A new species of Gyroporus (Gyroporaceae, Boletales) from Atlantic Forest in Southern Brazil

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A new species of *Gyroporus* (Gyroporaceae, Boletales) from Atlantic Forest in Southern Brazil

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With 4 figures and 1 table

Abstract: Surveys of boletoid fungi in the Brazilian Atlantic Forest resulted in the discovery of a new species similar to Gyroporus castaneus named Gyroporus austrobrasiliensis. Macro- and microscopic descriptions, molecular data (nrDNA ITS and LSU sequences), photographs of the basidiomata, and scanning electron microscopy images of the basidiospores are provided.

Key words: boletoid fungi, chestnut bolete, diversity, Sclerodermatineae, taxonomy.

Introduction

Gyroporus Quél. typified by *G cyanescens* (Bull.) Quél., is a small genus of boletoid fungi in the monogeneric family Gyroporaceae in Sclerodermatineae (Boletales) comprising no more than twenty species (Kirk et al. 2008, Wu at al. 2014, Vizzini et al. 2015, Moreno et al. 2016, Das et al. 2017). Known originally from the temperate zone of north hemisphere, and found usually in coniferous and broadleaf forests, like pines (*Pinus* L.), oaks (*Quercus* L.), beech (*Fagus* L.), sweet chestnut (*Castanea* Mill.) or birch (*Betula* L.) as putative ectomycorrhizal host trees but possibly only saprobic, or merely facultatively mycorrhizal (Wilson et al. 2012). There are also reports of the genus in tropical Central and South Americas following the distribution of its possible hosts (Singer et al. 1983, Halling & Mueller 2005, Ortiz-Santana et al. 2007, Flores et al. 2010, Vizzini et al. 2015).

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The genus is perhaps one of the easiest genera of boletes to identify at genus level, characterized by basidiomata epigeous, fleshy, small to medium sized. Pileus surface dry, usually minutely velvety, subtomentose to floccose, firm and leathery, microscopically trichodermal or a cutis of appressed hyphae. Hymenophore tubular, free, with minute, simple and small pores, white then yellowish. Stipe surface villose to velvety, sometimes with horizontal fissures or cracks, context white then straw colored, unchanging or strongly turning blue to violaceus, cavernous or completely hollow at maturity, firm and brittle (breaking like chalk), due to the arrangement of the hyphae that are transversely instead of being vertically arranged like in the most mushrooms. Spore print pale yellow, spores shortly-ellipsoid, smooth, inamyloid, cheilocystidia and pleurocystidia present, clamp-connections usually present (Smith & Thiers 1971, Singer 1986, Watling 2008, Bessette et al. 2010).

Subsequent investigation on boletes diversity in the Brazilian Atlantic Forest revealed a taxon morphologically similar to *G. castaneus*, but differing in some morphological as well as molecular characters and is here proposed as a new species, *G. austrobrasiliensis*.

Material and methods

Collection sites and morphology: Collections were conducted during rainy summer season between December to March of 2015 and 2016 at Morro Santana, metropolitan area of Porto Alegre, Southern Region of Brazil. The collecting area is a remaining of seasonal semideciduous forest of about 321 ha, that reaches an altitude of 311m above sea level and is part of the northeast chain of granite hill of Serra do Sudeste, as part of the Atlantic Forest biome. The most representative arborescent families at Morro Santana are Rubiaceae, Myrtaceae, Lauraceae, Sapindaceae, Euphorbiaceae and Fabaceae (Vargas & Oliveira 2007, Silveira & Miotto 2013). Collections were also made at Campeche Island in Florianópolis, Santa Catarina state in March of 2014. The island is covered by Atlantic Forest vegetation and is 1.8 km far from Florianópolis. The first collection of *Gyroporus* from Southern Brazil in Rio Grande do Sul and the collection from the Amazon forest, both initially identified as *G. castaneus*, were also included in this study (Singer et al. 1983, Putzke et al. 1994).

Macroscopic features were described from fresh basidiomata. Color codes in brackets (e.g., OAC742) are based on the Online Auction Color Chart (Kramer 2004). Micromorphological features were examined with an Olympus CX21 microscope and descriptive terms follow Largent et al. (1977). For the basidiospores measurements and length/width ratios (Q) are presented here as (minimum)–mean minus standard deviation–**mean**–mean plus standard deviation–(maximum); n, the number of basidiospores measured per specimen; and s, the number of specimens studied. For scanning electron microscopy (SEM) of the basidiospores, fragments of the hymenophore were removed from dried basidiomata, mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a scanning electron microscope (operating at 10KeV) at Centro de Microscopia e Microanálise (CMM) at Universidade Federal do Rio Grande do Sul. Voucher materials were deposited at Herbarium ICN from the Universidade Federal do Rio Grande do Sul.

Sequencing and phylogenetic analysis: DNA extraction from dried basidiomata follows Góes-Neto et al. (2005). The primer pairs ITS6-R - ITS8-F and LR0R - LR7 were used to amplify the ITS (ITS1-5.8S-ITS2) and LSU (28S) regions following the cycling conditions in Dentinger et al. (2010). Sequencing was performed with BigDye Terminator 3.1 Cycle Sequencing Kit following the manufacturer's procedure, using the same primers cited above. Sequence chromatograms were manually checked and edited in Geneious 6.1.8 (Kearse et al. 2012) and deposited in GenBank with the accession numbers listed in Table 1.

Alignments were generated using MAFFT v. 7 (Katoh & Standley 2013), following the L-INS–i and G-INS-i criteria (for ITS and LSU, respectively), and then cleaned using Gblocks v0.91b (Talavera &

Castresana 2007), allowing smaller final blocks and gap positions within the final blocks alignments were deposited in TreeBASE (http://www.treebase.org/treebase/index.html). Phylogenetic trees were reconstructed with two phylogenetic criteria, Maximum likelihood (ML) and Bayesian Inference (BI). ML was carried out with RAxML-HPC v.8 (Stamatakis 2014), available in the CIPRES science gateway (Miller et al. 2010, http://www.phylo.org/), using GTRGAMMA as evolutive model (Stamatakis 2006), choosing the rapid bootstrap analysis (command –f a) with a random starting tree and 1000 maximum likelihood bootstrap replications. BI was conducted on MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) as implemented on the CIPRES Science Gateway 3.1 (Miller et al. 2010), utilizing four parallel MCMC chains, which were allowed to run for 10 million generations, with sampling every 1000 generations. The best-fitted substitution model for for nrLSU gene marker was determined through jModeltest v2 (Nylander 2004) by using Akaike Information Criterion (AIC).

A node was considered well supported if it showed a a bootstrap (BS) ≥ 80% and/or Bayesian posterior probability (BPP) ≥ 0.96. *Scleroderma verrucosum* was defined as outgroup, based on Wu et al. (2014) and Vizzini et al. (2015). All phylogenetic trees were visualized using FigTree (Morariu et al. 2009).

Results

Molecular analysis

Five new sequences of *Gyroporus* (3 ITS, 2 LSU) were generated during this study, these are the first sequences of *Gyroporus* for South America. Both Maximum Likelihood (ML) and Bayesian Inference (BI) analyses produced similar topology; however, only the ML trees with both bootstrapping (BS) and Bayesian posterior probability (BPP) values are shown (Figs 1, 2). The ITS data matrix comprised a total of 33 sequences (including 30 from GenBank and 3 new generated), resulted in an aligned matrix of 468 bp. The LSU matrix consisted of 39 sequences (including 37 from GenBank and 2 new generated), resulted in an aligned matrix of 910 bp. In the ITS analysis, the sequences of *G. austrobrasiliensis* form a clade of tropical taxa, together with Zimbabwe collection (BS = 94%, BPP = 0.8). In the LSU analysis *G. austrobrasiliensis* form like in the ITS analyses, a sister group with a clade including *Gyroporus* sp. from Zimbabwe and a collection identified as *G. castaneus* (BS = 89%, BPP = 1. The molecular phylogenies presented in Figs 1 and 2 provide strong evidence that, along with our morphological studies, indicate *G. austrobrasiliensis* are distinct taxa in *Gyroporus*.

Taxonomy

Gyroporus austrobrasiliensis A.C.Magnago Figs 3–4

MycoBank 821923

Holotype: BRAZIL. Rio Grande do Sul: Porto Alegre, Universidade Federal do Rio Grande do Sul, Morro Santana (30°03'48.0"S, 51°07'48.0"W), 12.I.2015, A.C.Magnago 1136 (ICN184399). GenBank accession: ITS = MF436999, LSU = MF437014.

Etymology: from the Latin *austro* = south, *brasiliensis* = Brazil. Referring to collection area in Southern Brazil.

Pileus 25–48 mm wide, convex to broadly plane convex, surface velvety to subtomentose, dry, orange-brown (OAC741, 748), margin straight, usually splitting

Table 1. GenBank accession numbers and voucher numbers of sequences used for the phylogenetic analysis. Unavailable sequences for individual taxa are indicated by –.

Taxon	Voucher No.	Locality	GenBank acession No.	
			ITS	LSU
Gyroporus ammophilus	AH45814	Spain	KX869878	KX869892
Gyroporus ammophilus	AH45843	Spain	KX869877	KX869891
Gyroporus ammophilus	AH45842	Spain	KX869876	KX869890
Gyroporus austrobrasiliensis	ACM1136	Brazil	MF436999	MF437014
Gyroporus austrobrasiliensis	ACM1144	Brazil	MF437000	MF437015
Gyroporus austrobrasiliensis	ACM1150	Brazil	MF437001	–
Gyroporus aff. *castaneus*	REH8804	Thailand	EU718101	EU718137
Gyroporus aff. *castaneus*	E4600	–	–	EU718169
Gyroporus aff. *castaneus*	E843c	–	–	EU718170
Gyroporus castaneus	AH45841	Spain	KX869875	KX869889
Gyroporus castaneus	AH45844	Spain	KX869874	KX869888
Gyroporus castaneus	JMP0028	USA	EU819468	–
Gyroporus castaneus	SGT 2012	USA	JX030211	–
Gyroporus castaneus	PRL5664	USA	GQ166901	–
Gyroporus castaneus	PRL5872MAN	USA	GQ166884	–
Gyroporus castaneus	Gc1	Germany	EU718099	AF336252
Gyroporus castaneus	Arora 01 512	–	–	FJ710209
Gyroporus castaneus	HKAS76672	China	–	KF112478
Gyroporus castaneus	NCJ16	–	–	AY612808
Gyroporus castaneus	239-97	USA	–	AF336253
Gyroporus castaneus	Gc2	Germany	–	EU718168
Gyroporus castaneus	F1086418	–	–	EU718167
Gyroporus castaneus	F:PRL5664MAN	USA	GQ166887	–
Gyroporus castaneus	239/97	–	EU718100	–
Gyroporus castaneus	F:PRL5948MAN	USA	GQ166885	–
Gyroporus castaneus	3861	Canada	KM248947	–
Gyroporus aff. *cyanescens*	REH8821	Australia	EU718103	EU718139
Gyroporus aff. *cyanescens*	E486	Australia	–	EU718173
Gyroporus aff. *cyanescens*	E5685	Australia	–	EU718174
Gyroporus aff. *cyanescens*	REH8819	USA	–	EU718172
Gyroporus cyanescens	AH535	Spain	KX869879	KX869893
Gyroporus cyanescens	Gcy2	USA	–	AF336254
Gyroporus cyanescens	17184	Italy	JF908785	–
Gyroporus cyanescens	2837	Canada	KM248948	–
Gyroporus cyanescens	MB05-04	USA	EU718102	–
Gyroporus cyanescens	REH8758	Australia	–	EU718171
Gyroporus cyanescens	MCVE:28580	Italy	KT363684	KT363685
Gyroporus cyanescens	MB05-001	USA	–	EU718138
Gyroporus cyanescens	NAMA 190	USA	EU819495	–
Gyroporus lacteus	MCVE:28582	Italy	KT363682	KT363683
Gyroporus longicystidiatus	2B10	Japan	AB973746	–
Gyroporus paramjitii	KD 16-002	India	MF120284	MF120285

Gyroporus pseudocyanescens	OKM23719	Australia	–	EU718140
Gyroporus purpurinus	PRL3737	USA	EU718105	EU718141
Gyroporus sp.	REH8805	–	–	EU718175
Gyroporus sp.	REH8799	Thailand	EU718106	EU718142
Gyroporus sp.	Arora00-029	Zimbabwe	EU718107	EU718143
Gyroporus sp.	HKAS:63505	China	–	KF112476
Gyroporus sp.	HKAS:52520	China	–	KF112475
Gyroporus subalbellus	OKM25477	USA	EU718108	EU718144
Gyrporus sp.	E8155	Australia	–	EF561627
Gyrporus sp.	E4879cRBG Kew	–	–	FJ710208
Scleroderma verrucosum	K(M)54413	–	EU784416	–
Scleroderma verrucosum	Isolate 5	–	–	AF336271

in age. Tubes 3–4 mm long centrally, whitish to light yellow (OAC815, 816), free, depressed around stipe; pores 2–4 per mm, circular, unchanging under pressure. Stipe 22–36 mm × 3–4 mm, central, equal, brittle, developing several cavities or becoming hollow, surface like the pileus, paler especially near the apex. Context whitish (OAC 816), brittle, unchanging when exposed. Macrochemical reactions: 10%NH_4OH on pileus staining pink-orange and on stipe surface orange. 5%KOH on pileus staining yellow-orange. Spore print pale yellow.

Basidiospores (6.0)–7.0–**7.5**–8.0 × 5.0–**5.5**–6.0 µm; Q = 1.2–**1.45**–1.6; n/s = 30; s = 11) ellipsoid to broad ellipsoid, hyaline in H_2O, inamyloid, smooth, thin walled. Basidia 21–27 × 11–12 µm, clavate, thin walled, hyaline, 4-sterigmate, 2–3 µm long. Pleurocystidia scattered or rarely present, usually not projecting over the hymenium, aculeate to fusoid, 26–31 × 10–12 µm, hyaline, inamyloid, smooth, thin walled. Cheilocystidia very abundant, 15–33 × 4–6 µm, fusoid, cylindrical to narrowly ventricose, hyaline, inamyloid, smooth, thin walled. Hymenophoral trama boletoid, mediostratum with narrow hyphae, individual hyphae 3–8 µm wide, slightly circumferentially incrusted; lateral stratum divergent, hyphae gelatinized. Pileipellis as a trichodermium composed of clusters of terminal elementes; these elements 28–56 × 9–15 µm, tapered to a subacute apex, most hyaline, however, some hyphae with yellowish contents, smooth, slightly thick-walled, inamyloid; inflated hyphae near the base of trichodermium. Pileus trama interwoven, individual hyphae 5–22 µm wide, hyaline. Stipitipellis a trichodermium composed by caulocystidia cylindrical, narrowed fusoid to clavate, like pileocystidia, 4–11 µm wide, hyaline, inamyloid. Stipe trama composed of hyphae circumferentially arranged (not longitudinal), more compacted near the stipitipellis and becoming loosely towards the center, hyaline, inamyloid. Clamp connection present, more visible in the trama.

Habit, habitat and distribution: the specimens examined were collected during rainy summer (Dec-Jan), growing gregarious to scattered on the ground, under broad-leaf trees (Myrtaceae and Lauraceae) in Southern Atlantic Forest in Rio Grande do Sul and Santa Catarina, and under leguminous trees in the Amazon forest (Singer et al. 1983).

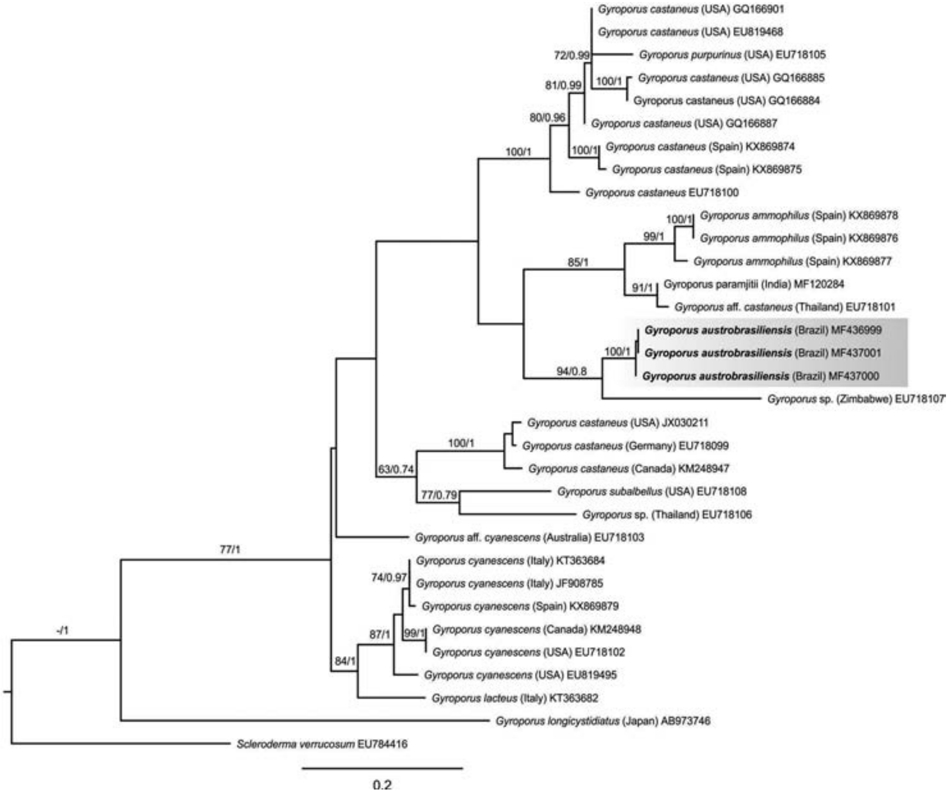

Fig. 1. Maximum likelihood tree of selected *Gyroporus* based on ITS sequences. Bootstrap values above 70% and Bayesian posterior probability above 0.9 are shown.

Collections examined: BRAZIL. Rio Grande do Sul: Porto Alegre, Morro Santana, 16.I.2015, A.C.Magnago 1144 (ICN184400), 1148 (ICN184401), 1150 (ICN184402), 28.XII.2015, A.C.Magnago 1251 (ICN192821), 1252 (ICN192822), 1253 (ICN192823), 4.I.2016, A.C.Magnago 1260 (ICN192824), 9.I.2017, A.C. Magnago 1335 (ICN192826); Pelotas, Horto Botânico, 6.IV.1960, Edemar C.Santos 161, det. Singer B. 7.XI.1961 (SP60960); Santa Catarina: Florianópolis, Ilha do Campeche, (27°41'57.6"S, 48°28'01.6"W) 24 Mar 2014, A.C.Magnago 1021 (ICN192825).

Additional collection examined: BRAZIL. Amazonas: São Gabriel da Cachoeira, 23.I.1979, Singer R B11487 (INPA82388).

Commentary: Morphologically and phylogenetically *G. austrobrasiliensis* falls into *G. castaneus* complex, in the non-cyanescent group of *Gyroporus* species. Sharing the orange brown tones of the pileus and stipe, white to yellowish hymenophore, pale yellow spore print, and unchanging context. Using those macrocharacters usually specimens are identified widely as *G. castaneus*, and its distribution considered widespread. Microscopically *G. austrobrasiliensis* differs from *G. castaneus* as it circumscribed by the presence of smaller basidiospores, usually *G. castaneus* in the north hemisphere has basidiospores larger (8–13 × 5–6 µm) (Quélet 1886), and the

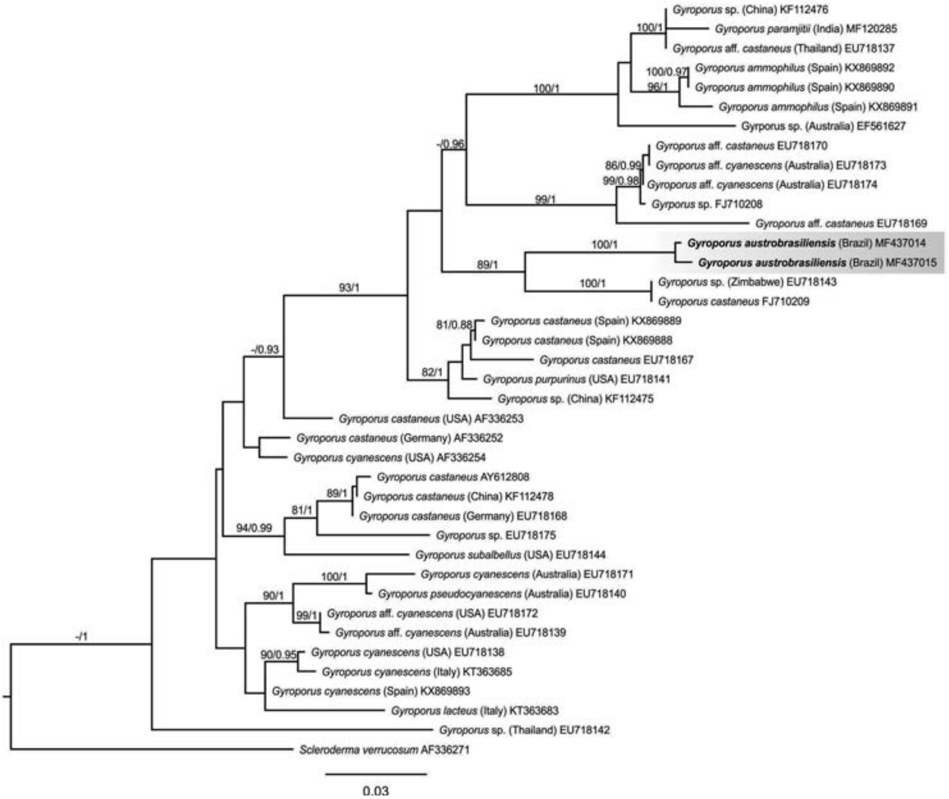

Fig. 2. Maximum likelihood tree of selected *Gyroporus* based on LSU sequences. Bootstrap values above 70% and Bayesian posterior probability above 0.9 are shown.

presence of aculeate to fusoid pleurocystidia in *G. austrobrasiliensis*. *Gyroporus castaneus* is mainly found associated with oaks (*Quercus* sp, Fagaceae) in the north hemisphere (Smith & Thiers 1971). This plant family do not occur naturally in Brazil, only as introduced plantations. The Southern Brazilian specimens were found in native forests under leguminous trees, Myrtaceae and Lauraceae as possible ectosymbionts.

A morphologically similar non cyanescent species in the genus is *G. purpurinus* (Snell) Singer that differs from *G. austrobrasiliensis* basically by the purplish red pileus and stipe colors and absence of pleurocystidia (Singer 1945).

The additional specimen from Brazilian Amazon forest identified by Singer et al. (1983) as *G. castaneus* was detail examined. The collection (INPA82388) has only one entire basidioma covered with mold. After examination, the microstructures observed, even mostly collapsed, match with *G. austrobrasiliensis* proposed here, however, we decided to keep the identity of this collection at genera level until more samples can be recollected to confirm the identity of the Amazonian species.

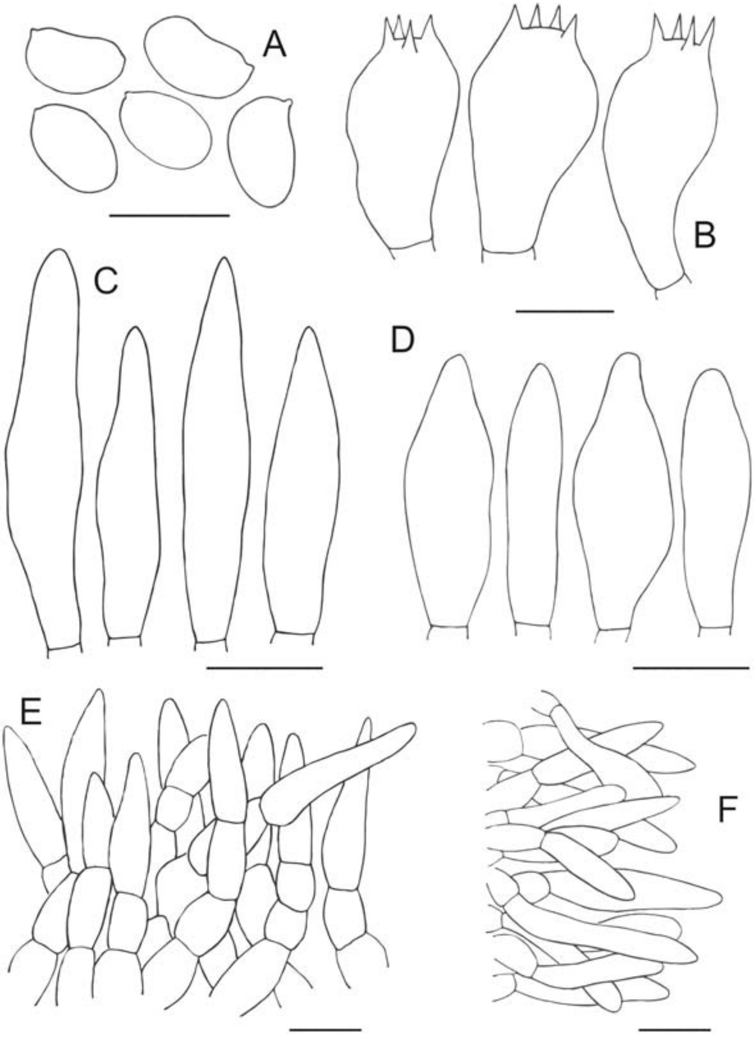

Fig. 3. *Gyroporus austrobrasiliensis*: micromorfological features. A. Basidiospores, B. Basidia, C. Pleurocystidia, D. Cheilocystidia, E. Pileipellis, F. Stipitipellis. Bar A–D = 10 µm, E–F = 25 µm.

Smith & Thiers (1971) commented that the range of colors and sizes presented in the collections of specimens identified as *G. castaneus* in the USA could cover more than one species. This idea was reinforced by molecular analyses by Wilson et al. (2012), Vizzini et al. (2015) and our studies, suggesting that the name *G. castaneus* as commonly ascribed to field and herbarium collections represents multiple cryptic taxa (complex of species), instead of a single species having a wide geographic distribution.

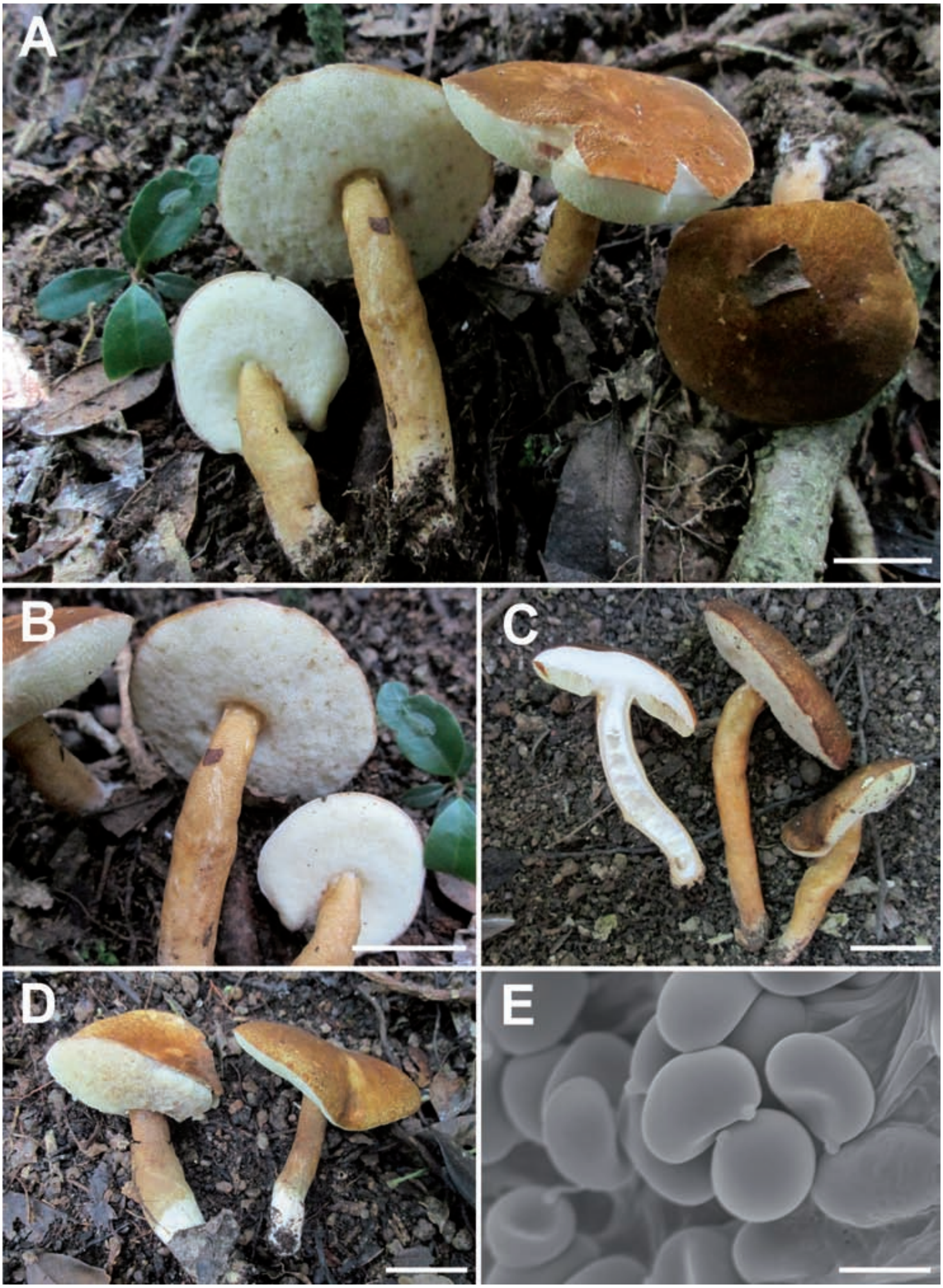

Fig. 4. *Gyroporus austrobrasiliensis*. A–D. Macromorphological features in the field. A–B. ACM1136, C. ACM1144, D. ACM1148. E. Scanning electron microscopy of the basidiospores. Bar A–D = 10 mm, E = 5μm.

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