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**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:** Austroboletoideae, Boletales, Neotropic, systematic

**1. Introduction**

*Fistulinella* Henne. was originally described from Cameroon with *F. staudtii* Henne. 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’ (bilaterial, divergent, boletoid).

The genus belongs to the subfamily Austroboletoideae G. Wu. & Zhu L. Yang (Boletaceae, Boletales), together with *Austroboletus* (Corner) Wolfe, *Mucilopilus* Wolfe and *Velorporphyrellus* 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) ± SD = average basidiospores length (width) with standard deviation, Q = the length: width ratio range as determined from all measured basidiospores, and Qm ± 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 sec, incubated in boiling water for 5 min, then vortexed again for 10 sec, 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′-CTTGGTCATTTAGGAAGTAA-3′) and ITS4 (5′-TCTCCGCTATTAGTATGC-3′) (White et al., 1990) and 28S rDNA gene bounded by primers LR0R (5′-ACCCGCTGAATTAGC-3′) and the reverse primer LR7 (5′-TACTACACACAGATCT-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 (Katoh 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 (v8.2.12) (Stamatakis, 2014) using GTR GAMMA 1 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.

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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.


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 subiculate, 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 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,
smooth, some with presence of granular refringent content inside. Cheilocystidia 23–70.4 × 5.1–17.9 µm, fusid-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. Stipitpellis two layered: suprastipitpellis a trichodermium, terminal cells 3.1–7.1 µm wide, cylindric-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; substipitpellis 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 2. Maximum likelihood phylogenetic tree of Fistulinella species based on 28S 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
2. Pileus or stipe not glutinous, but dry to somewhat viscid ........................................................................... 5
3. Pileus typically darker, mouse gray or slate gray to brown, dark brown or blackish brown, but pure white, 

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

5. Pileus dark brown closer to center to brown on a light brownish to beige background, surface bearing small brownish frivillose-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 4. Bayesian phylogenetic tree of Fistulinella species based on 28S rDNA fragments.
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–)15.3–18.4(–18.9) × 5.1–6.1 µm, cystidia ranging 23–79.1 µm long, and the two layered stipitpellis with a trichodermal suprastipitpellis and the substipitpellis 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–)12–15(–17.3) × 4–5(–5.3) µm (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 12.4–19.8(–24.8) × 3.7–4.9 µm, septe cheilocystidia, and a trichodermal palisade pileipellis (Fulgenzi et al., 2010). These authors did not define the morphology of stipitpellis 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. gloecarpa* 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 scrobulate pileus, finely pruinose to smooth and glabrous mostly whitish stipe, and its surface 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 (11.5–16.5 × 4–6 µm), 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 12.4–19.8(–24.8) × 3.7–4.9 µm, septate cheilocystidia, and a trichodermal palisade pileipellis (Fulgenzi et al., 2010).
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. rushii* 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. aurantioklava* T.H.G. Pham, A.V. Alexandrova & O.V. Morozova, *F. olivaceolba* T.H.G. Pham, Y.C. Li & O.V. Morozova, *F. prunicolor* (Cooke & Masssee) 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 & Masssee; McNabb 1967, as *Porphyrellus viscidas* 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.

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**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 stipitpellis, **G.** Caulocystidia. Scale bars = 10 µm.
cinereoalba, F. distromatica, F. gloecarpa 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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