New records of Phallales (Basidiomycota) from Brazilian semi-arid region

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Abstract  
Molecular studies have shown that Phallales constitutes a distinct clade within Phallomycetidae and morphologically comprises a wide variety of patterns of shapes and colors, producing expanded basidiomata, commonly known as stinkhorns, which may be free, latticed or sequestrate. Aiming to increase the knowledge of these fungi in the Brazilian semi-arid region, several field trips were conducted in the rainy season of 2014 and 2018 in the Araripe National Forest, which covers an area of about 38,262 ha and is located in the State of Ceará. Several specimens were macro and micro morphologically described, and five species were identified. *Laternea dringii* is the second record outside of Mexico (locality type), *Mutinus albotruncatus* is the second record for science, *Mutinus argentinus* and *Phallus indusiatus* are the first records for the Brazilian semi-arid.

Key words – Biodiversity – cage-fungi – gasteromycetes – stinkhorns – taxonomy

Introduction  
Phallales E. Fisch. is a molecularly well-defined order within Phallomycetidae Hosaka, Castellano & Spatafora (Hosaka et al. 2006, Hibbett et al. 2014) and morphologically comprises a wide variety of patterns of shapes and colors, producing expanded basidiomata, commonly known as stinkhorns, which may be free, latticed or sequestrate, such as *Claustula* K.M. Curtis, *Gelopellis* Zeller, *Protubera* Möller and *Restingomyces* Sulzbacher, Grebenc & Baseia (Sulzbacher et al. 2016). Basidiospore dispersion generally occurs through a variety of mycophagous animals, especially flies that are attracted by the odor of the mature gleba (Tuno 1998).

According to Dring (1980), the tropics are considered the center of diversity for many of the species in this group. In particular, Brazil is represented by approximately 51 taxa of phalloid fungi. However, some of these records are considered synonyms or doubtful records because the voucher specimens are not well preserved (Cortez et al. 2011a, b).

Covering nine states, the Brazilian semi-arid occupies 982,563 km² of the national territory...
(Pereira-Junior 2007), an area larger than countries such as Nigeria, Tanzania and Venezuela. In this area there is a predominance of the Caatinga, an exclusively Brazilian biome (Leal et al. 2005), as well as other vegetative enclaves that make the Brazilian semi-arid present a great diversity of plant species, many of them endemic to the biome. Through mycobiota studies in this region, it has been shown to be an interesting environment for the development of phalloid fungi, including the discovery of new taxa for science (Fazolino et al. 2010, Silva et al. 2015, Crous et al. 2017). The main goal of the present study is to contribute to an increase in the knowledge about gasteroid fungi in the Brazilian semi-arid region, based on morphological data.

Materials & Methods

Studied area
The Araripe National Forest covers an area of about 38,262 ha and has a perimeter of about 138 km, occupying part of the municipalities of Barbalha, Crato, Jardim and Santana do Cariri, all located in the State of Ceará (MMA 2006). The collections were carried out in the municipality of Crato (latitude 7°13'46" S and longitude 39°24'32" W), where habitat fragmentation is observed on roads and trails that cut through the preserved area (IBGE 2010). Fieldtrips occurred during the rainy season (February to May) of the years 2014 and 2018 (Fig. 1).

Collection methods
The specimens were collected following the methodology used by Baseia et al. (2014), in which the basidiomata were photographed with scales and geographical data and were removed from the substrate with a pocketknife. In the Biology of Fungi lab at the Federal University of Rio Grande do Norte the basidiomata were dried in an electric dehydrator and packaged with a ziplock bag; these packs were then placed together with an information sheet from the collection. The specimens were deposited in the Fungi Collection of the Federal University of Rio Grande do Norte (UFRN), Natal, Brazil.

Morphological observations
The morphological analysis was conducted according to Baseia et al. (2014). Macroscopic characteristics are based on observations of the fresh basidiomata, including characteristics of the
peridium and gleba. The size, shape and color of these characters were analyzed. Microscopic observation was performed using an optical Nikon Eclipse Ni (LM) microscope with Nikon DS–R1 camera attached, and all measurements were carried out using the NIS–Elements AR v.4.51.00 software. Slides for microscopic analyses were mounted in 5% KOH and Congo red containing portions of the fertile part, volva, receptacle, pseudostipe and rhizomorph. For each specimen, 30 basidiospores and 20 hyphae (volva, receptacle, pseudostipe and rhizomorph) were randomly selected and measured. The statistical data used in basidiospores were performed according to Sousa et al. (2014), which determined the variation of the length ($H_{\text{Min}}$ and $H_{\text{Max}}$) and width ($W_{\text{Min}}$ and $W_{\text{Max}}$), the mean of the two dimensions ($xH$ and $xW$), the standard deviation of these ($\pm \text{SD}$) and the height ($h$) by the width ($w$) quotient represented by $Q_m$ 

$$Q_m = \frac{H_{\text{Min}} - H_{\text{Max}} \times W_{\text{Min}} - W_{\text{Max}}}{xH \pm \text{SDH} \times xW \pm \text{SDW}}$$

always observed under 100× objective. The color was coded following Küppers (2002).

Results & Discussion

Taxonomy

**Clathrus columnatus** Bosc, Mag. Gesell. Naturf. Freunde, Berlin 5: 85 (1811) Fig. 2a–c

Facesoffungi number: FoF 05677

Description – Immature basidiomata 10–30 mm diam., globose to subglobose, epigeous, white (N00M00C00). Expanded basidiomata 30–60 mm high × 10–25 mm diam. Receptacle orange (N00Y80M50), formed by 4 columnar arms, spongy, free at the base, merged at the apex. Volva 10–30 diam., white (N00Y00M00), subglobose, papery. Gleba covering the inner upper arch of the columns, olive green (N99C80Y80), mucilaginous, fetid. Basidiospores 3.3–5.5(–6) × 1.2–2.5 μm ($x = 4.5 \pm 0.4 \times 1.9 \pm 0.2$, $Q_m = 2.37$), elongate to cylindrical, hyaline in 5% KOH, smooth. Receptacle composed of pseudoparenchymatous hyphae, 21.5–60 × 14–42 μm, globose, subglobose to ovoid, hyaline, thin walls (<1 μm). Volva composed of two layers: external layer composed of filamentous hyphae, 2.6–5.4 μm, straight, with inflated parts, irregular, hyaline, thin walls (<1 μm), with septa and branches; gelatinous internal layer, composed of filamentous hyphae, 2.9–4.5 μm, straight, hyaline, thin walls (<1 μm). Rhizomorph composed of filamentous hyphae, 1.3–3.4 μm, hyaline, thin walls (<1 μm), with inflated tips connected by pores.

Habitat – This species has a solitary habit and can be found in soil covered with leaves, sandy soil or decaying wood, in partially or totally shaded areas.


Material examined – Brazil, Ceará, Crato, Araripe National Forest (7°14′55.7″S, 39°29′42.5″W), on soil covered with leaves and decaying wood, 16 Mar 2014, M.M.B. Barbosa, R.H.S.F. Cruz, A100 (UFRN-Fungos 2419). Brazil, Ceará, Crato, Araripe National Forest (7°14′55.7″S, 39°29′42.5″W), on sandy soil, 16 Mar 2014, M.M.B. Barbosa, R.H.S.F. Cruz, A105 (UFRN-Fungos 2424). Brazil, Ceará, Crato, Araripe National Forest (7°14′55.7″S, 39°29′42.5″W), on soil covered with leaves and decaying wood, 17 Mar 2014, M.M.B. Barbosa, R.H.S.F. Cruz, R.J. Ferreira, A115 (UFRN-Fungos 2434).

Notes – Occurring in several parts of Brazil and of the world, this species is common in the Araripe National Forest, Ceará State (Baseia et al. 2014). **Clathrus columnatus** is characterized by presenting basidiomata with 3–4 columnar arms, free at the base and joined at the apex, and gleba scattered at the upper inner part of the arms. It resembles the *Laternea triscapa* Turpin, but the latter presents a gleba confined in a glebifer. **Pseudocolus fusiformis** (E. Fisch.) Lloyd also resembles *C. columnatus*, but the former is distinguished by having arms joined at the base. According to Dring (1980), Cunningham (1944), this species may present regional variants regarding the number of arms, color and shape of the receptacle. In the literature it is recorded
under different names: *Colonnaria* Rafinesque-Schmaltz, *Laternea* Turpin, *Linderia* G. Cunningham, and *Linderiella* G. Cunningham. This is the second record for the Brazilian semi-arid region.


Fig. 2d–f

Facesoffungi number: FoF 05678

Description – Immature basidiomata 8–12 mm high × 7–10 mm diam., subglobose, epigeous, white (N00Y00M00) at the base to yellowish gray (N60C00M30) at the apex, surface glabrous to cracked in the apical portion, not encrusted. Expanded basidiomata 26–40 mm high. Receptacle yellowish white (N00Y10M00) at the base, orange (N00Y80M50) at the apex, formed by 3–4 columnar arms, spongy, free at the base and joined by a transverse arm at the apex, with reniform tubes in transverse section. Absence of transverse arm in some basidiomata. Volva 10–13 mm high × 11–13 wide, white (N00Y00M00) to yellowish gray (N60C00M30), globose to subglobose, surface glabrous to cracked in the apical portion, papery. Rhizomorph up to 40 mm, white (N00Y00M00), adhered at the base of the volva. Glebifer reddish orange (N00Y90M50) in the lower inner part of the arms union. Gleba confined in glebifer, olive green (N00C10Y99), mucilaginous, fetid. Basidiospores 3.5–4.6 × 1.1–2 μm (x = 4.1 ± 0.3 × 1.5 ± 0.3, Qm = 2.69), cylindrical to bacilliform, hyaline in 5% KOH, smooth. Receptacle composed of pseudoparenchymatous hyphae, 15.7–48.8 × 14–38.7 μm, subglobose to ellipsoid, hyaline, thin walls (<1 μm). Volva composed of two layers: external layer composed of filamentous hyphae, 3–9 μm, straight, with inflated parts, hyaline, thin walls (<1 μm), with septa and branches; gelatinous internal layer, composed of filamentous hyphae, 2–3.3 μm, straight, hyaline, thin walls (<1 μm), with septa and branches. Rhizomorph composed of filamentous hyphae, 1.8–6.3 μm, hyaline, thin walls (<1 μm), septate, with inflated tips connected by pores.

Habitat – This species has a solitary or gregarious habit and can be found in soil covered with litter in totally shaded area.


Fig. 2 – *Clathrus columnatus*. a Expanded basidiomata in situ. b Gleba covering the inner upper arch of the columns. c Basidiospores. *Laternea dringii*. d Immature and expanded basidiomata in situ. e Glebifer in the lower inner part of the arm joints. f Basidiospores. Scale Bars: a, b, d, e = 10 mm; c, f = 10 μm.
Notes – According to López et al. (1981), *Laternea dringii* is characterized by basidiomata 13 to 15 mm in height, red-orange to yellowish orange, formed by 3–4 columns free at the base, joined at the apex by a transverse arm, a glebifer well defined in the interior of the columns and basidiospores measuring 4.2–5(–5.6) × 1.4 μm. The morphological characteristics of the specimens of the present study are very similar to those cited by López et al. (1981). However, present variations: basidiomata larger (26–40 mm high), basidiospores slightly smaller (3.5–4.6 × 1.1–2 μm) and absence of a transverse arm in some basidiomata. It is similar to *Laternea triscapa*, but the latter presents larger basidiomata (up to 70 mm), receptacle vermilion at the apex to pinkish at the base and arms with tubes trapezoid in transverse section (Dring 1980). *Laternea pusilla* Berk. & M.A. Curtis differs from *L. dringii* by presenting columns with crests and receptacle ochraceous to salmon pink. Calone et al. (2004) considered *L. dringii* a synonym of *L. triscapa*. The present study considered the classification proposed by López et al. (1981). Therefore, more detailed research is needed considering the Brazilian collection, to better delimit the informative characters of this species. In Brazil, Magnago et al. (2013) reported *L. dringii* for Paraíba State. However, only the macroscopic analysis was performed based on a sample that was lost. This is the second record outside Mexico and the first record for the Brazilian semi-arid region.

*Mutinus albotruncatus* B.D.B. Silva & Baseia, in Silva, Cabral, Martín, Marinho, Calone & Baseia, Phytotaxa 236: 241 (2015)  Fig. 3a–d

Facesoffungi number: FoF 05675

Description – Immature basidiomata 15–18 mm high × 4–7 diam., fabiform to reniform, epigeous, yellowish white (N00Y10M00), with rhizomorph adhered at the base. Expanded basidiomata formed by pseudostipe and volva. Pseudostipe 40–65 mm high × 5–10 mm diam., cylindrical, apically perforate, hollow, spongy; sterile portion white (N00Y00M00), straight, not chambered; fertile portion light brown (N30Y70M40), well-defined, cylindrical, truncate, slightly rugulose, 15–23 mm high, covering about 1/3 of the total length of pseudostipe. Volva 8–20 mm high × 8–10 mm wide, yellowish white (N00Y10M00), with rhizomorph adhered to the base. Rhizomorph up to 40 mm long, yellowish white (N00Y10M00). Gleba covering and confined to the upper region of the pseudostipe, mucilaginous, olive gray (N90C00Y40). Basidiospores 2.8–4.7 × 1.6–3 μm (x = 3.9 ± 0.3 × 2.2 ± 0.2, Qm = 1.79), ellipsoid to elongate, smooth, hyaline in 5% KOH. Pseudostipe composed of pseudoparenchymatous hyphae, 16.5–48.7 × 12–43 μm hyaline, irregular shaped, thin walls (<1 μm). Volva formed by filamentous hyphae, 2–4.5 μm diam., hyaline, septate, branched. Rhizomorphs composed of filamentous hyphae, 0.3–3.4 μm diam., hyaline, septate.

Habitat – This species has a solitary or gregarious habit and can be found in soil covered with litter or decaying wood, in partially or totally shaded areas.

Known distribution – Brazil – Ceará (Silva et al. 2015, present study).


Notes – This species is common in the Araripe National Forest, Ceará State. This species is characterized by presence of a white pseudostipe, a brown, doliform to cylindrical and truncate fertile portion, and the lignicolous habitat (Silva et al. 2015). Despite having a lignicolous habitat, in our collections it was verified that this species also occurs in soil covered with litter. This species resembles *M. zenkeri* Henn. E. Fischer, but the latter is differentiated by having an incomplete and brittle membrane on fertile portion (Dring & Rose 1977, Degreef et al. 2013). *Mutinus boninensis* Lloyd and *M. borneensis* Lloyd also have a pseudostipe with a white sterile portion. However,
*Mutinus boninensis* has a fertile portion with a pointed apex, and it is completely annulate (Lloyd 1909, Kobayasi 1937), while *M. borneensis* has a shorter fertile portion, and is salmon pink or red (Lloyd 1909, Grgurinovic 1997). This is the second record for science and the Brazilian semi-arid region.


Facesoffungi number: FoF 05676

Description – Immature basidiomata 25 mm high × 13 diam., ellipsoid, semi-hypogeous, white (N<sub>00</sub>Y<sub>00</sub>M<sub>00</sub>) to yellowish brown (N<sub>60</sub>Y<sub>90</sub>M<sub>60</sub>), with rhizomorph adhered at the base. Expanded basidiomata formed by pseudostipe and volva. Pseudostipe 104 mm high × 8 mm diam., cylindrical, apically not perforate, hollow, spongy; sterile portion whitish (N<sub>00</sub>Y<sub>00</sub>M<sub>00</sub>) to light pink (Y<sub>30</sub>M<sub>40</sub>C<sub>00</sub>), slightly curved, not chambered; fertile portion reddish pink (Y<sub>60</sub>M<sub>99</sub>C<sub>40</sub>), well-defined, subulate, slightly rugulose, 30–36 mm high, covering about 1/3 of the total length of pseudostipe. Volva 24 mm high × 14 mm wide, white (N<sub>00</sub>Y<sub>00</sub>M<sub>00</sub>) to yellowish brown (N<sub>60</sub>Y<sub>90</sub>M<sub>60</sub>), with rhizomorph adhered to the base. Rhizomorph up to 70 mm long, white (N<sub>00</sub>Y<sub>00</sub>M<sub>00</sub>). Gleba covering and confined to the upper region of the pseudostipe, mucilaginous, olive brown (N<sub>90</sub>C<sub>00</sub>Y<sub>40</sub>). Basidiospores 3.7–5 × 1–2 μm (x = 4.4 ± 0.3 × 1.4 ± 0.2, Q<sub>m</sub> = 3.12), cylindrical to bacilliform, smooth, hyaline in 5% KOH. Pseudostipe composed of pseudoparenchymatous hyphae, 21.8–71 × 13.5–64.5 μm hyaline, irregular shaped, thick walls (>1 μm). Volva formed by filamentous hyphae, 2–3.7 μm diam., hyaline, septate, branched. Rhizomorphs composed of filamentous hyphae, 1.5–7.5 μm diam., hyaline, septate.

Habitat – This species has a gregarious habit and can be found in soil covered with litter in totally shaded areas.

Known distribution – Pantropical (Gube & Piepenbring 2009). Brazil – Paraná (Meijer 2006, Alves & Cortez 2016), Paraíba (Magnago et al. 2013) and Ceará (present study).

Notes – This species is recognized by its receptacle with a reddish-pink and well-defined fertile portion, with rugulose surface and pseudostipe the white to light pink color. *Mutinus argentinus* resembles *M. bambusinus* (Zoll.) E. Fisch. However, *Mutinus bambusinus* differs by having a sterile portion occupying 2/3 of the basidiomata, presence of pseudoparenchymatous processes, and a small sterile apical portion with a verrucose surface (Dring & Rose 1977). Due to strong similarities between these taxa, there are some doubts about their taxonomy (Alves & Cortez 2016). Due to their similarity, these two species were treated as synonyms by different authors (Kobayasi et al. 1938, Cunningham 1944, Liu 1984). Dring & Rose (1977) discussed the morphological difference between the two taxa. Gube & Piepenbring (2009) discussed the possibility that some records of *M. bambusinus* in the Neotropics belong to *M. argentinus*. According to Silva et al. (2015), several species of the genus *Mutinus* need to be reviewed. This is the first record for the Brazilian semi-arid region.

**Phallus indusiatus** Vent., Mém. Inst. nat. Sci. Arts 1: 520 (1798)

**Facesoffungi number:** FoF 01080

**Description** – Immature basidiomata 10–30 mm diam., globose to subglobose, white (N00M00C00) to brown (Y60M60C50). Expanded basidiomata 100–160 mm high. Receptacle 2–4 mm high × 24 mm wide, white (N00M00C00), campanulate, reticulate surface with apical pore. Pseudostipe 135 mm high × 13 mm wide, white (N00M00C00), cylindrical, spongy, hollow. Indusium well developed extending to the ground, white (N00M00C00), polygonal meshes, joined to the apex of the pseudostipe. Volva 23–30 mm high × 20–35 wide, white (N00M00C00) to brown (Y60M60C50), with pink pigments (N00Y20M20), globose, smooth surface, papery. Rhizomorph white (N00M00C00), adhered to the base of volva. Gleba olive green (N80C00Y99), mucilaginous, fetid. Basidiospores 2.8–4.2 × 1.1–1.9 μm (x = 3.4 ± 0.3 × 1.4 ± 0.2, Qm = 2.41), elongated to cylindrical, hyaline in 5% KOH, smooth, thin walls (<1 μm). Pseudostipe composed of pseudoparenchymatous hyphae, 16–40.8 × 14.5–36 μm, globose to subglobose, hyaline, thin walls (<1 μm). Indusium composed of pseudoparenchymatous hyphae, 18.3–19.1 × 19–32 μm, globose to subglobose, hyaline, thin walls (<1 μm). Volva composed of three layers: outer layer composed of filamentous hyphae, 2.5–9.2 μm, straight, with inflated parts, hyaline, thin walls (<1 μm), septate, with connection clamps; intermediate layer gelatinous, composed of filamentous hyphae, 2.1–4.2 μm, straight, hyaline, septate; internal layer composed of filamentous hyphae, 2.5–8 μm, straight, with inflated parts, hyaline, thin walls (<1 μm), septate. Rhizomorph composed of filamentous hyphae, 2.6–9.6 μm, hyaline, thin walls (<1 μm), with inflated tips connected by pores.

**Habitat** – This species has a solitary habit and can be found in clay or sandy soil covered with litter, in partially shaded areas.


from the others because it presents reticulate receptacle, well-developed white indusium, white pseudostipe and volva. *Phallus aureolatus* Trierveiler-Pereira & De Meijer also exhibits white indusium and pseudostipe but differs in its pinkish volva, merulioid to rugulose receptacle and white pseudoparenchymatous cap on the top of the receptacle. Trierveiler-Pereira et al. (2017), in their analyses, indicated that this morphological species probably corresponds to a complex of phylogenetic taxa. *Phallus indusiatus* has been well reported in Brazil. This is the first record for the Brazilian semi-arid region.

**Fig. 4** – *Phallus indusiatus*. a Expanded basidioma *in situ*. b Reticulate receptacle. c Volva. d Basidiospores. Scale Bars: a–c = 10 mm; d = 10 μm.

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