



## ***Boletellus nordestinus* (Boletaceae, Boletales), a new species from Northeastern Atlantic Forest, Brazil**

**Magnago AC<sup>1</sup>, Neves MA<sup>2</sup>, Silveira RMB<sup>1</sup>**

<sup>1</sup>Programa de Pós-Graduação em Botânica, Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

<sup>2</sup>Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas, Departamento de Botânica, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

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### **Abstract**

*Boletellus nordestinus* is described as a new species from white sandy dunes in the Northeastern Brazilian Atlantic Forest. The area is known as a potential ectotrophic forest, since some ectomycorrhizal fungi had been found in the same area. The new species is characterized by dry, velutinous and olivaceous to chocolate brown pileus, context and hymenophore non-cyanescent when exposed or pressured, short and longitudinally ridged basidiospores, fusiform to ventricose cystidia, and a trichodermal pileipellis with disarticulated, acute terminal elements. A full description, line drawings, color photographs and molecular phylogenetic data of the new species are provided.

**Key words** – Agaricomycetes – boletoid fungi – Neotropical fungi – taxonomy

### **Introduction**

*Boletellus* Murrill (Boletaceae, Boletales, Basidiomycota) is a small group of ectomycorrhizal fungi forming mutualistic relationship with different host trees (Fulgenzi et al. 2008, Tedersoo et al. 2010). Morphologically the genus encompasses around 72 described species worldwide, the majority reported from tropical regions (Heinemann & Goossens-Fontana 1954, Singer 1970, Snell & Dick 1970, Smith & Thiers 1971, Corner 1972, Horak 1977, Singer 1986, Singer et al. 1992, Gómez 1996, Watling 2001, Halling & Mueller 2005, Ortiz-Santana et al. 2007, Fulgenzi et al. 2008, Halling & Ortiz-Santana 2009, Halling et al. 2015, Sato & Hattori 2015, Barbosa-Silva) Murrill (Murrill 1909). According to authors, *Boletellus* has been variably defined, having in et al. 2017). The type species designated for the genus was the North American *B. ananas* (M.A. Curtis common: basidiospores olivaceous brown in deposit, yellow hymenophore becoming olivaceous with maturity and boletoid tube trama. Singer (1986) in the traditional morphological concept adhered a broad definition for *Boletellus* including seven sections based by the basidiospores ornamentation: smooth basidiospores (section *Mirabilis*); basidiospores with imbedded short spines or pits (section *Allospori*); reticulate basidiospores (section *Retispori*) and longitudinally winged or ridged basidiospores (sections *Boletellus* Murrill, *Chrysenteroidei* Singer, *Ixocephali* Singer and *Dictyopodes* Singer) using morphological characters of pileus and stipe to separate these sections. However, recent molecular phylogenetic studies indicate that such characters may not delimit

clades in Boletaceae and *Boletellus* as morphologically circumscribed is polyphyletic (Dentinger et al. 2010, Nuhn et al. 2013, Wu et al. 2014, Halling et al. 2015). *Boletellus* s.str. may only include the two sections identified by Singer (1986), sect. *Boletellus* and sect. *Chrysenteroidei* Singer, with longitudinally winged or ridged basidiospores and a length/width ratio over two. Many species that were traditionally classified in *Boletellus* s.l., especially those without longitudinally winged or ridged basidiospores have been recently accommodated into new genera such as *Aureoboletus* Pouzar, *Heimioporus* E. Horak, and *Hemileccinum* Šutara.

For South America, few species are registered, and for Brazil only four *Boletellus* species are cited: *B. ananas* for the Amazon Forest (Singer et al. 1983), *B. lepidospora* E.-J. Gilbert ex Heinem., *B. pustulatus* (Beeli) E. J. Gilbert and *B. cremeovelosus* Barbosa-Silva & Wartchow for the Atlantic Forest (Oliveira & Sousa 1995, Barbosa-Silva & Wartchow 2017). Here we describe *B. nordestinus* sp. nov. discovered in ectomycorrhizal white sandy dunes in the extreme north Atlantic Forest, as part of the project Diversity of boletoid fungi in the Brazilian native forests, where new species has been described recently (Magnago et al. 2014, Magnago et al. 2017a, 2017b, 2018a, 2018b). In the same area where the new species was found, other ectomycorrhizal fungi (e.g. *Amanita* Pers., *Coltricia* Gray, *Lactifluus* (Pers.) Roussel, *Russula* Pers., *Scleroderma* Pers., *Tylopilus* P. Karst.) have been collected and described recently (Baltazar et al. 2010, Sá et al. 2013, Sulzbacher et al. 2013, Wartchow et al. 2013, Barbosa-Silva et al. 2017, Magnago et al. 2017b).

As part of our ongoing boletoid diversity surveys in the Brazilian Atlantic Forest, *Boletellus nordestinus* sp. nov. is described and proposed as new to science.

## Materials & Methods

### Sampling

Collections were made in 2009 and 2016 in the Northeastern region of Brazil in the coastal Atlantic Forest, at “Reserva Biológica Guaribas”, municipally of Mamanguape, Paraíba State and at “Parque Estadual Dunas de Natal”, municipally of Natal, Rio Grande do Norte State. In both areas, the specimens were collected in white sandy soil. Macroscopic features were described from fresh basidiomata and dried with a food dehydrator (Total Chef TCFD-05 Deluxe) at about 40°C. 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 (Largent et al. 1977). 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 da Universidade Federal do Rio Grande do Sul”. All microscopic features were line drawings by hand using digital photographs from the specimens examined. Voucher materials were deposited at UFRN, FLOR and ICN (Thiers, continuously updated).

### Molecular data

DNA was extracted from dried specimens followed Doyle & Doyle (1987) protocol adapted by Góes-Neto et al. (2005). 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 (Dentinger et al. 2010) and LR0R/LR7 (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 with accession numbers listed in materials examined.

## Results

*Boletellus nordestinus* A.C. Magnago, sp. nov.

MycoBank number: MB823951

Figs 1–2

Etymology – In homage to the Northeastern region (“Nordeste” in Portuguese) where the specimens were collected.

Diagnosis – Differs from the other *Boletellus* non-cyanescent species with the combination of dry, velutinous, chocolate brown pileus, short basidiospores, pleurocystidia and cheilocystidia fusiform to ventricose, and pileipellis trichodermal with acute terminal elements.

Holotypus – Brazil, Rio Grande do Norte, Natal, Parque Estadual das Dunas de Natal, 5°50'40"S, 35°11'25,2"W, Trilha da Perobinha, 08 June 2016, Fazolino, E.P. & Assis, N.M. s/n (UFRN-Fungos 2726), GenBank accessions: ITS = MG760443, nrLSU = MG760444).

Description – Pileus 38–66 mm wide, parabolic at first to convex when mature, velutinous to slightly fibrillose towards the margin, olivaceous to chocolate brown (OAC 733,735), dry; margin slightly uplifted when mature; context whitish to cream yellow (OAC 812), solid, unchanging when exposed. Hymenophore tubular, depressed around stipe, with decurrent tooth, tubes 5–8 mm long centrally, bright yellow (OAC 854, 895) when young maturing to olive yellow (OAC 10, 19), pores concolorous with tubes, not bluing under pressure, mostly 1–2 mm wide, subangular. Stipe 28–47 mm × 5–8 mm central to eccentric, equal, surface longitudinally slightly rugulose with short squamules, light yellow to pinkish (OAC 12,14), some specimens more brownish to vinaceous brown (OAC 636, 638), context solid, fibrillose, cream yellow (OAC 812) near the apex becoming vinaceous brown downwards (OAC 638), unchanging when exposed, basal mycelium white. Basidiospores olivaceous-brown to dark olive-green (OAC 866, 867) in deposit, (7.5) 8–10 (11) × 6–7 (8) µm (Qm=1.40), broadly ellipsoid, longitudinally ridged, dichotomously forked, occasionally non-forked, terminating in unequal lengths near apex, ridges converging at hilar appendage, pale yellow, inamyloid, thick-walled, hilar appendage 0.5–1 µm long. Basidia 27–42 × 12–14 µm, clavate, thin-walled, hyaline, inamyloid; 4-sterigmate, 2–3 µm long. Pleurocystidia and cheilocystidia not differentiated from each, 36–56 × 7–12 µm, fusiform to ventricose, hyaline, inamyloid. Hymenophoral trama boletoid, hyphae 4–12 µm wide, hyaline, inamyloid, smooth and thin-walled. Pileipellis trichodermal, hyphae disarticulated, terminal hyphae acute, brownish, inamyloid, terminal hyphae 8–15 µm wide; oiliferous hyphae present. Pileus tramainterwoven, hyphae 6–16 µm wide, light yellow. Stipitipellis repent, with projecting tufts of caulobasidia and caulocistidia, clavate, fusiform to sphaeropedunculate, 28–38 × 13–15 µm, hyaline, inamyloid. Stipe trama parallel to subparallel, hyphae 5–12 µm wide, light yellow, inamyloid. Clamp connections absent.

Habitat – Solitary to scattered, growing on white sandy soil and dunes in restinga vegetation in the far north of coastal Atlantic Forest. The vegetation is dominated by species of Leguminosae, Myrtaceae, Poaceae, Asteraceae and Euphorbiaceae (Freire 1990).

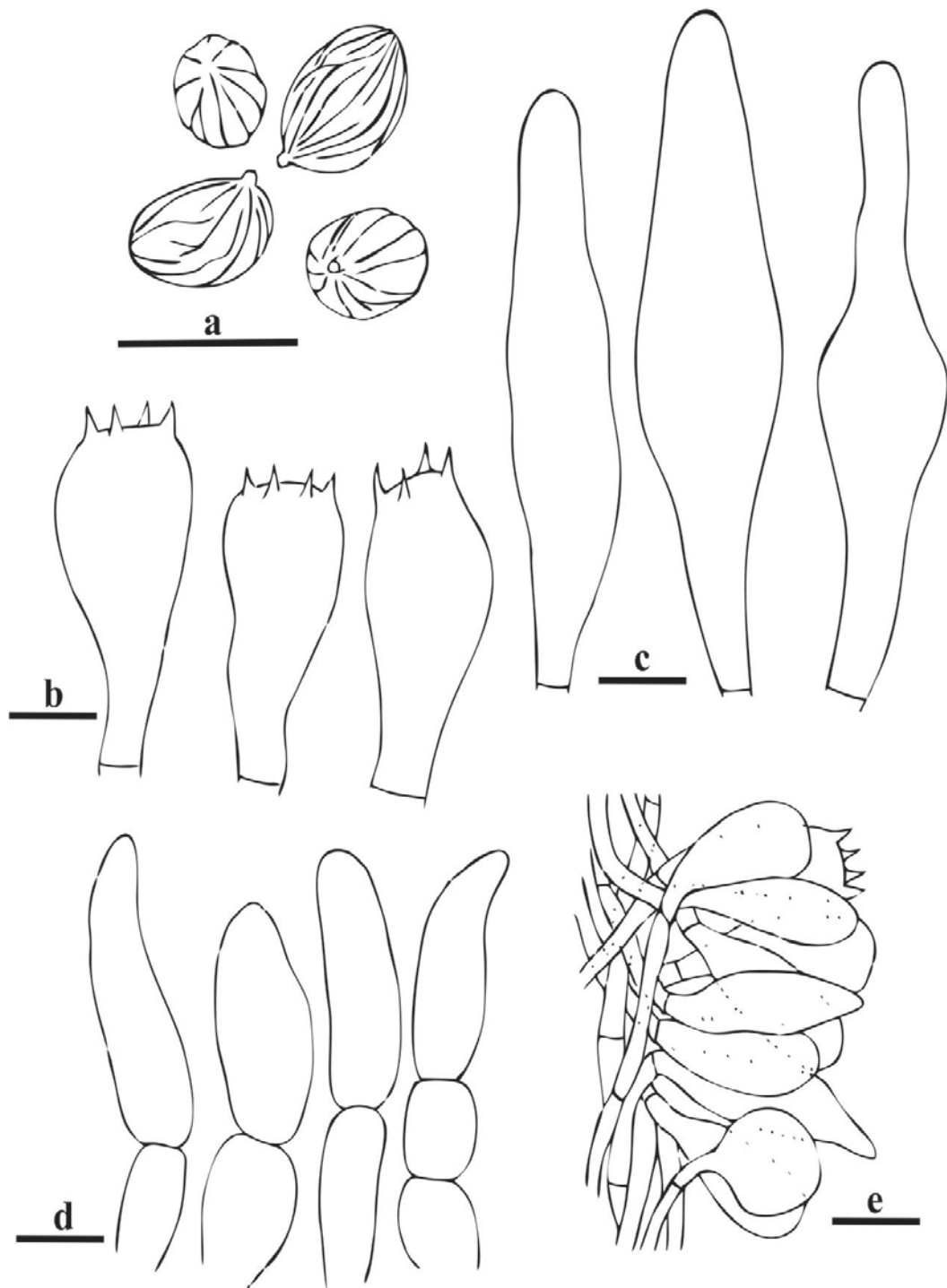
Geographic distribution – Known from Northeastern Brazilian region in the states of Paraíba and Rio Grande do Norte.

Material examined (paratypes) – Brazil, Paraíba, Mamanguape, Reserva Biológica Guaribas, SEMA II, 6°44'14"S, 35°8'55"W, 15 August 2009, coll. Neves M.A. MAN481 (FLOR51603); 23 September 2009, coll. Neves, M.A., Barbosa, M.R.V. & Thomas, W. MAN502 (FLOR51604); Rio Grande do Norte, Natal, Parque Estadual das Dunas de Natal, 5°50'40"S, 35°11'25,2"W, Trilha da Geologia, 07 June 2016, coll. Fazolino, E.P. & Neto, J. F. F. s/n (UFRN-Fungos 2725) GenBank accessions: ITS = MG760442; 5°50'39"S, 35°11'25,4' Trilha da Perobinha, 08 June 2016, coll. Fazolino, E.P & Assis, N.M. s/n (UFRN-Fungos 2729).

Molecular data – Three new sequences of *B. nordestinus* sp. nov. were generated (two nrITS and one nrLSU). ITS and nrLSU BLASTn queries of the new taxon on GenBank indicated affinities with specimens in the genera *Boletellus*.

## Discussion

*Boletellus nordestinus* is best disposed infragenerically in *Boletellus* section *Chrysenteroidei* Singer based on the longitudinally winged spore, perpendicular striations absent, pileus lacking red colors, veil absent, pileus and stipe not glutinous or viscid, stipe not lacunose-alveolate and clamp connections absent (Singer 1986). *Boletellus fibuliger* Singer from Venezuela has pileus brownish



**Fig. 1** – Micromorphology of *Boletellus nordestinus* (UFRN-Fungos 2726, holotype). a Basidiospores. b Basidia. c Cystidia. d Terminal cells of pileipellis from the pileus center. e Stipitipellis, cluster of caulobasidia and caulocystidia. Scale Bars: 10  $\mu$ m.

fibrillose over a yellowish ground, less fibrillose towards the margin, not or scarcely appendiculate margin, larger basidiospores (17–20  $\times$  9.5–11  $\mu$ m), cystidia ampullaceous, and clamp connections present. *Boletellus cubensis* (Berk. & M.A. Curt.) Singer from the eastern Caribbean, has squamulose pileus, red annular band on the stipe apex, bluing hymenophore when bruised, and the basidiospores are larger (20–22.5  $\times$  7.5–10  $\mu$ m). *Boletellus chrysenderoides* (Snell) Snell registered from Canada, USA and Honduras has larger basidiospores (9.5–18.2  $\times$  5.3–9.8  $\mu$ m) longitudinally ridged or winged, which are intervenose or themselves transversely striate, and is usually found

growing under conifers. *Boletellus exiguus* T.W. Henkel and Fulgenzi from Guyana differs by the velutinous, finely squamulose and areolate pileus when mature, margin slightly inrolled when young, context and hymenophore bluing rapidly with exposure or pressure; pleurocystidia with golden incrustations in Melzer's, and a pileipellis of angular cells with tufts of erect inflated terminal elements (Fulgenzi et al. 2008). *Boletellus dicymbophilus* Fulgenzi and T.W. Henkel from Guyana has red-brown to yellow-tan, rugulose to rugose pileus, hymenophore instantly bluing on exposure or pressure, and larger basidiospores ( $12\text{--}15 \times 8\text{--}11 \mu\text{m}$ ) (Fulgenzi et al. 2008). Among the two Congolian *Boletellus* spp. cited by Oliveira & Sousa (1995) for Atlantic Forest, Brazil, *B. pustulatus* possesses ventricose-rostrate cystidia and a bluing reaction in the exposed context (Gilbert 1931) and *B. lepidospora* has a rugulose brown to blackish pileus, stipe hollow towards the base, context yellow to light brown that becomes blue when exposed, basidiospores  $11\text{--}12.5 \times 6\text{--}7.4 \mu\text{m}$ , cystidia lanceolate ( $45\text{--}53 \times 12\text{--}13 \mu\text{m}$ ) and pileipellis composed by subglobose terminal elements (Heinemann & Goossens-Fontana 1954). These two last species cited for the Atlantic Forest should be recollected in the area to confirm its identification, because in the description of the Brazilian species by Oliveira & Sousa (1995) the context when exposed are non-cyanescent, and this is an important character of both species cited by Gilbert (1931) and Heinemann & Goossens-Fontana (1954). *Boletellus cremeovelosus* differs by the beige and squamose pileus, margin strongly appendiculate, context and hymenophore bluing when exposed and pressured, and subfusoid, longitudinally ribbed ( $15.8\text{--}20.4 \times 6.6\text{--}9.2$ ) basidiospores.



**Fig. 2** – Macromorphology in the field and SEM of the basidiospores of *Boletellus nordestinus*. a Voucher UFRN-Fungos2721 (holotype). b Detail of pileus surface (UFRN-Fungo 2725). c Detail of the hymenophore (UFRN-Fungos 2725). d Basidiomata in cross section (UFRN-Fungo 2729) (Scale Bar: 3 cm). e SEM micrograph of basidiospores (FLOR51604). Scale Bars: 5  $\mu\text{m}$ .

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