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Short note

Singerocomus guadelupae (Boletaceae, Agaricomycetes): morphology and phylogeny reveal a widely distributed neotropical bolete discovered in Northeast Brazil

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Abstract

Singerocomus guadelupae is characterized by velutinous and red to pink pileus surface, cream to red or both colors mixed in stipe surface, unchanging white to cream pileus and stipe context, broadly ellipsoid inamyloid basidiospores, and fusoid to ventricose-rostrate cystidia. Morphological, molecular and phylogenetic analyses were performed on materials collected in Paraíba state, demonstrating that they belong to *S. guadelupae* with some characteristics not previously described for the species, suggesting a somewhat intraspecific phenotypic and phylogenetic plasticity. Also, it has a wide distribution, occurring from the Lesser Antilles towards South Brazil, and now is recorded for the first time from Northeast Brazil, in two conservation units from the state of Paraíba.

Keywords
Atlantic Forest, Basidiomycota, Boletales, distribution, systematic, taxonomy

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Introduction

Singerocomus T.W. Henkel & M.E. Sm. is a neotropical genus of boletoid fungi recently described from Brazil and Guyana with *S. inundabilis* (Singer) T.W. Henkel as type species (Henkel et al., 2016). It was based after the combination of *Xerocomus inundabilis* Singer into *Singerocomus* from

Brazilian Amazon and the description of *S. rubriflavus* T.W. Henkel & Husbands from the Pakaraima Mountains in Guyana and adjacent lowlands forests (Henkel et al., 2016). This genus is macromorphological characterized by the pinkish red to red and tomentose pileus with white to light yellow changing context; tubulose hymenophore depressed around the stipe, bearing yellow, subangular tubes with unchanging pores; equal in length, concolorous or lighter stipe, with glabrous or squamulose surface, with a yellow to dull yellow tomentose base, and unchanging white to light yellow context when cut. Microscopically it is characterized by olivaceous brown spore print, smooth and inamyloid ellipsoid basidiospores, presence (in *S. rubriflavus* and *S. guadelupae*) or absence (in *S. inundabilis*) cheilocystidia, mostly parallel (i.e., phylloporoid) hymenophoral trama, pileipellis a trichodermium with cylindrical terminal cells, and clamp connections absent (Henkel et al., 2016). There are only three known taxa of *Singerocomus*: *S. inundabilis*, *S. rubriflavus*, and more recently *S. atlanticus* A.C. Magnago from Brazil (Magnago et al., 2018), which was recombined into *S. guadelupae* by Gelardi et al. (2023). *Singerocomus rubriflavus* was also recorded from this country, in the Atlantic Forest of the state of Bahia (Magnago et al., 2018). This work aims to describe the first record of *S. guadelupae* from Northeast Brazil, collected in two Atlantic Forest conservation units from the state of Paraíba, with the description of morphological, molecular and phylogenetic data.

Materials and Methods

Specimens were collected from the ‘Reserva Biologica (REBio) Guaribas’, an Atlantic Forest reserve located in the municipalities of Mamanguape, state of Paraíba, Northeast Brazil (Barbosa and Leal, 2022). The collections were made in a ‘tabuleiro savanna’ forest (Thomas and Barbosa, 2008) in the ‘SEMA 2’, the largest forest fragment of this full protected conservation unit (Barbosa et al., 2011; Barbosa and Leal, 2022). The ‘Área de Proteção Ambiental (APA) Barra do Rio Mamanguape’ is another conservation unit, but with sustainability purpose and is composed by Atlantic Forest remnants with 14,460 ha, formed by the estuaries of the Mamanguape, Miriri and Estivas rivers, covering some areas in the state of Paraíba, Northeast Brazil (Alves and Nishida, 2003; Pereira and Alves, 2007). Color codes follow Kramer-oac (2004).

Microscopic observations were made from dried material mounted in 3% KOH and Congo red solutions, while the photomicrographs were taken using a camera and software connected to a microscope. Biometric statistics of the basidiospoores were based on 60 measurements. For other microstructures (basidia, basidioles, pileipellis, hymenophoral trama, cystidia), 20-25 measurements were realized for each one. Abbreviations include Qm = the Q value averaged from all basidiospores measured. The analyzed materials are deposited in the herbarium Lauro Pires Xavier-JPB (Thiers, 2024).

To confirm our specimens identification, the whole genomic DNA of two dried specimens was extracted from the hymenium using DNeasy Plant Mini Kit (Qiagen, Germany). Partial sequences were obtained from the nuclear internal transcribed spacer (ITS rDNA) with the primer pairs ITS1F/ITS4 (White et al., 1990; Gardes and Bruns, 1993). PCR reactions were performed according to Barbosa-Silva et al. (2022). PCR products were unidirectional sequenced in an ABI 3130 Genetic Analyzer (Applied Biosystems®).

We used GENEIOUS v 9.1.3 (Kearse et al., 2012) to check the sequence quality of the strands by comparison to their respective chromatograms. To identify our samples we assigned it to a species through a nucleotide BLAST approach conducted in GenBank (http://blast.ncbi.nlm.nih.gov; November 19, 2024). Sequences of *Singerocomus* present in GenBank were incorporated to analyses for the ITS rDNA region. In the phylogenetic analysis, we aligned sequences with MAFFT v7.017 (Katoh et al., 2002), using the G-INS-I algorithm.

The best nucleotide substitution model was selected using jModelTest 2v.1.6. (Darriba et al., 2012), and Maximum Likelihood (ML) analysis was performed in RAxML v8.2.X (Stamatakis, 2014) using GTRGAMMA as model of substitution for bootstrapping phase (rapid bootstrap analysis with 1000 replicates). These softwares are implemented in the CIPRES Science Gateway 3.1 (Miller et al., 2010).

Bayesian inferences (BI) were performed using MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003), with the best nucleotide substitution model selected automatically according to the BIC (Bayesian Information Criterion) for the ITS rDNA dataset (K80+G), with a module implemented in TOPALi v. 2 (Milne et al., 2008). Two independent parallel runs were run, sampling every 100 MCMC generation for 1 million total generations. After discarding the first 25% of the sampled trees as burn-in, a majority rule consensus tree and posterior probabilities (PP) were computed using the remaining trees. *Butyriboletus* and *Rubroboletus* species, and *Bothia castanella* (Peck) Halling, T.J. Baroni & Manfr. Binder were used as the outgroup taxa. Our sequences were deposited in GenBank (NCBI) under accession OQ288919 and OQ288920.

Results and Discussion

Comments on the type specimen of Boletus guadelupae

Boletus guadelupae Singer & J.P. Fiard, 1976

Holotype/paratype (Gelardi et al., 2023; 31 of 36): Guadelupe, Matouba, Basse Terre, 700 m, 31.vii.1975, leg. J.P. Fiard 563 (F 1020987).

Notes: Gelardi et al. (2023) discussed about the type material of *B. guadelupae* mentioning a brief confusion regarding the typification status of the specimens. The browsed image from the botanical collection of the herbarium F website (https://fm-digital-assets.fieldmuseum.org/1861/225/C0326156F_SPECIMEN_b.jpg) shows two different sheets, 'Fiard 563' and 'Fiard 563B'. Indeed, they really came from the two localities mentioned by Singer and Fiard (1976), in which 'Fiard 563' is from Matouba (the holotype) and 'Fiard 563B' from Sofaïa (paratype). However, that image showed three basidiomata and a single metallic pot, on which we can suppose that all were placed inside. Unfortunately, apparently both collections are mixed into this pot, making it unable to correlate which specimen belongs to which place. Thus, the best solution is to assume all exsiccatum with barcode C0326156F simultaneously corresponding to holotype and paratype of *B. guadelupae* (Gelardi et al., 2023), since the holotype was well defined in the protologue of the species (Greuter et al., 2025; Art. 9.2).

Phylogenetic analysis

In the species identification, our nucleotide BLAST analysis of the two sequences (OQ288919 and OQ288920) showed that the specimens correspond to *S. guadelupae* (nuc-ITSrDNA, identity 99.35–100%, E-value 0.00). Both ML and Bayesian analyses produced the same topologies. Therefore, only the ML trees with both MLB and BPP values are shown (Fig. 1). The ITS dataset of *S. guadelupae* comprises 32 sequences and 820 characters. The ITS sequences cluster our specimens together with

S. guadelupae collections from Guadeloupe (including the holotype-OQ108304) and Martinique, with two *Xerocomus* sp. from Guyana, and five Brazilian *S. atlanticus* corresponding to the "guadelupae clade" obtained by Gelardi et al. (2023) with high support. We also observed two well characterized subclades, one formed by Brazilian and Lesser Antilles sequences, and the second by continental sequences (Brazil and Guyana). It apparently reflects a probable genotypic variation among the populations of *S. guadelupae*, with the occurrence of both in the Atlantic Forest of Paraíba.

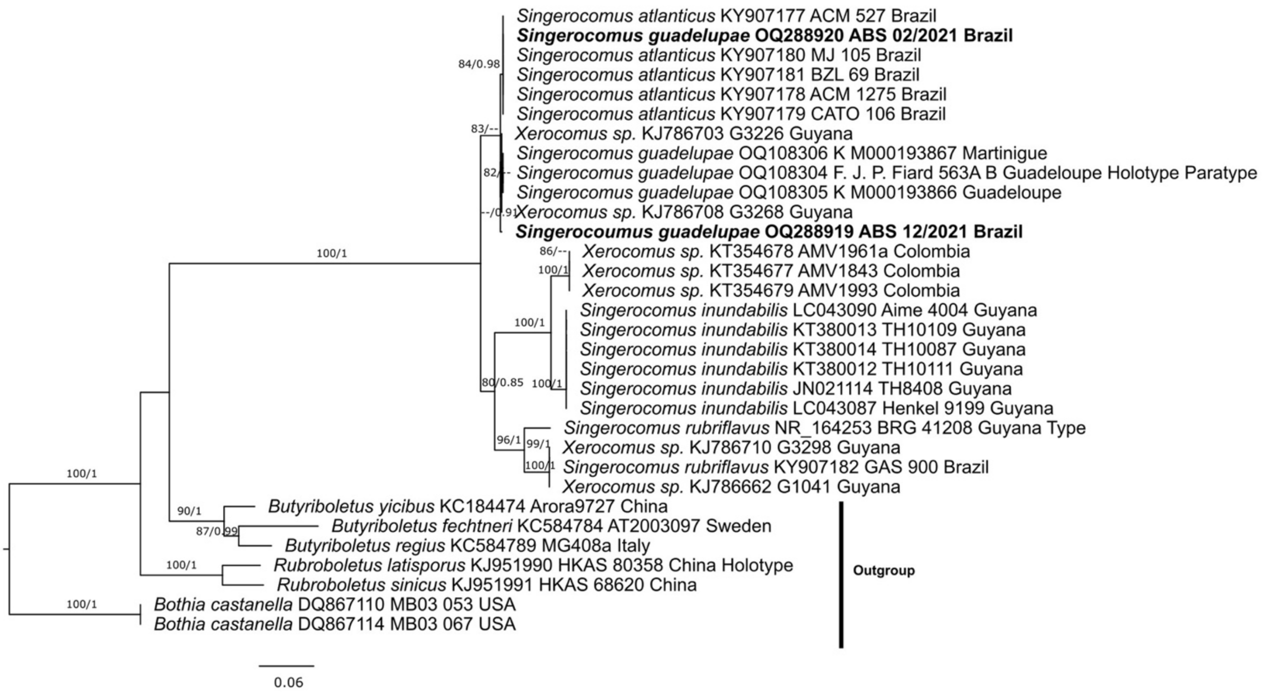

Fig. 1 – Phylogenetic tree of *Singerocomus* spp. based on ML and BI analyses for the nrDNA-ITS region. Sequences generated in this study are in bold. Bootstrap support values (MLB ≥ 0.70, right) and Posterior Probabilities values (BPP ≥ 0.80, left) are shown above the supported branches.

Description of the new collection

Singerocomus guadelupae (Singer & Fiard) Gelardi, Biketova, Suz & Vizzini, Mycol. Prog. 22: 29. 2023.

≡ *Boletus guadelupae* Singer & Fiard, Bull. Soc. Mycol. Fr. 92: 445. 1976.
≡ *Xerocomus guadelupae* (Singer & Fiard) Pegler, Kew Bull. Add. Ser. 9: 575. 1983.
= *Singerocomus atlanticus* A.C. Magnago, Acta Bot. Bras. 32: 224. 2018.
Figs. 2–3

Pileus 31–45 mm in diam., convex to plano-convex, dark wine (oac622) to dark red (oac608,609, 622) or pinkish (oac500) where can show pale yellowish cream tones (oac899), surface light velutinous, minutely rimulose-areolate, dry, margin entire and plane; context 4–8 mm thick near center, cream (oac858) to pale light cream (oac7) or white (oac909), solid, soft, unchanging.

Hymenophore tubulose, green (oac75, 96) light green (oac47, 48) ranging to 3–5 mm long, adnate, can be depressed around the stipe; pores rounded to subrounded to angular (some), more radially elongate near to stipe, 0.4–1.5 mm diam., concolor with the tubes, unchanging when injured.

Stipe 54–73 × 7–8 mm, central, equal, cream (oac814, 815) to pale cream (oac900) with pink tones (oac484, 499) where in some materials the apex can be pinkish (oac500) with the base yellowish cream (oac5, 6); surface longitudinally appressed fibrillose, and small patches of appressed squamules on apex, tapering towards the base, dry; context cream (oac814) to white (oac909), solid, fibrous, unchanging, some materials show partially consumed with visible yellowish worm trails. Odor pleasant, slightly sweet. Taste none. Spore print not performed.

Macrochemical chemical tests: in the presence of KOH 3-4% pileus surface turns beige greenish to light ocher and context light ocher, hymenophore greenish ocher to ocher, stipe surface ocher to negative and context negative; in the presence of NH_4OH pileus surface turns beige green to negative and context, hymenophore, stipe surface and context negative.

Basidiospores [60,2,2] (5.3–)5.8–7.8(–8.3) × (3.9–)4.4–5.3 µm, L = 7.1 µm, W = 4.7 µm, Q = (1.18–)1.27–1.77(–1.88), Qm = 1.52, ellipsoid to broadly ellipsoid, smooth, pale in KOH 3-4%, inamyloid, thin-walled, hilar appendix prominent and sublateral. **Basidia** 24.0–36.7 × 7.8–10.8 µm, clavate, 4-sterigmata, hyaline in KOH 3%, thin-walled. **Pleurocystidia** 33.3–81.3 × 5.8–9.8 µm, fusoid to subfusoid to subventricose-rostrate, sometimes with long neck and obtuse apex, hyaline in KOH 3%, thin-walled. **Cheilocystidia** 36.2–53.9 × 5.8–8.8 µm, subfusoid to subventricose-rostrate, sometimes with long neck and obtuse apex, hyaline in KOH 3%, thin-walled. **Hymenophoral trama** barely divergent to phylloporoid, gelatinized, with hyphae 3–9 µm wide, hyaline in KOH 3%, thin-walled, presence of oleiferous hyphae. **Pileipellis** trichodermal, terminal elements and cylindrical terminal cells with obtuse apex, 5.4–13.8 µm wide, thin-walled, light brownish in KOH 3%. Pileus trama with interwoven hyphae 4.4–11.7 µm wide, hyaline in KOH 3%, gelatinized, presence of oleiferous hyphae. **Stipitipellis** predominantly a cutis, but in a few segments a trichodermium with cylindrical to elongated-cylindrical terminal cells with obtuse apex, 4.4–8 µm wide, pale to hyaline in KOH 3%; scattered tufts composed of caulobasidioles; caulobasidia broadly clavate, 18.6–30.4 × 7.8–11.3 µm, thin-walled, and a few caulocystidia subventricose-rostrate with obtuse apex 25.5–37.2 × 5.8–11.8 µm, all pale to hyaline in KOH 3%, thin-walled. Stipe trama longitudinally oriented, with hyphae 5.4–10.8 µm wide, hyaline in KOH 3%, presence of oleiferous hyphae. Clamp connections absent.

Habit and habitat: Epigeous, solitary or gregarious on sandy soil in ‘tabuleiro savannas’ of Atlantic Forest fragments belonging to two conservation units in Northeast Brazil. In the collected area there are some tree species belonging to Nyctaginaceae and Polygonaceae families, reported to contain species forming ectomycorrhiza (Smith and Read, 2008): *Guapira opposita* (Vell.) Reitz, *G. pernambucencis* (Casar.) Lundell, *Coccoloba alnifolia* Casar., *C. arborescens* (Vell.) R.A. Howard, *C. laevis* Casar., *C. mollis* Casar., *C. ramosissima* Wedd. and *C. scandens* Casar. occur in the REBio Guaribas (Barbosa et al., 2011), whereas *C. alnifolia* and *C. laevis* are referred from the APA Barra do Rio Mamanguape (Pereira and Alves, 2007).

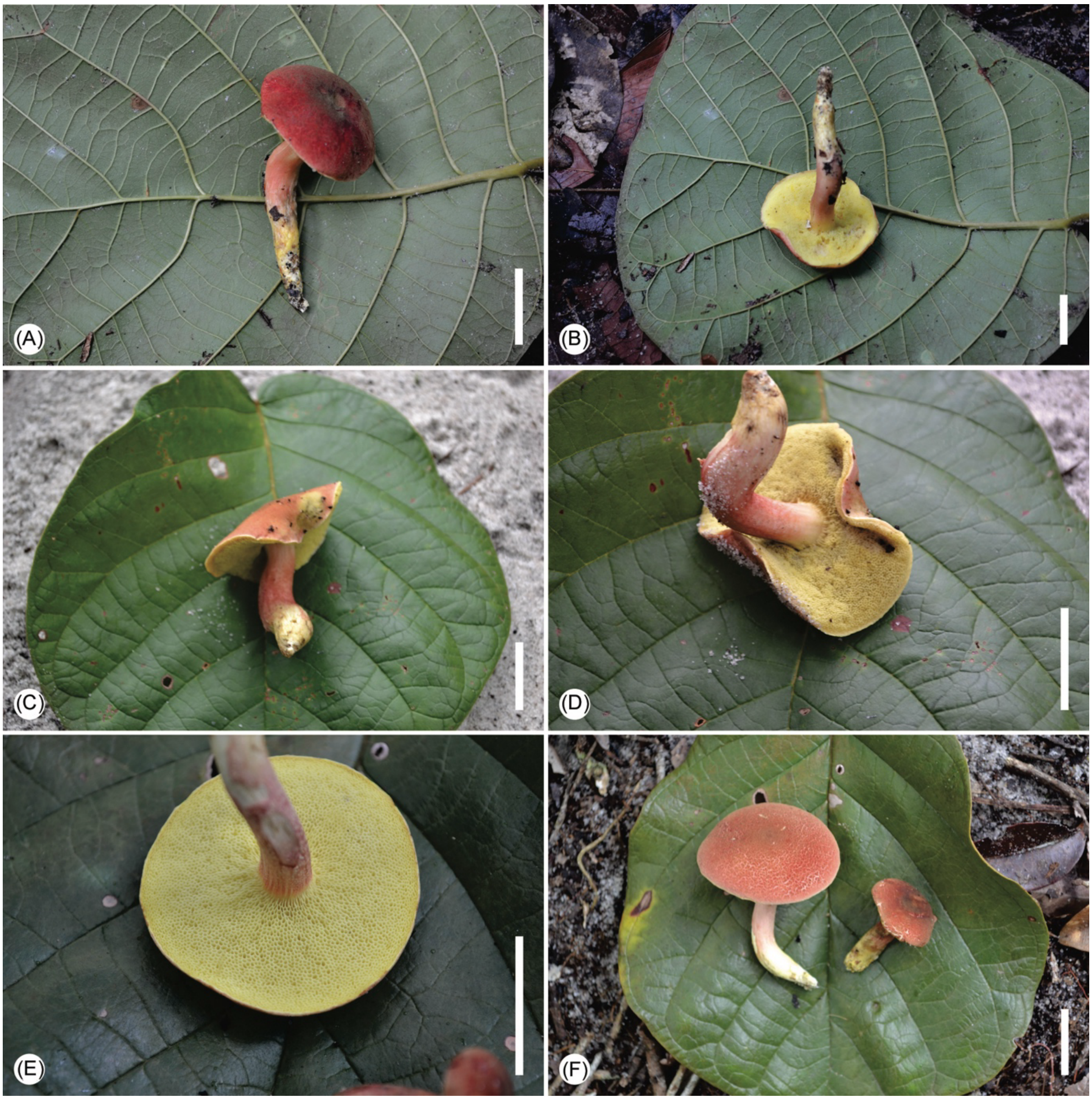

Fig. 2 – Basidiomes of *Singerocomus guadelupae*. A-B) ABS 02/2021 (JPB 67187); C-D) ABS 12/2021 (JPB 67194); E-F) FW 64/2021 (JPB 67191). Scale bars = 20 mm.

Know distribution: Brazilian Atlantic Forest of States of Espírito Santo and Santa Catarina (Magnago et al., 2018 as *S. atlanticus*), and now from the Atlantic Forest of the state of Paraíba. It was also recorded from Martinique, Guadeloupe and Dominica in the Lesser Antilles (Singer and Fiard, 1976; Pegler, 1983; Gelardi et al., 2023).

Material examined: Paraíba, Mamanguape, REBio Guaribas, SEMA II, 28.vii.2021, A. Barbosa-Silva ABS 12/2021 (JPB 67191) GenBank ITS OQ288919-LSU OQ288923; same place, same date, A. Barbosa-Silva ABS 19/2021 (JPB 67192); same place, 04.viii.2021, F. Wartchow FW 64/2021(JPB 67194); Rio Tinto, APA Barra do Rio Mamanguape, Mata do Oiteiro, 26.vii.2021, A. Barbosa-Silva ABS 02/2021 (JPB 67187) GenBank ITS OQ288920-LSU OQ288924; same place, same date, A. Barbosa-Silva ABS 03/2021 (JPB 67188), A. Barbosa-Silva ABS 05/2021 (JPB 67189), A. Barbosa-Silva ABS 06/2021 (JPB 67190); same place, 02.viii.2021, A. Barbosa-Silva ABS 22/2021 (JPB 67193).

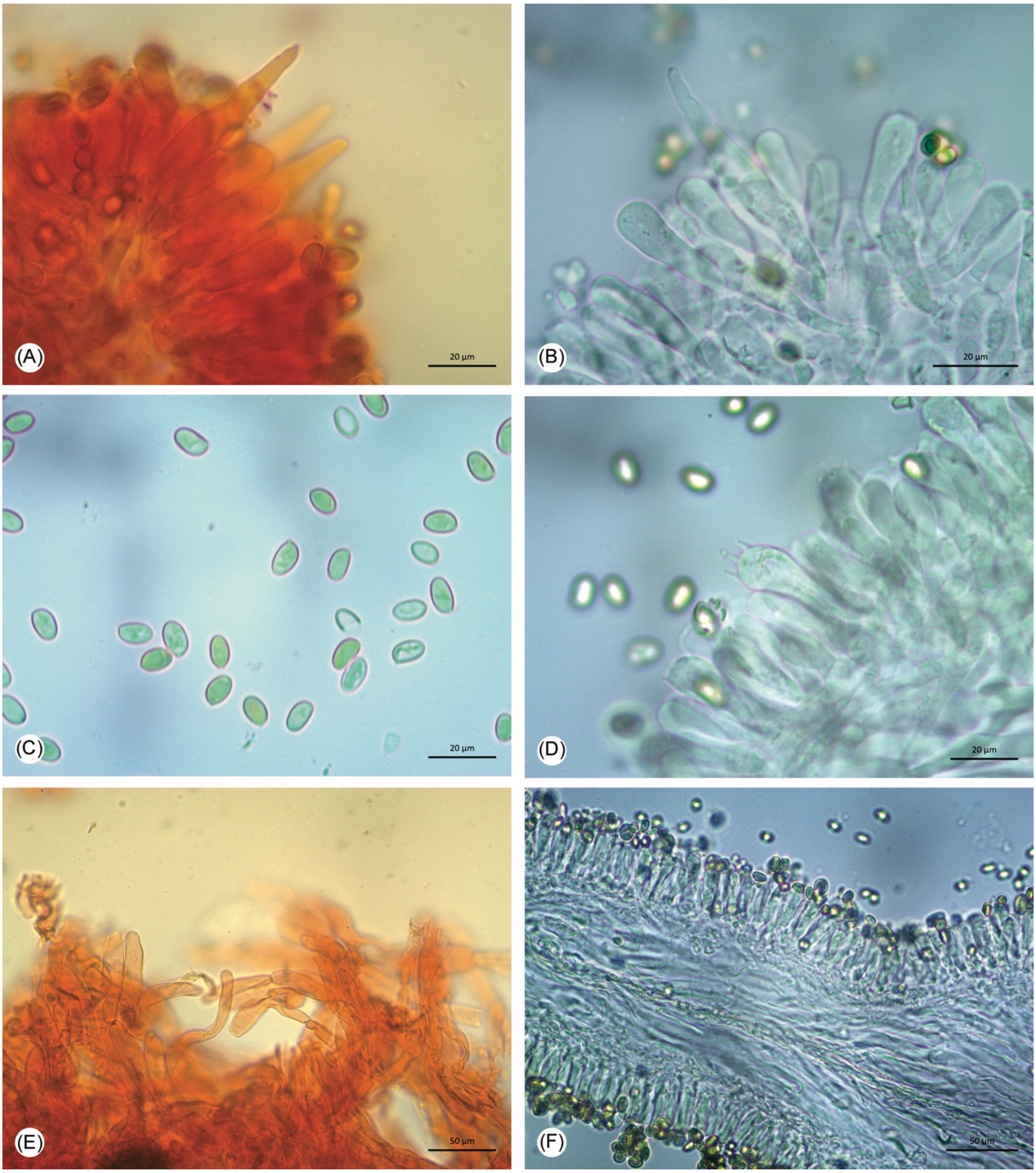

Fig. 1 – Micromorphological character of *Singerocomus guadelupae*. A) Cheilocystidia in Congo red; B) Cheilocystidia and basidioles in 4% KOH; C) Basidiospores in 4% KOH; D) Basidia in 4% KOH; E) Terminal elements of the trichodermal pileipellis in Congo red; F) Barely divergent to phylloporoid hymenophoral trama in 4% KOH.

Our materials match morphologically with the description by Singer and Fiard (1976), Magnago et al. (2018) and Gelardi et al. (2023), e.g., velutinous and red to pink tones on pileus surface, cream to red color or both mixed in stipe surface, white to cream color of pileus and stipe context and unchanging when injured, and the micromorphology, dimensions and color of the structures are similar (Figs 2–3). Therefore, macrochemical reactions among specimens can vary, on which the presence of KOH 3–4% on pileus can turn the surface beige green to light ocher and context light ocher; the hymenophore greenish ocher to ocher, and stipe surface ocher to negative and context negative. When using NH_4OH in the pileus surface, it turns beige green to negative; and context, hymenophore, stipe surface and context the reaction is solely negative. Despite these differences among specimens, we conclude that, based in the morphological, molecular and phylogenetic evidence, the materials studied here correspond to *S. guadelupae*, but demonstrating some variable characteristics, suggesting a somewhat intraspecific phenotypic and genotypic plasticity among the populations. The other two species also present reddish tints on pileus and are somewhat difficult to differentiate basing solely on macro- and micromorphological characters. Anyway, *S. imundabilis* and *S. rubriflavus* can be readily separated in their microscopic features, i.e. the first species lacks cheilocystidia (Singer et al., 1983; Henkel et al., 2016) while *S. rubriflavus* is characterized by having larger basidiospores 9–11(–12) × 4.5–6.5 mm (Henkel et al., 2016).

Regarding ecological aspects, *S. guadelupae* was discovered on ‘the ground of a degraded primary rainforest, and once on dead wood’ in the island of Guadeloupe (Singer and Fiard, 1976), and later ‘on soil among leaves’ and ‘solitary on soil in primary hygrophytic forest’ from Martinique and Guadeloupe, respectively (Pegler, 1983). Its synonym *S. atlanticus* was referred as growing ‘on soil in proximity to putative ECM host plants of the Nyctaginaceae and Fabaceae’ (Magnago et al., 2018). Actually, none of these studies mentioned the type of soil. Thus, in this study we inform that *S. guadelupae* from Paraiba was collected on a white-sand soil in forests called ‘tabuleiro savanna’, that is characterized by patches of savannas formation with well-drained white sandy soils that are often intercalated by semideciduous forests (Thomas and Barbosa, 2008).

Previous studies already affirmed that the preference of ectomycorrhizal fungi in white-sand environments may deeply to influence the tree community structure and diversity (Roy et al., 2016). These ecosystems are described as having frequent ECM fungi probably in association with a few host trees including members of Polygonaceae and Nyctaginaceae (Smith and Read, 2008; Roy et al., 2016). The phytophysiognomy of the collections places includes individuals of these possible hosts as *Guabira* and *Cocolloba* tree species (Pereira and Alves, 2007; Barbosa et al., 2011).

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