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Gloeocantharellus aculeatus (Gomphaceae), a new neotropical species in the gomphoid-phalloid clade

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Abstract

Gloeocantharellus has an amphi-Pacific distribution, with five known neotropical species. *Gloeocantharellus corneri* was the only species in the genus recorded from Brazil. A new species, *Gloeocantharellus aculeatus*, was collected in the Atlantic Forest in the states of Espírito Santo and Santa Catarina of Brazil, and is described based on morphological and molecular characteristics. It is distinguished from other species in the genus by the orange to salmon pileus with slightly fibrillose margin, squamulose to pulverulent stipe, and aculeate basidiospores. The results of molecular analyses show that *G. aculeatus* is a distinct species and is closely related to *G. corneri* and *G. echinosporus*.

Keywords: Agaricomycotina, Gomphales, mushroom, taxonomy

Introduction

Gloeocantharellus (Gomphaceae, Gomphales) was proposed by Singer (1945), who based the genus on the presence of gloeocystidia, warty spores and lamellae with venose reticulation, and designated *Gloeocantharellus purpurascens* (Hesler) Singer as the type species. Corner (1969) reviewed the morphological characteristics and the phylogenetic relationships of *Gloeocantharellus* with closely related genera and proposed new combinations and new species for the genus. Since then, only a few taxonomic studies (Petersen 1971; Joseph & Manimohan 1998; Giachini 2004; Vasco-Palacios & Franco-Molano 2005; Deng & Li 2008; Giachini & Castellano 2011) have focused on the genus. Currently, the genus includes species with an agaricoid-gomphoid habit, decurrent lamellae, ornamented spores, and the presence of many gloeoplerous hyphae and gloeocystidia (Giachini 2004).

Presently, *Gloeocantharellus* (Giachini & Castellano 2011) comprises 11 species and has an amphi-Pacific and mostly tropical distribution, with records from the Neotropics (South America), Holartic (North America), Paleotropics (mostly Southeast Asia) and Australian regions (Oceania). Until recently in Brazil there were only two records for the genus, both identified as *G. corneri* (Singer) Corner and collected in the Atlantic Forest in Rio de Janeiro (Singer 1961) and Paraná (Watling & De Meijer 1997).

Due to shared morphological features, *Gloeocantharellus* is considered as a part of *Gomphus s.l.*, together with *Gomphus* Pers., *Phaeoclavulina* Brinkmann, and *Turbinellus* Earle. Giachini *et al.* (2010), with molecular analyses using nuclear (nuc-LSU rDNA) and mitochondrial (mit-SSU rDNA and mit-*atp*6 DNA) markers, proposed its phylogenetic relationships among Gomphales, Geastrales, Hysterangiales and Phallales (subclass Phallomycetidae). In this study, *Gloeocantharellus* was found to be monophyletic and sister to *Gomphus*, *Gautieria* Vittad., *Ramaria* Fr. ex Bonord. subg. *Ramaria*, *Ramaria* subg. *Laeticolora* Marr & D.E. Stuntz, and *Turbinellus*.

The present work describes a new species of *Gloeocantharellus* from the Atlantic Forest of Brazil, based on morphological and molecular analyses.

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Material and methods

Morphological data:—Fieldwork was conducted during the rainy season (January and December 2012) in Atlantic Forest in Lagoinha do Leste Municipal Park, Florianópolis, in southern Brazil (27°46'43"S, 48°29'51"W), and the Augusto Ruschi Biological Reserve, Santa Teresa, Espírito Santo, in southeastern Brazil (19°54'19.6"S, 40°34'8.2"W). Macro- and micromorphological analyses were conducted with traditional methods used to describe mushrooms (Largent 1986; Largent *et al.* 1977). Color codes were based on the Online Auction Color Chart (Kramer 2004). All microscopic observations and measurements were made in 3% KOH and Congo Red with an optical microscope (Olympus CX22LED OM). Microscopic illustrations were made using a drawing tube mounted on a Nikon Labophot-2 OM. Basidiospore measurements were based on 30 profile basidiospores per specimen, and excluded the apiculus and ornamentation. The following abbreviations were used for the basidiospore measurements: ($\overline{X}$ = arithmetic average) ($\overline{Q}$ = arithmetic average of ratio length/width). This work follows Stalpers (1996) and the Resupinate Russulales species database (http://www.cbs.knaw.nl/russulales/) for the terminology used to describe basidiospore shape, which is based on the value and symmetry. For scanning electron microscopy (SEM) of the basidiospores, hymenophore fragments were removed from dried material, mounted on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a JEOL JSM-6390LV SEM operating at 10 Kev. The SEM analysis was conducted at the Laboratório Central de Microscopia Eletrônica at the Universidade Federal de Santa Catarina (Brazil). Voucher specimens were deposited in FLOR and herbarium abbreviations follow Thiers (continuously updated).

Genomic DNA extraction, PCR and Sequencing:—Genomic DNA was extracted from dried material using a modified CTAB extraction method (Romano & Brasileiro 1998) or a Magnex DNA Kit, following the manufacturer's protocol adapted to fungi. DNA sequence data were obtained from two loci: the internal transcribed spacer of ribosomal nuclear DNA (nrITS) and ATPase subunit 6 of mitochondrial DNA (mit-*atp*6). The nrITS and mit-*atp*6 regions were amplified using primers ITS8F and ITS6R (Dentinger *et al.* 2010) and *atp*6-2 and *atp*6-3 (Kretzer & Bruns 1999), respectively. PCRs were performed using ABM® mix following the protocol recommended by the manufacturer. The DNA was amplified using a thermocycler, according to the cycle parameters described in Dentinger *et al.* (2010) for nrITS and Kretzer & Bruns (1999) for mit-*atp*6. PCR products were purified using PEG (polyethylene glycol; Sambrook *et al.* 1989). Sequencing was performed with a BigDye Terminator 3.1 Cycle Sequencing Kit following manufacturer procedures, using the same primer pairs cited above, at Fundação Oswaldo Cruz (Fiocruz), Minas Gerais State, Brazil. The generated sequences and their respective chromatograms were manually checked and edited with Geneious 6.1.8 (Kearse *et al.* 2012).

Phylogenetic analyses:—Specimens and Genbank accession numbers used in this study are shown in Table 1. Eleven sequences of nrITS and 16 of mit-*atp*6, from Genbank and generated in this study, and representing 12 putative species (including the outgroup), were used to construct two distinct datasets. DNA sequences were aligned in MAFFT v7 (Katoh & Standley 2013) following the G-INS-I and Q-INS-I criteria for the mit-*atp*6 and nrITS datasets, respectively. Sequences were then manually corrected using the software MEGA 6.06 (Tamura *et al.* 2013). Indels present in the nrITS dataset were recoded as binary characters according to the "simple indel coding method" (SIC, Simmons & Ochoterena 2000) implemented in SeqState (Müller 2005). The resulting binary characters were joined in the final matrix as distinct partitions. The final alignments (as well the final topologies) were logged in TreeBASE (http://www.treebase.org/treebase/index.html) under ID19141. The final nrITS, including the recoded indels, and mit-*atp*6 alignments had lengths of 747 and 686 characters, respectively. New sequences generated for this work were included in Genbank (Sayers *et al.* 2009).

Maximum Likelihood (ML) and Bayesian Inference (BI) methods were applied to the two datasets, which were divided into three partitions for nrITS (ITS1/2, 5.8S and indels) and according to codon position for mit-*atp*6. The best model of nucleotide evolution for each nucleotide partition was determined using BIC (Bayesian Information Criterion) with the software jModelTest v2.1.6 (Guindon & Gascuel 2003; Darriba *et al.* 2012). ML analyses were performed using RAxML v. 8.2.4 software (Stamatakis 2014) available on the CIPRES portal (Miller *et al.* 2010, http://www.phylo.org/). The analysis first involved 100 ML searches, each starting from one randomized stepwise addition parsimony tree, under a GTRGAMMA model (estimating the proportion of invariant sites only for the nrITS dataset, according to the calculated models), with all other parameters estimated by the software. To assess the reliability of the nodes, multi-parametric bootstrapping replicates under the same model were computed, allowing the program halt bootstrapping automatically with the autoMRE option. The BI was performed with the software Mr. Bayes 3.2.6 (Ronquist & Huelsenbeck 2003) implemented with the CIPRES Science Gateway 3.1 (Miller *et al.* 2010). BI was implemented using two independent runs, each starting from random trees, with four simultaneous independent chains, and performed 10,000,000 generations, keeping one tree every 1000th generation. Four rate categories were

used to approximate the gamma distribution. Of all trees sampled, 20% were discarded as burn-in and checked by the convergence criterion (frequencies of average standard deviation of split <0.01) with Tracer v.1.6 (Rambaut *et al.* 2014), while the remaining were used to reconstruct a 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities (BPP) of the branches. A node was considered to be strongly supported if it had a BPP ≥ 0.95 and/or BS ≥ 90%, while moderate support was considered when BPP ≥ 0.9 and/or BS ≥ 70%. *Gomphus clavatus* (Pers.) Gray was defined as the outgroup based on a previous study that recovered it as a sister group of *Gloeocantharellus* (Giachini *et al.* 2010).

TABLE 1. Species, vouchers and accession numbers used in the phylogenetic analyses.

Taxon	Voucher	Genbank accession number	
		nrITS	mit-*atp*6
Gloeocantharellus aculeatus	FLOR47977	KU884895	KU884887
Gloeocantharellus aculeatus	FLOR49692	KU884896	KU884888
Gloeocantharellus aculeatus	FLOR59113	KU884897	KU884889
G. corneri	FLOR47978	KU884898	KU884890
G. echinosporus	CGE16040	-	KU884891
G. echinosporus	CGE16041	KU884899	KU884892
G. novae-zelandiae	PDD44960	-	AY574809
G. okapaensis	CGE16046	KU884900	KU884893
G. pallidus	BPI54917	-	AY574815
Gloeocantharellus papuanus nom. nud.	PERTH4549	-	AY574810
Gloeocantharellus persicinus	GDGM21480	EU118161	-
G. purpurascens	TUB19102	-	KF147737
G. purpurascens	TENN14265	-	AY574824
G. purpurascens	TENN12793	-	AY574823
G. purpurascens	TENN60053	AY872281	-
G. purpurascens	REH 6904	KU884901	KU884894
Gloeocantharellus sp. 1	OSC122875	-	DQ218903
Gloeocantharellus sp. 2	TH8525	KT339202	-
Gomphus clavatus	-	DQ365637	-
Gomphus clavatus	OSC97616	-	AY574807

Results

Molecular Phylogenetic Analyses:—Seven sequences of nrITS and eight of mit-*atp*6 were generated in this study. The aligned nrITS and mit-*atp*6 data matrix consisted of 628 and 686 nucleotide positions, respectively. The best models of nucleotide substitution estimated for each partition in the datasets were the following: HKY+G, F81+G and TrN+G for the first, second and third positions of mit-*atp*6, respectively, and TrNef+I and K80+I for ITS1/2 and 5.8s, respectively. Only the topologies from the BI analyses are exhibited, while both BPP and BS values are shown (Fig. 1, 2).

The ML and BI analyses based on mit-*atp*6 (Fig. 1) generated trees with a few distinct topologies. The ML tree showed that *G. corneri* is more related to *G. echinosporus*, while in the BI *G. corneri* clustered with *G. aculeatus*. Both arrangements are, however, not significantly supported. *Gloeocantharellus pallidus*, *G. novae-zelandiae* and *G. papuanus nom. nud.* arise as early branches in the trees. Two other clades are formed by the remaining species.

The clade including *G. purpurascens* was strongly supported (BPP=1; BS=100). *Gloeocantharellus okapaensis* and *Gloeocantharellus sp*. 1 are shown as sister taxa of *G. purpurascens*. The Brazilian specimens clustered in a clade that included *G. aculeatus* with *G. corneri* as a sister branch. *Gloeocantharellus echinosporus* is a sister group of the Brazilian specimens, with a high support value (BPP=1; BS=100) for both mit-*atp*6 and nrITS analyses. Both the nrITS and the mit-*atp*6 analyses presented phylograms with similar topologies. The nrITS analyses (Fig. 2) resulted in two main well-supported clades (BPP=1; BS=100). The first clade includes *G. purpurascens*, *G. okapaensis*, *Gloeocantharellus sp*. 2 and *G. persicinus*. The second clade comprises *G. aculeatus*, *G. corneri* and *G. echinosporus*, a result that was also retrieved in the mit-*atp*6 analyses. The phylogenies presented in Figs. 1 and 2 show that the molecular nrITS (BPP=1; BS=100) and mit-*atp*6 (BPP= 0.98; BS=78) sequences, of the three specimens of *G. aculeatus*, were recovered with significant support and were distinct from other known *Gloeocantharellus* species.

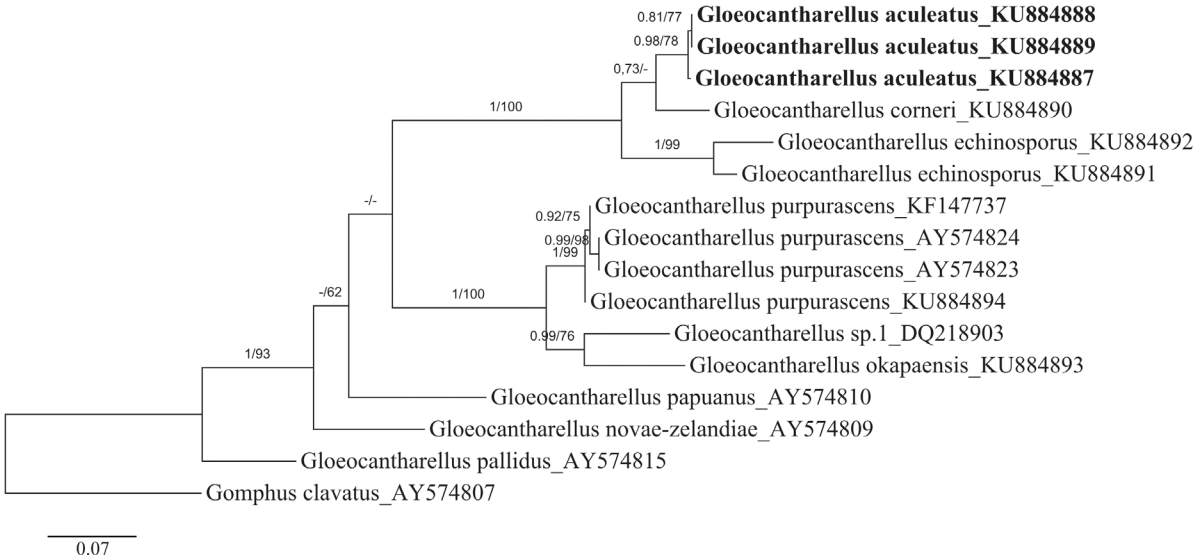

FIGURE 1. Fifty-majority rule consensus tree from Bayesian Inference (BI) of *Gloeocantharellus*, based on dataset of 16 mit-*atp*6 sequences. Bayesian posterior probability above 0.7 and Bootstrap values above 50% are shown.

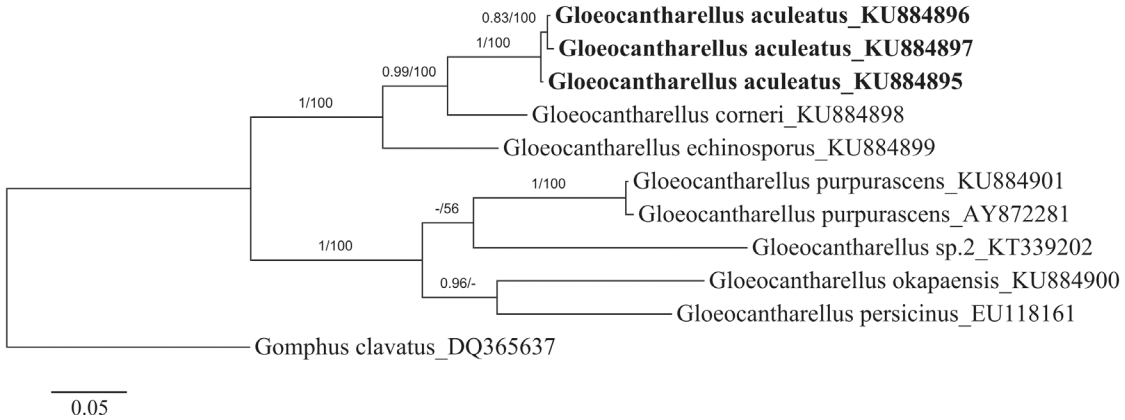

FIGURE 2. Fifty-majority rule consensus tree from Bayesian Inference (BI) of *Gloeocantharellus*, based on dataset of 11 nrITS sequences. Bayesian posterior probability above 0.7 and Bootstrap values above 50% are shown.

Taxonomy

Gloeocantharellus aculeatus Linhares, Daniëls & M.A.Neves *sp. nov.* (Figs. 3, 4, 5)
Mycobank: MB 816104

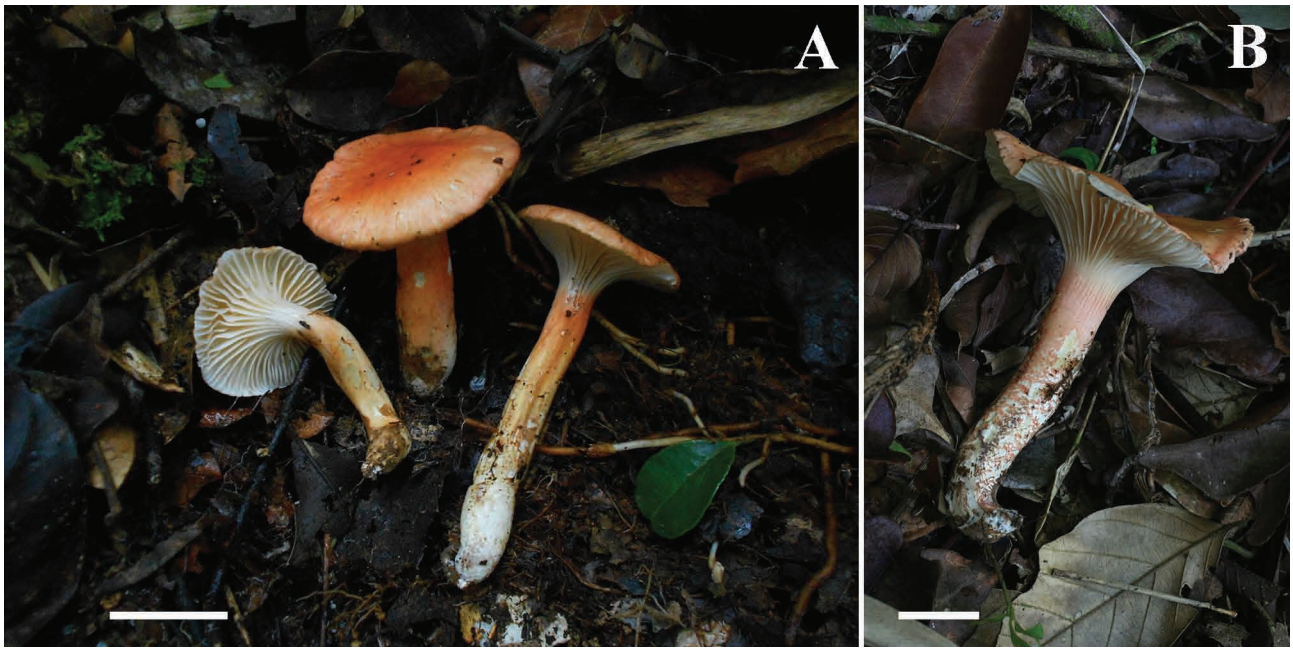

FIGURE 3. Basidiomata of *Gloeocantharellus aculeatus* in the field. **A.** FLOR 59113 (by A.C. Magnago); **B.** FLOR 47977 (by M.A. Neves). (Bar = 2 cm).

Diagnosis:—Basidiomata light salmon to dark orange; lamellae pale cream, forming longitudinal striae on stipe apex; basidiospores 9.0–10.5 × 5.0–6.0 µm, hyaline to light brown, aculeate; basidia with 2–4 sterigmata; gloeocystidia lanceolate to subventricose; pileipellis as an ixocutis; context with clamped hyphae. Similar to *Gloeocantharellus echinosporus* but differing in the wrinkled basidiospore surface with rounded aculei.

Etymology:—"*aculeatus*" refers to the aculeate ornamentation on the basidiospores.

Holotype:—BRAZIL. Santa Catarina: Florianópolis, Pântano do Sul, Lagoinha do Leste Municipal Park trail, 27°46'43"S, 48°29'51"W, 04 January 2012, *F.T.F. Linhares 18* (FLOR 47977).

Description:—*Pileus* 35–75 mm diam., plane, sometimes with slight depression in center, margin plane to decurved and entire; surface dry, smooth to velutinous and dark orange (OAC756 to OAC631) at center, slightly fibrillose and light salmon to light orange (OAC632 to OAC634) towards margin; context 1–2 mm thick, soft, spongy, white. *Lamellae* deeply decurrent, close, sometimes bifurcate and slightly intervenose, smooth to slightly fimbriate at edges, pale cream (OAC815 to OAC816), sometimes extended to stipe apex forming pinkish longitudinal striae up to 12 mm long; lamellulae present. *Stipe* 50–80 × 8–17 mm, central, solid, terete to slightly compressed, equal to clavate-bulbous, base obtuse and sometimes tapered and bent; surface pulverulent, cracking irregularly and exposing context toward base when mature, pale salmon to light orange (OAC619 to OAC631 or OAC682) over entire surface when immature, lighter salmon to lighter orange (OAC578 to OAC696) basally when mature; context soft, spongy, white. Basidiomata becoming purplish brown (OAC526 to OAC640) where bruised.

Basidiospores (8.5–) 9.0–10.5 (–11.0) × 5.0–6.0 µm ($\overline{X}$ = 9.43 × 5.50 µm) ($\overline{Q}$ = 1.73), ellipsoid to narrowly ellipsoid, thick-walled (<2 µm), hyaline to light brown in H_2O, IKI-, cyanophilic, strongly ornamented with ornamentation up to 2 µm, aculeate under OM; ornamentation with apically rounded aculei, ventricose, sometimes somewhat tuberculate, with outgrowths eventually connected at base and wrinkled surface between them under SEM; apiculus 1–2 µm, hyaline. *Basidia* 45–75 × 10–16 µm, clavate, deeply tapering towards base, hyaline, with 2–4 sterigmata up to 8 µm long. *Gloeocystidia* on the sides and margin of the lamellae, 43–142 × 4–12 µm, subventricose, cylindrical to lanceolate with obtuse to subacute apex, deeply tapering and often becoming sinuous towards the base, thin-walled; hyaline and without content when young, then with refringent yellowish brown and densely granular content when old; base inserted in subhymenium or in lamellar trama, rarely protruding above the hymenium. *Hyphidia* 17–40 × 2–5 µm, filiform, sinuous, inconspicuous, scattered in hymenium. *Generative hyphae* clamped through all parts of basidioma and basal mycelium. *Gloeoplerous hyphae* with refringent or granular content, sinuous, sometimes branched, 2–10 µm diam. *Lamellar trama* partially to completely gelatinized, composed of hyphae 2–10 (–16) µm diam., interwoven, sometimes slightly inflated, mediostratum with subparallel hyphae and some hyaline gloeoplerous

hyphae. *Pileipellis* an ixocutis composed of hyphae 2–5 µm diam., loose, interwoven, sinuous, thin-walled, sometimes with granular content. *Pileus context* with hyphae 4–36 µm diam.; context hyphae interwoven, thin-walled becoming slightly thick in some parts, often inflated, hyaline, intermixed with some gloeoplerous refringent hyphae. *Stipitipellis* a trichodermial palisade on the pulverulent portions, with thin-walled hyphae 3–8 µm diam.; gloeoplerous elements present as pseudocaulocystidia. *Stipe context* with hyphae 2.5–9 µm diam., which are parallel, thin-walled to slightly thick-walled, hyaline; some gloeoplerous hyphae present. *Basal mycelium* composed of hyphae 2–5 µm diam., which are interwoven, slightly thick-walled, hyaline; gloeoplerous hyphae scattered; cysts 11–20 × 8–18 µm, globose to amorphous, with granular content, sometimes with bipyramidal crystals up to 1 µm wide.

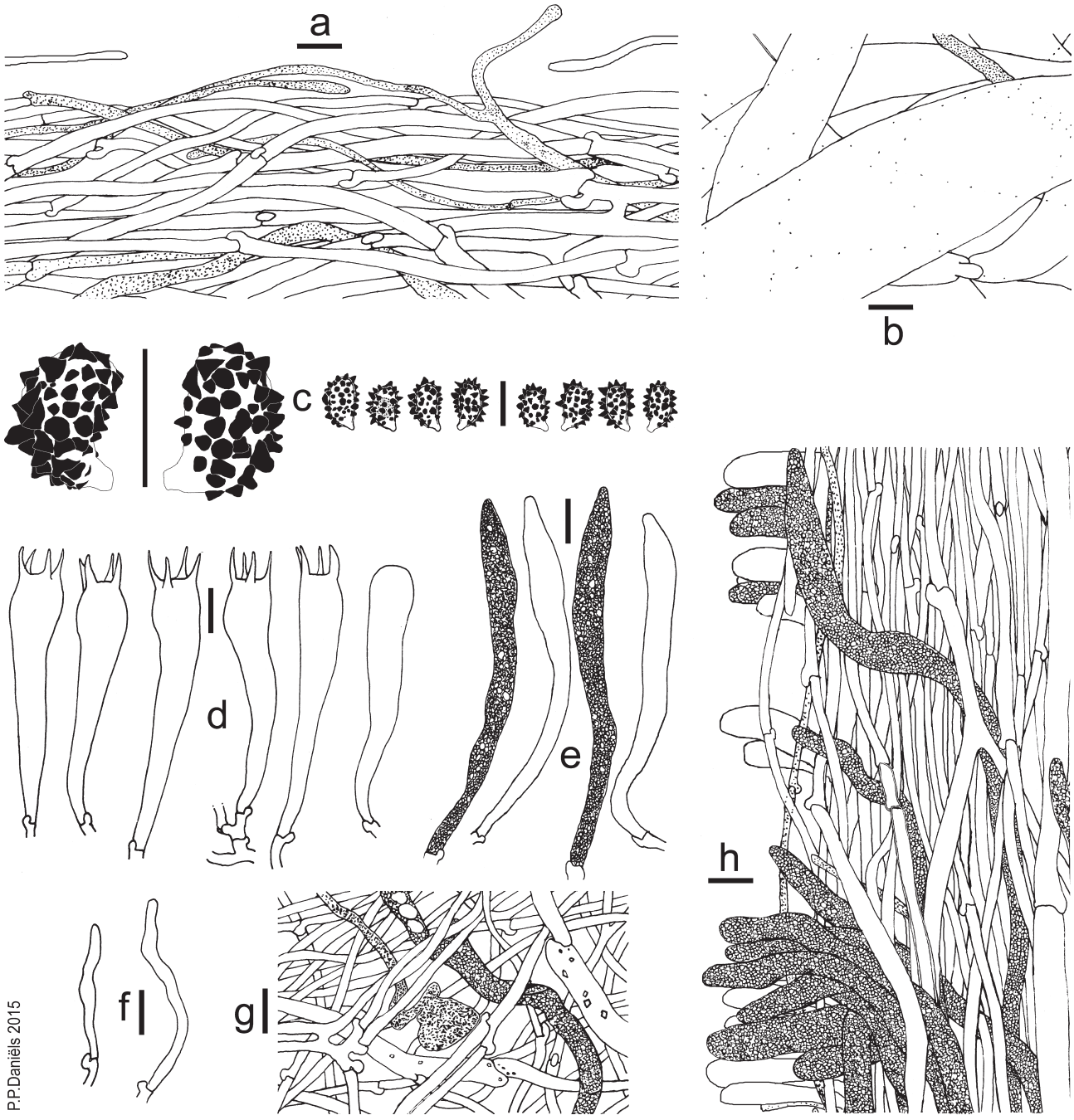

FIGURE 4. Microcharacters of *Gloeocantharellus aculeatus* (FLOR 47977, holotype). **a** Pileipellis; **b** Pileus context; **c** Basidiospores; **d** Basidia; **e** Gloeocystidia; **f** Hyphidia; **g** Basal mycelium; **h** Stipitipellis. (Bar = 10 µm). Drawings by P.P. Daniëls

Habit, habitat and distribution:—Solitary to scattered, sometimes forming fairy rings. Brazil, Atlantic Forest (Espírito Santo and Santa Catarina states).

Specimens examined:—BRAZIL. Santa Catarina: Florianópolis, Pântano do Sul, Lagoinha do Leste Municipal Park trail, 27°46'43"S, 48°29'51"W, 04 January 2012, *F.T.F. Linhares 18* (FLOR 47977—holotype); Espírito Santo:

Santa Teresa, Augusto Ruschi Biological Reserve, Waterfall trail, 19°54'19.6"S, 40°34'8.2"W, 04 December 2012, *A.C. Magnago 507* (FLOR 49692); Road near Casa da Pedra, 05 December 2012, *A.C. Magnago 524* (FLOR 59113).

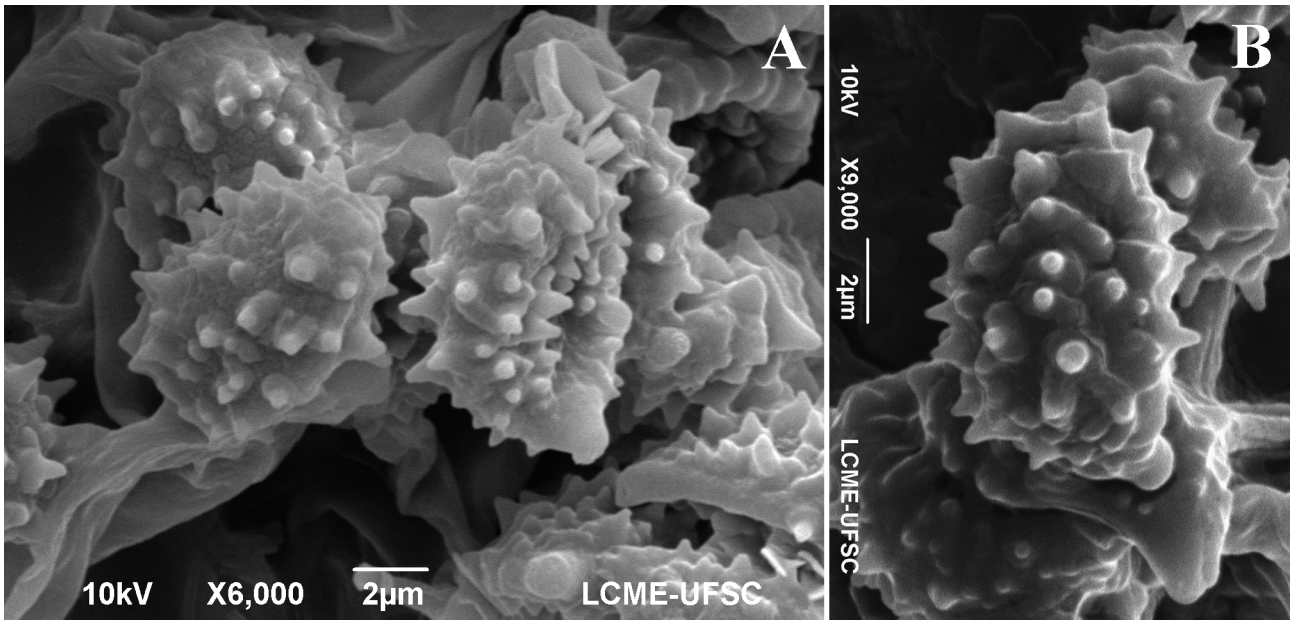

FIGURE 5. *Gloeocantharellus aculeatus*: Basidiospores (SEM)**. A.** FLOR 49692; **B.** FLOR 59113. Photos by F.T.F. Linhares

Additional specimens examined:—***Gloeocantharellus corneri***: BRAZIL. Paraná: Piraquara, Morro do Canal, 25°30'55"S, 48°58'53"W, 12 November 2010, *M.A. Neves 750* (collected by P. Santos) (FLOR 47978); ***G. echinosporus***: MALAYSIA. Sabah (N-Borneo): Mt Kinabalu N.P., 14 April 1964, *RSNB 8275* (CGE 16040); PAPUA NEW GUINEA. Morobe: Bulolo district, Manki, 22 March 1972, *Horak 72-266* in ZT; SINGAPORE. Central Region: Bukit Timah, 19 August 1939, *Corner s.n.* (CGE 16048—holotype); SOLOMON ISLANDS. Kolombangara, 23 August 1965, *RSS 1028A* (CGE 16041); ***G. okapaensis***: PAPUA NEW GUINEA. Okapa: 11 October 1960, *Corner s.n.* (CGE 16045—holotype, as *Gomphus okapaensis*); SOLOMON ISLANDS. San Cristobal: July 1965, *RSS 731* (CGE 16044); Kolombangara: 23 August 1965, *RSS 1028B* (CGE 16046).

Remarks:—*Gloeocantharellus aculeatus* is macromorphologically characterized by the orange to salmon color of the pileus with plane to slightly depressed shape and slightly fibrillose margin surface, the cream color of the lamellae that form longitudinal striae on the stipe apex, and the squamulose to pulverulent stipe. The basidiomata become purplish brown after a few minutes when bruised, which is a common characteristic in the genus. Micromorphologically, the basidiospores have aculeate to tuberculate ornamentation with a wrinkled surface between the outgrowths (this last feature can only be seen under SEM). *Gloeocantharellus aculeatus* is also characterized by its clavate basidia that are deeply tapered at the base, stipitipellis trichodermial palisade with caulocystidia, and subventricose to lanceolate gloeocystidia present throughout the hymenium. The immature hyaline gloeocystidia could be mistaken for another type of cystidia in the hymenium, but the content of gloeocystidia changes during maturation (Stalpers 1996). The presence of a robust basidioma, decurrent lamellae, ornamented basidiospores, gloeocystidia and gloeoplerous hyphae make it a species of *Gloeocantharellus*. The pattern of basidiospore ornamentation has never been recorded for the genus; the basidiospores of most other species have a verrucose to warty ornamentation.

Gloeocantharellus aculeatus is morphologically similar to *Gloeocantharellus echinosporus* Corner (Corner 1969). Both species have a robust basidioma, orange-pink pileus, bifurcate and slightly intervenose lamellae that are cream colored and attenuate on the stipe apex forming ribs, yellowish to pinkish stipe with pulverulent surface, almost the same sized basidiospores, and gelatinized pileipellis. However, the type of *G. echinosporus* does not have hyaline cystidia (even though hyaline cystidia have been observed in one specimen from Papua New Guinea, Horak 72-266 in ZT), and has ellipsoid to subfusiform basidiospores with echinulate ornamentation (Corner 1969). The distinct basidiospore ornamentation patterns between the two species can be observed under OM, which are more remarkable under SEM (Fig. 6) where the ornamentation of *G. echinosporus* has very acute apices. The distribution of the two species is also very distinct because *G. echinosporus* is known only from Southeast Asia and Oceania.

A pileus with an orange-pink color and dry surface is frequent in species of *Gloeocantharellus*, such as *G. dingleyae* (Segedin) Giachini, *G. novae-zelandiae* (Segedin) Giachini, *G. okapaensis* (Corner) Corner, and *G. purpurascens*.

However, all of those species have basidiospores with verruculose to verrucose ornamentation and lack a gelatinized pileipellis (Corner 1966; Corner 1969; Petersen 1971; Segedin 1984). *Gloeocantharellus uitotanus*, from Colombia, has a gelatinized pileipellis, but possesses a reddish brown pileus and verruculose basidiospores (Vasco-Palacios & Franco-Molano 2005).

Gloeocantharellus corneri, the other species reported for Brazil, is different from the new species in the more robust basidiomata (up to 160 mm high), reddish orange pileus, cream-colored stipe and amygdaliform to subfusiform basidiospores with verrucose ornamentation.

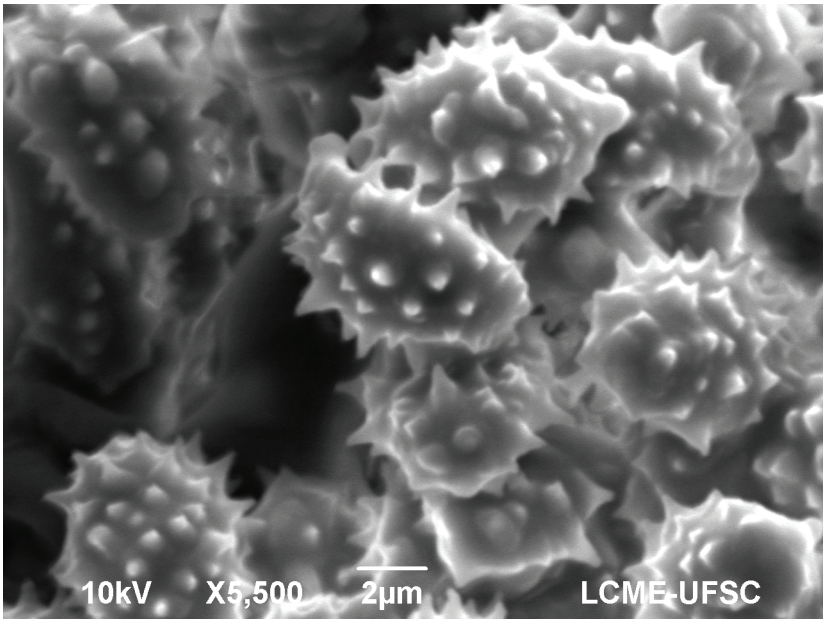

FIGURE 6. *Gloeocantharellus echinosporus*: Basidiospores (SEM). CGE 16040. Photos by F.T.F. Linhares

Discussion

Gloeocantharellus is a well-circumscribed genus in Gomphaceae, although collections are rare worldwide. There are no previous studies about the phylogenetic relationships among the species in the genus. In this article a molecular analysis based on two markers (nrITS and mit-*atp*6) was conducted, which placed the species within *Gloeocantharellus* and showed two main clades in the genus.

Although the datasets do not include all the described species of the genus, our phylogenetic results support the separation of *Gloeocantharellus aculeatus* from other species (for which DNA sequences are available), corroborating the morphological data previously discussed. The topologies with independent genes showed that *G. echinosporus* grouped with *G. corneri* and *G. aculeatus*. *Gloeocantharellus echinosporus* and *G. aculeatus* have a similar ornamentation pattern on the basidiospore walls, which is a morphological evidence of their close relationship. *Gloeocantharellus echinosporus* grows in Southeast Asia and Oceania, where the majority of the species in the genus were found. The genus has an amphi-Pacific distribution with no species known for Europe and Africa. Additional biogeographical studies about *Gloeocantharellus* would help clarify the dispersal patterns of the genus.

As for the mit-*atp*6 analyses, *G. pallidus* and *G. novae-zelandiae* represent the two most distant branches from other species, as recovered in a phylogenetic study by Giachini *et al.* (2010). These species have some unusual morphological characteristics. *Gloeocantharellus pallidus* is from Japan, and the combination of wrinkled hymenophore, absence of gloeocystidia, and absence of clamp connections can separate it from other species (Petersen 1971; Giachini 2004). *Gloeocantharellus novae-zelandiae* is from New Zealand and it has a smooth hymenophore (Giachini 2004).

Mit-*atp*6 is a widely used molecular marker in phylogenetic studies of Gomphales (Hosaka *et al.* 2006; Giachini *et al.* 2010). Despite the fact that it is a coding gene, it has sufficient interspecific variation to differentiate species and to reconstruct infrageneric relationships. Further analyses that use more samples and additional molecular markers are needed to elucidate the relationships among other taxa within the genus, including the undetermined taxa (*Gloeocantharellus spp*. 1 and 2) presented in this work.

The description of this new species from the Atlantic Forest, a world hotspot (Myers *et al.* 2000; Mittermeier *et al.* 2005), corroborates the importance of conserving this biome.

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References

Corner, E.J.H. (1966) A monograph of cantharelloid fungi. *Annals of Botany Memoir* 2: 1–255.

Corner, E.J.H. (1969) Notes on cantharelloid fungi. *Nova Hedwigia* 18 (2–4): 783–818.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9 (8): 772.
http://dx.doi.org/10.1038/nmeth.2109

Deng, C.-Y. & Li, T.-H. (2008) *Gloeocantharellus persicinus*, a new species from China. *Mycotaxon* 106: 449–453.

Dentinger, B.T.M., Margaritescu, S. & Moncalvo, J.M. (2010) Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. *Molecular Ecology Resources* 10: 628–633.
http://dx.doi.org/10.1111/j.1755-0998.2009.02825.x

Giachini, A.J. (2004) *Systematics, Phylogeny, and Ecology of Gomphus sensu lato*. Ph.D. Dissertation, Oregon State University. 446 pp.

Giachini, A.J. & Castellano, M.A. (2011) A new taxonomic classification for species in *Gomphus* sensu lato. *Mycotaxon* 115: 183–201.
http://dx.doi.org/10.5248/115.183

Giachini, A.J., Hosaka, K., Nouhra, E., Spatafora, J. & Trappe, J.M. (2010) Phylogenetic relationships of the Gomphales based on nuc-25S-rDNA, mit-12S-rDNA, and mit-atp6-DNA combined sequences. *Fungal Biology* 114: 224–234.
http://dx.doi.org/10.1016/j.funbio.2010.01.002

Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696–704.
http://dx.doi.org/10.1080/10635150390235520

Hosaka, K., Bates, S.T., Beever, R.E., Castellano, M.A., Colgan III, W., Domínguez, L.S., Nouhra, E.R., Geml, J., Giachini, A.J., Kenney, S.R., Simpson, N.B., Spatafora, J.W. & Trappe, J.M. (2006) Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. *Mycologia* 98 (6): 949–959.
http://dx.doi.org/10.3852/mycologia.98.6.949

Joseph, A.V. & Manimohan, P. (1998) Rediscovery of two rare agaricoid basidiomycetes. *Mycological Research* 102 (4): 476–478.
http://dx.doi.org/10.1017/S0953756297005169

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
http://dx.doi.org/10.1093/molbev/mst010

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28 (12): 1647–1649.
http://dx.doi.org/10.1093/bioinformatics/bts199

Kramer, L.A. (2004) *The online auction color chart*. Online Auction Color Chart Company, Stanford.

Kretzer, A.M. & Bruns, T.D. (1999) Use of atp6 in fungal phylogenetics: an example from the Boletales. *Molecular Phylogenetics and Evolution* 13(3): 483–492.
http://dx.doi.org/10.1006/mpev.1999.0680

Largent, D.L. (1986) *How to identify mushrooms to genus I: macroscopic features. I*, 2nd ed., Mad River Press Inc., Eureka, California, USA, 166 pp.

Largent, D.L, Johnson, D. & Watling, R. (1977) *How to identify mushrooms to genus III: microscopic features*, 3rd ed., Mad River Press Inc., Eureka. California, USA, 148 pp.

Miller, M.A., 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)*. New Orleans, Louisiana, pp. 1–8.
http://dx.doi.org/10.1109/GCE.2010.5676129

Mittermeier, R.A., Gil, P.R., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreux, J. & Fonseca, A.B.G. (2005) *Hotspot revisited: Earth's biologically richest and most endangered terrestrial ecoregions*. University of Chicago Press. Chicago, USA, 392 pp.

Müller, K. (2005) SeqState - primer design and sequence statistics for phylogenetic DNA datasets. *Applied Bioinformatics* 4: 65–69.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858.
http://dx.doi.org/10.1038/35002501

Petersen, R.H. (1971) The genera *Gomphus* and *Gloeocantharellus* in North America. *Nova Hedwigia* 21: 1–118.

Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. (2014) *Tracer v1.6*, U.K. Available from: http://beast.bio.ed.ac.uk/Tracer (Accessed 5 May 2016).

Romano, E. & Brasileiro, A.C.M. (1998) Extração de DNA de plantas. *Biotecnologia: Ciência & Desenvolvimento* 2 (9): 40–43.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
http://dx.doi.org/10.1093/bioinformatics/btg180

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual. Second Edition. Volumes 1, 2, and 3*. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York, USA, 1828 pp.

Sayers, E.W., Barrett, T., Benson, D.A., Bryant, S.H., Canese, K., Chetvernin, V., Church, D.M., DiCuccio, M., Edgar, R., Federhen, S., Feolo, M., Geer, L.Y., Helmberg, W., Kapustin, Y., Landsman, D., Lipman, D.J., Madden, T.L., Maglott, D.R., Miller, V., Mizrachi, I., Ostell, J., Pruitt, K.D., Schuler, G.D., Sequeira, E., Sherry, S.T., Shumway, M., Sirotkin, K., Souvorov, A., Starchenko, G., Tatusova, T.A., Wagner, L., Yaschenko, E. & Ye, J. (2009) Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research* 37: D5–D15.
http://dx.doi.org/10.1093/nar/gkn741

Segedin, B.P. (1984) Two new species of *Gomphus* Pers. (Aphyllophorales) from New Zealand. *New Zealand Journal of Botany* 22 (4): 533–537.
http://dx.doi.org/10.1080/0028825X.1984.10425286

Simmons, M.P. & Ochoterena, H. (2000) Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.
http://dx.doi.org/10.1093/sysbio/49.2.369

Singer, R. (1945) New genera of fungi. *Lloydia* 8(3): 139–144.

Singer, R. (1961) Two genera of fungi new for South America. *Vellozia* 1: 14–19.

Stalpers, J.A. (1996) The aphyllophoraceous fungi II. Keys to the species of the Hericiales. *Studies in Mycology* 40: 1–185.

Stamatakis, A. (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* 30(9): 1312–1313.
http://dx.doi.org/10.1093/bioinformatics/btu033

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
http://dx.doi.org/10.1093/molbev/mst197

Thiers, B. (2016) [continuously updated]. *Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium*. Available from: http://sweetgum.nybg.org/ih/ (accessed 19 February 2016).

Vasco-Palacios, A.M. & Franco-Molano, A.E. (2005) A new species of *Gloeocantharellus* (Fungi-Basidiomycetes) from Colombian Amazonia. *Mycotaxon* 91: 87–92.

Watling, R. & De Meijer, A.R. (1997) Macromycetes from the state of Paraná, Brazil. *Edinburgh Journal of Botany* 54 (2): 231–251.
http://dx.doi.org/10.1017/S0960428600004042