

Fistulinella distromatica (Boletaceae, Basidiomycota), a new bolete from the Atlantic Forest of Bahia, Brazil

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Abstract: *Fistulinella distromatica* sp. nov. is described based on morphological and molecular data. It is characterized by the following features: pileus surface that is subviscid with appressed, small brownish fibrillose-squamulose; subviscid, minutely subviscid, minutely punctate stipe that is yellow at the base; the relatively large inamyloid spores; hymenial cystidia up to 70 µm long; two-layered stipitipellis with a trichodermal suprastipitipellis and interwoven substipitipellis that is interwoven in a strongly gelatinized matrix. *Fistulinella distromatica* clusters phylogenetically with a sequence of *F. campinaranae* var. *scrobiculata* recorded from Bahia, and in a distinct branch from Colombian Amazon material. Since Bahian specimens are morphologically and molecularly different from Amazonian ones, a new species is described. Description, drawings, photographs, discussion with comparison to similar taxa are provided.

Key words: Austroboletoidae, Boletales, Neotropic, systematic

1. Introduction

Fistulinella Henn. was originally described from Cameroon with *F. staudtii* Henn. as type species, and was characterized by the “flesh pileus with margin wrapped by a membranous veil, stipitate, and poroid (tubular) hymenophore with free and separate cylindrical tubes” (Hennings, 1901: 43). Recently, Gelardi et al. (2021: 25) gave the most modern definition of the genus: pileus and stipe usually viscid to strongly glutinous, furthermore the pileus can be sometimes scrobiculate and the slender stipe can be smooth to rarely reticulate, tubular hymenophore with whitish to pinkish or brownish tones, surfaces and context unchanging when injured; microscopically by the narrowly elongated/fusoid basidiospores with pink to brown pigment, smooth, inamyloid to dextrinoid, pileipellis a trichoderm to ixotrichoderm or ixocutis, and hymenophoral trama as strongly gelatinized ‘boletus-type’ (bilateral, divergent, boletoid).

The genus belongs to the subfamily Austroboletoidae G. Wu. & Zhu L. Yang (Boletaceae, Boletales), together with *Austroboletus* (Corner) Wolfe, *Mucilopilus* Wolfe and *Veloporphyrellus* L.D. Gómez & Singer (Wu et al., 2014, 2016). Some studies indicated that the genus might be polyphyletic, demonstrating that the Neotropical

Fistulinella sequences form a branch apart from other taxa around the world (Vasco-Palacios et al., 2014; Magnago et al., 2017; Gelardi et al., 2021). Thus, *Fistulinella* comprises about 27 names with mostly tropical distribution in Africa, Americas, Southeast Asia and Australia (Singer, 1978, 1986; Singer et al., 1983, 1991; Neves and Capelari, 2007; Ortiz-Santana et al., 2007; Fulgenzi et al., 2010; Vasco-Palacios et al., 2014; Magnago et al., 2017).

In Brazil, only four taxa are known from Amazonia and Atlantic Forest biomes (IBGE, 2004, 2012): *F. campinaranae* var. *campinaranae* Singer and *F. campinaranae* var. *scrobiculata* Singer from state of Amazonas, *F. violaceipora* (G. Stev.) Pegler & T.W.K. Young sensu Oliveira and Sousa from Paraíba and *F. ruschii* A.C. Magnago from Bahia, Espírito Santo, Paraíba and Santa Catarina (Singer, 1978; Oliveira and Sousa, 2002; Magnago et al., 2017). The recently cited *F. campinaranae* var. *scrobiculata* from Atlantic Forest of Northeast Brazil (Magnago et al., 2017) would be an additional record from this region. However, additional specimens collected by us in the same region were analyzed in the light of morphological and molecular evidences, and correspond actually to an undescribed species that is fully described here.

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Since our specimen is phylogenetically and morphologically (see below) distinct from the material protologued by Singer (1978) and recent molecular studies by Vasco-Palacios et al. (2014), we describe here a new species that is related to *Fistulinella campinaranae* var. *scrobiculata* sensu Magnago et al. (2017).

2. Materials and methods

2.1. Collection area

Inaugurated in 1997 in the southern region of the state of Bahia the 'Parque Estadual do Conduru' (PESC) covers about 7000 ha, extending through the municipalities of Uruçuca, Itacaré and Ilhéus, 14°23'07"S, 39°04'43"W (Araújo et al., 1998; Ângelo, 2003; Martini et al., 2007). It is characterized by a dense submontane ombrophilous forest vegetation type, and tropical humid climate with mean annual temperatures up to 24 °C with an average annual precipitation over than 1300 mm (Sá et al., 1982; Thomas et al., 1998; Thomas et al., 2009; Sambuichi, 2002; Sambuichi et al., 2008).

2.2. Morphological analysis

Macroscopic analyses follow Singer (1986). Color codes follow Online Auction Color (2004). Microscopic observations were made from sections of dried material mounted in 3% KOH and Congo red solutions. Measurements and statistics are based on 50 basidiospores. Abbreviations include $L(W) \pm SD$ = average basidiospores length (width) with standard deviation, Q = the length: width ratio range as determined from all measured basidiospores, and $Q_m \pm SD$ = the Q value averaged from all basidiospores measured with standard deviation. The identification key is based in Singer & Digilio (1960), Singer (1978), Singer et al. (1983), Fulgenzi et al. (2010), Magnago et al. (2017) and Gelardi et al. (2021). The holotype is deposited at herbarium JPB (Universidade Federal da Paraíba) (Thiers, 2022). The new species name and typification are registered with MycoBank (Robert et al., 2005).

2.3. Phylogenetic analysis

The whole genomic DNA of one specimen was extracted as follow. About 0.125 cm³ was homogenized with a pestle for 60 seg in 150 uL of 5% Chelex 100 (Bio-Rad, USA). The tissue is vortexed for 10 seg, incubated in boiling water for 5 min, then vortexed again for 10 seg, and centrifuged at 10,000 rpm for 90 seg. This method was adapted from (HwangBo et al., 2010). The supernatant was used as template for PCR amplification. PCR amplifications were done for complete internal transcribed spacers 1 and 2 and the 5.8S rDNA (nuc-ITSrDNA) bounded by primers ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) and 28S rDNA gene bounded by primers

LR0R (5'-ACCCGCTGAACTTAAGC-3') and the reverse primer LR7 (5'-TACTACCACCAAGATCT-3') (Moncalvo et al., 2000). PCR conditions for amplification consisted of 1× buffer, dNTP at 0.2 mM, each primer at 0.2 μM, MgCl₂ at 2mM, 1U Taq polymerase and 2 μL of template DNA, in a total reaction volume of 25 μL. The PCR cycling program was used for both primer sets: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 51 °C for 40 s, and 72 °C for 1 min and concluding with a 10 min extension at 72 °C. PCR products were bidirectional sequenced in 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 and to assemble and edit if necessary. Sequences of *Fistulinella* present in GenBank were incorporated to analyses for both genetic regions (GenBank accession numbers and specimens countries are available at Table). In our analysis, we included 28S rDNA sequences with more than 400 bp. In the phylogenetic analysis, we aligned sequences using MAFFT v.7.017 (Kato and Standley, 2013), a module implemented in GENEIOUS v.9.1.3 using G-INS-I algorithm. We constructed maximum likelihood (ML) trees with RAxML (v.8.2.12) (Stamatakis, 2014) using GTR GAMMA I model and 1000 bootstrap (BP) replicates. A Bayesian tree (BS) was constructed with MrBayes v.3.2.6 (Ronquist et al., 2012). The best available model of evolution selected by jModel Test v.3.0.4 (Posada, 2008) (nuc-ITSrDNA, HKY+I+G; 28S rDNA, GTR+I+G). Two independent parallel runs were run, sampling every 1000th generations for 40 million total generations. The convergence of the parameters was assessed with Tracer v.1.6.082. Effective sample sizes (ESS) were well within acceptable ranges (ESS >> 200). After discarding the first 10% of the sampled trees as burn-in, a majority rule consensus tree and posterior probabilities (PP) were computed using the remaining trees. Our sequences were deposited in GenBank (NCBI) under accession OM670210 and OM630459.

3. Results

3.1. Genetic analysis

A total of 23 sequences of nuc-ITSrDNA region, and 32 sequences of 28S rDNA gene were downloaded from GenBank. The ML and BS trees are shown in Figures 1–2 and 3–4, respectively. Both phylogenetic analyses showed that the specimen of *Fistulinella* sampled by us is closed related with the sequence named as '*Fistulinella campinaranae*' collected in the same place, into strongly supported clades (BP > 99%; PP > 98%). Also, these specimens are genetically distant from sequences of *Fistulinella campinaranae* var. *scrobiculata* recently collected in the Amazon region, such as Colombia (see Figures 1–4).

Table. Details of taxon, GenBank accession numbers (ITS and LSU), voucher, country and references of the sequences used in the phylogenetic analyses.

Taxon	ITS	LSU	Voucher	Country	References
<i>Austroboletus amazonicus</i>	NR_153523	NG_058569	HUA:2032 AMV	Colombia	Vasco-Palacios et al. (2014)
<i>Austroboletus festivus</i>	KY886202	KY888001	FLOR 51599	Brazil	Magnago et al. (2017)
<i>Bothia castanella</i>	DQ867113	-	28002	USA	Halling et al. (2007)
<i>Bothia castanella</i>	-	DQ867115	MB03-067	USA	Halling et al. (2007)
<i>Bothia fujianensis</i>	KM269196	KM269194	HKAS82693	China	Zeng et al. (2015)
<i>Fistulinella aurantioflava</i>	MW784159	MW760388	LE315616	Vietnam	Crous et al. (2021)
<i>Fistulinella aurantioflava</i>	MW784160	MW760389	LE315617	Vietnam	Crous et al. (2021)
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	KY886204	KY888003	ACM484	Brazil	Magnago et al. (2017)
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	KT724090	KT724100	AMV1513	Colombia	Bidartondo and Doring (Unpub.)
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	-	KF714520	1980 AMV	Colombia	Vasco-Palacios et al. (2014)
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	-	KJ195892	HUA:AMV1783	Colombia	Vasco-Palacios et al. (2014)
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	-	KT724099	AMV1486	Colombia	Vasco-Palacios et al. (Unpub.)
<i>Fistulinella campinaranae</i> var. <i>scrobiculata</i>	-	KT724101	AMV1535	Colombia	Vasco-Palacios et al. (Unpub.)
<i>Fistulinella cinereoalba</i>	KT339237	GQ477439	TH8471	Guyana	Fulgenzi et al. (2010)
<i>Fistulinella distromatica</i> sp. nov.	OM670210	OM630459	JPB66574	Brazil	This study
<i>Fistulinella gloeocarpa</i>	GQ981503	-	KM162946	Guyana	Bidartondo and Doring (Unpub.)
<i>Fistulinella gloeocarpa</i>	KF878352	-	AMV511 root	Colombia	Vasco-Palacios et al. (2014)
<i>Fistulinella gloeocarpa</i>	MT581527	MT580906	JBSD130769, ANGE969 and MG777	Dominican Republic	Gelardi et al. (2021)
<i>Fistulinella gloeocarpa</i>	-	MT580904	CFMR:B4	Bahamas	Gelardi et al. (2021)
<i>Fistulinella gloeocarpa</i>	-	MT580905	CFMR:B10	Bahamas	Gelardi et al. (2021)
<i>Fistulinella olivaceoalba</i>	NR_163311	MH718396	LE312004	Vietnam	Crous et al. (2018)
<i>Fistulinella olivaceoalba</i>	-	MH745969	HKAS 53432	Vietnam	Crous et al. (2018)
<i>Fistulinella prunicolor</i>	-	JX889648	REH9502	Australia	Halling et al. (2012)
<i>Fistulinella ruschii</i>	KY886205	KY888004	FLOR 51609	Brazil	Magnago et al. (2017)
<i>Fistulinella ruschii</i>	KY886208	-	ICN 192819	Brazil	Magnago et al. (2017)
<i>Fistulinella ruschii</i>	KY886209	KY888005	ICN 192818	Brazil	Magnago et al. (2017)
<i>Fistulinella ruschii</i>	KY886210	-	ICN 192820	Brazil	Magnago et al. (2017)
<i>Fistulinella ruschii</i>	NR_156320	KY888006	FLOR:51611	Brazil	Magnago et al. (2017)
<i>Fistulinella ruschii</i>	-	MT580907	CORT:TJB-8329	Virgin Islands (USA)	Gelardi et al. (2021)
<i>Fistulinella viscida</i>	-	AF456826	238	New Zealand	Binder and Bresinsky (2002)
<i>Fistulinella viscida</i>	-	HM624052	PDD 25185 clone 1	New Zealand	Li and Yang (Unpub.)
<i>Fistulinella viscida</i>	-	HM624053	PDD 25185 clone 2	New Zealand	Li and Yang (Unpub.)
<i>Fistulinella viscida</i>	-	HM624054	PDD 25185 clone 3	New Zealand	Li and Yang (Unpub.)
<i>Mucilopilus castaneiceps</i>	MN622732	-	HFJAU-TD356	China	Wang (Unpub.)
<i>Mucilopilus castaneiceps</i>	-	KT990547	HKAS71039	China	Wu et al. (2016)
<i>Soliococcus polychromus</i>	JX888459	-	J. Trappe 15399	Australia	Trappe et al. (2013)
<i>Soliococcus polychromus</i>	-	JQ287642	R.E. Halling 9417	Australia	Trappe et al. (2013)
<i>Veloporphyrellus latisporus</i>	MZ079120	-	SWAT001360	Pakistan	Khan et al. (2021)
<i>Veloporphyrellus pseudovelatus</i>	-	JX984541	KUN:HKAS52673	China	Li et al. (2014)
<i>Veloporphyrellus vulpinus</i>	MN511177	MN511170	LE315544	Vietnam	Crous et al. (2019)

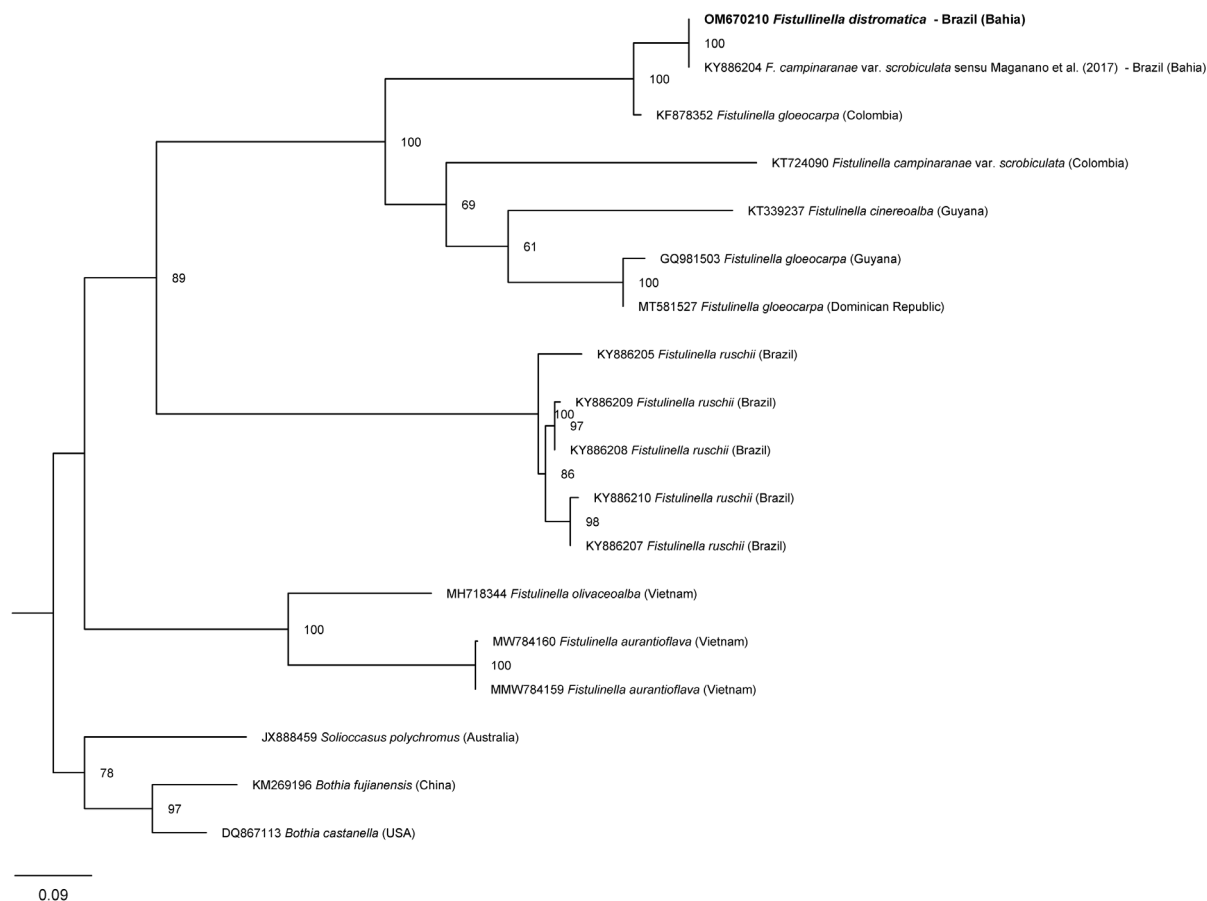


Figure 1. Maximum likelihood phylogenetic tree of *Fistulinella* species based on nuc-ITS rDNA fragments.

3.2. Morphological analysis

Fistulinella distromatica Barbosa-Silva & Wartchow, **sp. nov.** (Figures 5–7)

Mycobank: MB 844081

Type: Brazil, Bahia, Uruçuca, Parque Estadual da Serra do Conduru, 29 Nov. 2012, F. Wartchow FW 139/2012 (JPB 66574, **holotype!**).

Etymology. From Greek, di (= two) and stroma (= layer); due the stipitipellis having two distinct layers.

Name misapplied to present species: *Fistulinella campinaranae* var. *scrobiculata* Singer sensu Magnago et al., Mycologia 109: 1006-1008. 2017.

Basidiomata in pairs, small. Pileus 29–37 mm diameter, convex to broadly convex, dark brown (OAC 636, 637) closer to centre to brown (OAC 702, 771) on a background light pale brownish (OAC 683) to light cream or light beige (OAC 697, 795); surface subviscid, shallowly subciliate, composed by small, tiny and appressed fibrillose squamules; margin entire nonsulcate nor striate, grayish with 3% KOH; context up to 4 mm thick in center, whitish (OAC 909) unchanging with 3% KOH or when bruised, worm hole color golden yellow. Hymenophore tubulose,

adnexed, tubes 2–6 mm long, light pale pinkish (OAC 256) to light pinkish (OAC 487), unchanging with 3% KOH or when bruised; pores angular to round or subrounded, 0.5–1 mm in diam., concolorous with tubes, unchanging with 3% KOH and when bruised. Stipe 70–78 × 4–6 mm, central, equal, slightly light pale brownish (OAC 674, 800) turns slightly brownish (OAC 778, 828) toward base with yellowish (OAC 854, 896) base; surface minutely punctate, slightly viscid; context solid, whitish (OAC 909), worm holes golden yellowish, unchanging with 3% KOH or when bruised. Odor mild. Taste bitter.

Basidiospores (14.8–)15.3–18.4(–18.9) × 5.1–6.1 μm (L = 17.3 ± 0.84 μm; W = 5.5 ± 0.56 μm; Q = (2.64–)2.91–3.40(–3.50), Qm = 3.15 ± 0.19, subfusiform, yellowish light brown in 3% KOH, inamyloid, thin-walled, smooth, hilar appendix sublateral. Basidia 24–37.7 × 10.7–11.7 μm, clavate, hyaline in 3% KOH, thin-walled, some with presence of granular refringent content inside. Pleurocystidia 30.6–79.1 × 7.7–14.3 μm, frequently protruding above the hymenium, fusoid-subventricose, subventricose-rostrate, lageniform, all with obtuse apex, and some clavate with a mucronate apex, hyaline with 3% KOH, thin-walled,

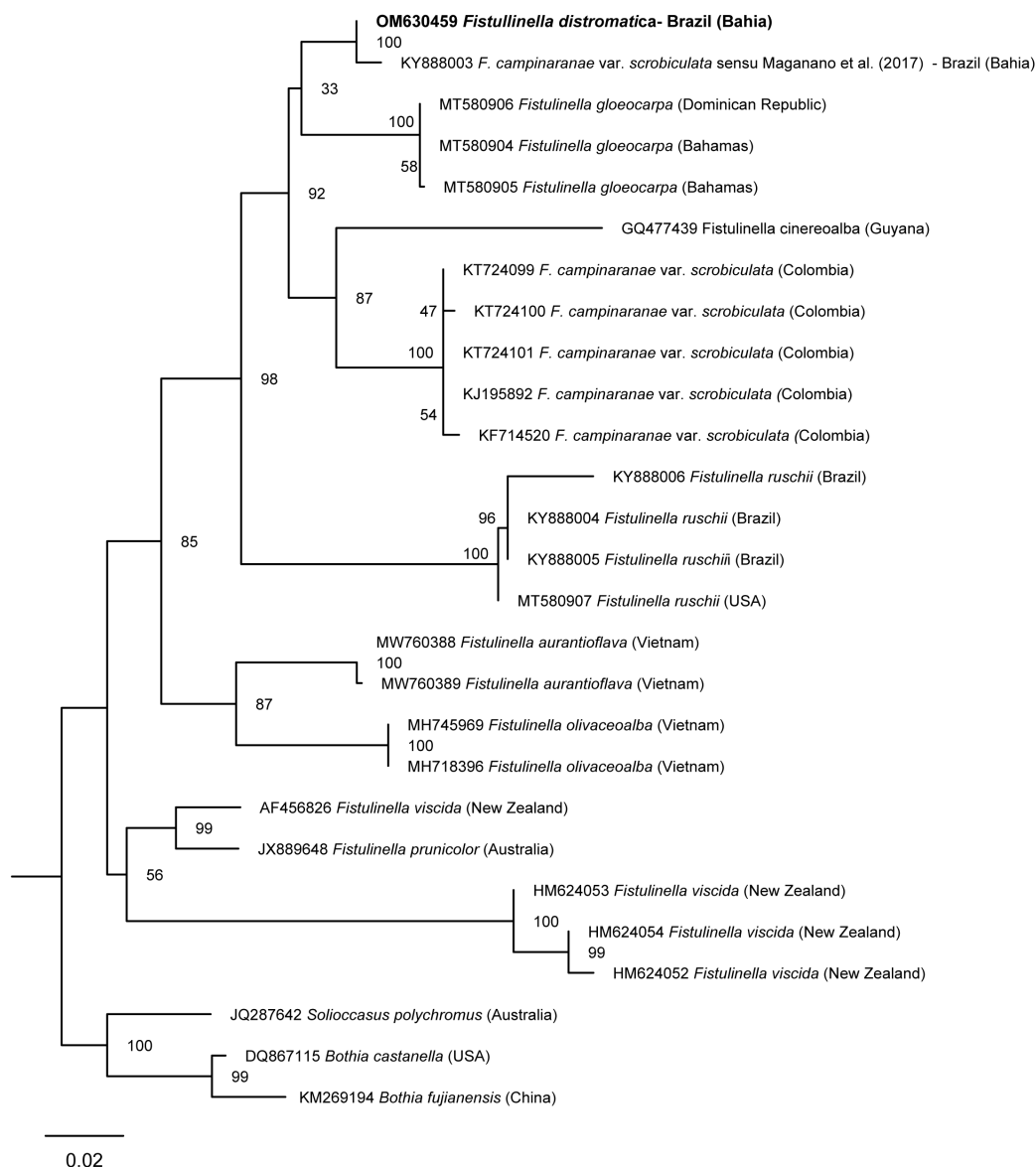


Figure 2. Maximum likelihood phylogenetic tree of *Fistulinella* species based on 28S rDNA fragments.

smooth, some with presence of granular refringent content inside. Cheilocystidia 23–70.4 × 5.1–17.9 µm, fusoid-subventricose, subfusoid, subventricose-rostrate with obtuse apex, hyaline with 3% KOH, thin-walled, smooth, some with presence of granular refringent content inside. Hymenophoral trama boletoid; hyphae 3.1–7.7 µm wide, immerse in a gelatinized matrix, hyaline with 3% KOH, oleiferous hyphae present. Pileipellis a trichodermium, terminal elements cylindrical with obtuse apex, when septate the terminal cells also are cylindric with obtuse apex, 6.1–8.2 µm wide, light yellowish brown (majority) to hyaline (minority) with 3% KOH, some with presence of granular refringent content. Pileus trama interwoven, immersed in a strongly gelatinized matrix, hyphae 4.6–

14.3 µm wide, hyaline with 3% KOH. Stipitipellis two layered: suprastipitipellis a trichodermium, terminal cells 3.1–7.1 µm wide, cylindrical-clavate, subfusoid to subventricose with obtuse apex, pale light brown to hyaline with 3% KOH, oleiferous hyphae present; caulobasidia, caulobasidiospores and caulocystidia present, with the latter scattered and/or close but not in tufts, 34.2–46.9 × 5.1–9.2 µm, pale light brown to hyaline with 3% KOH, thin-walled, smooth, with granular refringent content; substipitipellis hyphae interwoven, 3.6–7.7 µm wide, hyaline with 3% KOH, immerse in a strongly gelatinized matrix, oleiferous hyphae present. Stipe trama with hyphae 5.1–20.4 µm wide, hyaline with 3% KOH, longitudinally oriented, parallel to subparallel. Clamp connections absent.

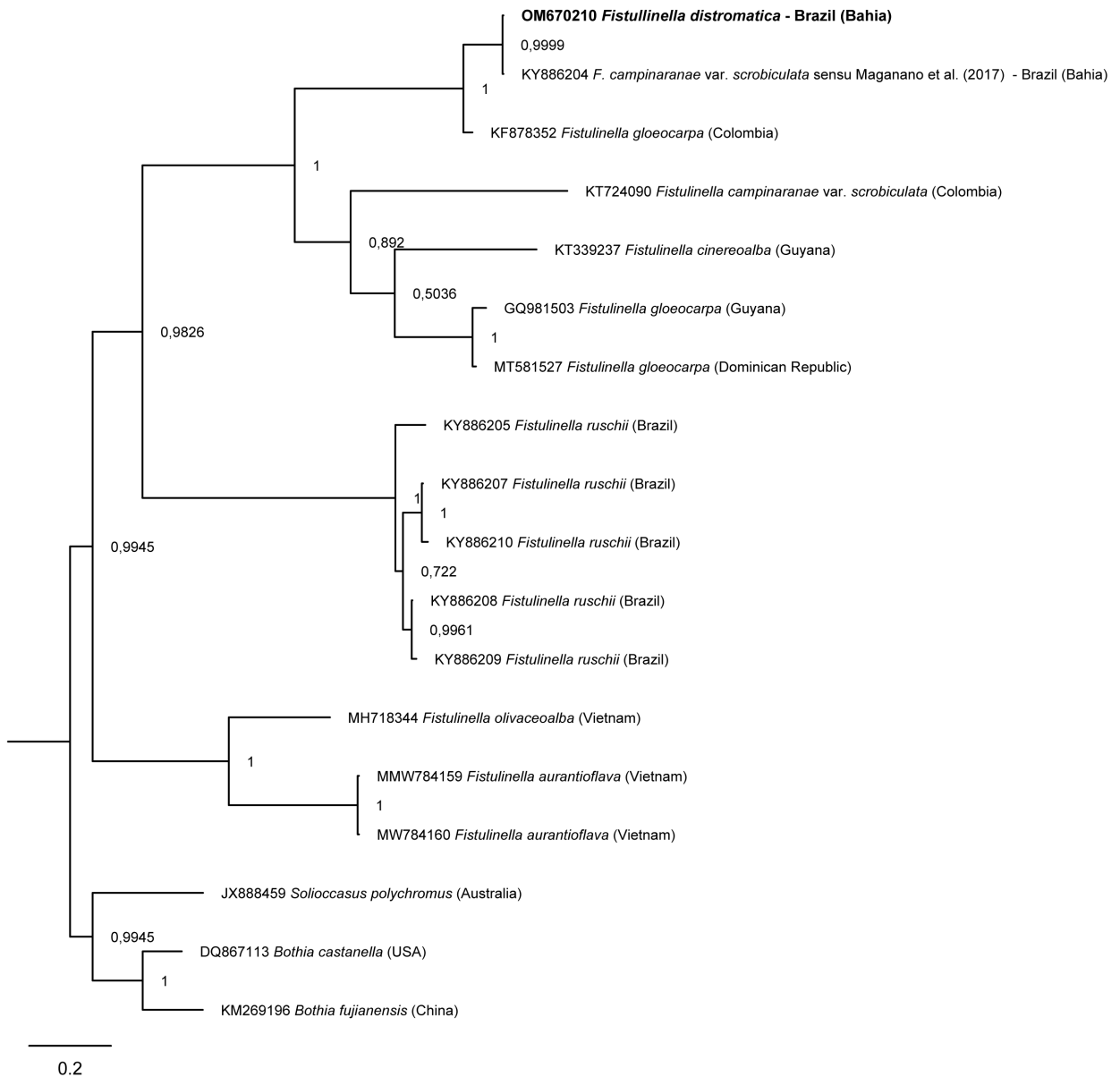


Figure 3. Bayesian phylogenetic tree of *Fistulinella* species based on nuc-ITS rDNA fragments.

Known distribution: Atlantic Forest from state of Bahia in Northeast Brazil.

Habitat: In pairs on soil under trees of the genera *Coccoloba* P. Browne, *Guapira* Aubl., *Neea* Ruiz & Pav., among other (Martini et al., 2007).

Conservation status: *Fistulinella distromatica* is only known from the type locality and was not abundant or widespread. Actually, no data about species density is fully known. Since it was collected once in a well-protected conservation unit (INEMA, 2004), it cannot qualify for ‘Critically Endangered’, ‘Endangered’, ‘Vulnerable’ or ‘Near Threatened’, but as Least Concern/LC until more information are gathered (IUCN, 2019).

4. Key for Neotropical lowland forest species of *Fistulinella*

- 1. Pileus and stipe strongly glutinous 2
- 1. Pileus or stipe not glutinous, but dry to somewhat viscid 5
- 2. Pileus mostly whitish or pallid or greyish white with brownish or fuscous area 3
- 2. Pileus typically dark colored 4
- 3. Pileus smooth *F. campinaranae* var. *campinaranae*
- 3. Pileus more or less coarsely and strongly scrobiculate-rugulose *F. campinaranae* var. *scrobiculata*
- 4. Pileus typically darker, mouse gray or slate gray to brown, dark brown or blackish brown, but pure white,

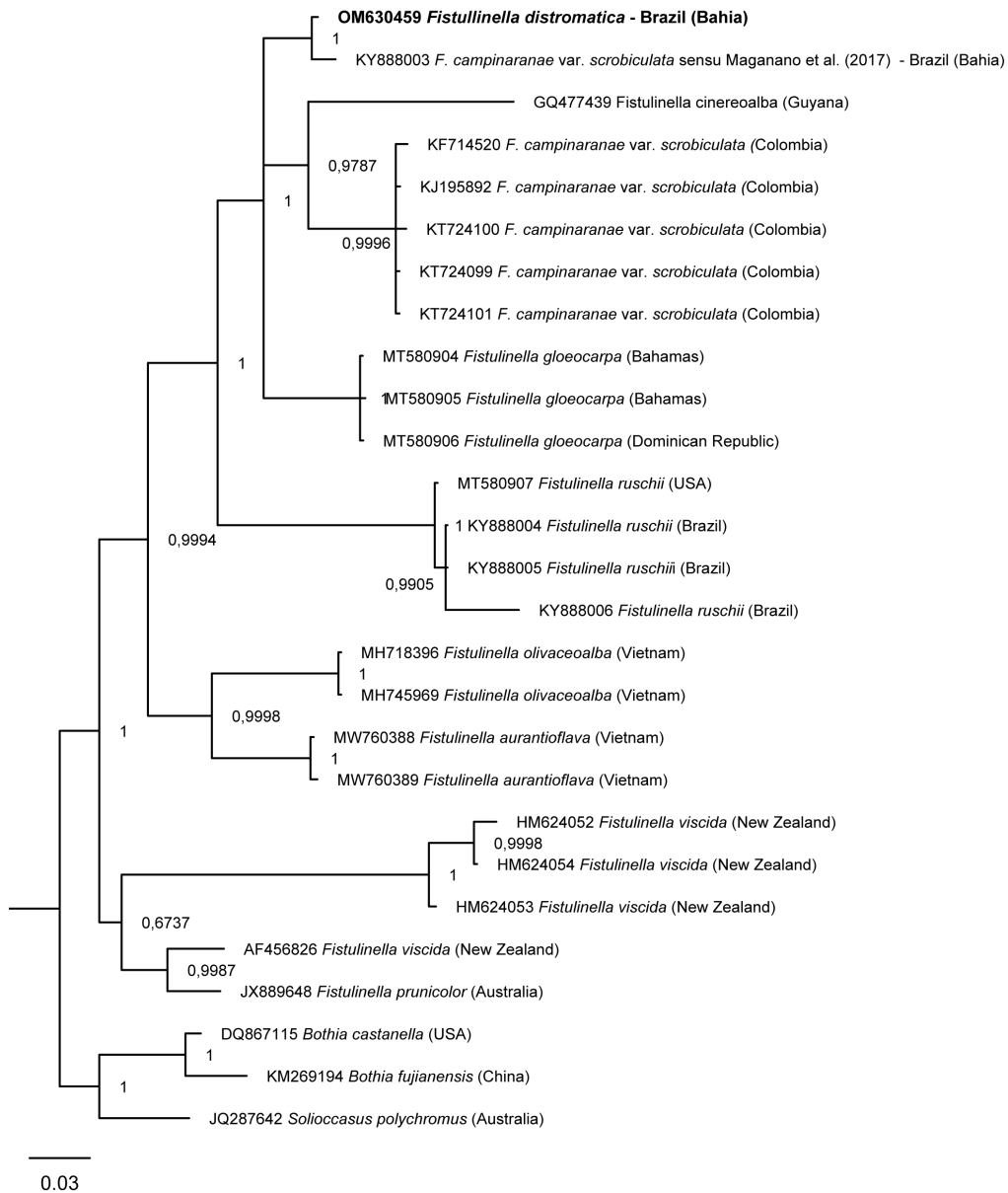


Figure 4. Bayesian phylogenetic tree of *Fistulinella* species based on 28S rDNA fragments.

whitish or pale grayish white to pale brownish gray when rainy; glutinous colorless membrane which soon disrupts in velar remnants forming an ascending, persistent glutinous annulus located in the upper part of the stipe present *F. gloeocarpa*
 4. Pileus dark gray then lighter gray with age; stipe without a trace of any velar-like structure ... *F. cinereoalba*
 5. Pileus dark brown closer to center to brown on a light brownish to beige background, surface bearing small brownish fibrillose-squamules; stipe surface minutely punctate; stipitipellis distinctly two-layered, composed by a strongly gelatinous subpellis and a trichodermal suprapellis *F. distromatica* sp. nov.

5. Pileus with some shade of orange or ochraceous tints, stipe surface not punctate 6
 6. Pileus chestnut brown to orange-brown; cystidia broadly cylindrical septate *F. ruschii*
 6. Pileus mostly lighter, orange or ochraceous; cystidia not septate 7
 7. Pileus ochraceous; hymenophore pinkish; stipe dry *F. venezuelae*
 7. Pileus light orange, yellowish red, orange white to brownish orange; hymenophore orange white to brownish orange; stipe bearing detachable gelatinous pellicle ... *F. violaceispora* sensu Oliveira & Sousa



Figure 5. Basidiomata of *Fistulinella distromatica* sp. nov. (JPB 66574, Holotype). Scale bars = 20 mm.

5. Discussion

Fistulinella distromatica is characterized by the finely ornamented and subviscid pileus surface with appressed small brownish fibrillose squamules, minutely subviscid punctate stipe surface with yellowish base, inamyloid basidiospores measuring $(14.8\text{--}15.3\text{--}18.4\text{--}(18.9) \times 5.1\text{--}6.1 \mu\text{m}$, cystidia ranging $23\text{--}79.1 \mu\text{m}$ long, and the two layered stipitipellis with a trichodermal suprastipitipellis and the substipitipellis bearing interwoven hyphae immersed in a strongly gelatinized matrix. The new species fits phylogenetically very well with *F. campinaranae* var. *scrobiculata* sensu Magnago et al. (2017: 1006) from Bahia.

The Amazonian *F. campinaranae* var. *scrobiculata* s.s. species is distinguished morphologically from *F. distromatica* by the pileus that is glabrous, viscid to glutinous, whitish to grayish white with some fuscous area, and by the pure white smooth/glabrous and viscid/glutinous stipe (Singer et al., 1983). The spores of the former are also shorter $(9.5\text{--}12\text{--}15\text{--}(17.3) \times 4\text{--}5\text{--}(5.3) \mu\text{m}$ (Singer et al. 1983: 148). Later, Vasco-Palacios et al. (2014) reported this variety from Colombian Amazon also with viscid pileus with grayish brown to dark brown or paler toward the margin, overall brownish with age on a white to beige background and finely rugulose and pruinose surface mainly in the center; white and fibrillose stipe with fine erect scales embedded in a thick gelatinous pellicle (matrix), smaller dextrinoid basidiospores $(12\text{--})13\text{--}15\text{--}(17) \times (3\text{--})4\text{--}5$, the pileipellis as an ixocutis, and the stipitipellis a cutis of compact hyphae. In addition, although forming a well-supported branch, Colombian Amazon and Bahian

specimens are phylogenetically distant, confirming that both are distinct taxa.

Fistulinella campinaranae var. *campinaranae* also from Amazon differs by the pallid white with brown areas and viscid and smooth pileus, and white and slightly viscid and smooth stipe. Microscopically it differs in the smaller dextrinoid basidiospores $(11.5\text{--}16.5 \times 4\text{--}6 \mu\text{m})$, and the pileipellis that is an ixotrichoderm (Singer, 1978; Singer et al., 1983).

Fistulinella cinereoalba Fulgenzi & T.W. Henkel from Guyana also has a pigmented pileus with finely rugulose and matted-fibrillose surface. However, it differs in the grayish and more glutinous pileus surface, tubes that discolor brownish or when bruised, entirely white to light pink-gray pores but slightly browning after handling, stipe bearing fine erect scales imbedded in a dense gelatinous pellicle throughout (Fulgenzi et al., 2010). Microscopically, the Guyanese taxon presents a slightly larger dextrinoid basidiospores $12.4\text{--}19.8\text{--}(24.8) \times 3.7\text{--}4.9 \mu\text{m}$, septate cheilocystidia, and a trichodermal palisade pileipellis (Fulgenzi et al., 2010). These authors did not define the morphology of stipitipellis with detail, but they mentioned the presence of concentrated tufts of slightly interwoven, inflated cylindrical elements concentrated in the stipe scales.

Another member of this clade, *F. gloeocarpa* Pegler from Martinique and Dominican Republic, differs in the Drab (=light yellowish brown) to Cinnamon-Drab (=light grayish reddish brown) strongly glutinous, smooth and glabrous or sometimes scrobiculate pileus, finely pruinose to smooth and glabrous mostly whitish stipe, and its surface

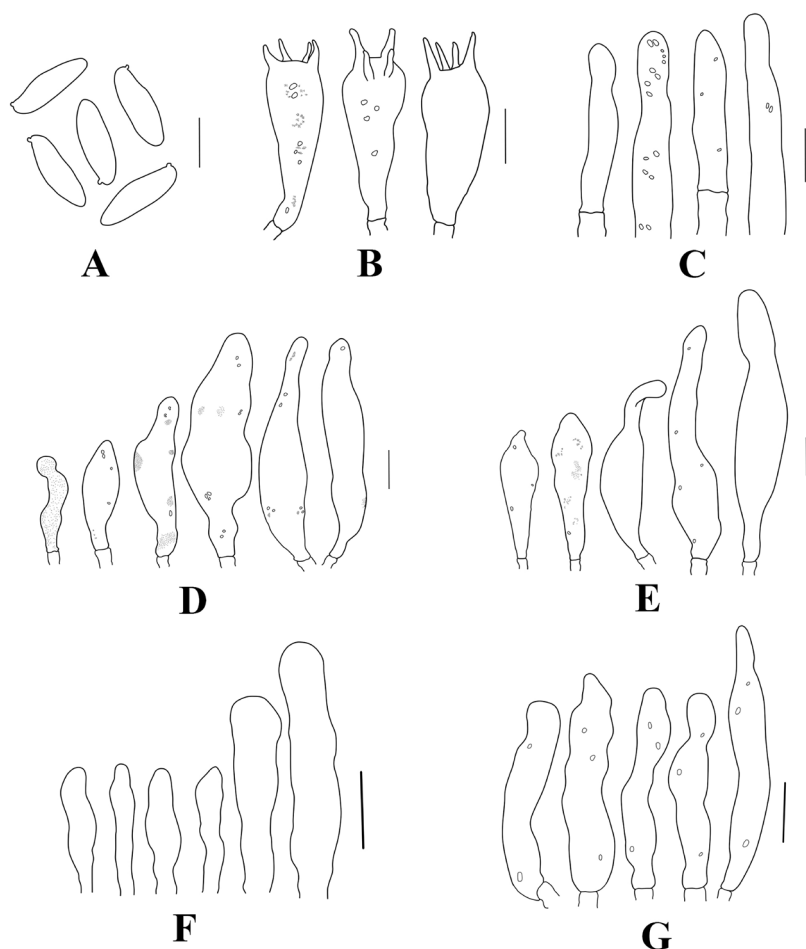


Figure 6. Micromorphological character of *Fistulinella distromatica* sp. nov. (JPB 66574, Holotype): **A.** Basidiospores, **B.** Basidia, **C.** Terminal elements and terminal cells of pileipellis, **D.** Cheilocystidia, **E.** Pleurocystidia, **F.** Terminal elements of stipitipellis, **G.** Caulocystidia. Scale bars = 10 μ m.

totally covered by glutinous membrane that soon disrupts into velar remnants forming an ascending, persistent glutinous annulus located in the upper part of the stipe (Pegler, 1983; Gelardi et al., 2021).

Other South American species are *F. rushii*, '*F. violaceipora*' (sic) sensu Oliveira and Souza and *F. venezuelae* (Singer & Digilio) Singer, which primarily differ in the basidiomata presenting ochraceous and orange to orange-brown colored pileus (Singer and Digilio, 1960 as *Tylopilus venezuelae* Singer & Digilio; Singer, 1978; Pegler, 1983; Singer et al., 1983; Oliveira and Souza, 2002; Magnago et al., 2017). An interesting issue to be highlighted would be the probable synonymy of *F. ruschii* with *F. venezuelae*, as briefly discussed by Gelardi et al. (2021: 40) in view of the data obtained in their study, but they emphasize that further studies are needed to elucidate this issue.

In addition, *F. aurantioflava* T.H.G. Pham, A.V. Alexandrova & O.V. Morozova, *F. olivaceoalba* T.H.G.

Pham, Y.C. Li & O.V. Morozova, *F. prunicolor* (Cooke & Masee) Watling and *F. viscida* (McNabb) Singer all share in the glutinous basidiomes, at least the pileus surface, but differ in many ways in their color (yellow, olive green, cinnamon brown or purple) (Cooke, 1887 as *Boletus prunicolor* Cooke & Masee; McNabb 1967, as *Porphyrellus viscidus* McNabb; Crous et al., 2018, 2021).

In view of all these morphological, molecular and phylogenetic characteristics described and discussed above, we conclude that the Bahia material corresponds to a distinct taxon from the other taxa of *Fistulinella* and therefore conclude that *F. distromatica* is a new species.

During our molecular analysis some *Fistulinella* clustered in branches distant from the Neotropical clade. Three distinct clades are identified in our phylogeny, clearly concerning sequences from three distinct geographic regions: (1) a clade with taxa originating from the Neotropics, i.e. *F. campinaranae* var. *scrobiculata*, *F.*

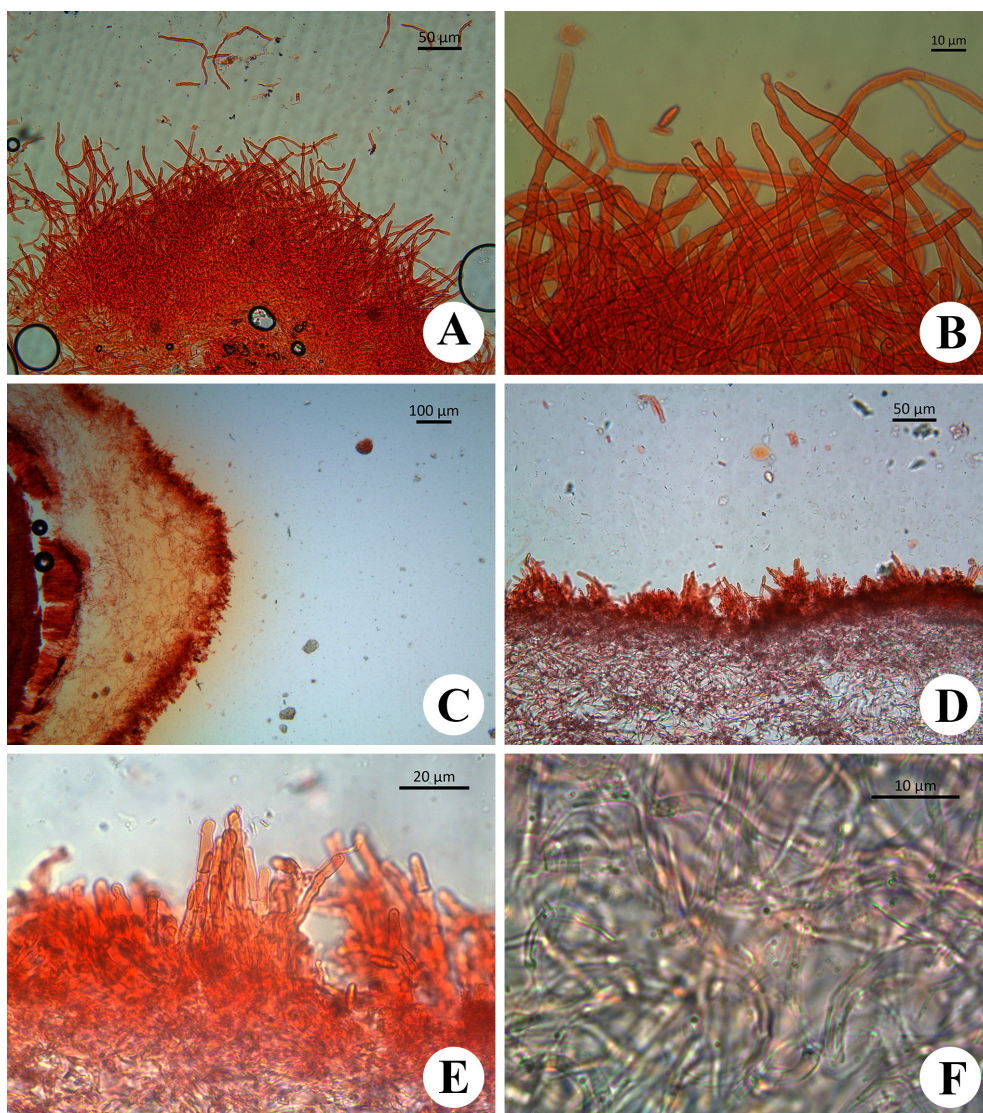


Figure 7. Micromorphological character of *Fistulinella distromatica* sp. nov. (JPB 66574, Holotype): A. Trichodermal pileipellis, B. Terminal elements of pileipellis, C–D. Two layered stipitipellis showing the suprastipitipellis and substipitipellis, E. Trichodermal suprastipitipellis of stipitipellis, F. Interwoven and strongly gelatinized substipitipellis of stipitipellis.

cinereoalba, *F. distromatica*, *F. gloeocarpa* and *F. ruschii*; (2) a clade referring to entities from the tropical region of Southeast Asia, more specifically from Vietnam (*F. aurantioflava* and *F. olivaceoalba*); and (3) a third clade corresponding to taxa from Australia and New Zealand: *F. prunicolor* and *F. viscida*. These results corroborate with previous studies that already demonstrated that *Fistulinella* is a possibly polyphyletic genus (Vasco-Palacios et al., 2014; Magnago et al., 2017; Crous et al., 2018, 2021; Gelardi et al., 2021). However, due to the scarcity of molecular samples available in genetic databases for the genus, for example GenBank, this hypothesis is currently inconclusive. This problem will persist unless the type species, *F. staudtii*, or

recent collection from the type locality from Cameroon in Africa, has the DNA sequenced for a better delimitation and resolution of *Fistulinella*. Thus, further molecular and phylogenetic analyzes are needed for the taxa currently included in the genus (including the type species) to infer a robust and conclusive elucidation of the taxonomy, geographic delimitation, relationship, and interspecific limits of *Fistulinella sensu lato*.

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Conflict of interest

For the present work, the authors declare that they have no conflict of interest.

References

- Ângelo JGM (2003). A conservação da biodiversidade através da gestão das unidades de conservação do estado da Bahia: atualidades, potencialidades e desafios. *Bahia Análise & Dados* 13 (3): 641–652 (in Portuguese with an abstract in English).
- Araújo M, Alger K, Rocha R, Mesquita CAB (1998). Mata Atlântica do Sul da Bahia: situação atual, ações e perspectivas. Caderno n° 8, Série Estados e Regiões da Reserva da Biosfera da Mata Atlântica. São Paulo, Brazil: Instituto Florestal do Estado de São Paulo (in Portuguese).
- Binder M, Bresinsky A (2002). *Retiboletus*, a new genus for a species-complex in the Boletaceae producing retipolides. *Feddes Repertorium* 113: 30–40.
- Cooke MC (1887). Australasian fungi. *Grevillea* 16 (18): 30–33.
- Crous PW, Luangsa-ard JJ, Wingfield MJ, Carnegie AJ, Hernández-Restrepo M et al. (2018). Fungal Planet description sheets: 785–867. *Persoonia* 41: 238–417. doi: 10.3767/persoonia.2018.41.12
- Crous PW, Wingfield MJ, Lombard L, Roets F, Swart WJ et al. (2019). Fungal Planet description sheets: 951–1041. *Persoonia* 43: 223–425. doi: 10.3767/persoonia.2019.43.06
- Crous PW, Cowan DA, Maggs-Kölling G, Yilmaz N, Thangavel R et al. (2021). Fungal Planet description sheets: 1182–1283. *Persoonia* 46: 313–528. doi: 10.3767/persoonia.2021.46.11
- Fulgenzi TD, Halling RE, Henkel TW (2010). *Fistulinella cinereoalba* sp. nov. and new distribution records for *Austroboletus* from Guyana. *Mycologia* 102 (1): 224–232.
- Gelardi M, Angelini C, Costanzo F, Ercole E, Ortiz-Santana B et al. (2021). Outstanding Pinkish Brown-Spored Neotropical Boletes: *Austroboletus subflavidus* and *Fistulinella gloeocarpa* (Boletaceae, Boletales) from the Dominican Republic. *Mycobiology* 49 (1): 24–45. doi: 10.1080/12298093.2020.1843221
- Halling RE, Baroni TJ, Binder M (2007). A new genus of Boletaceae from eastern North America. *Mycologia* 99 (2): 310–316.
- Halling RE, Nuhn M, Osmundson T, Fechner N, Trappe JM et al. (2012). Affinities of the *Boletus chromapes* group to *Royoungia* and the description of two new genera, *Harrya* and *Australopilus*. *Australian Systematic Botany* 25: 418–431. doi: 10.1071/SB12028
- Hennings P (1901). Fungi camerunensis novi. III. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 30: 35–57.
- HwangBo K, Son SH, Lee JS, Min SR, Ko SM et al. (2010). Rapid and simple method for DNA extraction from plant and algal species suitable for PCR amplification using a chelating resin Chelex 100. *Plant Biotechnology Reports*. 4 (1):49–52. doi: 10.1007/s11816-009-0117-4
- IBGE (2004). Mapas de Cobertura Vegetal dos Biomas Brasileiros. Primeira Aproximação. Ministério do Meio Ambiente, Ministério do Planejamento, Orçamento e Gestão, Instituto Brasileiro de Geografia e Estatística, Diretoria de Geociências, Brasília.
- IBGE (2012). Mapa da Área de Aplicação da lei N° 11.428 de 2006. 2a edição. Ministério do Meio Ambiente; Instituto Brasileiro de Geografia e Estatística; Diretoria de Geociências, Brasília.
- INEMA (2004). Plano de Manejo do Parque Estadual Serra do Conduru. Instituto do Meio Ambiente e Recursos Hídricos, Salvador.
- IUCN Standards and Petitions Subcommittee (2019). Guidelines for Using the IUCN Red List categories and Criteria. Version 14. Prepared by the Standards and petitions Subcommittee. Website <https://www.iucnredlist.org/resources/redlistguidelines> [accessed 05 March 2022]
- Katoh K, Standley DM (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* 30 (4): 772–780. doi: 10.1093/molbev/mst010
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M et al. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28 (12): 1647–1649. doi: 10.1093/bioinformatics/bts199
- Khan J, Ullah S, Sher H, Fiaz M, Khalid NA (2021). *Veloporphyrellus latisporus* (Boletaceae), a new species from moist temperate forests of Pakistan. *Nordic Journal of Botany* 39 (9): 1–7. doi: 10.1111/njb.03178
- Li Y-C, Ortiz-Santana B, Zeng N-K, Feng B, Yang ZL (2014). Molecular phylogeny and taxonomy of the genus *Veloporphyrellus*. *Mycologia* 106 (2): 291–306. doi: 10.3852/106.2.291
- Magnago AC, Neves MA, Silveira RM (2017). *Fistulinella ruschii*, sp. nov., and a new record of *Fistulinella campinaranae* var. *scrobiculata* for the Atlantic Forest, Brazil. *Mycologia* 109 (6): 1003–1013. doi: 10.1080/00275514.2018.1431503

- Martini AMZ, Fiaschi P, Amorim AM, Paixão JL (2007). A hot-point within a hot-spot: a high diversity site in Brazil's Atlantic Forest. *Biodiversity and Conservation* 16: 3111–3128. doi: 10.1007/s10531-007-9166-6
- McNabb RFR (1967) The Strobilomycetaceae of New Zealand. *New Zealand Journal of Botany* 5 (4): 532–547. doi: 10.1080/0028825X.1967.10428772
- Moncalvo J-M, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R (2000). Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. *Systematic Biology* 49 (2): 278–305. doi: 10.1093/sysbio/49.2.278
- Neves MA, Capelari M (2007). A preliminary checklist of Boletales from Brazil and notes on Boletales specimens at the Instituto de Botânica (SP) Herbarium, São Paulo, SP, Brazil. *Sitientibus Série Ciências Biológicas* 7 (2): 163–169 (in Portuguese with an abstract in English).
- Oliveira IC, Sousa MA (2002). Boletales (Hymenomycetes) no Campus I da Universidade Federal da Paraíba, João Pessoa: III – Strobilomycetaceae. *Revista Nordestina de Biologia* 16: 43–53 (in Portuguese with an abstract in English).
- Online Auction Color (2004). Online Auction Color Chart. Stanford, Online Auction Color Co.
- Ortiz-Santana B, Lodge DJ, Baroni TJ, Both EE (2007). Boletes from Belize and the Dominican Republic. *Fungal Diversity* 27: 247–416.
- Pegler DN (1983). Agaric Flora of the Lesser Antilles. *Kew Bulletin Additional Series* 9: 1–668.
- Posada D (2008). jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution* 25 (7): 1253–1256. doi: 10.1093/molbev/msn083
- Robert V, Stegehuis G, Stalpers J (2005). The MycoBank engine and related databases [online]. Website <https://www.mycobank.org/> [accessed 07 March 2022].
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A et al (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539–42. doi: 10.1093/sysbio/sys029
- Sá DF, Almeida HA, Silva LF, Leão AC (1982). Fatores edafoclimáticos seletivos ao zoneamento de cacauicultura no Sudeste da Bahia. *Revista Theobroma* 12 (3): 169–187 (in Portuguese with an abstract in English).
- Sambuichi RHR (2002). Fitossociologia e diversidade de espécies arbóreas em cabruca (Mata Atlântica raleada sobre plantação de Cacau) na região sul da Bahia, Brasil. *Acta Botanica Brasílica* 16 (1): 89–101 (in Portuguese with an abstract in English). doi: 10.1590/S0102-33062002000100011
- Sambuichi RHR, Oliveira RM, Neto EM, Thévenin JMR, Júnior CPJ et al. (2008). Conservation status of ten endemic trees from the Atlantic Forest in the south of Bahia - Brazil. *Natureza & Conservação* 6(1): 208–225.
- Singer R (1978). Notes on bolete taxonomy - II. *Persoonia* 9: 421–438.
- Singer R (1986). *The Agaricales in Modern Taxonomy*. 4th ed. Koenigstein, Germany: Koeltz Scientific Books.
- Singer R, Digilio APL (1960). Las Boletaceas de Sudamerica tropical. *Lilloa* 30: 141–164 (in Spanish with an abstract in English).
- Singer R, Araujo IJ, Ivory MH (1983). The Ectotrophically Mycorrhizal Fungi of the Neotropical Lowlands, Especially Central Amazonia (Litter decomposition and ectomycorrhiza in Amazonian forests 2.). *Beihfte zur Nova Hedwidia* 77: 1–352.
- Singer R, García J, Gómez LD (1991). The Boletineae of Mexico and Central America III. *Beihfte zur Nova Hedwigia* 102: 1–97.
- Stamatakis A (2014). RAXML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* (30): 1312–1313. doi: 10.1093/bioinformatics/btu033
- Thiers B (2022). (continuously updated). Index Herbariorum: A global directory of public herbaria and associated staff. New York Garden's Virtual Herbarium [online]. Website <http://sweetgum.nybg.org/science/ih/> [accessed 06 March 2022].
- Thomas WW, Carvalho AMV, Amorim AMA, Garrison J, Arbeláez AL (1998). Plant endemism in two forests in southern Bahia, Brazil. *Biodiversity and Conservation* 7: 311–322.
- Thomas WW, Jardim JG, Fiaschi P, Neto EM, Amorim AM (2009). Composição florística e estrutura do componente arbóreo de uma área transcional de Floresta Atlântica no sul da Bahia, Brasil. *Revista Brasileira de Botânica* 32(1): 65–78 (in Portuguese with an abstract in English). doi: 10.1590/S0100-84042009000100007
- Trappe JM, Castellano MA, Halling RE, Osmundson TW, Binder M et al. (2013). Australasian sequestrate fungi 18: *Soliococcus polychromus* gen. & sp. nov., a richly colored, tropical to subtropical, hypogeous fungus. *Mycologia* 105 (4): 888–895. doi: 10.3852/12-046
- Vasco-Palacios AM, López-Quinteros C, Franco-Molano AE, Boekhout T (2014). *Austroboletus amazonicus* sp. nov. and *Fistulinella campinaranae* var. *scrobiculata*, two commonly occurring boletes from a forest dominated by *Pseudomonotes tropenbosii* (Dipterocarpaceae) in Colombia Amazonia. *Mycologia* 106 (5): 1004–1014. doi: 10.3852/13-324
- White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (editors). *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press, pp. 315–322. doi: 10.1016/B978-0-12-372180-8.50042-1
- Wu G, Feng B, Xu J, Zhu X-T, Li Y-C et al. (2014). Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. *Fungal Diversity* 69: 93–115. doi: 10.1007/s13225-014-0283-8
- Wu G, Li Y-C, Zhu X-T, Zhao K, Han L-H et al. (2016). One hundred noteworthy boletes from China. *Fungal Diversity* 81 (1): 25–188. doi: 10.1007/s13225-016-0375-8
- Zeng N-K, Su M-S, Liang Z-Q, Yang Z-L (2015). A geographical extension of the North American genus *Bothia* (Boletaceae, Boletales) to East Asia with a new species *B. fujianensis* from China. *Mycological Progress* 14: 1–6. doi: 10.1007/s11557-014-1015-x