MYCOLOGIA
2018, VOL. 110, NO. 5, 985–995
https://doi.org/10.1080/00275514.2018.1511339

Taylor & Francis
Taylor & Francis Group

Check for updates

A global view of *Gyroporus*: molecular phylogenetics, diversity patterns, and new species

Naveed Davoodian[a,b], Sarah E. Bergemann[c], Kentaro Hosaka [d], Olivier Raspé [e], Neale L. Bougher[f], Nigel A. Fechner[g], Terry W. Henkel[h], Matteo Gelardi[i], Kasem Soytong[j], Arooj Naseer[k], Beatriz Ortiz-Santana [l], Timothy J. Baroni[m], Eiji Nagasawa[n], Matthew E. Smith [o], and Roy E. Halling [a]

[a]Institute of Systematic Botany, The New York Botanical Garden, Bronx, New York 10458; [b]The Graduate Center, City University of New York, 365 5th Avenue, New York, New York 10016; [c]Department of Biology, Middle Tennessee State University, P.O. Box 60, Murfreesboro, Tennessee 37132; [d]Department of Botany, National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan; [e]Botanic Garden Meise, Nieuwelaan 38, B-1860 Meise, Belgium; [f]Department of Biodiversity, Conservation and Attractions, Western Australian Herbarium, Locked Bag 104, Bentley Delivery Centre, Western Australia 6983, Australia; [g]Queensland Herbarium, Mount Coot-tha Road, Toowong, Brisbane, Queensland 4066, Australia; [h]Department of Biological Sciences, Humboldt State University, Arcata, California 95521; [i]Via Angelo Custode 4a, I-00061 Anguillara Sabazia, Italy; [j]Faculty of Agricultural Technology, King Mongkut's Institute of Technology, Ladkrabang, Bangkok 10520, Thailand; [k]Centre for Undergraduate Studies, University of the Punjab, Lahore, Pakistan; [l]Center for Forest Mycology Research, Northern Research Station, US Forest Service, One Gifford Pinchot Drive, Madison, Wisconsin 53726; [m]Department of Biological Sciences, State University of New York–College at Cortland, Cortland, New York 13045; [n]Tottori Mycological Institute, 211 Kokoge, Tottori 689-1125, Japan; [o]Department of Plant Pathology, University of Florida, Gainesville, Florida 32611

ABSTRACT

Gyroporus (Gyroporaceae, Boletales) is a highly diverse genus of poroid ectomycorrhizal mushrooms with a nearly worldwide distribution. Previous attempts to unravel the diversity within this genus proved difficult due to the presence of semicryptic species and ambiguous results from analysis of ribosomal RNA markers. In this study, we employ a combined morphotaxonomic and phylogenetic approach to delimit species and elucidate geographic and evolutionary patterns in *Gyroporus*. For phylogenetic analyses, the protein-coding genes *atp6* (mitochondrial adenosine triphosphate [ATP] synthase subunit 6) and *rpb2* (nuclear second largest subunit of RNA polymerase II) were selected based on their utility in studies of Boletales. We infer several distinct clades, most notably one corresponding to *G. castaneus* as a speciose Northern Hemisphere group, another unifying *G. cyanescens* and like entities, and a third group unifying *G. longicystidiatus* and a New World sister species. Also notable is the recovery of a sister relationship between the *cyanescens* and *longicystidiatus* clades. We formally describe five new species of *Gyroporus*, outline a number of provisional species, and briefly discuss distributional patterns. This study provides an important scaffold for future work on this well-known but poorly understood genus of fungi.

ARTICLE HISTORY
Received 4 September 2017
Accepted 10 August 2018

KEYWORDS
atp6; Boletales; ectomycorrhizal fungi; *rpb2*; systematics; 5 new taxa

INTRODUCTION

Gyroporus Quélet (Gyroporaceae, Boletales) is a genus of ectomycorrhizal boletes with representatives on every major continent except Antarctica. These fungi are strongly implicated as symbionts with an array of ectotrophic plants, including species of Pinaceae, Fagaceae, Myrtaceae, Fabaceae, and Phyllanthaceae, among others (Singer et al. 1983; Agerer 1999; Raidl et al. 2006; Ramanankierana et al. 2007; Yuwa-amornpitak 2009). Taxa within this group have been known since the 18th century (Bulliard 1787, 1788). *Gyroporus* sensu Quélet (1886) included some members that have since been excluded (Patouillard 1900; Singer 1945). Its modern circumscription encompasses boletes with a yellow spore print, clamp connections, and circumferential to variously arranged (i.e., not longitudinal) stipe hyphae (Singer 1986; Watling 2008). The monophyly of this genus has been corroborated in previous studies (Binder and Bresinsky 2002; Wilson et al. 2011, 2012).

Despite the distinctiveness of this genus, its wide geographic distribution, and long formal history, much of the diversity of *Gyroporus* has yet to be elucidated. Previous phylogenetic studies on *Gyroporus* emphasized loci (i.e., ribosomal RNA markers) that yielded equivocal results at the infrageneric level (e.g., Wilson et al. 2011; Das et al. 2017). Imprecise taxonomy has also been a hindrance to progress on this genus, further confounded by the presence of many

CONTACT Naveed Davoodian ndavoodian@nybg.org
Supplemental data for this article can be accessed on the publisher's Web site.
© 2018 The Mycological Society of America

Published online 10 Oct 2018

semicryptic species. Collections from various parts of the world have generally been interpreted in relation to European species concepts (i.e., *G. cyanescens, G. castaneus*) or, to a lesser extent, North American ones (i.e., *G. subalbellus*). Here, we present a global phylogenetic overview of *Gyroporus*. Major results include the delimitation of multiple species and a sister relationship between the *cyanescens* and *longicystidiatus* clades. Five new species are described, three of which are known only from Australia and are closely related to *G. cyanescens*.

MATERIALS AND METHODS

Morphology, vouchers, and names.—Micromorphological, macromorphological, and ecological notes were made from fresh collections and herbarium material. Slides for microscopic study were prepared with water or 3% potassium hydroxide. Pileipellis and hymenium sections were studied. Stipitipellis morphology was observed but is not reported here, since it is highly variable and of limited diagnostic value in this genus. Q represents the mean length/width quotient of the spores. Specimens in this study are identified by field collection numbers and/or herbarium accession numbers (e.g., NY, TNS, MICH). Unless otherwise noted, the collections used here are housed at the following herbaria: NY, BR, TNS, TMI, MICH, and CFMR. Some collections may have duplicates housed at other institutions (e.g., CORT, FLAS, BRI, HMAS, MFLU, SDBR-CMU). Names that do not appear in MycoBank or are not formally described represent provisional names and are indicated as nom. prov.; these need additional study. Names given as *Gyroporus* sp. are based on two or fewer specimens.

The following geographic abbreviations are utilized in figures: USA (United States of America) with standard abbreviations for states (VT, FL, etc.), COL (Colombia), CR (Costa Rica), BLZ (Belize), CHN (China), JP (Japan), SKOR (South Korea), THAI (Thailand), PK (Pakistan), IT (Italy), BELG (Belgium), CMRN (Cameroon), SEN (Senegal), QLD (Queensland, Australia), and WAUS (Western Australia, Australia). Exemplars labeled with "gb" in figures were acquired from GenBank.

DNA extraction, amplification, and DNA sequence alignment.—Phylogenetic analyses included partial sequences of two protein-coding genes often used for systematic studies of the Boletales (Kretzer and Bruns 1999; Dentinger et al. 2010): the mitochondrial gene *atp6*, encoding the sixth subunit of adenosine triphosphate (ATP) synthase, and the nuclear gene *rpb2*, encoding the second largest subunit of RNA polymerase II. A list of *Gyroporus* specimens/sequences used in this study is provided (see SUPPLEMENTARY TABLE 1). DNAs were extracted from homogenized tissues of dried basidiocarps based on extraction protocols outlined in Hosaka and Uno (2011) and Kluting et al. (2014). DNA sequences were aligned, and new primers designed for *Gyroporus* using the default parameters in PRIMER 3 (Rozen and Skaletzky 1999). The partial mitochondrial *atp6* gene was polymerase chain reaction (PCR)-amplified with ATP6-1 (or ATP6-3) and ATP6-2 (Kretzer and Bruns 1999) or using primers designed for specific amplification of *Gyroporus* (ATP6gyro-F1: 5′-TTGAAGTTACAAGTTTATTAAG-3′ and ATP6gyro-R1: 5′-CTGCTAATTCTARSCCTGTA-3′). The partial *rpb2* gene was initially PCR-amplified with degenerate primer sets fRPB2-5F/bRPB2-7R, fRPB2-5F/bRPB2-7R2 (Liu et al. 1999), and bRPB2-6F/bRPB2-7.1R (Matheny 2005). PCR amplification of *rpb2* also included primers designed specifically for the amplification of *Gyroporus* (rpb2gyro-F1: 5′-AAGAAGCTTACYAGGGATGT-3′ and rpb2gyro-R1: 5′-YCCYTTRACSARNTGYTCC-3′). A total of 55 *atp6* and 38 *rpb2* sequences for *Gyroporus* were generated and have been deposited in GenBank under accession numbers MF818144–MF818236 (SUPPLEMENTARY TABLE 1).

PCR cycling conditions for *atp6* included an initial denaturation at 95 C for 5 min, followed by 40 cycles of 95 C for 30 s, 45 C for 90 s, and 72 C for 2 min, and a final extension at 72 C for 10 min. The cycling conditions for *rpb2* used an initial denaturation at 95 C for 5 min, followed by 35 cycles of 95 C for 30 s, 58 C for 1 min, and 72 C for 1 min, and a final extension at 72 C for 7 min.

Phylogenetic analyses.—Alignments of *atp6* and *rpb2* were performed using MUSCLE via the European Molecular Biology Laboratory European Bioinformatics Institute (EMBL-EBI) Web site (http://www.ebi.ac.uk/). Introns were removed from the *rpb2* alignment prior to phylogenetic analysis. The total lengths of the alignments for phylogenetic analyses were 710 and 922 bp for *atp6* and *rpb2*, respectively. Bayesian analysis for each gene was performed with MrBayes 3.2.6 (Ronquist et al. 2012) on the CIPRES Science Gateway 3.1 (Miller et al. 2010) using a generalized time-reversible model with gamma-distributed rate variation between sites. The priors utilized were defaults in MrBayes. Metropolis-coupled Markov chain Monte Carlo analyses were run with default settings (nruns = 2, nchains = 4, swapfreq = 1, nswaps = 1) for 20 million generations, which was

sufficient for convergence (convergence diagnostic: 0.0015–0.0023). The proportion of samples discarded as burn-in was 25%. A secondary Bayesian analysis was conducted for *atp6* with a reduced number of terminals (analysis identical except the alignment was reduced from 59 to 36 terminals). Maximum likelihood (ML) analyses were conducted, following procedures outlined in Kluting et al. (2014), although *atp6* and *rpb2* were analyzed separately, not combined. ML analyses were conducted using 1000 bootstrap replications. Alignments are deposited in TreeBASE (study 21499).

RESULTS

Bayesian analysis of *atp6* sequences (FIG. 1) inferred two major clades of *Gyroporus*: one unifying *G. cyanescens, G. longicystidiatus*, and like entities (including the lineage represented by OR182 and BOS472), the other unifying *G. castaneus, G. mcnabbii*, sp. nov., and the clade consisting of *G. naranjus*, nom. prov., and African exemplars (TH9913, Thoen7634). The secondary Bayesian *atp6* analysis recovered the same major relationships and increased the posterior probabilities at several nodes, two of which are included and indicated with asterisks (FIG. 1). Bayesian analysis of *rpb2* sequences (FIG. 2) inferred nested clades of *Gyroporus* and also recovered the clade unifying *G. cyanescens, G. longicystidiatus*, and like entities (including the lineage represented by OR182 and REH8805). The clade unifying *G. cyanescens, G. longicystidiatus*, and like entities is denoted as clade b in the phylograms (FIGS. 1, 2). In the *rpb2* analysis, the *G. castaneus, G. mcnabbii*, sp. nov. (+ *G. subalbellus* + OR801, OR870), and *G. naranjus*, nom. prov. (+ TH9913, Thoen7634), clades were each maintained as distinct similar to the *atp6* analysis, but unlike the *atp6* analysis they did not together form a larger clade distinct from clade b. The *G. castaneus* clade is denoted as clade a in the phylograms (FIGS. 1, 2). ML analyses were comparable to the Bayesian analyses, recovering the same species and key clades, although in the ML *rpb2* analysis *G. mcnabbii*, sp. nov., *G. subalbellus*, and OR801/OR870 were not recovered together in a single clade as in the Bayesian *rpb2* analysis.

TAXONOMY

Gyroporus australiensis Davoodian, Fechner & Halling, sp. nov. FIGS. 3–5
MycoBank MB822346

Typification: AUSTRALIA. QUEENSLAND: Great Sandy National Park, Fraser Island, near Lake McKenzie, 25°27′50″S, 153°04′14″E, 97 m, 18 May 2011, *R.E. Halling 9501* (**holotype** BRI, **isotype** NY).

Diagnosis: Differs from other species of *Gyroporus* in Australia by the brown to light brown pileus and gradual cyanescent reaction that is present in the flesh and on the pores but absent from the tubes.

Etymology: *australiensis* (Latin), in reference to the place (Australia) of origin.

Pileus 3.5–8.5 cm broad, convex to plane, margin even to slightly projecting, sometimes slightly incurved when young, dry, matted fibrillose to tomentose to squamulose/subscaly, brown to cinnamon to light brown to light peach brown (brown in dried condition), often over whitish to yellowish background. Flesh white, slowly bluing (pale blue very gradually to darker blue), although rarely rapidly and intensely cyanescent (REH 9492), with mild odor and taste. Tubes adnexed, white to off-white to yellowish at first, light and clear yellow to yellowish white to creamish with age, generally not cyanescent, although sometimes bluing slightly in areas, with pores concolorous and cyanescent (rarely intensely so: REH 9492). Stipe 2–7 cm long, 0.7–2 cm broad, straight or curved, subequal to subclavate, sometimes ventricose, sometimes pinched or tapered at base, dry, finely subtomentose to matted tomentose, sometimes appearing with annular zone, subconcolorous to concolorous with pileus over white to off-white to yellowish background, slowly bluing when injured, pithy at first, becoming cavernous-hollow.

Spores (6.5–)7.2–9(–11.9) × (4–)4.5–5.4(–6.3) µm (Q = 1.8), smooth, yellow-hyaline, ellipsoid to subovoid to subreniform to reniform (the larger spores sometimes elongate), sometimes tapered toward hilar end, often appearing apiculate. Basidia 23–31 × 8–12 µm, clavate. Cystidia inconspicuous or absent (appearing like basidioles or subclavate to subcylindrical elements). Pileipellis of elongated, repent hyphae that are usually clamped and faintly tinged light melleous brown, (6–)8–10(–14) µm wide. Clamp connections present.

Ecology and distribution: Gregarious to scattered to solitary. On sand in sclerophyll habitat with species of Myrtaceae and Casuarinaceae. Known only from Fraser Island and nearby Cooloola. Mar to Jun.

Other specimens examined: AUSTRALIA. QUEENSLAND: Great Sandy National Park, Cooloola, Freshwater Road, 25°57′04.0″S, 153°08′06.0″E, 150 m, 25 May 2011, *R.E. Halling 9559* (NY, BRI); Fraser Island, 4.8 km along Woralie Road, 25°13′07.0″S, 153°13′22.8″E, 171 m, 18 May 2010, *R.E. Halling 9312* (NY, BRI); Road from Eurong to Central Station, 25°30′01.1″S, 153°06′07.2″E, 82 m, 17 May 2011, *R.E. Halling 9492* (NY, BRI); Cornwell's Road, 25°24′40.3″S, 153°02′42.0″E, 68 m, 10 Jun 2009, *R.E. Halling 9226* (NY, BRI); 1–2 km SE of Kingfisher Bay, 25°23′38.8″S, 153°01′48.0″E, 30–35 m, 9

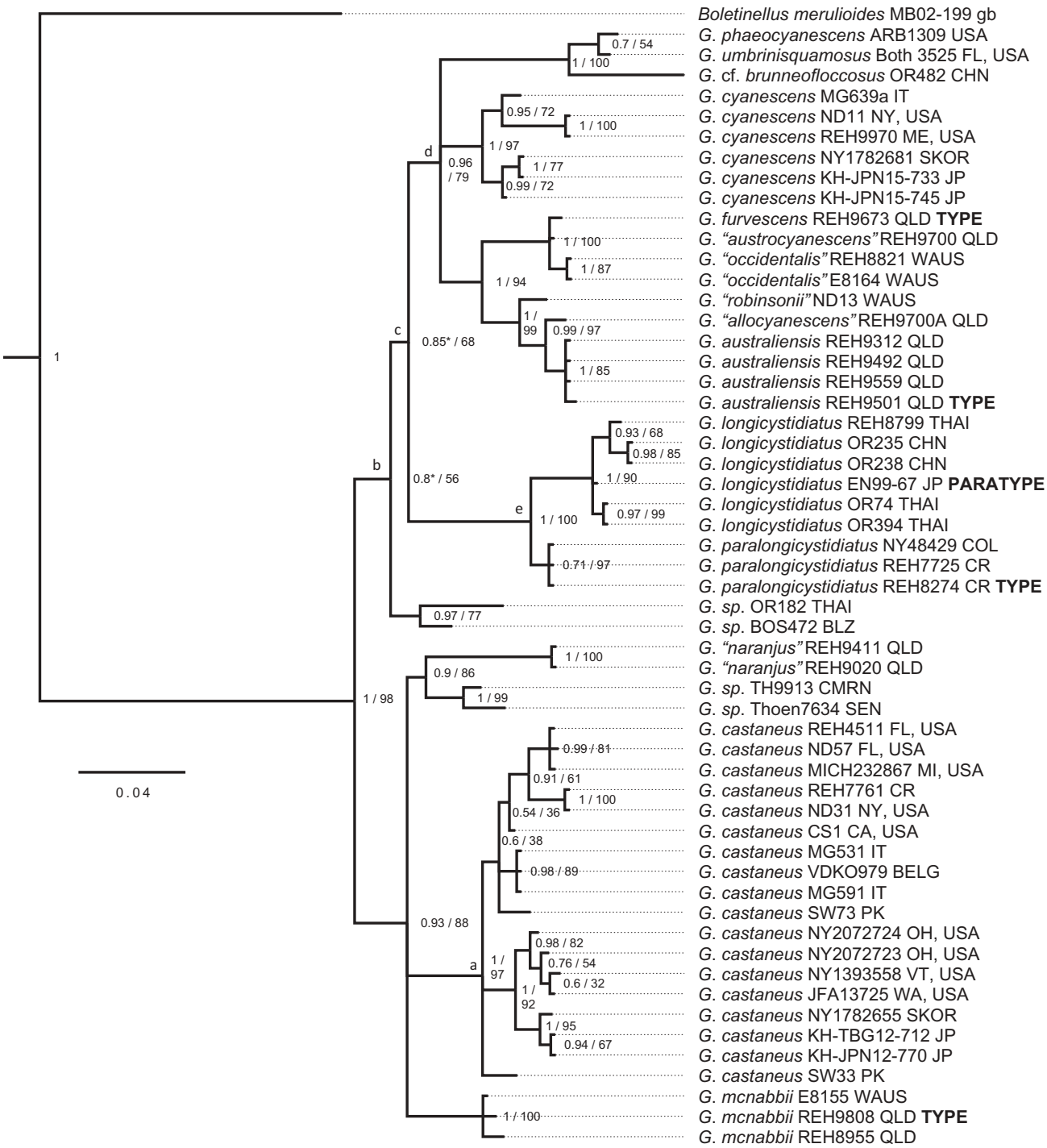

Figure 1. Consensus phylogram from Bayesian analysis of *atp6* sequences. Bayesian posterior probabilities are at the nodes (values with asterisks are from the secondary analysis). Bootstrap values from ML analysis of *atp6* sequences are included after the posterior probabilities. Included in the analysis but cropped from this image are outgroup sequences for *Hygrophoropsis aurantiaca* (GLM45936) and *Suillus spraguei* (MB03-93) acquired from GenBank and *Suillus umbonatus* (REH8715). Scale bar shows substitutions/site. Letters at nodes indicate the following clades: a = *G. castaneus* clade; b = clade unifying the *cyanescens* clade, *longicystidiatus* clade, and a clade of undescribed species including OR182; c = *cyanescens* clade + *longicystidiatus* clade; d = *cyanescens* clade, containing *G. cyanescens* and related clades, including a clade of new species from Australia; e = *longicystidiatus* clade, containing *G. longicystidiatus* and *G. paralongicystidiatus*, sp. nov.

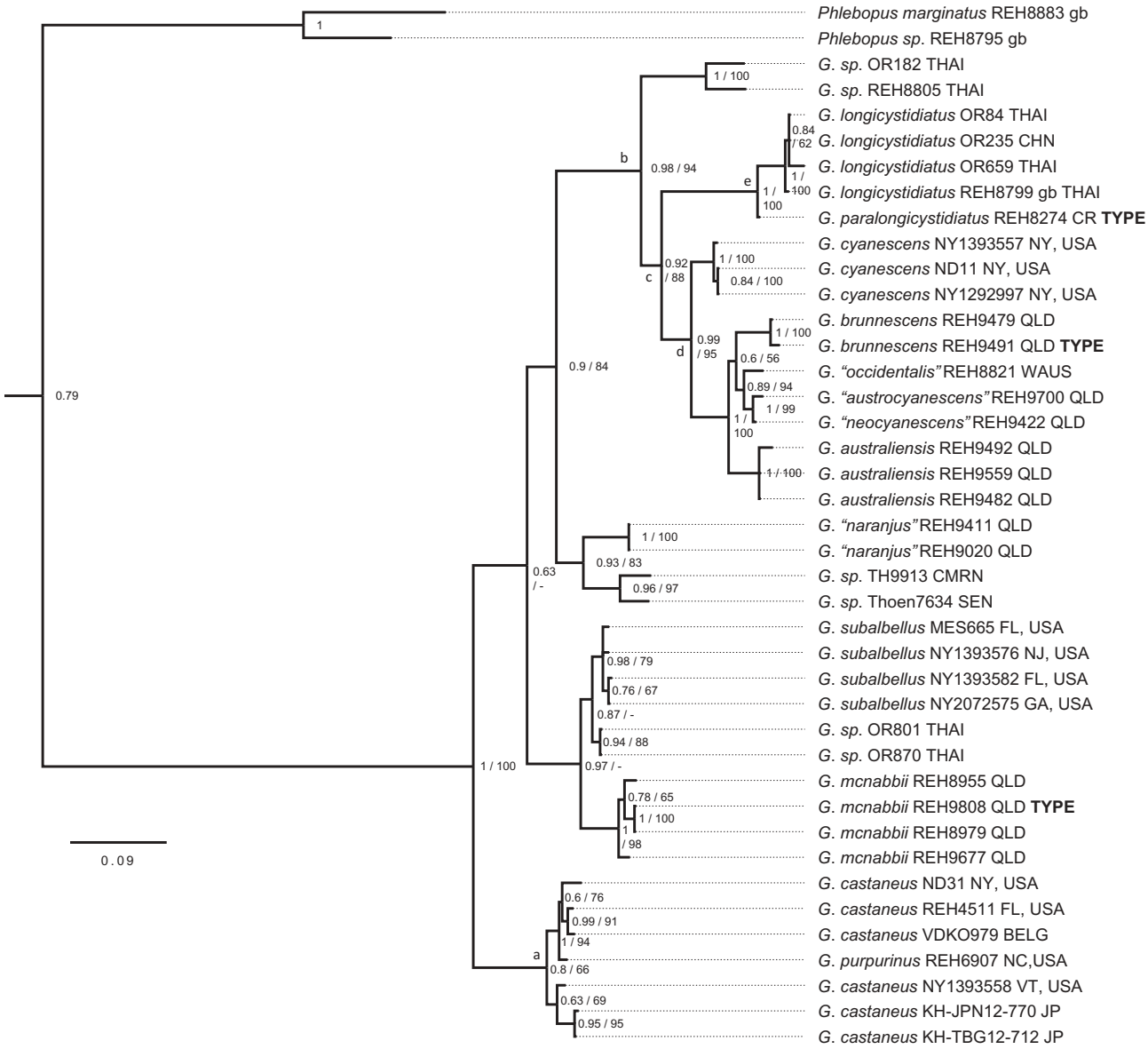

Figure 2. Consensus phylogram from Bayesian analysis of *rpb2* sequences. Bayesian posterior probabilities are at the nodes. Bootstrap values from ML analysis of *rpb2* sequences are included after the posterior probabilities. Included in the analysis but cropped from this image are outgroup sequences for *Hygrophoropsis aurantiaca* (MB03-127) and *Suillus spraguei* (MB03-002) from GenBank. Scale bar shows substitutions/site. Letters at nodes indicate the following clades: a = *G. castaneus* clade; b = clade unifying the *cyanescens* clade, *longicystidiatus* clade, and a clade of undescribed species including OR182; c = *cyanescens* clade + *longicystidiatus* clade; d = *cyanescens* clade, containing *G. cyanescens* and relatives; e = *longicystidiatus* clade, containing *G. longicystidiatus* and *G. paralongicystidiatus*, sp. nov.

Mar 2011, *R.E. Halling 9482* (NY, BRI); Road from Eurong to Central Station, 25°30′14.0″S, 153°06′57.6″E, 30 m, 6 Mar 2011, *R.E. Halling 9460b* (NY, BRI); Cornwell's Road, 25°24′06.8″S 153°01′51.6″E, 48 m, 24 May 2010, *R.E. Halling 9361* (NY, BRI); track from Central Station to Lake Birrabeen, 25°29′42.7″S, 153°03′54.0″E, 138 m, 4 Jun 2009, *R.E. Halling 9151* (NY, BRI).

Comments: Gyroporus australiensis is the only cyanescent *Gyroporus* known so far from Australia with a distinctly brown pileus, which is especially apparent in dried condition. Many other *G. cyanescens*–like entities in Australia exhibit a straw yellow pileus suggestive of "classical" *G. cyanescens* of Europe and the Northern Hemisphere (e.g., *G. austrocyanescens*, nom. prov. [FIGS. 1, 2], *G.*

Figure 3. *Gyroporus australiensis* (holotype R.E. Halling 9501). Photo: Roy Halling.

Figure 4. *Gyroporus australiensis* (R.E. Halling 9361). The annular zone/velar ridge is apparent in the basidiocarp on the left. Photo: Roy Halling.

Figure 5. *Gyroporus australiensis* (R.E. Halling 9312). Photo: Roy Halling.

Figure 6. *Gyroporus brunnescens* (R.E. Halling 9414). Photo: Roy Halling.

allocyanescens, nom. prov. [FIG. 1], and *G. neocyanescens*, nom. prov. [FIG. 2]).

Gyroporus brunnescens Davoodian, Fechner & Halling, sp. nov. FIG. 6
MycoBank MB822347

Typification: AUSTRALIA. QUEENSLAND: Great Sandy National Park, Fraser Island, road from Eurong to Central Station, 25°30′01″S, 153°06′07″E, 82 m, 17 May 2011, *R.E. Halling 9491* (**holotype** BRI, **isotype** NY).

Diagnosis: Differs from other species of *Gyroporus* by exhibiting a distinct brown oxidation reaction in exposed tissues.

Etymology: brunnescens (Latin), in reference to the brown oxidation reaction where tissues are exposed.

Pileus 2–6 cm broad, convex to plano-convex to uplifted, dry, heavily appressed fibrillose to tomentose to felty, sometimes subsquamulose, yellowish brown to grayish yellow to light yellow, staining light brown or unstaining. Flesh white to pale yellow, staining pale pinkish brown to dark brown, with mild odor and taste. Tubes adnexed to adnate, white to whitish to very pale greenish yellow to yellow, staining pale pinkish brown to brown (especially on pores). Stipe 1.5–6 cm long, 0.9–2.5 cm broad, equal, tapered at base, nearly concolorous with pileus, although white background often more apparent on stipe, dry, appressed fibrillose to finely matted subfelty with matted squamules at base, sometimes appearing with annular zone, staining brown, pithy becoming chambered.

Spores (6.4–)8–11.2(–12) × (4–)4.4–4.8(–5.6) µm (Q = 2.05), smooth, yellow-hyaline to nearly so, elliptical to subelliptical to subreniform to subovoid, generally elongate, sometimes appearing apiculate. Basidia 28–33 ×

8–10 µm, clavate. Cheilocystidia 32–48 × 9–11 µm, versiform (ventricose, fusiform, or tapering toward the apex). Undifferentiated hymenial elements 27–30 × 8–9 µm, cylindrical to clavate. Pileipellis of repent, tangled elements, elements sometimes appearing organized in bundles. Clamp connections present.

Ecology and distribution: Gregarious to scattered to solitary in association with species of Myrtaceae and Casuarinaceae. Known from localities in Great Sandy National Park and Davies Creek National Park, Queensland. Feb to May.

Other specimens examined: AUSTRALIA. QUEENSLAND: Davies Creek National Park, Davies Creek Road, 17°01′36.1″S, 145°36′03.6″E, 713 m, 11 Mar 2007, *R.E. Halling 8908* (NY, BRI); Great Sandy National Park, Cooloola, Freshwater Road, 25°56′37.0″S, 153°07′22.8″E, 154 m, 23 May 2011, *R.E. Halling 9545* (NY, BRI); Fraser Island, road from Pile Valley to Lake McKenzie, 25°28′12.0″S, 153°04′12.0″E, 90 m, 9 Mar 2011, *R.E. Halling 9479* (NY, BRI); Wanggoolba Road west of Central Station, 25°28′41.5″S, 153°02′49.2″E, 54 m, 15 Feb 2011, *R.E. Halling 9414* (NY, BRI).

Comments: This is the only *Gyroporus* known to have a distinct brown oxidation reaction with no cyanescent stage. It is phylogenetically embedded in the clade unifying *G. cyanescens* and relatives.

Gyroporus furvescens Davoodian & Halling, sp. nov. FIGS. 7, 8

MycoBank MB822348

Typification: AUSTRALIA. QUEENSLAND: D'Aguilar National Park, vicinity of Mt. Glorious, Maiala area walking tracks, 27°20′00″S, 152°45′48″E, 680 m, 8 Mar 2012, *R. E. Halling 9673* (**holotype** BRI, **isotype** NY).

Diagnosis: Differs from other cyanescent species of *Gyroporus* in Australia by the oxidation reaction that is deep blue then nearly black and unique appearance in dried condition (surfaces blackish to deep blue to sometimes purplish).

Figure 7. *Gyroporus furvescens* (holotype R.E. Halling 9673). Photo: Roy Halling.

Figure 8. *Gyroporus furvescens* (R.E. Halling 9807). Photo: Roy Halling.

Etymology: *furvescens* (Latin), becoming dark, in reference to the deep blue to nearly black oxidative reaction.

Pileus 2–12 cm broad, convex to plano-convex, dry, appressed squamulose-scaly to flattened lanose to matted fibrillose, yellow-white to yellow-brown to cinnamon brown, quickly cyanescent (eventually to nearly black or very dark brown). Flesh white, quickly and intensely deep blue sometimes with lilac and/or violet, with mild odor and taste. Tubes adnexed, whitish at first, becoming pale yellow, with pores likewise and only the pores cyanescent, although tubes occasionally patchily cyanescent. Stipe 3–9 cm long, 0.8–3 cm broad, subclavate to clavate, dry, often with annular zone (sometimes very faint to nearly absent), finely matted pubescent above, appressed fibrillose to appressed sublanose below, yellowish white above, brownish below, quickly cyanescent (eventually to nearly black), chambered.

Spores (5.6–)6.8–8.1(–9) × (3.2–)3.4–4.7(–4.8) µm (Q = 1.71), smooth, yellow-hyaline, subreniform to reniform to ellipsoid to subovoid, sometimes appearing apiculate. Basidia 30–33 × 9.5–12 µm, clavate. Cheilocystidia 22–24(32) × (5)5.5–6.5(9.5) µm, fusoid. Undifferentiated hymenial elements 16–24 × 3–8 µm, clavate to cylindrical. Pileipellis of elongated, repent elements; elements compactly to loosely bundled; terminal cells with more or less rounded or tapering ends. Clamp connections present.

Ecology and distribution: Solitary or cespitose on soil with species of *Eucalyptus* and *Lophostemon*. Thus far known only from three collections in southeastern Queensland. Feb to Mar.

Other specimens examined: AUSTRALIA. QUEENSLAND: D'Aguilar National Park, vicinity

of Mt. Glorious, 27°17′45.2″S, 152°43′40.8″E, 601 m, 20 Feb 2013, *R.E. Halling 9807* (NY, BRI); Main Range National Park, Cunningham's Gap, 28°02′58.9″S, 152°23′38.4″E, 760–770 m, 3 Mar 2012, *R. E. Halling 9662* (NY, BRI).

Comments: Gyroporus furvescens is especially distinguishable by the appearance in dried condition, where the pileus and stipe surfaces are stained black, deep blue, slate blue, or sometimes purplish over gray to very pale yellow ground. This appearance seems to persist for at least several years in herbarium specimens.

Gyroporus mcnabbii Davoodian, Bougher & Halling, sp. nov. FIG. 9

MycoBank MB822349

Typification: AUSTRALIA. QUEENSLAND: D'Aguilar National Park, vicinity of Mt. Glorious, 27°17′45.2″S, 152°43′40.8″E, 601 m, 20 Feb 2013, *R.E. Halling 9808* (**holotype** BRI, **isotype** NY).

Diagnosis: Differs from other species of *Gyroporus* in Australasia by the darkly colored subvelutinous pileus and stipe surfaces, which sometimes appear mottled.

Etymology: mcnabbii (Latin), named in honor of New Zealand mycologist, R. F. R. McNabb.

Pileus 2–9 cm broad, convex to plano-convex, dry, dark chestnut brown to cinnamon brown to brownish orange to brownish red, with these colors sometimes forming a mottled appearance, subvelutinous to finely subtomentose to roughly furfuraceous. Flesh white, unchanging, with mild odor and taste. Tubes adnexed, 4–8 mm long, white to creamish becoming pale yellow, sometimes discoloring to orange. Stipe 2.5–6 cm long, 0.5–2.9 cm broad, equal to subclavate, sometimes pinched at the base, dry, subvelutinous to finely subtomentose to subpruinose, subconcolorous to concolorous with pileus, white to faint orange to faint pinkish at base, with interior white, unchanging, pithy to chambered to hollow, with annular zone especially apparent when a button (faint to absent in maturity). Spore print yellow to bright yellow.

Figure 9. *Gyroporus mcnabbii* (holotype R.E. Halling 9808). Photo: Roy Halling.

Spores (7.6–)8.4–10.7(–11.9) × (4.9–)5.5–6.7(–7) µm (Q = 1.61), smooth, yellow-hyaline, ellipsoid to subovoid, sometimes subreniform, sometimes appearing minutely apiculate. Basidia 24–30 × 9.5–11.5 µm, clavate. Cheilocystidia 25–40 × 6–9 µm, versiform (fusoid, elongated ventricose-rostrate, narrow subcylindrical). Pileipellis a dense trichoderm. Clamp connections present.

Ecology and distribution: Solitary to cespitose to gregarious to scattered with species of Myrtaceae (*Eucalyptus, Leptospermum*) and possibly Casuarinaceae and Fabaceae (*Acacia*). Known from Australia and New Zealand. Jan to Jun.

Other specimens examined: AUSTRALIA. QUEENSLAND: Great Sandy National Park, Cooloola, Freshwater Road, 25°57′13.3″S, 153°06′00.0″E, 152 m, 11 Mar 2012, *R.E. Halling 9677* (NY, BRI); Davies Creek National Park, Davies Creek Road, ±8.5 km from Kennedy Highway, 17°01′36.1″S, 145°35′24.0″E (coordinates are of area formerly known as Rope Swing Lunch Site), 670 m, 22 Mar 2007, *R.E. Halling 8955* (NY, BRI); Route 97, near Queensland–New South Wales border, 28°15′27.0″S, 153°14′09.6″E, 160 m, 5 Jun 2007, *R.E. Halling 8979* (NY, BRI); NEW SOUTH WALES: Gibraltar Range National Park, along Gwydir Highway, 2 May 1992, leg. *M.A. Castellano E 4600* (PERTH); Central Coast, Palm Grove, 7 km W of intersection of Pacific Highway and Ourimbah Creek Road, O'Sullivan's Way, 33°19′54.8″S, 151°18′28.8″E, 64 m, 10 Mar 2017, *R.E. Halling 10126* (NY, DAR); WESTERN AUSTRALIA: Perth, Victoria Road, ±31°52′53.8″S, ±116°07′37.4″E, 14 Apr 2005, leg. *K. Griffiths E8155* (PERTH); TASMANIA: Forest Resources Mycorrhiza Site, 20 Mar 1993, leg. *N. Malajczuk E843* (PERTH). NEW ZEALAND. AUCKLAND: Titirangi, Clark Bush, 13 Jun 1973, leg. *S. Haydon 30836* (PDD); Atkinson Park, 11 Jan 1966, leg. *R.F.R. McNabb 25065* (PDD).

Comments: Gyroporus mcnabbii is a distinctive species that appears to have a wide range through Australia and New Zealand. The deep, sometimes mottled colors and subvelvety texture distinguish it from other superficially *castaneus*-like entities in the region. Other New Zealand specimens referenced for this description but not studied in detail were PDD 25066 (leg. *R.F.R. McNabb*) and PDD 29553 (leg. *P.J. Brook*). McNabb (1968) treated his New Zealand collections as *G. castaneus*, and his description of the material agrees strongly with the material examined here.

Gyroporus paralongicystidiatus Davoodian, sp. nov.

MycoBank MB822350

Typification: COSTA RICA. SAN JOSÉ: Dota, ±5 km SW of Cerro de la Muerte, Albergue de Montaña, Savegre, 2200 m, 9 Jul 2001, *R.E. Halling 8274* (**holotype** USJ, **isotype** NY).

Diagnosis: Differs from other species of *Gyroporus* in the Americas by the combination of the tomentose to finely matted pileus, brown to light brown to pinkish brown coloration, and conspicuous cheilocystidia in early maturity.

Etymology: paralongicystidiatus (Greek), near *longicystidiatus*.

Pileus 4–5 cm broad, plane to slightly convex, sometimes slightly concave with maturity, dry, brown to light brown to pinkish brown, woolly tomentose to fine matted, sometimes forming very fine squamules or furfur. Flesh white, unchanging. Tubes adnexed, whitish to cream to yellow, with concolorous pores. Stipe 3–6.5 cm long, 1–1.5 cm broad, more or less equal, slightly tapered at base, more or less concolorous with pileus or paler, subtomentose to roughly glabrous, pithy to chambered to hollowing.

Spores (7–)8.2–9.8(–10.5) × (4.4–)5.1–5.7(–6.4) µm (Q = 1.63), smooth, yellow to yellow-hyaline, ellipsoid to subovoid to subreniform, occasionally shaped more or less like an unshelled peanut, sometimes appearing apiculate. Basidia 27–33 × 9–12 µm, clavate. Cheilocystidia 17–55 × 6–13 µm, versiform (fusoid, tapered toward apex, ventricose-rostrate, obclavate, cylindrical, subutriform, sublageniform, roughly cylindrical with slightly inflated apex), especially noticeable in early maturity, somewhat obscured with advanced maturity as other hymenial elements develop. Pileipellis an elongated, dense, tangled, collapsing trichodermium. Clamp connections present.

Ecology and distribution: Solitary to scattered on soil. Known from Costa Rica under *Quercus seemannii* and *Quercus copeyensis* and Colombia in mixed forest with *Quercus humboldtii*. May, Jul, and Nov.

Other specimens examined: COSTA RICA. SAN JOSÉ: Dota, ±5 km SW of Cerro de la Muerte, Albergue de Montaña, Savegre, 2200 m, 10 Jul 2000, *Halling 8002* (NY); same locality as previous, 18 Jun 1997, *Halling 7725* (NY); same locality as previous, 24 Nov 1993, *Halling 7190* (NY). COLOMBIA. CAUCA: Tunia, Corregimiento El Mango, Reserva Natural "El Guayabo," 1640 m, 28 May 1991, *A.E. Franco-Molano 498* (NY).

Comments: Gyroporus paralongicystidiatus, known only from three collections in the northern neotropics, is phylogenetically sister to *G. longicystidiatus* Nagasawa & Hongo from East Asia (Nagasawa 2001) and geographically separated. Several exemplars of the latter were examined, including a paratype (EN99-67). The Colombian specimen roughly agrees with the type morphology, and its *atp6* sequence is identical to the type, although the surfaces of the Colombian specimen are lighter in color and the pileipellis is shorter. Additional collections are needed to ascertain the geographic extent and morphological variation of this species.

DISCUSSION

The sister relationship between the *cyanescens* and *longicystidiatus* clades is a key result (node c in FIGS. 1, 2). Given the subdued brownish colors of *G. longicystidiatus*, along with the lack of oxidation reactions, previous researchers have assumed this and similar species to be closer to *G. castaneus*. The evidence presented here, however, places *G. longicystidiatus* phylogenetically closer to *G. cyanescens*. This is likely one of the reasons for confusion in previous systematic studies of *Gyroporus*, since many collections of *G. longicystidiatus* and similar entities (e.g., *G. paralongicystidiatus, Gyroporus* sp. BOS472) were originally determined as *G. castaneus*.

Species in the *cyanescens, longicystidiatus*, and "OR182" clades (together constituting clade b in FIGS. 1, 2) are morphologically comparable in their pileipellis structure, which is always a dense elongated trichodermium, i.e., the pileus is a mat of fibrils. This is most apparent in the *cyanescens* clade (node d in FIGS. 1, 2), where species are often very strongly tomentose, the fibrillosity being greatly elongated and somewhat clumped. In *G. longicystidiatus* and like entities (i.e., *G. paralongicystidiatus* and the clade including OR182, which is somewhat comparable to *G. longicystidiatus* in appearance), the dense fibrillosity is generally shorter than in the *cyanescens* clade and can be clearly visualized with a dissecting microscope; *G. longicystidiatus* was originally described as "tomentose to floccosely squamulose" (Nagasawa 2001). The trichodermium is often intensely tangled in the *longicystidiatus* clade (node e in FIGS. 1, 2). The remaining *Gyropori* (*G. castaneus, G. naranjus*, nom. prov., etc.) display a variety of trichoderms: more or less palisadal, erect/tangled, short, or elongated as discussed above, with rounded, tapered, or cystidioid end cells.

The Australian *cyanescens* group (i.e., the Australian clade within clade d in FIGS. 1, 2) is composed of two major clades, one of light-oxidizing and one of dark-oxidizing species. The light-oxidizing species are represented by the clade including *G. australiensis, G. allocyanescens*, nom. prov., and *G. robinsonii*, nom. prov. In these species the bluing reaction on injured flesh is gradual, initially turning slowly light blue then slightly intensifying over time. The dark-oxidizing species are represented by the clade including *G. furvescens, G. austrocyanescens*, nom. prov., *G. neocyanescens*, nom.

prov., and *G. occidentalis*, nom. prov. In these species, the flesh stains immediately and intensely deep blue. *Gyroporus brunnescens*, the only species of *Gyroporus* known to have a brown oxidation reaction, is placed with the dark-oxidizing clade (FIG. 2). The placement of western Australian exemplars *G. robinsonii*, nom. prov. (FIG. 1), and *G. occidentalis*, nom. prov. (FIGS. 1, 2), suggests a level of east/west continental signal within each of these clades. Ladiges et al. (2010) detected east/west phylogenetic signal in Australian *Eucalyptus* species, which are mycorrhizal with *Gyroporus* (Bougher 1995; Yuwa-amornpitak 2009).

Biogeographic links between Eurasia and North America (including Central America) are apparent in several clades inferred here (FIGS. 1, 2) and are suggestive of boreotropical scenarios in which extensive biotic exchange occurred between Eurasia and North America apparently starting in the Cenozoic (Wolfe 1975; Lavin and Luckow 1993; Sánchez-Ramírez et al. 2015; Han et al. 2017). The Australian species display various biogeographic affinities: *G. naranjus*, nom. prov., is sister to African *Gyropori* (FIGS. 1, 2) and the Australian *cyanescens* group is sister to its Northern Hemisphere relatives (FIGS. 1, 2). Further sampling of *G. cyanescens* in the Northern Hemisphere is needed to confidently determine where *G. cyanescens* sensu stricto should apply.

The *G. castaneus* clade (node a in FIGS. 1, 2) is a species complex that is widespread in the Northern Hemisphere. It includes representatives from Europe and North America (including Central America) as well as eastern Eurasia. *Gyroporus purpurinus*, an iconic purplish red North American species, is also a member of this clade (FIG. 2). The *G. castaneus* clade is highly diverse in North America, with at least three distinct undescribed lineages represented here. *Gyroporus castaneus* sensu stricto would appropriately be applied to the European lineage represented by the Italian and Belgian exemplars (FIGS. 1, 2), although it should be noted that the presence of other *G. castaneus* lineages in Europe is possible. The species has been neither neo- nor epitypified. *Gyroporus castaneus* sensu lato can be applied to clade a in its entirety (FIGS. 1, 2). Our study has excluded from *Gyroporus castaneus* sensu lato several lineages previously assumed to be *G. castaneus* or an infraspecific taxon, among them *G. mcnabbii, G. naranjus*, nom. prov., the African exemplars TH9913 and Thoen7634, and the lineages represented by OR182 and BOS472. Further studies with additional sampling are needed to fully reveal the diversity of the *G. castaneus* clade in North America and worldwide.

Much of the diversity of *Gyroporus* remains formally undescribed. Some provisional taxa appearing here will remain undescribed until additional exemplars are collected; others will be described in forthcoming publications.

ACKNOWLEDGMENTS

The staff at MICH, especially P. Rogers and M. Foltz, were instrumental in facilitating work at the herbarium. The curators and staff at TNS are greatly thanked for their support. Study of specimens at TMI and KPM, coordinated by E. Nagasawa and T. Orihara, was also of great assistance to this project. D. H. Pfister, G. E. Tocci, and others at FH are sincerely thanked for facilitating the study of the R. Singer and F. X. R. von Höhnel material housed there. We are very grateful to FLAS for extensive cooperation in securing fresh *Gyroporus* samples from Florida as well as dried material from Pakistan. Cooperation from CFMR in sending material on loan to NY, including E. E. Both's collections, is greatly appreciated. R. M. Robinson, J. Borovička, and R. E. Tulloss are thanked for sharing specimens and data early in this study's development. The following individuals generously provided specimens that appear in this study: J. F. Ammirati, A. R. Bessette, T. P. Delaney, C. Schwarz, I. G. Safonov, W. E. Sturgeon, J. Burghardt, A. R. Franck, and B. E. Overton. K. Soytong and King Mongkut's Institute of Technology are gratefully thanked for providing Material Transfer Agreements to study material from Thailand.

FUNDING

The Mycological Society of America provided support from the Alexander H. and Helen V. Smith Research Fund, which made study of specimens at MICH possible. This research was also supported in part by a National Science Foundation EAPSI fellowship in 2015 (NSF award: 1515344; institution: Department of Botany, National Museum of Nature and Science, Japan; host: K. Hosaka). A Friends of the Farlow Graduate Student Fellowship supported a week of study at the Farlow Reference Library and Herbarium in 2014. Participation by M. E. Smith was made possible in part by the Ordway-Swisher Biological Station seed grant program at the University of Florida. O. Raspé is grateful to the Fonds National de la Recherche Scientifique (Belgium) for travel grants to Thailand and China. Many specimens and sequences used in this study were generated with support from the following National Science Foundation awards: DEB 9972018, DEB 0414665, DEB 1020421, DEB 9300798, DEB 9972027, DEB 0103621, DEB 1556338. Support from the National Geographic Society was provided by grants 8457-08, 7341-02, and 9235-13. T. Henkel is Principal Investigator (PI) on NSF DEB 1556338 and NGS CRE 9235-13. Collaboration of J. Carranza at Universidad de Costa Rica in support of NSF DEB grants 9300798, 9972018, and 9972027 is greatly appreciated and contributed to discovery of *G. paralongicystidiatus*, sp. nov.

ORCID

Kentaro Hosaka http://orcid.org/0000-0002-4469-8303
Olivier Raspé http://orcid.org/0000-0002-8426-2133
Beatriz Ortiz-Santana http://orcid.org/0000-0003-4545-7162
Matthew E. Smith http://orcid.org/0000-0002-0878-0932
Roy E. Halling http://orcid.org/0000-0002-2610-3062

LITERATURE CITED

Agerer R. 1999. *Gyroporus cyanescens* (Bull.: Fr.) Quél. + *Pinus sylvestris* L. Descriptions of Ectomycorrhizae 4:43–47.

Binder M, Bresinsky A. 2002. Derivation of a polymorphic lineage of Gasteromycetes from boletoid ancestors. Mycologia 94:85–98.

Bougher NL. 1995. Diversity of ectomycorrhizal fungi associated with eucalypts in Australia. In: Brundrett M, Dell B, Malajczuk N, Gong M, eds. Mycorrhizal research for forestry in Asia. Canberra, Australia: Australian Centre for International Agricultural Research. p. 8–15.

Bulliard JBF. 1787. Herbier de la France. Paris. 7:tab. 328.

Bulliard JBF. 1788. Herbier de la France. Paris. 8:tab. 369.

Das K, Chakraborty D, Vizzini A. 2017. Morphological and phylogenetic evidences unveil a novel species of *Gyroporus* (Gyroporaceae, Boletales) from Indian Himalaya. Nordic Journal of Botany. 35:669–668.

Dentinger BT, Ammirati JF, Both EE, Desjardin DE, Halling RE, Henkel TW, Moreau PA, Nagasawa E, Soytong K, Taylor AF, Watling R. 2010. Molecular phylogenetics of porcini mushrooms (*Boletus* section *Boletus*). Molecular Phylogenetics and Evolution 57:1276–1292.

Han LH, Feng B, Wu G, Halling RE, Buyck B, Yorou NS, Ebika STN, Yang ZL. 2017. African origin and global distribution patterns: evidence inferred from phylogenetic and biogeographical analyses of ectomycorrhizal fungal genus *Strobilomyces*. Journal of Biogeography, doi:10.1111/jbi.13094

Hosaka K, Uno K. 2011. Assessment of the DNA quality in mushroom specimens: effect of drying temperature. Bulletin of the National Museum of Nature and Science, Series B 37:101–111.

Kluting, KL, Baroni TJ, Bergemann SE. 2014. Toward a stable classification of genera within the Entolomataceae: a phylogenetic re-evaluation of the *Rhodocybe-Clitopilus* clade. Mycologia 106:1127–1142.

Kretzer AM, Bruns TD. 1999. Use of *atp6* in fungal phylogenetics: an example from the Boletales. Molecular Phylogenetics and Evolution 13:483–492.

Ladiges PY, Bayly MJ, Nelson GJ. 2010. East-west continental vicariance in *Eucalyptus* subgenus *Eucalyptus*. In: Williams DM, Knapp S, eds. Beyond cladistics: the branching of a paradigm. Berkeley, California: University of California Press. p. 267–302.

Lavin M, Luckow M. 1993. Origins and relationships of tropical North America in the context of the boreotropics hypothesis. American Journal of Botany 80:1–14.

Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16:1799–1808.

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*, Agaricales). Molecular Phylogenetics and Evolution 35:1–20.

McNabb RFR. 1968. The Boletaceae of New Zealand. New Zealand Journal of Botany 6:137–176.

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

Nagasawa E. 2001. Taxonomic studies of Japanese boletes. I. The genera *Boletinellus*, *Gyrodon* and *Gyroporus*. Reports of the Tottori Mycological Institute 39:1–27.

Patouillard NT. 1900. Essai taxonomique sur les familles et les genres des Hyménomycètes. Lons-le-Saunier: Lucien Declume. 124 p.

Quélet L. 1886. Enchiridion fungorum in Europa media et praesertim in Gallia vigentium. Paris. 161 p.

Raidl S, Scattolin L, Agerer R. 2006. *Gyroporus castaneus* (Bull.: Fr.) Quél. + *Quercus robur* L. Descriptions of Ectomycorrhizae 9:39–44.

Ramanankierana N, Ducousso M, Rakotoarimanga N, Prin Y, Thioulouse J, Randrianjohany E, Ramaroson L, Kisa M, Galiana A, Duponnois R. 2007. Arbuscular mycorrhizas and ectomycorrhizas of *Uapaca bojeri* L. (Euphorbiaceae): sporophore diversity, patterns of root colonization, and effects on seedling growth and soil microbial catabolic diversity. Mycorrhiza 17:195–208.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

Rozen S, Skaletsky H. 1999. Primer3 on the WWW for general users and for biologist programmers. In: Misener S, Krawetz SA, eds. Bioinformatics methods and protocols. Methods in Molecular Biology 132. Totowa, New Jersey: Humana Press. p. 365–386.

Sánchez-Ramírez S, Tulloss RE, Amalfi M, Moncalvo JM. 2015. Palaeotropical origins, boreotropical distribution and increased rates of diversification in a clade of edible ectomycorrhizal mushrooms (*Amanita* section *Caesareae*). Journal of Biogeography 42:351–363.

Singer R. 1945. The Boletineae of Florida with notes on extralimital species. II. The Boletaceae (Gyroporoideae). Farlowia 2:223–303.

Singer R. 1986. The Agaricales in modern taxonomy, fourth ed. Koenigstein, Germany: Koeltz Scientific Books. 981 p.

Singer R, Araujo I, Ivory HM. 1983. The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia. Beihefte zur Nova Hedwigia 77:1–352.

Watling R. 2008. A manual and source book of the boletes and their allies. Oslo, Norway: Fungiflora. 250 p.

Wilson AW, Binder M, Hibbett DS. 2011. Effects of gasteroid fruiting body morphology on diversification rates in three independent clades of fungi estimated using binary state speciation and extinction analysis. Evolution 65:1305–1322.

Wilson AW, Binder M, Hibbett DS. 2012. Diversity and evolution of ectomycorrhizal host associations in the Sclerodermatineae (Boletales, Basidiomycota). New Phytologist 194:1079–1095.

Wolfe JA. 1975. Some aspects of plant geography of the Northern Hemisphere during the late Cretaceous and Tertiary. Annals of the Missouri Botanical Garden 62:264–279.

Yuwa-amornpitak T. 2009. Molecular characterization of ectomycorrhizal fungi associated on *Eucalyptus* root system in Maha Sarakham Province. Journal of Science and Technology Mahasarakham University 28:98–105.