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Singerocomus atlanticus sp. nov., and a first record of *Singerocomus rubriflavus* (Boletaceae, Boletales) for Brazil

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ABSTRACT

Ongoing surveys of macrofungi in the Brazilian Atlantic Forest ecoregion continue to uncover a diverse assemblage of ectomycorrhizal fungi. A new species of Boletaceae, *Singerocomus atlanticus* sp. nov., is described. *Singerocomus rubriflavus*, previously known only from Guyana, is recorded for the first time from the Brazilian Atlantic Forest. Phylogenetic data, macro- and microscopic illustrations, and comments are presented for each species.

Keywords: Atlantic Forest, boletoid fungi, ITS and LSU sequences, Neotropics, taxonomy

Introduction

The Atlantic Forest ecoregion of Brazil is a globally important biodiversity hotspot, harboring many endemic species across multiple taxonomic groups (Olson & Dinerstein 2002; Paese *et al.* 2010; Joly *et al.* 2014). Ectomycorrhizal (ECM) fungi were traditionally poorly known from the Atlantic Forest, although this situation is changing (Meijer 2008; Maia *et al.* 2015; Barbosa-Silva *et al.* 2017; Magnago *et al.* 2017). In particular, macrofungi in ECM basidiomycete lineages of the Agaricales, Boletales, Russulales, and Cantharellales appear to be widespread in the Atlantic Forest region and new species and distribution records are being rapidly described (e.g. Sulzbacher *et al.* 2013a; b; Roy *et al.* 2017).

In Brazil new species of Boletaceae (Boletales, Agaricomycetes, Basidiomycota) have been described from the Amazon rainforest (e.g. Singer & Digilio 1957; 1960; Singer *et al.* 1983) as well as the Atlantic Forest (e.g. Rick 1960; Vinha 1988; Putzke *et al.* 1994; Oliveira & Sousa 1995; 1996; 2002; Watling & Meijer 1997; Neves & Capelari 2007; Meijer 2008; Magnago & Neves 2014; Barbosa-Silva *et al.* 2017; Magnago *et al.* 2017). Most of these new species were placed in traditional, morphology-defined genera of Boletaceae in the absence of corroborative DNA sequence data with the exception of the three last works cited.

The genus *Singerocomus* was proposed by Henkel *et al.* (2016) to accommodate a new species from Guyana, *Singerocomus rubriflavus*, and a new combination for *Xerocomus inundabilis*, originally described from the Brazilian Amazon (Singer *et al.* 1983). Diagnostic features for *Singerocomus* include a pileate-stipitate, epigeous basidioma, pinkish red to red, tomentose pileus, yellow, tubulose, non-cyanescent hymenophore depressed at the stipe, subangular pores, concolorous or lighter stipe with or without squamules, and olivaceous brown basidiospore deposit. The combination of smooth basidiospores, phylloporoid tube trama, trichodermial pileipellis, and lack of clamp connections characterize the genus micromorphologically. The new genus was justified by a multi-locus, taxon-dense phylogenetic analysis across the Boletaceae which demonstrated that *Singerocomus* species

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had no close relatives at the generic level within the family (Henkel *et al.* 2016).

As part of our ongoing macrofungal surveys in the Atlantic Forest, *Singerocomus atlanticus* sp. nov. is described, and *S. rubriflavus*, previously known only from Guyana, is recorded for the first time for Brazil.

Materials and methods

Collections

Collections of *Singerocomus* T.W. Henkel & M.E. Sm. were made between 2011 and 2016 at multiple sites in the Brazilian Atlantic Forest, including Reserva Biológica Augusto Ruschi in Espírito Santo, Parque Estadual da Serra do Conduru in Bahia, and Unidade de Conservação Ambiental do Desterro and Plaza Caldas da Imperatriz in Santa Catarina.

Macroscopic features were described from fresh basidiomata and specimens were dried with a food dehydrator (Total Chef TCFD-05 Deluxe) at about 40 ºC. Color codes (e.g. OAC 640) were based on the Online Auction Color Chart (Kramer 2004). Macrochemical tests were performed according to Singer (1986). Microscopic structures were rehydrated in 3 % KOH and dyed with Congo Red. Melzer´s reagent was used for testing amyloidity. To observe the surface of the basidiospores in scanning electron microscopy (SEM), small fragments of the hymenophore were taken from dried specimens and mounted directly on aluminum stubs using carbon adhesive tabs, coated with 30 nm of gold, and examined with a JEOL JSM-6390LV scanning electron microscope, operating at 10KeV, at the Centro de Microscopia e Microanálise of the Universidade Federal do Rio Grande do Sul. Line drawings of microscopic features were made using digital photographs from the specimens examined. Voucher materials were deposited at ICN and FLOR Herbaria and duplicates will be sent to VIES (Thiers 2017).

DNA extraction, amplification and sequencing

DNA was extracted from dried specimens using the Doyle & Doyle (1987) protocol adapted by Góes-Neto *et al.* (2005). Full nuclear ribosomal internal transcribed spacer nrITS (ITS1-5.8S-ITS2) and nuclear ribosomal large subunit (nrLSU) were PCR-amplified with primer pairs ITS6-R/ITS8-F, and the nuclear LSU rDNA (28S) were PCR-amplified with LR0R/LR7 following Dentinger *et al.* (2010) and Vilgalys & Hester (1990) respectively. Complementary unidirectional sequence reads were aligned and edited in Geneious 6.1.8 (Kearse *et al.* 2012) and deposited in GenBank (Tab. 1). The ITS ribosomal DNA sequence from the new taxon was initially subjected to a BLASTn query against GenBank to explore its putative phylogenetic relationships. A total dataset of 38 sequences (18 nrITS and 20 nrLSU) were used in the analyses, both newly generated in this study or downloaded from Genbank database. Five genera (*Butyriboletus*, *Lanmaoa*, *Rugiboletus*, *Rubroboletus*, *Singerocomus*) were included in the phylogenetic analyzes based on Henkel *et al.* (2016) with *Bothia* included as the outgroup.

Sequence alignment, and phylogenetic analysis

Each dataset was automatic alignment separately using MAFFT (Katoh & Standley 2013), following the L-INS–i and G-INS-i criteria (for nrITS and nrLSU, respectively). Gblocks (Talavera & Castresana 2007) was used to exclude ambiguous portions of the alignment, producing a final aligned dataset of 775 bp for ITS and 841 bp for 28S. Alignments were deposited in TreeBASE (http://www.treebase.org/treebase/index.html) under accession no. ID 21441. Maximum likelihood (ML) analysis was performed separately on ITS and 28S to determine whether there were any well-supported phylogenetic incongruencies between these two loci. Because no supported incongruence was detected we conducted maximum likelihood and Bayesian inference (BI) analysis based on the concatenated ITS+28S dataset. The dataset was subdivided into four partitions: ITS1, 5.8S, ITS2, nrLSU. Maximum likelihood 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 the model of evolution (Stamatakis 2006), choosing the rapid bootstrap analysis (command –fa) with a random starting tree and 1000 maximum likelihood bootstrap replications. Bayesian inference was conducted on MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) as implemented on the CIPRES Science Gateway 3.1 (Miller *et al.* 2010), using four parallel MCMC chains, which were allowed to run for 20 million generations, with sampling every 1000 generations. The best-fitted substitution models were set to each partition calculated by MrModeltest 2.3 (Nylander 2004). The Akaike information criterion was chosen to select the most appropriate model of DNA substitution for each data set or data partition used in the analyses. The convergence diagnostic was calculated every 10^4 generations and its critical value was set in order to automatically stop the analysis when the standard deviation of the split frequencies had reached the value defined by the stopval command (stoprule = yes stopval = 0.01). In all analyses, the first 25 % trees from each run were discarded as burnin. Resulting trees from the two independent runs were then pooled to produce one 50 % majority-rule consensus tree and Bayesian posterior probabilities were generated for the resulting tree. A node was considered well supported if it showed a Bayesian Posterior Probabilities (BPP) ≥ 0.95 and/or Bootstrap (BS) ≥ 80 %, while moderate support was considered BS ≥ 70 %, and non-supported BPP < 0.95. All phylogenetic trees were visualized using FigTree (Morariu *et al.* 2009).

Table 1. GenBank accession numbers and voucher numbers of sequences used for the phylogenetic analysis.

Species	Collection No.	Origin	GenBank accesion No.	
			ITS	LSU
Bothia castanella	MB03 067	USA	DQ867114	DQ867115
Butyriboletus fechtneri	AT2003097	-	KC584784	KF030270
Butyriboletus regius	MG 408a	-	KC584789	KC584790
Butyriboletus yicibus	Arora 9727	China	KC184474	KC184475
Lanmaoa asiatica	HKAS 54094	China	-	KF112353
Lanmaoa carminipes	MB06 001	USA	-	JQ327001
Rubroboletus latisporus	HKAS 80358	China	KJ951990	KP055023
Rubroboletus sinicus	HKAS 68620	China	KJ951991	KF112319
Rugiboletus brunneiporus	HKAS 83209	China	-	KM605134
Rugiboletus extremiorientalis	HKAS 563591	China	-	KM605136
Singerocomus atlanticus	ACM 527	Brazil	KY907177	KY926776
Singerocomus atlanticus	CATO 106	Brazil	KY907179	KY926778
Singerocomus atlanticus	ACM 1275	Brazil	KY907178	KY926777
Singerocomus atlanticus	BZ L69	Brazil	KY907181	-
Singerocomus atlanticus	MJ 105	Brazil	KY907181	-
Singerocomus inundabilis	TH8408	Guyana	JN021114	HQ161863
Singerocomus inundabilis	Henkel 9199	Guyana	LC043087	LC043087
Singerocomus inundabilis	Aime 4004	Guyana	LC043090	LC043090
Singerocomus inundabilis	TH10087	Guyana	KT380014	KT380016
Singerocomus inundabilis	TH10109	Guyana	KT380013	KT380015
Singerocomus rubriflavus	MCA 5472	Guyana	LC043093	-
Singerocomus rubriflavus	Henkel 9585	Guyana	-	KP941572
Singerocomus rubriflavus	GAS 900	Brazil	KY907182	KY926779

Results

BLASTn queries and phylogenetic analysis

Ten new sequences of *Singerocomus* from Brazil were generated (6 nrITS and 4 nrLSU). ITS BLASTn queries of each of the new taxa on GenBank indicated affinities with specimens in the genera *Xerocomus* and *Singerocomus*. LSU BLASTn queries indicated affinities with Boletaceae, but were uninformative at the genus level.

All rDNA markers were combined into a single matrix, including 38 sequences from 23 specimens representing 13 putative species, resulting in an aligned matrix of 1116 bp. The respective ML tree showing BS and BPP values on branches are shown in Figure 1. All phylogenetic analysis performed showed that specimens of *Singerocomus* grouped into one distinct, well supported clade (BS = 0.99, BPP = 1 %) that included the new species, *S. inundabilis* (Singer) T.W. Henkel, and *S. rubriflavus* T.W. Henkel & Husbands. The *S. inundabilis* clade contained only specimens from Guyana (BS = 1.0, BPP = 100 %). The Brazilian *S. rubriflavus* specimen clustered with others from Guyana in a well-supported clade (BS = 0.92, BPP = 98 %), although the variability in branch lengths among the specimens suggests intraspecific variability. The other specimens collected in the south of the Atlantic Forest clustered in a distinct well-supported clade (BS = 1.0, BPP = 100%) described below as the new species *Singerocomus atlanticus*.

Taxonomy

Singerocomus atlanticus A.C. Magnago **sp. nov.** (Figs. 2A-F, 3A-D)

MycoBank MB822576

Etymology. from the Latin *atlanticus* = referring to the Atlantic Forest where the new species was collected.

Diagnosis. Differs from other known *Singerocomus* species by the combination of the following characters: pileus red-orange to pink-red, velutinous with olivaceous yellow pruina, stipe glabrous to vertically rugulose, instantly bluing reaction on pileus surface and ferrugineous-orange on the stipe with NH_4OH, basidiospores broadly ellipsoid, 6–8 × 4–5 µm, cystidia in the tubes and pores fusoid to ventricose-rostrate.

Type. BRAZIL, Espírito Santo: Santa Teresa, Reserva Biológica Augusto Ruschi, Trilha Casa da Pedra, 19° 54' 19.5" S; 40° 34' 08.4" W, 05 December 2012, *A.C. Magnago 527* (ICN 188266!; GenBank accessions: ITS = KY907177, 28S = KY926776).

Description. *Pileus* 16–37 mm wide, parabolic at first to convex to planoconvex when mature, velutinous, red-orange to pink-red (OAC 629, 642), darkest centrally

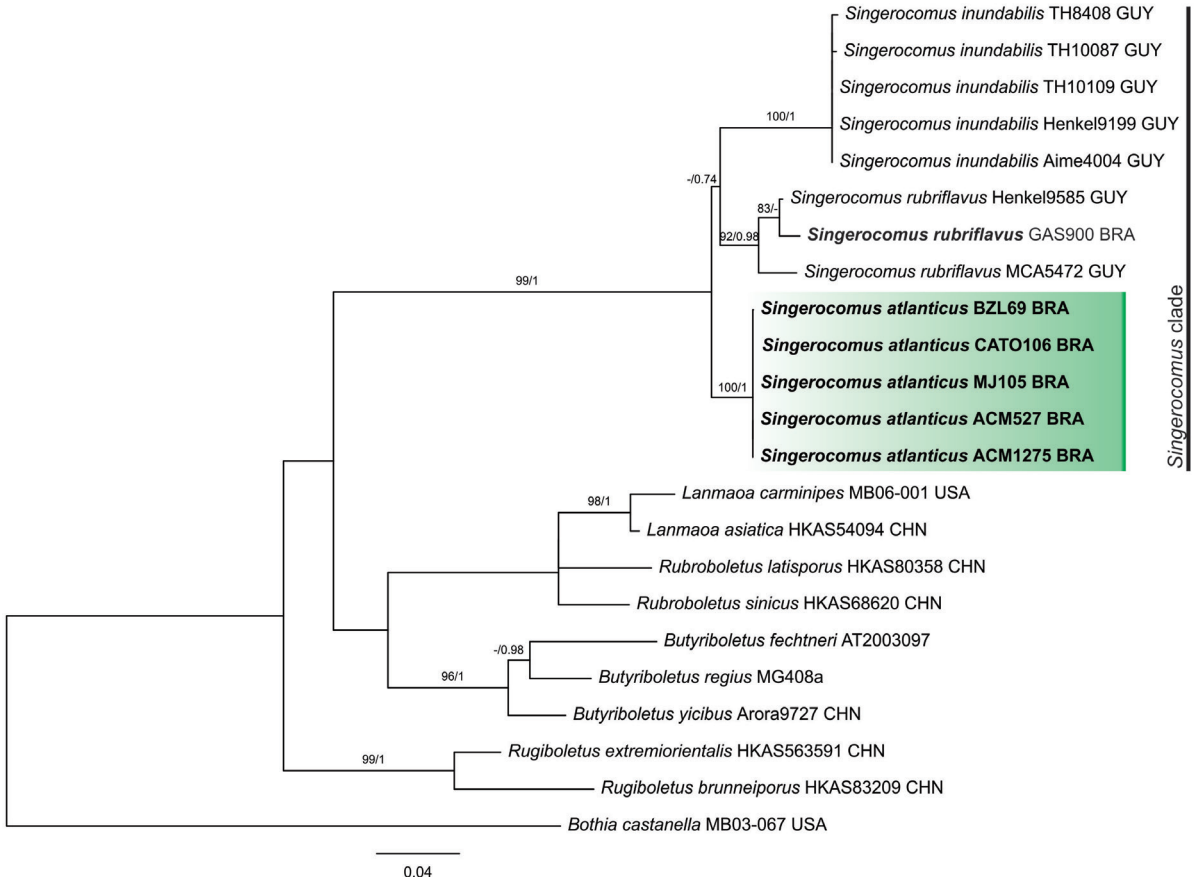

Figure 1. Maximum likelihood (ML) tree based on nrITS and nrLSU sequences. Bootstrap values above 70% and Bayesian posterior probability above 0.9 are shown.

lighter progressively toward the margin; surface initially with olivaceous yellow (OAC 831, 838) pruina throughout (more visible under lens) with age becoming matted-velutinous and exposing a light pink to light yellow (OAC 855, 858) ground, dry; margin even; context whitish to cream yellow (OAC 812), solid, unchanging when exposed. *Tubes* 2–5 mm long centrally, sulphureus yellow (OAC 002, 895), depressed around stipe; pores mostly 2–3 per mm, subangular, unchanging under pressure. *Stipe* 26–54 mm × 3–5 mm centrally, subequal, glabrous to vertically rugulose to reticulate, off white to light yellow (OAC 003) over apical 1/5, red to pink (OAC 628, 629, 631) over central 3/5, and citrine yellow (OAC 010) over basal 1/5; *basal mycelium* white at extreme base; context cream yellow (OAC812), solid, unchanging when exposed. *Odor* and *Taste* not obtained. *Macrochemical reactions*: NH4OH instantly blue on pileus surface and ferrugineous-orange on the stipe; KOH on pileus and stipe surface becoming instantly yellow. *Spore print* olive brown (OAC 867), light deposit. *Basidiospores* 6–8 × 4–5 µm (Qm=1.60), broadly ellipsoid, olivaceous in H2O, paler in 3% KOH, inamyloid, smooth, thin walled; hilar appendage 0.5–1 µm long. *Basidia* 28–40 × 8–11 µm, narrowly clavate to clavate, thin-walled, hyaline, 4-sterigmate; sterigmata 2–5 µm long. *Pleurocystidia* and *cheilocystidia* similar in size and shape, 34–62 × 8–11 µm, fusoid to ventricose-rostrate, usually with a long neck, hyaline, inamyloid, smooth, thin-walled. *Hymenophoral trama* slightly divergent (phylloporoid); individual hyphae of mediostratum narrow, 3–6 µm wide, yellowish, inamyloid; oleiferous hyphae present; lateral stratum with individual hyphae more inflated, 6–10 µm wide, slightly divergent. *Pileipellis* a semi-erect to erect trichodermium; terminal elements 5–12 µm wide, cylindrical, rounded at apex, light brown to yellowish in H2O, inamyloid, slightly incrusted circumferentially under phase contrast. *Pileus trama* interwoven to subparallel; individual hyphae 4–14 µm wide, hyaline to light yellow in H2O. *Stipitipellis* repent with discontinuous clusters of suberect, clavate to cylindrical terminal elements, 24–28 × 6–12 µm, hyaline to pale yellow in H2O, inamyloid, thin walled. *Stipe trama* of longitudinally arranged hyphae; individual hyphae 5–7 µm wide, uninflated, hyaline to light yellow in H2O, inamyloid. *Clamp connections* absent.

Habit, habitat, and distribution. Solitary to gregarious on soil in proximity to putative ECM host plants of the Nyctaginaceae and Fabaceae; known only from the Brazilian Atlantic Forest.

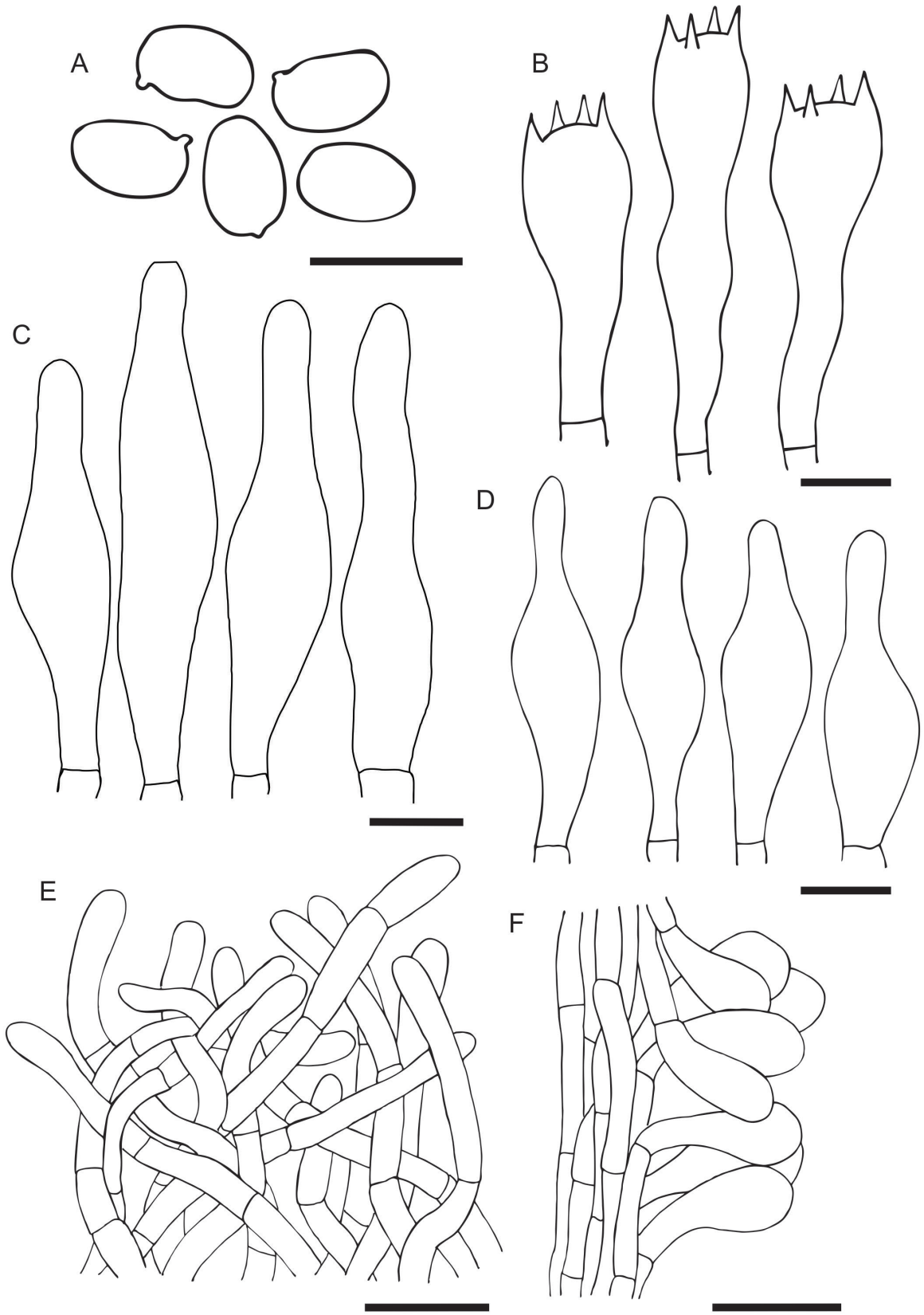

Figure 2. Microscopic features of *Singerocomus atlanticus* (holotype, *ACM 527*). **A.** Basidiospores. **B.** Four-sterigmate basidia. **C.** Pleurocystidia. **D.** Cheilocystidia. **E.** Pileipellis. **F.** Stipitipellis. Bars A–D = 10 µm, E–F = 25 µm.

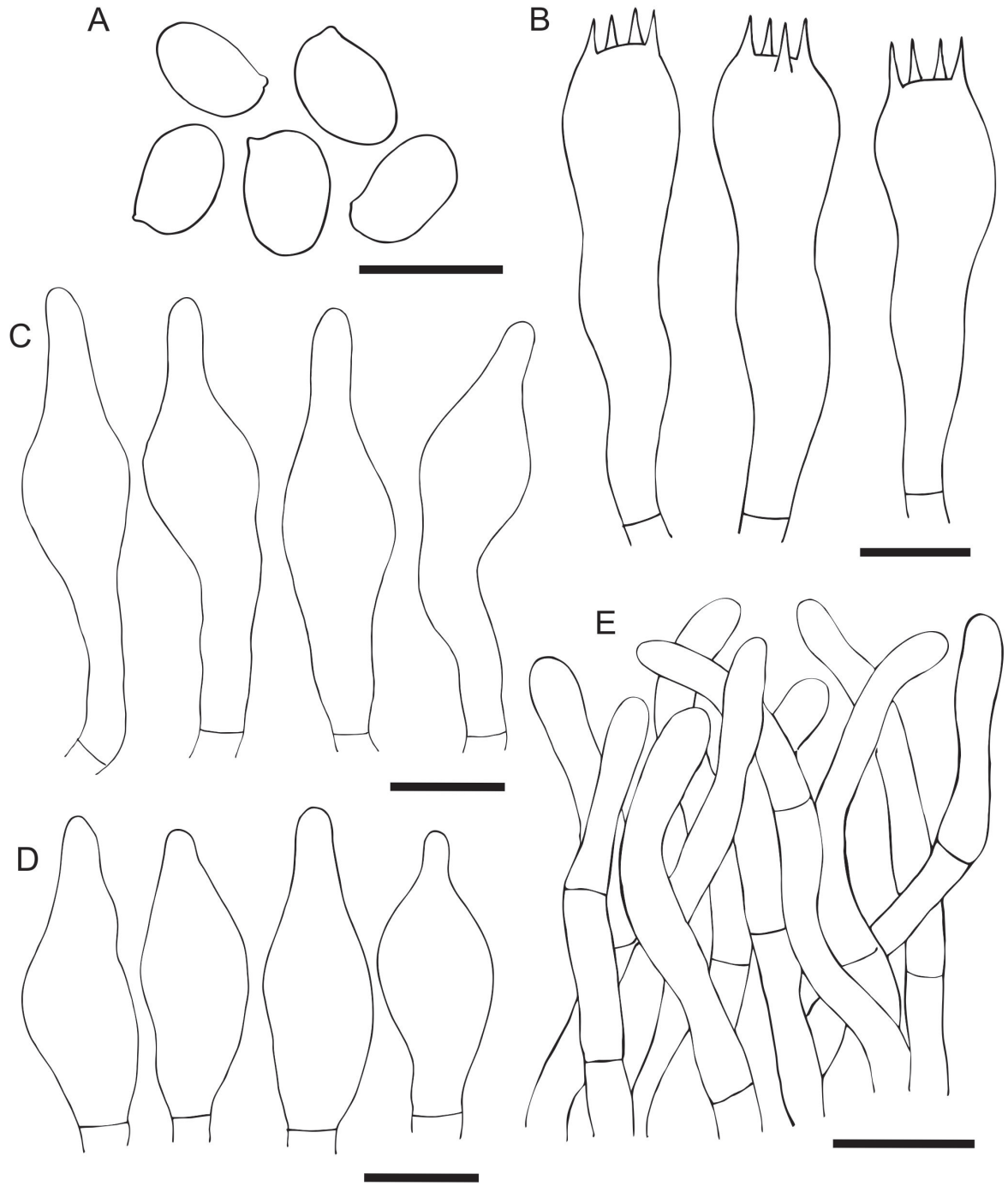

Figure 3. Macromorphology and MEV of the basidiospores. **A–D.** *Singerocomus atlanticus*. A,B,D (holotype, *ACM 527), C* (*ACM 1275*). **E–F.** *Singerocomus rubriflavus* (*GAS 900*). Bar = 15 mm.

Additional specimens examined. BRAZIL, Santa Catarina: Florianópolis, Saco Grande, Unidade de Conservação Ambiental do Desterro, 27°31'52.9"S 48°30'45.3"W (Park headquarters coordinates) 20 December 2011, *M. Jaeger 105* (ICN 188267!; GenBank accession: ITS = KY907180); 21 December 2011, *B. Dentinger 69* (ICN 188268!; GenBank accession: ITS = KY907181); 15 January 2015, *C. Oliveira 106* (ICN 188269!; GenBank accession: ITS = KY907179, 28S = KY926778); 13 April 2017, Trilha do Pântano do Sul, *M.A. Neves 1216* (FLOR!); Santo Amaro da Imperatriz, Plaza Caldas da Imperatriz, 27°44'02.3"S; 48°48'48.8"W, 12 January 2016, *A.C. Magnago 1275* (ICN 188270!; GenBank accession: ITS = KY907178, 28S = KY926777). *Singerocomus inundabilis*. BRAZIL.

Amazonas: Manaus, Igarapé do Turumãzinho, 09 October 1978, *Singer RB* B11432 (INPA-Fungos 82822 – holotype!). *Xerocomus chapinii*. **Brazil:** Amazonas, Manaus, Igarapé do Turumãzinho, 12 March 1980, *Chapin 12027* (INPA-Fungos 101995 – holotype!).

Commentary. *Singerocomus atlanticus* is morphologically similar to *S. inundabilis* (Tab. 2) but differs in its richer red pileus color and its NH4OH macrochemical reactions of instantly blue on the pileus and ferrugineous-orange on the stipe, features lacking in the other known species of the genus (Singer *et al.* 1983; Henkel *et al.* 2016). From a molecular standpoint *Singerocomus atlanticus* is also clearly different at the species level from *S. inundabilis* (Fig. 1). *Singerocomus atlanticus* has a broad distribution in the Atlantic Forest region, with conspecific specimens documented from the Espírito Santo type locality and from Santa Catarina ca. 1.700 km distant.

The combination of pink to red pileus and stipe colors and yellow hymenophore of *S. atlanticus* is reminiscent of several boletes described outside of the genus, including *Xerocomus chapinii*, *Hortiboletus campestris* and *H. rubellus*. *Xerocomus chapinii*, described from *igapó* vegetation in the Brazilian Amazon, differs from *S. atlanticus* in its mostly cylindrical, narrower basidiospores (6–7.8 × 2.7–3.2 µm), frequent ampullaceous hymenial cystidia with obtuse necks, and ixocutis pileipellis when young (Singer *et al.* 1983). The North American *H. campestris* and *H. rubellus* differ from *S. atlanticus* by their greenish-blue autoxidation reaction of exposed context and tubes, and elliptic to subfusoid basidiospores that are considerably longer (10–15 µm vs. 6–8 µm) (Smith & Thiers 1971; Vizzini 2015).

Singerocomus rubriflavus T.W. Henkel & Husbands Mycologia 108: 169. 2016 (Figs. 3E-F, 4A-E)

Description. *Pileus* 35 mm wide, convex to plane convex when mature, matted-tomentose, dark red (OAC 600, 656) over a pinkish (OAC 850) ground, dry, lighter towards the margin and darker in the center, margin entire, straight to slightly wavy. *Context* yellowish (OAC 812), solid, unchanging when exposed. *Tubes* 5–6 mm long centrally, olivaceous yellow (OAC 008, 019), depressed around stipe, sublamellate at stipe slightly decurrent; pores mostly 1–2 per mm, subangular, unchanging under pressure. *Stipe* 42 mm × 7–9 mm, central, subequal, pinkish (OAC 665, 672) covered by yellowish (OAC 811) depressed squamules. *Basal mycelium* a dense, bright yellow (OAC 001) tomentum, extreme base white. *Macrochemical reactions* not observed. *Spore print* olivaceous brown. *Basidiospores* 8–10 × 5–6 µm (Qm=1.69), ellipsoid, with a prominent suprahilar depression, hilar appendage 0.5–1 µm long, olivaceous brown in H2O, paler in 3 % KOH, inamyloid, smooth, thin walled. *Basidia* 29–40 × 9–11 µm, clavate, tapering toward the base, thin walled, hyaline, 4-sterigmate, 2–7 µm long. *Pleurocystidia* 33–66 × 11–12 µm, ventricose to ventricose-rostrate, hyaline, inamyloid, smooth, thin walled. *Cheilocystidia* 29–42 × 8–10 µm, broadly ventricose to obclavate, hyaline, inamyloid, smooth, thin walled. *Hymenophoral trama* subparallel to divergent (phylloporoid), mediostratum with narrow

Table 2. Comparison among taxa of *Singerocomus* (Singer *et al.* 1983; Henkel *et al.* 2016).

	S. atlanticus	*S. inundabilis*	*S. rubriflavus*
Pileus	16–37 mm wide, convex to plane convex, velutinous to matted-velutinous, reddish to pinkish, with olive yellowish pruina all over	25–33(–47) mm wide, broadly convex to planate, areolate, occasionally with a low umbo, pinkish red	37–77 mm broad, broadly convex, areolate to matted-tomentose, dark red
Hymenophore	Sulphureus yellow, unchanging with pressure	Olivaceous, unchanging with pressure	Olivaceous yellow, darkening slightly with pressure
Stipe	26–54 mm × 3–5 mm, glabrous to vertically rugulose to reticulate, whitish to light yellow at the top, reddish to pink in the center, and citrine yellow in the base	37–63 × 5–7 mm, pinkish red over upper two-thirds, lower third light yellow, glabrous, sligtly pulverulent and faintly longitudinally striate	39–70 × 9–14 mm, light yellow squamules on concolorous ground
Basidiospores	6–8 × 4–5 µm (Qm=1.60), broadly ellipsoid, olivaceous	6–9 × 4–5.5(–7) µm (Qm= 1.38), ellipsoid, with proemiment suprahilar depression, olivaceous brown	9–12 × 4.5–6.5 µm (Qm= 1.94), ellipsoid, with a proeminent suprahilar depression, olivaceous brown
Pleurocystidia	Similar in shape and size, 34–62 × 8–11 µm, fusoid to ventricose-rostrate, usually with a long neck, conspicuous	34.5–61 × 7.5–10µm, narrowly to broadly ventricose rostrate, infrequent	32–64 × 10–15 µm, ventricose to ventricose-rostrate, infrequent to scattered
Cheilocystidia		Absent	29.5–39 × 67–10 µm, broadly ventricose-rostrate or obclavate, infrequent
Reaction with NH_4OH	Bluing on pileus surface and ferrugineous-orange on the stipe	Slightly yellowing on pileus, unreactive elsewhere	Slightly yellowing or unreactive on all surfaces

Figure 4. Microscopic features of *Singerocomus rubriflavus* (GAS 900). **A.** Basidiospores. **B.** Four-sterigmate basidia. **C.** Pleurocystidia. **D.** Cheilocystidia. **E.** Pileipellis. Bars A–D = 10 µm, E = 25 µm.

hyphae, individual hyphae 3–7 µm wide, yellow, inamyloid, oleiferous hyphae absent; lateral stratum with hyphae somewhat inflated, individual hyphae 6–13 µm wide, divergent. *Pileipellis* a trichodermium, in mass brownish in H2O, terminal individual hyphae 4–11 µm wide, round apex, with minute incrustations circumferentially, inamyloid. *Pileus trama* interwoven, individual hyphae 4–11 µm wide, hyaline to light yellow in H2O. *Stipitipellis* hymenidermous, sterile terminal elements clavate to cylindrical, 16–28 × 7–11 µm, clusters of caulobasidia present, hyaline to pale yellow in H2O, inamyloid, thin walled. *Stipe trama* slightly interwoven to vertically arranged hyphae, cylindrical,

individual hyphae 4–8 µm wide, hyaline to light yellow in H20, inamyloid. *Clamp connection* absent.

Specimen examined. BRAZIL, Bahia: Uruçuca, Parque Estadual da Serra do Conduru, 14°29'40.9"S 39°08'01.8"W, 19 September 2016, G. *Alves-Silva 900* (ICN 188271!; GenBank accession: ITS = KY907182, 28S = KY926779).

Habit, habitat, and distribution. Solitary on soil, in the north region of the Atlantic Forest in Bahia. The species was previously known only from Guyana in association with ECM *Pakaraimaea dipterocarpacea* (Cistaceae).

Commentary. *Singerocomus rubriflavus* is a highly distinctive bolete recognized by its medium to large basidiomata with dark red, tomentose-pitted and centrally areolate pileus, and olivaceous yellow, non-cyanescent tubes. and unique squamulose surface of the concolorous, solid stipe. Micromorphologically the species combines ellipsoid basidiospores, a trichodermial pileipellis, and hymenidermous stipitipellis (Henkel *et al.* 2016).

Only one single-basidiome collection of *S. rubriflavus* has so far been made in Brazil, approximately 3.000 km from the type locality in Guyana. While the Brazilian specimen agrees well both macro- and micromorphologically with the Guyana type specimen, minor differences include the smaller stipe squamules and slightly shorter basidiospores (8–10 *µm* vs. 9–11(–12) *µm*) of the Brazilian specimen. Although the Brazilian specimen and two specimens from Guyana formed a well-supported monophyletic group in our phylogenetic analysis (Fig. 1), the variability in branch lengths among the specimens suggests intraspecific variability. As noted by Henkel *et al.* (2016) similar genetic variability was found among multiple morphologically identical specimens from Guyana, and more research is needed to determine whether *S. rubriflavus* may encompass a species complex.

Discussion

Morphological and molecular analyses corroborate the placement of our boletoid specimens in *Singerocomus*. Given that *Singerocomus* was first recognized from an Amazonian species described in *Xerocomus*, renewed taxonomic and phylogenetic scrutiny could be profitably applied to Neotropical Boletaceae. In this respect special attention should be given to the numerous Amazonian boletes described in *Xerocomus* by Rolf Singer (Singer & Digilo 1957; 1960; Singer *et al.* 1983). Most boletoid specimens deposited at INPA are poorly preserved, and many species have only the holotype, these were observed when INPA Herbarium was visited.. Recollections of these species will help to confirm the occurrence of *Xerocomus* species in Brazil and phylogenetic analysis may uncover more new genera.

While the Brazilian Atlantic Forest has long been recognized for its unique plants and animals, macrofungal surveys in the region continue to uncover a wealth of undescribed species. In the present study our discoveries of a new bolete species and a large range extension for another highlight the importance of continued mycological exploration in this unique region.

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