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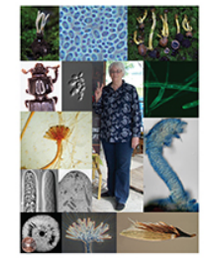

Mycologia

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

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To cite this article: M. Catherine Aime, Hector Urbina, Julian A. Liber, Gregory Bonito & Ryoko Oono (2018) Two new endophytic Atractiellomycetes, Atractidochium hillariae and Proceropycnis hameedii, Mycologia, 110:1, 136-146

To link to this article: https://doi.org/10.1080/00275514.2018.1446650

MYCOLOGIA
2018, VOL. 110, NO. 1, 136–146
https://doi.org/10.1080/00275514.2018.1446650

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Two new endophytic Atractiellomycetes, *Atractidochium hillariae* and *Proceropycnis hameedii*

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ABSTRACT

Sterile fungal isolates are often recovered in leaf and root endophytic studies, although these seldom play a significant role in downstream analyses. The authors sought to identify and characterize two such endophytes—one representing the most commonly recovered fungal isolate in recent studies of needle endophytes of *Pinus taeda* and the other representing a rarely isolated root endophyte of *Populus trichocarpa*. Both are shown by DNA sequencing to be undescribed species of Atractiellomycetes (Pucciniomycotina, Basidiomycota), a poorly characterized class of mostly plant-associated and presumably saprobic microfungi. The authors describe the new genus and species *Atractidochium hillariae* (Phleogenaceae) and the new species *Proceropycnis hameedii* (Hoehnelomycetaceae), both in the Atractiellales, to accommodate these unusual isolates. Following incubations of 1–2 mo, *A. hillariae* produces minute white sporodochia, similar to those produced by several other members of Atractiellales, whereas *Pr. hameedii* forms conidia singly or in chains in a manner similar to its sister species *Pr. pinicola*. Additionally, we provide a taxonomic revision of Atractiellomycetes based on multilocus analyses and propose the new genera *Neogloea* (Helicogloeaceae) and *Bourdotigloea* (Phleogenaceae) to accommodate ex-*Helicogloea* species that are not congeneric with the type *H. lagerheimii*. Atractiellomycetes consists of a single order, Atractiellales, and three families, Hoehnelomycetaceae, Phleogenaceae, and Helicogloeaceae. Accumulated evidence suggests that Atractiellomycetes species are common but infrequently isolated members of plant foliar and root endobiomes.

ARTICLE HISTORY
Received 12 July 2017
Accepted 26 February 2018

KEYWORDS
Atractiella; Helicogloeaceae; *Hobsonia*; *Leucogloea*; orchid mycorrhizae; *Phleogena*; *Saccoblastia*; 7 new taxa

INTRODUCTION

Most recovered foliar fungal endophytes are species of Ascomycota, with members of the Sordariomycetes, Dothideomycetes, Leotiomycetes, and Eurotiomycetes being predominant (e.g., Arnold and Lutzoni 2007; Arnold et al. 2007). Although fungal species from the Basidiomycota can also be common in the endophyte biome, especially in woody tree trunks (e.g., Crozier et al. 2006; Thomas et al. 2008; Vega et al. 2010), few are reported abundantly or consistently in leaves or in association with a specific plant host (but see Pinruan et al. 2010). Within Pucciniomycotina (one of three subphyla of Basidiomycota), only some yeast species in the genera *Rhodotorula* and *Sporobolomyces* appear to have been infrequently recovered as foliar endophytes (Bura et al. 2012; Urbina and Aime 2018; Zhao et al. 2002), although environmental sequences indicative of *Mixia* (Mixiomycetes) were detected from leaves in Europe and Asia, which may suggest a potential but unlikely endophytic role for these (Toome et al. 2014). Known root associates in Pucciniomycotina are likewise sparsely reported and primarily consist of the root pathogen *Helicobasidium* (Valder 1958), unnamed orchid-mycorrhiza formers in Atractiellales (Kottke et al. 2010), and the *Populus* root associate *Atractiella rhizophila* (Bonito et al. 2017).

Atractiellomycetes constitute one of nine classes of Pucciniomycotina (Aime et al. 2006, 2014; Schell et al. 2011). Species are primarily known for forming inconspicuous basidiomata or conidiomata on woody or herbaceous material, where they are assumed saprobic (Bauer et al. 2006). However, recent studies, spurred by environmental sequencing or molecular identifications of nonsporulating cultures, show that members of the class may be associated with plant roots and are presumably mycorrhizal or endorhizal (Kottke et al. 2010; Bonito et al. 2016, 2017).

The most frequently cultured fungus (30.7% of isolates) from a recent study of *Pinus taeda* (loblolly pine)

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Seven new taxa reported: *Atractidochium*, gen. nov.; *Atractidiochium hilleriae*, sp. nov.; *Proceropycnis hameedii*, sp. nov.; *Bourdotigloea*, gen. nov.; *Neogloea*, gen. nov.; *Neogloea variabilis*, comb. nov.; *Bourdotigloea vestita*, comb. nov.

Published online 04 Jun 2018

needle endophytes belonged to a single unknown, sterile operational taxonomic unit (Oono et al. 2015). DNA sequence analyses indicated a basidiomycetous affinity for this species, with possible placement in the Atractiellomycetes. Additionally, during ongoing efforts to document endorhizal associates of *Populus trichocarpa*, a single (from >1800 isolations) sterile strain of what appeared to be an Atractiellomycete, but one not congeneric with the more frequently isolated and recently described *A. rhizophila*, was discovered (Bonito et al. 2016, 2017). The goals of the present study are to completely characterize these inhabitants of *Pi. taeda* needles and *Po. trichocarpa* roots, described here as *Atractidochium hillariae*, gen. et sp. nov., and *Proceropycnis hameedii*, sp. nov., respectively. To this end, a resolved multilocus phylogenetic analysis of the Atractiellomycetes is presented and the genera *Neogloea* gen. nov. and *Bourdotigloea* gen. nov., are proposed to accommodate some species of *Helicogloea* that are not congeneric with the type, *H. lagerheimii*.

MATERIALS AND METHODS

Strain isolation and morphological characterization.—Cultures from needles of adult *Pi. taeda* trees were isolated from surface sterilized mature needles (second year) in the Blackwood Forest Division of Duke Forest in Orange County, North Carolina, USA (approx. 35.97°N, 79.09°W). Cultures were prevalent in needles collected during both winter and summer months and were isolated over multiple years (Arnold et al. 2007; Oono et al. 2015); details of isolation and culturing are outlined in Oono et al. (2015). From roots of natural *Po. trichocarpa* stands in southern Washington, an isolate of Atractiellomycetes (PMI 927; GenBank accession number KF428609; Bonito et al. 2016, supplementary material) was obtained among ca. 1800 fungal isolates as part of the Plant Microbial Interfaces (PMI) project; details of isolation and culturing are explained in Bonito et al. (2016).

Dried biologically inert cultures to serve as holotypes were deposited in the Kriebel Herbarium at Purdue University (PUL). Living cultures were deposited in the United States Department of Agriculture (USDA) Agriculture Research Service Culture Collection (NRRL; Peoria, Illinois), the Cheadle Center for Biodiversity and Ecological Restoration at the University of California, Santa Barbara (UCSB) (*Pi. taeda*), and the laboratory culture collections of G. Bonito (MSU) (*Po. trichocarpa*), and M. C. Aime (MCA).

Morphological characterization was carried out using Difco oatmeal agar (OA; BD Difco, Houston, Texas, USA), Difco potato dextrose agar (PDA), Difco yeast extract-peptone-dextrose (YPD) agar, and walnut agar (WA; 8 whole blended walnuts with shells in 500 mL of Millipore water and 1.5% of agar, autoclaved at 121 C and 15 psi for 20 min). Cultures were incubated at room temperature and examined every 2 wk for 2 mo for signs of differentiated hyphae or sporulating structures. Cultures were photographed with a SZ61-Olympus dissecting microscope with SC30-Olympus camera and the software cellSens Olympus 1.14 (Olympus Life Science Solutions, Waltham, Massachusetts, USA). Water mounts were examined with a BH2-Olympus research microscope and photographed with the same camera. To ascertain the nuclear status of *Pr. hameedii*, slide cultures were prepared and visualized following the methods of Fisher et al. (2018) by growing mycelium across a thin layer of nutrient agar, made by dipping sterile glass microscope slides into liquid medium (10 g/L malt extract, 1 g/L yeast extract, 10 g/L Bacto agar; BD Biosciences, San Jose, California, USA) and allowing this to solidify. Plugs with mycelium measuring 0.5 cm × 1 cm were placed on one side of the slide, then slides were placed in a moist chamber, prepared in glass Petri dish with a wet, sterile wipe and bent glass rod to elevate the slide. After 1 wk of growth, plugs were removed from the slides with a razor. Fungal cell walls and nuclei were stained following a protocol adapted from Robin et al. (1986) and Chazotte (2011). Briefly, slides with growing mycelium were submerged for 15 min in phosphate-buffered saline (PBS) solution with bovine serum albumin (BSA) (PBS^+; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1mg/mL BSA). Then slides were placed in 0.2% Triton X-100 for 5 min, then for 10 min in PBS^+ without BSA. The mycelium on the slides was inundated in 50 µL of 10 µg/mL fluorescein-labeled wheat germ agglutinin (λ_{ex} ~ 494 nm, λ_{em} ~ 518 nm) and placed in a humid Petri dish in the dark for 25 min. Next, to label nuclei, 50 µL of 200 µg/mL 4',6-diamidino-2-phenylindole (DAPI; λ_{ex} ~ 359 nm, λ_{em} ~ 461 nm) was applied to the mycelium, which was then placed in the dark for 5 min before adding coverslips and viewing. Micrographs were obtained with an Olympus Fluoview FV10i confocal laser scanning microscope. Measurements of microscopic characters are based on a minimum of 30 measurements for each structure.

Molecular characterization and phylogenetic analysis.—Five cultures of *A. hillariae*, isolated from different needle samples and different time periods, were chosen for DNA extraction and initial sequencing of the nuc rDNA internal transcribed

spacers (ITS1-5.8S-ITS2 = ITS); three were selected for additional sequencing along with the only isolate of *Pr. hameedii* (TABLE 1). Methods for DNA extraction and amplification of three nuc rDNA loci, ITS plus the 28S subunit (28S) and 18S subunit (18S), follow Oono et al. (2015) and Goodwin and Lee (1993). These loci were chosen for sequencing because they represent vast majority of available reference data for Atractiellomycetes. Sequence contigs were assembled and edited in Sequencher 5.2.1 (Gene Codes, Ann Arbor, Michigan). To compile a data set comprising most of the known species and all of the known genera in the class, sequences were downloaded from GenBank or newly generated from vouchered material (TABLE 1). Three outgroups were chosen from the genus *Symmetrospora* (Cystobasidiomycetes, Basidiomycota), because Cystobasidiomycetes is one potential sister lineage to Atractiellomycetes (Aime et al. 2014).

For phylogenetic analyses, data sets for each locus were assembled individually in Mesquite 3.2 (Maddison and Maddison 2017), aligned with the online version of MAFFT (Katoh and Standley 2013), and concatenated in Mesquite. Maximum likelihood (ML) phylogenetic inference was performed in raxmlGUI (Silvestro and Michalak 2012), using a partitioned data set under the GTRGAMMA nucleotide evolution model; node support values were computed using 1000 bootstrap replications. The best bipartitions tree was imported into the R package phytools for editing (Revell 2012). The tree and alignments were deposited in TreeBASE (study no.: S21300); newly generated sequence data were deposited in GenBank.

RESULTS

Fungal cultures of the abundant *Pi. taeda* endophyte were obtained from the study of Oono et al. (2015), originating from 2-y-old needles collected during both summer and winter months in North Carolina. Five of these were randomly selected for sequencing at the ITS and found to share 99% identity (data not shown); further analyses were conducted only on a subset of these (TABLE 1). In contrast, among the >1800 fungal isolates derived from surface sterilized *Populus* roots by Bonito et al. (2016), only one isolate of *Proceropycnis* (as "Atractiellales sp.") was identified. Although initially sterile, both species developed conidia, and in the case of *A. hillariae*, sporodochia, after 1–2 mo incubation; these were best induced at room temperature

Table 1. Origin of isolates and sequences used in this study.

Species	Voucher/Strain number	SSU	LSU	ITS
Atractidochium hillariae	cas001(65b)[T]	**MF461288**	**MF461291**	KM519195
Atractidochium hillariae	cas012(72a)	**MF461289**	**MF461292**	KM519202
Atractidochium hillariae	caw001(1.1.6b)	**MF461290**	**MF461293**	**MF461287**
Atractidochium hillariae	caw004(1.2.11a)	—	—	KM519229
Atractidochium hillariae	caw008(8.1.10)	—	—	KM519230
Atractiella rhizophila	CBS 137288	**MF476083**	JX243797	JX243797
Atractiella solani	Strain TUB F107	DQ198797	DQ198781	AY512831
Atractiella sp.	P30	KX812532	KX812533	**MG765268**
Atractiellales sp.	WIM(M)1397	EF406118	EF406119	EF406119
Basidiopycnis hyalina	TUB FO44664	DQ198795	DQ363322	DQ198779
Bourdotigloea (as *Helicogloea*) *vestita*	TUB FO39734	AY124480	AY512872	—
Helicogloea lagerheimii	TUB FO36341	DQ198794	AY512849	—
Helicogloea sp.	TUB Chen1178	—	AY512848	—
Helicogloea sp.	TUB FO42773	DQ198793	AY512847	—
Helicogloea sp.	TUB 020325	KF061294	KF061297	—
Helicogloea sp.	RJB 6478-5	KX812537	KX812536	**MF476085**
Hobsonia mirabilis	ATCC 18961	AF289663	—	—
Infundibura adhaerens	TUB 020326	KF061295	KF061296	—
Infundibura adhaerens	F029224	—	AJ406404	—
Leucogloea (as *Pleurocolla*) *compressa*	RoKi 640, TNM	—	AY382581	—
Leucogloea sp.	JAC13103	—	KP191766	KP191965
Neogloea (as *Helicogloea*) *variabilis*	KW 1540	U78043	L20282	**MF476084**
Phleogena faginea	TUB FO30201	DQ198798	AY512869	—
Phleogena faginea	AFTOL-ID 1889	DQ831022	DQ831021	—
Proceropycnis hameedii	PMI 927[T]	**MG765269**	**MG765270**	KF428609
Proceropycnis pinicola	AH33906	DQ198796	DQ363323	DQ198780
Saccoblastia farinacea	GEL4771	—	AJ406401	—
Symmetrospora symmetrica	SA107	**KJ701209**	**KJ701208**	**KJ701207**
Symmetrospora symmetrica	P118	**KJ701212**	**KJ701211**	**KJ701210**
Symmetrospora symmetrica	CBS 9727	—	AB279627	KY105573
Uncultured Atractiellales	Clone 1EC2-1	—	GU079580	GU079580
Uncultured Atractiellales	Clone 1ED2-11	—	GU079581	GU079581
Uncultured Atractiellales	Clone 3TC2-2	—	GU079597	GU079597
Uncultured Atractiellales	Clone 4TG1-9	—	GU079612	GU079612
Uncultured Atractiellales	Clone 3TH1-5	—	GU079600	GU079600

Note. Sequences generated in this study in bold. [T] indicates type specimens.

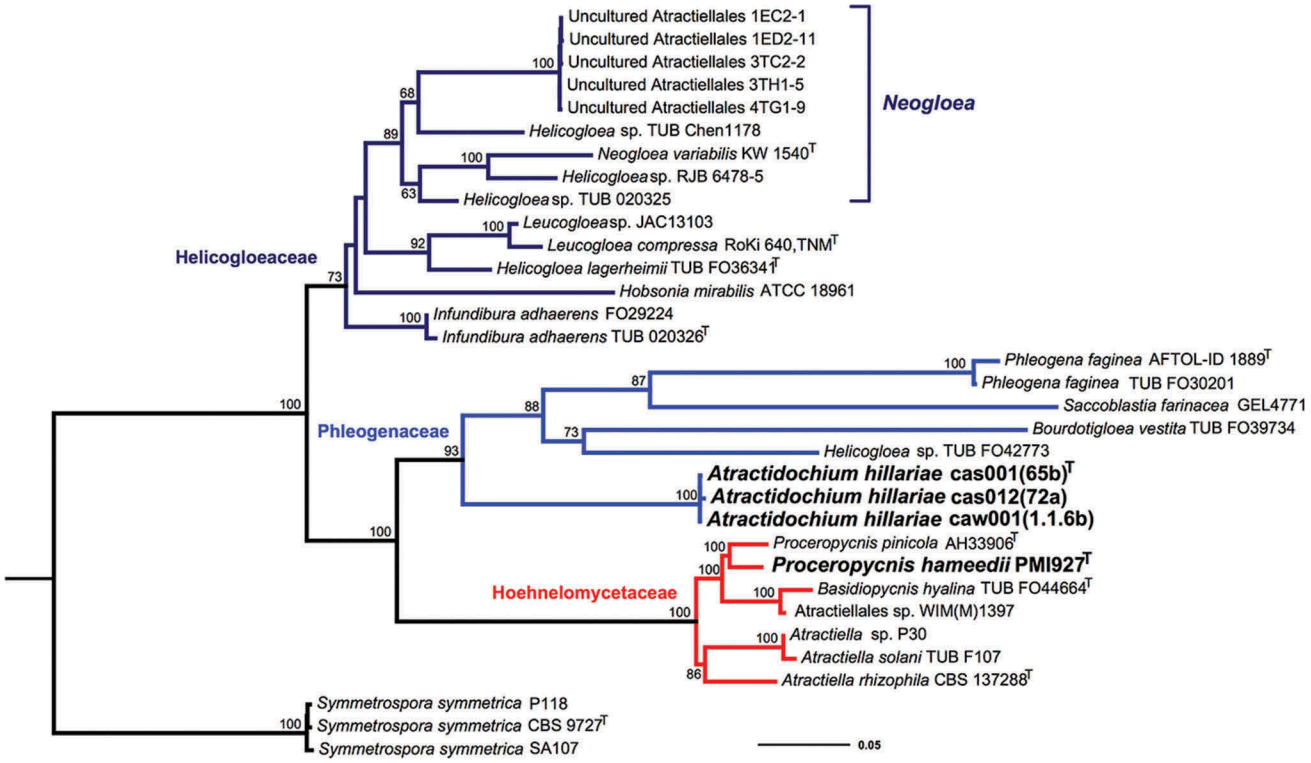


Figure 1. Maximum likelihood tree for the Atractiellomycetes from 18S, 28S, and ITS data (in order of concatenation). ML score = −14721.74. *Symmetrospora symmetrica* isolates were selected as outgroup for rooting purposes. Numbers above the branches indicate bootstrapping values. Three supported family-level lineages are recovered, indicated by color. New species are indicated in bold. T indicates type species.

under ambient light, as further discussed for each species below.

Our phylogenetic analysis of Atractiellomycetes shows that the class is naturally subdivided into three supported lineages that roughly correspond to the described families Hoehnelomycetaceae, Phleogenaceae, and Helicogloeaceae (FIG. 1). Isolates identified as species of *Helicogloea* do not appear monophyletic (FIG. 1). *Helicogloea variabilis* is not congeneric with the type, *H. lagerheimii*, in these analyses, and an isolate of *H. alba* (GenBank GQ411522), included in the original analyses, was later removed when preliminary data showed that it was not a member of Atractiellomycetes. *Helicogloea vestita* (TUB FO39734) is supported within the Phleogenaceae and not the Helicogloeaceae. The uncultured Atractiellales associated with orchid roots (Kottke et al. 2010) are supported as congeneric allies of *H. variabilis*.

TAXONOMY

Atractidochium Oono, Urbina & Aime, gen. nov.
MycoBank MB824356

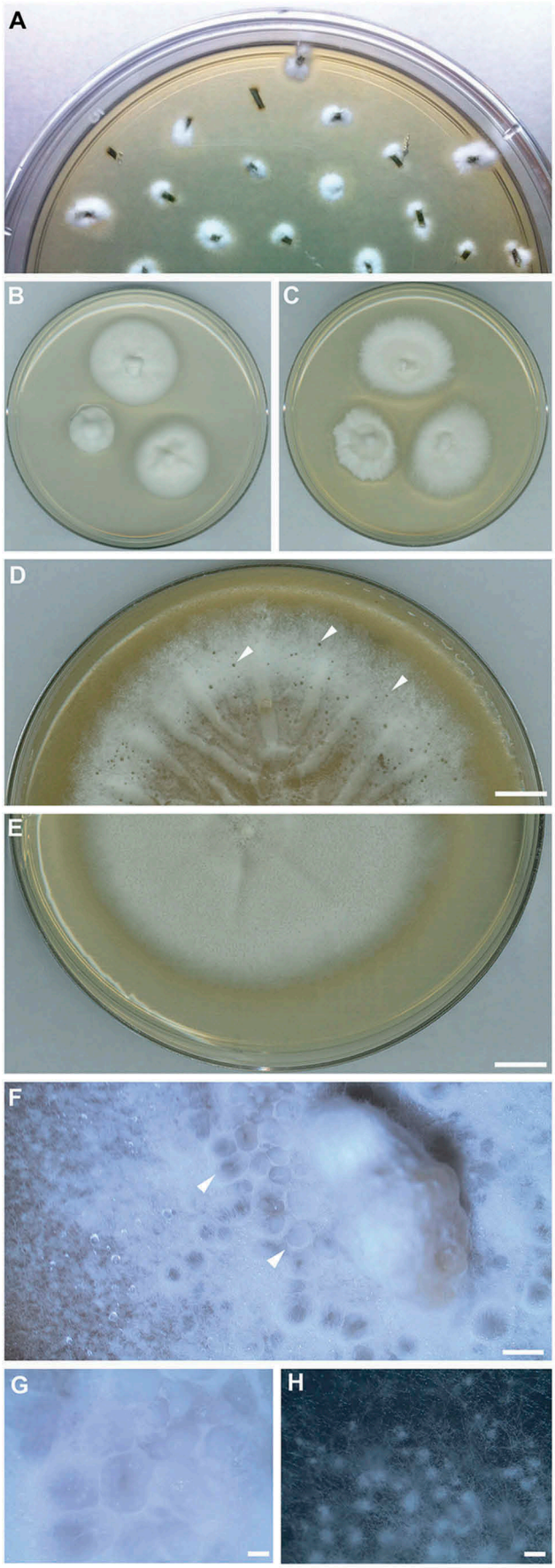
Typification: Atractidochium hillariae Oono, Urbina & Aime.

Etymology: Atracti- from *Atractiella*, and *-dochium* for sporodochium.

Colonies on PDA growing slowly; white, hyphal, with irregular borders. Conidiomata sporodochial, white, gelatinous. Hyphae hyaline; clamp connections present. Condiophores branching at the apex, organized in a hymenium-like layer within the center of the sporodochium. Conidia hyaline, falcate, aseptate at first becoming phragmoseptate after maturation; secession schizolytic. Teleomorph unknown. Phylogenetically distinct from other members of Atractiellomycetes, belonging to Phleogenaceae (Atractiellales). Known only from the type species.

Atractidochium hillariae Oono, Urbina & Aime, sp. nov. FIGS. 2–3
MycoBank MB823927

Typification: USA. NORTH CAROLINA: Orange County, Blackwood Forest Division of Duke Forest, general vicinity of 35.975°N, 79.094°W, a dried, inert, culture isolated from an adult tree in Ring 5 of FACE facility at Duke University, Jun 2012, *R. Oono cas001 (65b)* (**holotype** PUL F20086). Ex-type culture: NRRL 66669 = UCSB044473 = P-130 (MCA). ITS: GenBank KM519195.



Etymology: "*hillariae*," honoring Hillary Rodham Clinton—a devoted public servant and first female candidate nominated by a major U.S. political party for president of the United States. By naming this species after Hillary Clinton, we honor her career fighting against gender inequality.

Colonies after 1 mo of incubation at room temperature white with radiating zones of dense hyphae and powdery irregular margins (FIG. 2B, D), producing abundant white, floccose tufts (FIG. 2H); 6.3 cm diam on PDA, 6 cm diam on YPD, and 5.2 cm diam on OA. Asexual state gelatinous, white sporodochia (FIG. 2F–G), gregarious and abundant; generated in single cultures and co-cultures between strains cas012(72a) and caw001(1.1.6b); produced initially at the center of colonies and then more sparsely toward the edge, never in the interface between co-cultures; produced on WA after a month of incubation and OA and PDA after 2 mo, both at room temperature; ellipsoidal to somewhat irregular in outline, (0.66–)0.7–1.24(–1.31) mm diam. Hyphae hyaline, thin, 1–2 µm diam, with abundant clamp connections (FIG. 3A–C). Conidiogenous cells holoblastic, subulate with antler-like branching from apical end; length greater than 80 µm, width at the broadest point 3–8 µm. Conidia abundant, produced at tips of branches of conidiogenous cells, basal cell somewhat flattened at point of attachment to conidiogenous cell (FIG. 3D, E); hyaline, falcate, fusiform and often sinuous; basal and apical cells longer than central cells; nonseptate at first, becoming phragmoseptate with 3–6 septa when mature; thin- and smooth-walled, (30.5–)32–46(–48) × 3–3.5(–4) µm (FIG. 3F–I); germination by germ tube from central or apical cells (FIG. 3J–K).

Habitat: Isolated abundantly as hyphal endophytes of *Pinus taeda* needles.

Distribution: North Carolina, USA.

Material examined: USA. NORTH CAROLINA: Orange County, Blackwood Forest Division of Duke Forest, general vicinity of 35.975°N, 79.094°W, Ring 5 of FACE facility, Jun 2012, *R. Oono cas001(65b)* (**holotype** PUL F20086; ex-type culture: NRRL 66669 =

Figure 2. Colony morphology of *A. hillariae*. A. Isolation plate on 2% MEA showing frequency of isolation. B–C. Colony of cas001(65b)$^{Ex\text{-}T}$ (top), caw001(1.1.6b) (left), and cas012(72a) (right) on PDA (B) and YGPA (C). D–E. cas001(65b)$^{Ex\text{-}T}$ 2-mo-old cultures on 10-cm Petri plates of PDA (D) and OA (E). F–G. cas001(65b)$^{Ex\text{-}T}$ gelatinous sporodochia (white arrowheads) produced in the colony center on WA. H. Hyphal conglomerates on the border of the colony of cas001(65b)$^{Ex\text{-}T}$. Bars: D, E = 1 cm; F = 1 mm; G, H = 200 µm. $^{Ex\text{-}T}$ indicates ex-type culture.

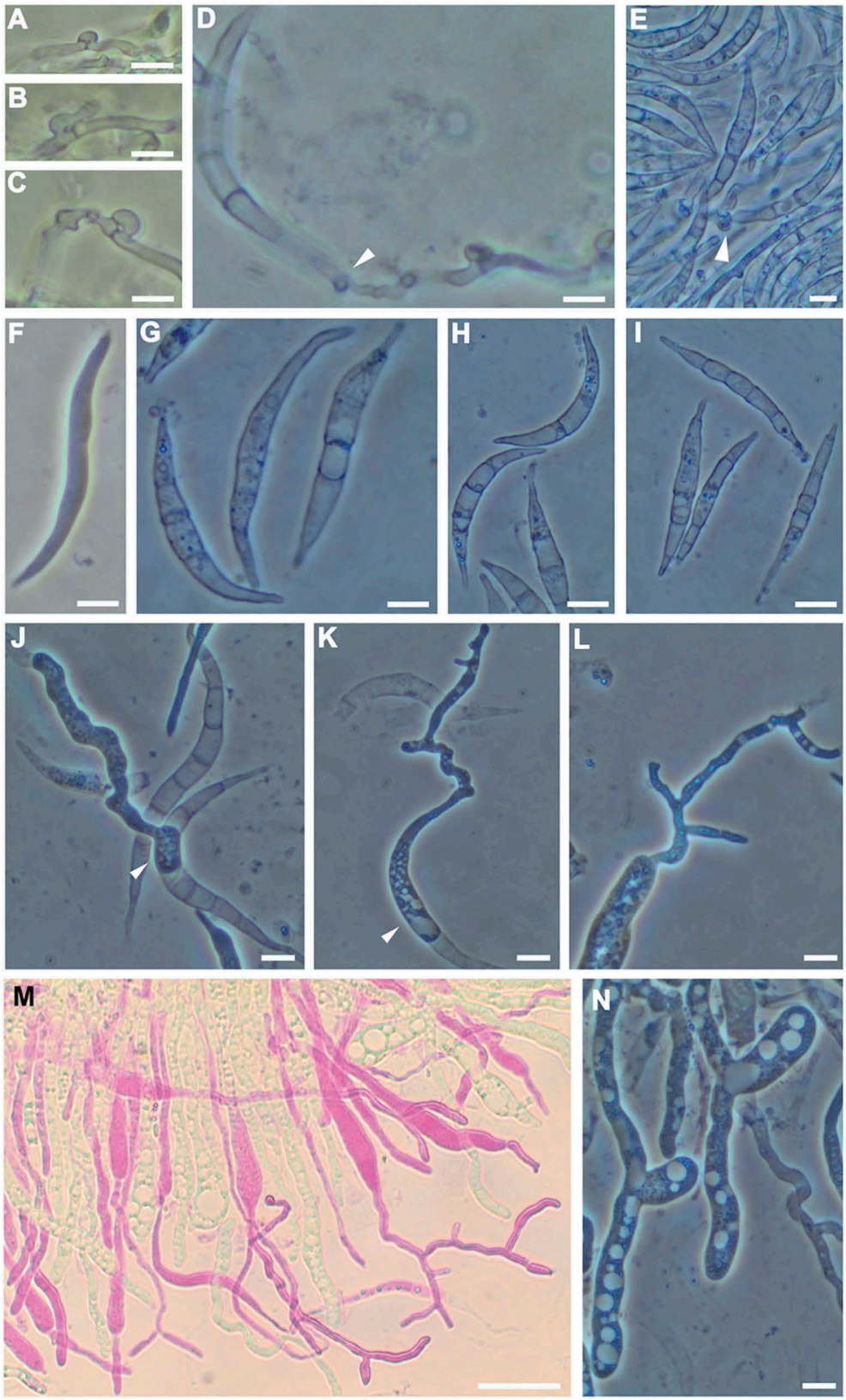


Figure 3. Micromorphology of *A. hillariae* cas001(65b)$^{Ex\text{-}T}$. A–C. Clamp connections. D–E. Conidium attached to a hypha. F. Young conidium. G–I. Mature conidia. J. Lateral conidium germination. K. Apical conidium germination. L. Conidiogenous hyphae. M. Conidiogenous structures stained with Congo red; 400×. N. Epibasidium-like cells. Bars: A–L, N = 5 µm; M = 20 µm. $^{Ex\text{-}T}$ indicates ex-type culture; white arrowheads indicate conidium attachment points.

UCSB044473 = P-130 (MCA); ITS: GenBank KM519195); Ring 6 of FACE facility, Jun 2012, *R. Oono cas012(72a)* (dried culture: PUL F20087; cultures: NRRL 66670, UCSB044474, P-131 (MCA); ITS: GenBank KM519202); Ring 1 of FACE facility, Dec 2012, *R. Oono caw001(1.1.6b)* (dried culture: PUL F20088; cultures: NRRL 66671, UCSB044475, P-133 (MCA)).

Notes: Atractidochium hillariae produces minute, white, gelatinous sporodochia similar to those produced by *Leucogloea, Hobsonia, Infundibura*, and some species of *Helicogloea* in culture. Despite long incubation times (up to 2 mo), teleomorph characters were not observed for *A. hillariae*, although cultures produced abundant clamp connections indicating a dikaryotic state, as well as a few elongated epibasidium-like cells that failed to further mature (FIG. 3N). *Hobsonia* and the monotypic *Leucogloea* and *Infundibura* were described as anamorphic genera. These can easily be distinguished from *A. hillariae* by the distinctive coiled, multicelled conidia of *Hobsonia* species (Martin 1959), the verticillate conidiogenous cells and ovoid aseptate conidia that are diagnostic for *L. compressa* (Baker 1936), and the production of setae in the sporodochia and aseptate conidia with fan-shaped appendages in *I. adhaerens* (Kirschner 2004). Among aquatic hyphomycetes, *Atractidochium* can be compared with two basidiomycetous conidiomata formers. *Fibulotaeniella canadensis* also produces sporodochia, but it differs in producing mostly aseptate falcate, fusiform or sinuous conidia, and clamp connections in a repetitive fashion at the base of conidia (Marvanová and Bärlocher 1988). *Anguillomyces acadiensis* produces sporodochial-like bodies in the center of cultures, but unlike in *A. hillariae*, these become brown with age and exude pigments into the culture, in addition to producing primarily aseptate condia that are clamped when septate (Marvanová and Bärlocher 2000).

Baker (1946) roughly divided known species of *Helicogloea* into two groups, those with floccose basidiomata and those of the mucous-gelatinous type seen

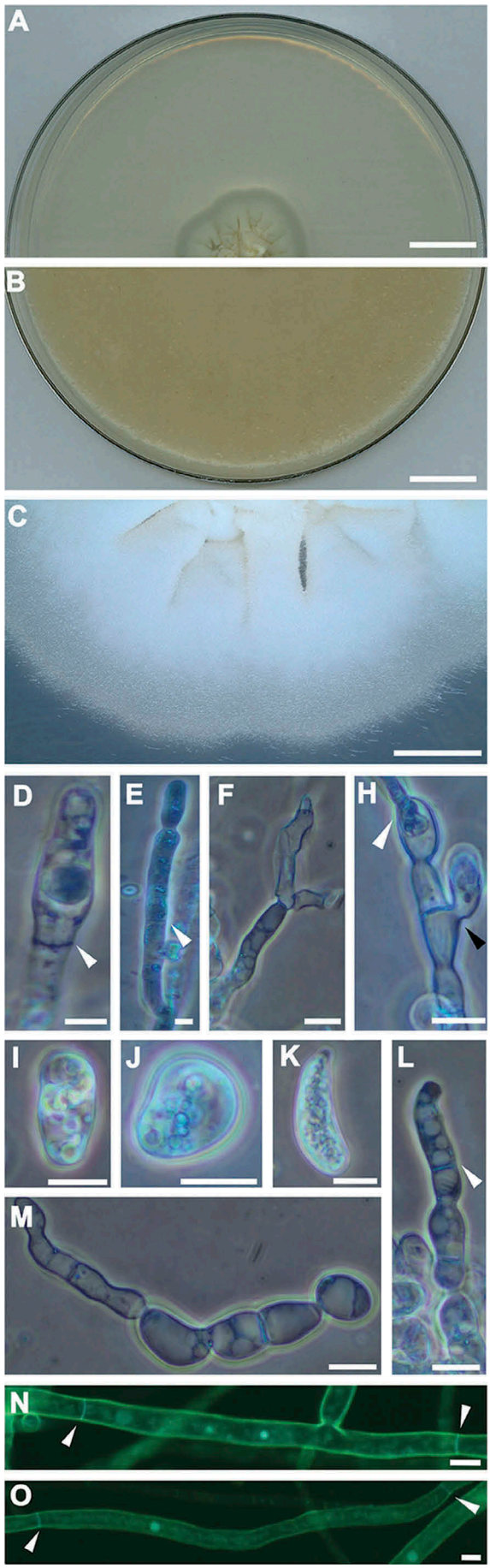


Figure 4. Colony morphology and micromorphology of *Pr. hameedii* PMI 927 [Ex-T]. A, B. Two-month-old colony on (A) PDA and (B) OA. C. Detail of the colony border on PDA. D. Terminal immature conidiogenous cell with basal simple septa. E. Simple septum generative hypha. F. Hypha with fibrillose wall remnants after cytoplasmic migration. H. Mature terminal and intercalary conidia (black arrowhead) in a spiral arrangement. I–K. Mature conidia. L. Mature conidiogenous cell. M. Immature conidia in chain formation. Culture conditions: in dark at 25 C. N–O. DAPI staining of hyphae showing cells with both single and binucleate cells. Bars: A, B = 1 cm; C = 200 µm; D–O = 5 µm. White arrowheads indicate septa. [Ex-T] indicates ex-type culture.

in *A. hillariae*. Of the various taxa described in *Helicogloea, H. pinicola* has also been associated with *Pinus* species, but, despite the species epithet, it is most commonly associated with a wide range of angiosperm substrata and is known only from its floccose, effused basidiomata (Baker 1936, 1946).

Most class 3 fungal endophytes (sensu Rodriguez et al. 2009) belong to the Ascomycota; members of Basidiomycota are much more infrequently isolated. Endophytic Basidiomycota may be more common in foliage than currently indicated, but biases in identification methods (e.g., primer bias, culturing vs. environmental sequencing) may mask their true diversity and abundance as endophytes. *Atractidochium hillariae* is a uniquely common Basidiomycota endophyte in *Pi. taeda* needles that remained unnamed and phylogenetically and morphologically uncharacterized for decades despite its abundance.

Proceropycnis hameedii Bonito, Urbina & Aime, sp. nov. FIG. 4
MycoBank MB824238

Typification: USA. OREGON: Along the Clatskanie River, a dried, inert culture isolated from roots of *Populus trichocarpa* (BESC 246 genotype), general vicinity of 46.15914°N, −123.25904°W, Jun 2012, *K. Hameed PMI 927* (**holotype** F21483). Ex-type culture: NRRL 66745 = PMI 927 (MSU) = P-140 (MCA). ITS: GenBank KF428609.

Etymology: "*hameedii*," named in honor of Dr. Khalid Hameed, who isolated the species, in recognition of his mentorship and lifetime of contributions to the international mycological community.

Colonies whitish to cream, raised, with wrinkled to sulcate center and entire margin; up to 2 cm diam after 2 mo incubation at 25 C on PDA (FIG. 4A, C); surface turning powdery after 2 mo of incubation at 4 C. *Hyphae* hyaline, smooth- and thin-walled, 1–2.5 µm diam; clamp connections not observed; cells uni- or binucleate (FIG. 4N–O). Conidiogenous hyphae hyaline, inflated, with abundant vacuoles; thin-walled, 3.5–5(–6) µm diam, with or without constricted septa. Conidiogenous cells terminal or intercalary, sinuous (FIG. 4L). Conidia 5–10(–11) × (8–)9–19(–23) µm, hyaline, globose to elongated, some with a droplet at the apex, 1-celled, thin-walled, somewhat truncate at the base, with abundant cytoplasmic vacuoles; holoblastic development, solitary or in chains; produced after 1 mo incubation at 4 C (FIG. 4I–K, M).

Habitat: Isolated from surface sterilized roots of *Populus trichocarpa*.

Distribution: Oregon, USA.

Material examined: USA. OREGON: Along the Clatskanie River, in Natural *Po. trichocarpa* stand, general vicinity of 46.15914°N, −123.25904°W, Jun 2012, *K. Hameed PMI 927*= (**holotype** F21483 as dried, inert, culture). Ex-type cultures: NRRL 66745, PMI 927, P-140 (MCA). ITS: GenBank KF428609).

Notes: Proceropycnis pinicola, the type and only other described species of *Proceropycnis*, was described from beetle galleries in a species of pine from China (Oberwinkler et al. 2006). In culture, it produces elongate pycnidial conidiomata with radiating hyphae around its ostiole, and ellipsoidal conidia from sympodial conidiogenous cells. In addition to the absence of conidiomata, *Pr. hameedii* is readily distinguished from *Pr. pinicola* in culture by the recalcitrant conidiation (conidia produced after 1 mo in the former and 2 wk in the latter at 24 C) and slow growth (reaching 20 mm after 1 mo in the former, vs. 39–46 mm after 2 wk in the latter at 24 C).

Neogloea Aime, gen. nov.
MycoBank MB823928

Typification: Neogloea variabilis (K. Wells) Aime.

Etymology: "Neo" (Latin), meaning new, referring both to the Neotropical location where the type species was found and for this new genus segregated from *Helicogloea.*

Fungi similar to *Helicogloea* sensu stricto, but phylogenetically distinct, belonging to Helicogloeaceae (Atractiellales, Atractiellomycetes, Pucciniomycotina, Basidiomycota). Basidiomata minute, gelatinous, grayish when fresh, crustose; basidia transversely septate, arising from saccate probasidia; clamp connections absent. Septal pores associated with crystal-containing microbodies rather than atractosomes.

Notes: At present, *Neogloea* contains only the type species. However, several unnamed "*Helicogloea*" or "Atractiellales" species (FIG. 1) represented by sequences in GenBank also belong here. These include one isolate, *Helicogloea* sp. TUB 020326, from roots of *Baccharis* (Asteraceae) (Reiss et al. 2014), and several from roots of neotropical orchids (Kottke et al. 2010). This may indicate a predominantly root-associated habit and Neotropical distribution for the genus. Two types of membrane-bound bodies are associated with the septal pore in Atractiellomycetes: (i) microbodies that act as plugs during cellular disruption, or (ii) atractosomes of unknown function (Weiss et al. 2004; Bauer et al. 2006). *Neogloea variabilis* and an undetermined *Neogloea* species (as *Helicogloea* sp. isolate RJB 6478-5; McLaughlin et al. 2017) and the orchid-associated species of *Neogloea* (as Atractiellales spp.; Kottke

et al. 2010) all have crystal-containing microbodies rather than atractosomes, which are otherwise present in all other examined members of the class, except *Saccoblastia farinacea* (Weiss et al. 2004).

Neogloea variabilis (K. Wells) Aime, comb. nov.
MycoBank MB823929

≡ *Helicogloea variabilis* K. Wells, Mycol Res 94:835. 1990. Basionym.

Notes: Neogloea variabilis was isolated from decaying wood in Brazil (Wells 1990). Although the habit and fructifications of *N. variabilis* are reminiscent of *H. lagerheimii*, basidial ontogeny is distinct because during development of some basidia a portion of the probasidium is converted into hypobasidial segments (Wells 1990).

Bourdotigloea Aime, gen. nov.
MycoBank MB824239

Typification: Bourdotigloea vestita (Bourdot & Galzin) Aime.

Etymology: Honoring Hubert Bourdot, one of the original authors of the type species.

Fungi similar to *Helicogloea* sensu stricto, but phylogenetically distinct, belonging to Phleogenaceae (Atractiellales, Atractiellomycetes, Pucciniomycotina, Basidiomycota). Basidiomata minute, gelatinous, grayish to hyaline when fresh, effused; basidia transversely septate; clamp connections absent.

Bourdotigloea vestita (Bourdot & Galzin) Aime, comb. nov.
MycoBank MB824240

≡ *Platygloea vestita* Bourdot & Galzin, Bull Soc Mycol Fr 39:261. 1924 ("1923"). Basionym.

≡ *Helicogloea vestita* (Bourdot & Galzin) P. Roberts, Windahlia 22:19. 1997 ("1995–1996").

≡ *Achroomyces vestitus* (Bourdot & Galzin) Wojewoda, Flora Polska, Grzyby (Mycota) 8:251. 1977.

DISCUSSION

Atractidochium hillariae is a sterile fungal endophyte abundantly cultured from the mature needles of *Pi. taeda* trees (FIG. 2A), and well represented in cloned environmental sequences of the same host. In Oono et al. (2015), this species represented 30.7% of isolates, making it the most frequently cultured endophyte in that study; it was also the third-most frequently sequenced clone. In Arnold et al. (2007), *A. hillariae* was the third-most frequently cultured endophyte species of *Pi. taeda*, represented by 7 of 145 isolates. Despite the frequency of isolation, the exact identity and phylogenetic placement of this isolate remained to be determined in prior studies. Of note is the fact that BLAST queries against GenBank and the UNITE (Kõljalg et al. 2013) databases do not produce any significant matches other than to the previously generated sequences of *A. hillariae* produced by Oono et al. (2015). However, an apparent sister species to *A. hillariae* was recently recovered from environmental sequencing studies of fungal endophytes of Bishop pines (*Pi. muricata*) in California (R. Oono et al. unpublished), suggesting that other species of *Atractidochium* are associated with pine species across the USA.

In contrast, our isolate of *Proceropycnis hameedii*, isolated from sterilized roots of *Populus trichocarpa*, is the only known strain of this species, despite ongoing long-term studies of fungal associates of *Populus* (Bonito et al. 2016). In both cases, isolates formed only somatic hyphae and required >1 mo incubation before sporulating structures were developed. The lack of diagnostic characters combined with relative rarity in the case of *Pr. hameedii* may indicate a potential for these and similar species to be overlooked or discarded in culture-based and high-throughput sequence–based studies.

Atractiellomycetes are a recently recognized class of inconspicuous Pucciniomycotina united by a unique subcellular character of precisely arranged microfilaments that cross-link the endoplasmic reticulum and mitochondria, called microscala (also known as symplechosomes) (McLaughlin 1990; Bauer et al. 2006). Although thus far no yeast-like or dimorphic fungi are known in this lineage, many species possess the yeast-like trait of basidiospores that are capable of secondary budding. Detailed ultrastructural analyses indicate a close affinity with Pucciniomycetes (McLaughlin et al. 2017), although resolution of the deeper nodes of Pucciniomycotina remains elusive (Aime et al. 2014).

Atractiellomycetes contains a single order, Atractiellales, but taxonomy at the subordinal level has been somewhat in flux (e.g., Oberwinkler and Bandoni 1982 and references therein). Our phylogenetic analyses indicate that the currently sequenced diversity can be circumscribed within three family-level lineages: *Helicogloeaceae* (containing *Helicogloea* sensu stricto, *Hobsonia, Infundibura, Leucogloea*, and *Neogloea*), *Hoehnelomycetaceae* (*Atractiella, Basidiopycnis*, and *Proceropycnis*), and *Phleogenaceae* (*Bourdotigloea, Phleogena, Atractidochium*, and *Saccoblastia farinacea*) (FIG. 1). *Leucogloea* and *Hobsonia*—previously classified as incertae sedis hyphomycetes (Bauer et al. 2006)—are resolved with other sporodochium-forming species within the *Helicogloeaceae*.

Species of *Helicogloea* are united by the production of minute gelatinous basidiocarps typically observed fruiting from decaying pinecones, wood, or leaf culms

(Kirschner 2004) and the production of characteristic sac-like probasidia, a characteristic also shared with members of *Saccoblastia* (Baker 1936; Bandoni 1956). However, as noted by Kirschner (2004), although all produce sporodochial anamorphs, the conidia of these species can be quite variable, from arthrosporic in *H. aurea* to globose with secondary spores in *H. lagerheimii*. Further phylogenetic analyses may show that these distinctions in conidial production are indicative of generic-level lineages.

Species of Atractiellomycetes are infrequently collected or isolated (Toome-Heller 2016); most often they are collected on woody or herbaceous substrata and presumed to be saprobic (Baker 1936; Kirschner 2004; Bauer et al. 2006). However, the growing body of evidence from environmental sequencing and culture studies of plant endobiomes, especially of roots, provides an emerging picture of these fungi as primarily symbiotic associates of a broad range of host plants, ranging from orchids to conifers and broad-leaved plants. The better-known fungal endophyte of *Pi. taeda, Lophodermium australe* (Leotiomycetes; Arnold et al. 2007; Oono et al. 2014), produces ascocarps on dead needles of their hosts, and other teleomorphs of Atractiellales have been observed fruiting, for example, from pine cones. Hence, a closer inspection of dead pine needles may reveal fruiting bodies of *A. hillariae* in nature. The superficial resemblance of *A. hillariae* conidia and sporodochia to some aquatic hyphomycetes such as *Anguillomyces acadiensis* may also indicate a more complex life history strategy for these fungi that includes an aquatic dispersal stage (Selosse et al. 2008).

ACKNOWLEDGMENTS

We are grateful to Shannon Newerth, John Klimek, Merje Toome-Heller, Alejandro Rojas, Khalid Hameed, and Rytas Vilgalys for useful discussions, assistance in culture maintenance and generation of some of the sequence data used in this study and two anonymous reviewers for their insight and suggested improvements to the manuscript.

FUNDING

This work was supported by the USDA National Institute of Food and Agriculture Hatch project 1010662, and funded in part by NSF DEB 1502887 & NSF DEB 0732968. GB acknowledges Michigan State University AgBioResearch NIFA project MICL02416 and Plant-Microbe Interfaces program (DE-AC05-00OR22725) for support.

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