should be done to test this. Xerotolerant ectomycorrhizal fungi so far are unknown and could be an interesting avenue to explore.

Xerophilic fungi grow at water activities below 0.85 under at least one set of environmental conditions (Pitt 1975). Most ascomycetes are able to tolerate water activities below $a_w = 0.90$ (Zak and Wildman 2004). Xerotolerance is presently considered a rare character in the basidiomycetes (Zak and Wildman 2004, Zalar et al. 2005). All *Basidioascus* and *Geminibasidium* strains are able to grow on low water activity media such as M40Y and DG18. Our data show that they can also grow below $a_w = 0.85$ (FIG. 8), the threshold defining a xerophilic fungus, but they grow better at slightly higher water activity. Thus, we consider them xerotolerant rather than xerophilic. They grow well in a high sucrose environment, and thus it is not surprising that *Geminibasidium* was isolated from blueberry jam (Y. Kikoku pers comm). The average NaCl concentration of seawater is 0.5 M (about 3.0%). *Basidioascus undulatus*, *G. donsium* and *G. hirsutum* are able to grow above this concentration, suggesting these fungi could live in the ocean as well as in normal or salty soils. Soil is probably their primary habitat because most of the environmental sequences from GenBank are from soil and we isolated them from soil in our study.

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