



***Phallus fluminensis* (Phallaceae, Basidiomycota), a new species of stinkhorn from the Brazilian Atlantic rainforest**

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Abstract

Basidiomata of a *Phallus* species were found near bamboo trees in the Rio de Janeiro Botanical Garden. This species was described as new to science and was named as *Phallus fluminensis* based on morphological characteristics. A detailed description, color images of microstructures and basidiomata of *P. fluminensis*, a comparison of *P. fluminensis* with its morphologically closely related taxa and a key to the genus *Phallus* in Brazil are given. A comparative analysis of *P. glutinolens* collections from the type locality was also made.

Key words – Biodiversity, Gasteromycetes, Neotropics, Phallales, Taxonomy

Introduction

The genus *Phallus* Junius ex L. is characterized by its phallic shape, with a cylindrical and hollow pseudostipe, bearing an apically attached receptacle that is smooth, rugose or reticulate (Cunningham 1944, Calonge 1998). According to Kreisel (1996), the most important morphological criteria for the taxonomy of *Phallus* are the shape and surface configuration of the receptacle, the coloration of the receptacle and volva, the size of the basidiomata and the presence or absence of an indusium. Molecular studies by Cabral et al. (2012) confirmed that the conspicuous indusium is apparently not informative for phylogenetic context.

Phallus comprises around 32 species with a worldwide distribution (Li et al. 2016), but 174 names are recorded in the Index Fungorum database (<www.indexfungorum.org>), including synonyms related to *Endophallus* M. Zang & R.H. Petersen, *Xylophallus* (Schltdl.) E. Fisch., *Mutinus* Fr., *Morchella* Dill. ex Pers. and others. Fungal taxonomic studies in Brazil have shown a rich diversity of this genus, and 11 species of *Phallus* have been reported for Brazil: *P. atrovolvatus*

Kreisel & Calonge, *P. callichrous* (Möller) Lloyd, *P. campanulatus* Berk, *P. cinnabarinus* (W.S. Lee) Kreisel, *P. glutinolens* (Möller) Kuntze, *P. granulosodenticulatus* B. Braun, *P. impudicus* L., *P. indusiatus* Vent., *P. merulinus* (Berk.) Cooke, *P. rubicundus* (Bosc) Fr., and *P. subtilis* (Möller) Lloyd (Trierweiler-Pereira & Baseia 2009, Cabral et al. 2014, Cabral et al. 2015, Trierweiler-Pereira et al. 2016).

Phallus glutinolens, a rare and interesting species originally described by Möller in 1895 as *Ithyphallus glutinolens*, was rediscovered and emended by Trierweiler-Pereira et al. (2009). Kuntze (1898) relocated *Ithyphallus balansae*, *I. cucullatus*, *I. glutinolens* and *I. lauterbachii* in the genus *Phallus*. Based on macro- and microscopic features, *P. glutinolens* was characterized mainly by a globose receptacle when young, and by a campanulate and perforate apex, observed at maturity according to the emendation made by Trierweiler-Pereira et al. (2009). The emended description of *P. glutinolens* presents a close resemblance to a new sample found in Rio de Janeiro, Brazil.

The Atlantic rainforest was one of the largest biomes in the Americas, originally covering around 150 million hectares, in highly heterogeneous environmental conditions. These geographical characteristics, combined with the large altitudinal range, have favored high diversity and endemism (Goerck 1997, Mittermeier et al. 1999, Ribeiro et al. 2009). Most of the remaining Atlantic rainforest exists in small fragments (Ranta et al. 1998) such as the Botanical Garden of Rio de Janeiro. Several species of gasteroid fungi were related to Atlantic rainforest, revealing existing areas that still have an unknown mycobiota (Baseia et al. 2003, Baseia & Calonge 2005, 2006, Rodrigues & Baseia 2013).

In this context, the present study aims to broaden the knowledge about the genus *Phallus* in the Brazilian Atlantic rainforest, through the description of a new species, as well as hoping to elucidate the knowledge about the great biodiversity of this striking genus.

Materials & Methods

Collection details

Collections were made in June 2012 at the Botanical Garden of Rio de Janeiro (JBRJ), located in Rio de Janeiro city, Brazil (Fig. 1). The complete collection is formed by three basidiomata, one of them perfectly preserved, and the others in small fragments. Basidiomata were examined and photographed in the field, observing all stages of development. The specimens were dehydrated in an electric dehydrator to 24-34°C for 24 hours and stored in ziplock bags.

Morphological analysis

The macroscopic analysis was performed in the Laboratory of Fungal Biology at the Federal University of Rio Grande do Norte (UFRN), Brazil. Macroscopic measurements of the samples were carried out with the aid of a stereomicroscope Leica EZ4, observing informative structures for the identification of taxa. Microscopic analysis was performed using an optical microscope Nikon Eclipse NiU with camera Nikon DS-Ri1 attached, and all the measurements were carried out using the NIS-Elements AR v.4.51.00 software. For the microscopic analysis, hand-cut sections of the peridium layers, pseudostipe and spores were mounted in 5% KOH and congo red. Taxonomic studies were based on Möller (1895), Calonge (2005) and Trierweiler-Pereira et al. (2009). Spore statistics (Qm) followed Bates (2004), using 30 structures. The values in parentheses are the mean of basidiospore width and length, and the standard deviation of these values. “Qm” is the mean of the quotient of basidiospores’ width and length, and “n” is the number of randomly measured basidiospores. Color standardization followed Kornerup & Wanscher’s color handbook (1978). The holotype of the species was deposited in the Rio de Janeiro Botanical Garden herbarium (RB728048) and the isotype in the Federal University of Rio Grande do Norte (UFRN) herbarium (UFRN-Fungos 2849). Specimens of *Phallus glutinolens* from the type locality deposited in the UFRN Fungal Collection were additionally analyzed.

Results

Mycobank: MB821095

Etymology – Named in reference to the type locality, Rio de Janeiro.

Diagnosis – Bigger basidiomata with 175–182 mm height, white pseudostipe, porose surface, receptacle globose with perforate and rugose apex, volva reddish-brown, areolate with longitudinal hyphae and thick rhizomorph reddish brown.

Description – Immature basidiomata subglobose. Expanded basidiomata 175–182 mm height. × 24–25 mm diam. Receptacle globose with apex perforate, 22 mm height × 20 mm diam., rugose, white. Pseudostipe spongy, cylindrical, 92–95 mm height × 24–25 mm diam., with pores, white (KW 3A1). Gleba mucilaginous, olive brown (KW 4F4), with presence of a discoid base with a single and thick rhizomorph, reddish brown (KW 9D5).

Basidiospores elongated, $3.6\text{--}4.8 \times 2.1\text{--}2.7 \mu\text{m}$ ($4.1 \pm 0.2 \times 2.4 \pm 0.3 \mu\text{m}$; $Q_m = 1.73$; $n = 30$ spores), smooth with two gutules at each extremity, hyaline in KOH. Pseudostipe exhibiting globose to subglobose and pyriform cells, $46.5\text{--}27.8 \times 41\text{--}25.9 \mu\text{m}$, wall $\leq 0.9 \mu\text{m}$, hyaline. Volva composed of filamentous hyphae, $3\text{--}5.9 \mu\text{m}$ diam., wall $\leq 0.4 \mu\text{m}$, septate, with inflated cells, hyaline in KOH and brownish in congo red. Rhizomorph composed of filamentous hyphae, $2.1\text{--}7.0 \mu\text{m}$ diam., wall $\leq 0.2 \mu\text{m}$, septate, with clamp connections, hyaline in KOH and brownish in congo red.

Known distribution – Brazil (designated here).

Material examined – Brazil, Rio de Janeiro State, Rio de Janeiro, Rio de Janeiro Botanical Garden, on soil, 16 Jul 2012, $22^\circ 57' 58.7''\text{S}$, $43^\circ 13' 26.1\text{W}$, A.A. Carvalho Jr 07/12 (RB728048, Holotype; UFRN-Fungos 2849, Isotype);

Additional materials examined – Brazil, Santa Catarina, Santo Amaro da Imperatriz, Hotel Plaza Caldas da Imperatriz Resort & Spa, Pousada da Mata, Estrada Velha Trail, on soil, 12 May 2007, L. Trierveiler-Pereira, J.M. Baltazar & Maccarini (UFRN-Fungos 834).

Key to *Phallus* species reported in Brazil

1. Basidiomata with indusium.....2
- 1.1 Basidiomata without indusium or with a rudimentary one.....6
2. Volva black becoming pale later.....*P. atrovolvatus*
- 2.1 Volva with different colors.....3
3. Pseudostipe cinnabar-red above, pale below, indusium and receptacle cinnabar-red.....*P. cinnabarinus*
- 3.1 Pseudostipe and indusium with other colors.....4
4. Receptacle surface rugose to meruloid.....*P. merulinus*
- 4.1 Receptacle surface not meruloid.....5
5. Pseudostipe yellow to orange, receptacle orange to pink.....*P. callichrous*
- 5.1 Pseudostipe and receptacle white.....*P. indusiatus*
6. Receptacle without a perforated apex.....*P. subtilis*
- 6.1 Receptacle with a perforated apex.....7
7. Basidiomata completely white.....*P. impudicus*
- 7.1 Basidiomata not completely white.....8
8. Pseudostipe reddish, orange to pinkish.....*P. rubicundus*
- 8.1 Pseudostipe with pale color (whitish).....9
9. Pseudostipe with granular surface, receptacle surface smooth.....*P. glutinolens*
- 9.1 Pseudostipe with porose surface, receptacle surface not smooth.....10
10. Receptacle globose throughout the development.....*P. fluminensis*
- 10.1 Receptacle campanulate throughout the development.....11
11. Receptacle surface rugose with ring-like apex.....*P. campanulatus*
- 11.1 Receptacle surface granular to slightly rugulose without ring-like apex.....*P. granulosodenticulatus*

Discussion

Phallus fluminensis was found growing near bamboo trees in the Rio de Janeiro Botanical Garden and is characterized by a globose receptacle, which initially resembles *P. glutinolens* (Möller) Kuntze emend. Trierveiler-Pereira, Calonge & Baseia in its young stage. According to Trierveiler-Pereira et al. (2009), *P. glutinolens* develops a campanulate and perforate apex at maturity, a fact already observed by Möller in his protologue “Sehr deutlich könnte in vielen Fällen bei dieser Form die erste Anlage der Gleba als einer glatten, glockenförmigen, nur am Scheitel unterbrochenen pallisadenschicht bestätigt werden.” (p.106) which means a soft and smooth bell-shaped gleba interrupted only at the apex. However, all stages of development of *P. fluminensis* were observed, and the globular shape of the receptacle remained unaltered.

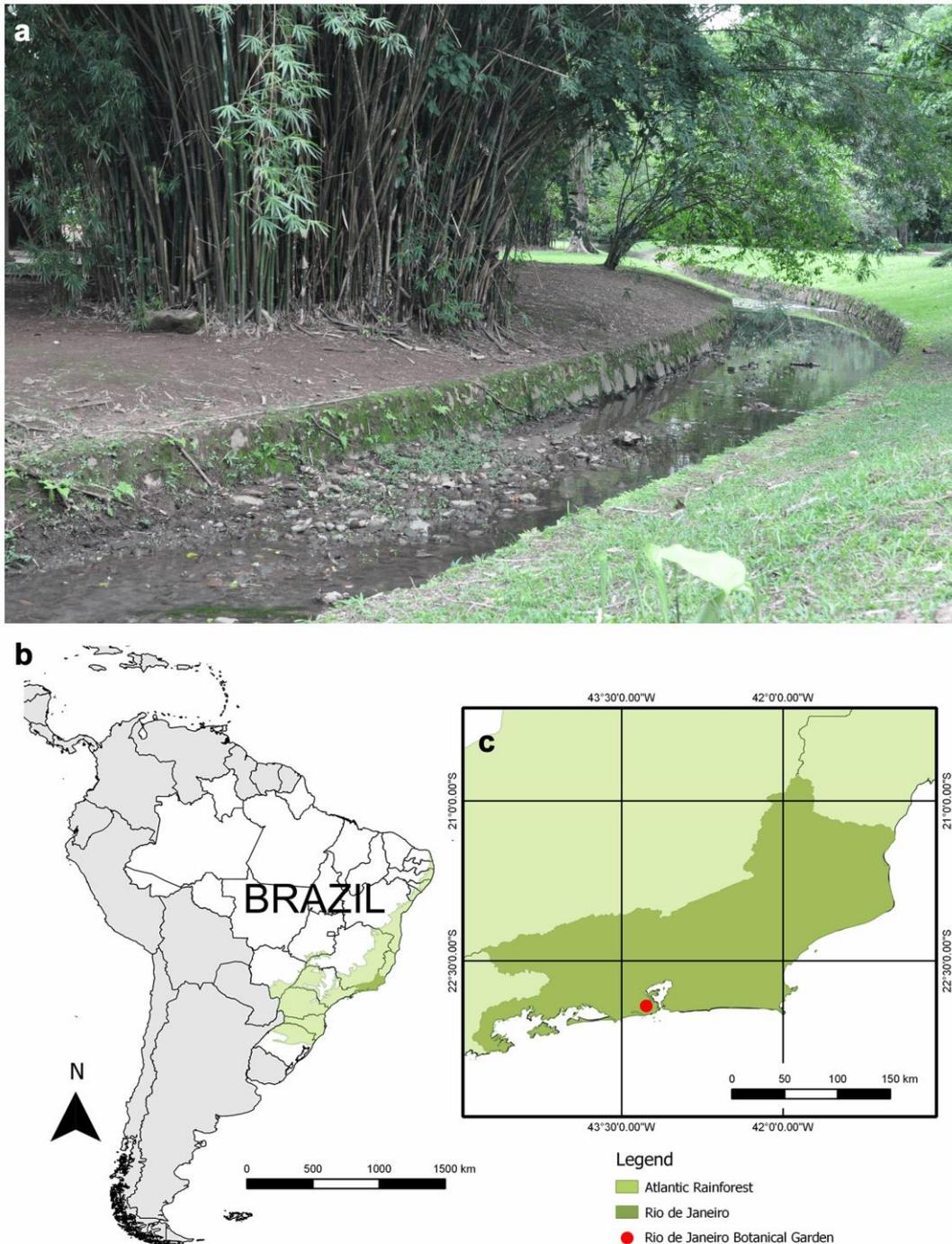


Fig. 1 – Details of collection site. a Collection site, Rio de Janeiro Botanical Garden. b, c Map showing the type location of *Phallus fluminensis*.

This species does not modify the receptacle shape according to the age of basidiomata, as seen in *P. glutinolens* emended by Trierweiler-Pereira et al. (2009). Comparing the exsiccates of *P. fluminensis* and *P. glutinolens* (UFRN-Fungos 834), the latter having been analyzed by Trierweiler-Pereira et al. (2009), some relevant differences were observed: *Phallus glutinolens* has small-sized basidiomata, reaching a maximum height of 60 mm, when compared to *P. fluminensis*, which has bigger basidiomata, reaching 182 mm in height. Moreover, our new species has a porose pseudostipe when compared with *P. glutinolens*, which reveals a granular surface.



Fig. 2 – *Phallus fluminensis* (RB728048, holotype). a, b Immature basidiomata in early stages of development. c Mature basidiomata fully developed showing the globose receptacle. Scale bars: a, b = 3 cm, c = 5 cm.

Another difference between these two species is the volva, which is reddish-brown, areolate with longitudinal hyphae in *P. fluminensis*, and white, smooth with pseudoparenchymatous cells in *P. glutinolens* (Table 1). The exsiccate FLOR 77079, cited in Trierweiler-Pereira et al. (2009) as an examined sample, was not found in this herbarium under this voucher number, but duplicates of this collection were deposited in the UFRN-Fungos collection and analyzed in detail. A remarkable peculiarity of *P. fluminensis* is a disciform volva base, also seen in *P. glutinolens* and *P. yunnanensis* (M. Zang & R.H. Petersen) Kreisel (Kreisel 1996). However, *P. yunnanensis* presents smaller basidiomata, a rugose to reticulate receptacle and an alveolate pseudostipe (Zang & Petersen 1989) when compared with *P. fluminensis* (Table 1). This new species is morphologically closely related to *P. granulosodenticulatus* B. Braun, redescribed by Cortez et al. (2011) by the granular to slightly rugose receptacle also seen in *P. fluminensis*. Nevertheless, the main feature of *P. granulosodenticulatus* is the receptacle with a conspicuously cogged margin, which is not found in *P. fluminensis*. Thus, we consider that these distinct morphological characteristics give sufficient support to establish *P. fluminensis* as a distinct and firmly identified species.

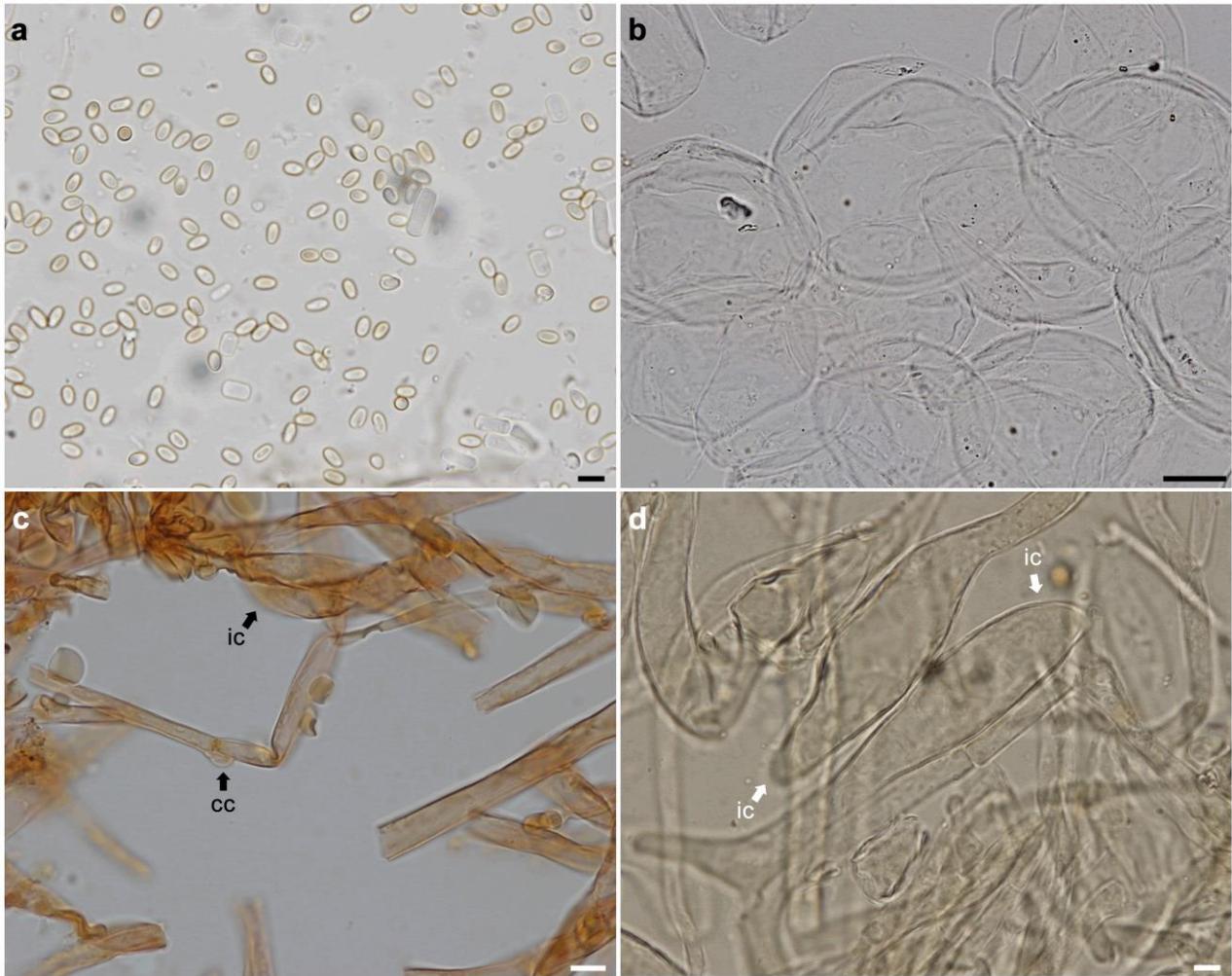


Fig. 3 – *Phallus fluminensis* microstructures (RB728048, holotype). a Basidiospores elongated. b Globose to subglobose cell on pseudostipe. c Filamentous hyphae in the volva, showing clamp connections (cc) and inflate cells (ic). d Inflate cells (ic) in the rhizomorphs. Scale bars: a, c, d = 5 μ m, b = 10 μ m.

Table 1 Comparison of macro- and micro morphology of *Phallus fluminensis* with *P. glutinolens*, *P. yunnanensis*.

	<i>Phallus fluminensis</i>	<i>Phallus glutinolens</i>	<i>Phallus yunnanensis</i>
Basidiomata	180–182 mm in height	40–60 mm in height	34–80 mm in height
Receptacle	Subglobose; rugose; 18–22 mm in height \times 20–30 mm in diam.; olive-brown gleba	Globose when young; campanulate when mature; rugose; 5–19 mm in height \times 17–20 mm in diam.; dark-olive gleba	Conical-campanulate; rugose-slightly reticulate; 30–50 mm in height \times 20–35 mm in diam.; brown gleba
Pseudostipe	Cylindrical; porose; white in color; pseudoparenchymatous cells	Cylindrical, granulose; white in color; pseudoparenchymatous cells	Cylindrical; alveolate; white in color; cells not observed

Table 1 Continued.

	<i>Phallus fluminensis</i>	<i>Phallus glutinolens</i>	<i>Phallus yunnanensis</i>
Volva	Not observed; areolate; totally reddish-brown in color; exoperidium and endoperidium with filamentous hyphae	Subglobose; smooth; totally yellowish-black in color; exoperidium pseudoparenchymatous; endoperidium with septate hyphae	Oblong-subspherical; glabrous; white with apex gray to olive in color; exoperidium and endoperidium with filamentous hyphae
Basidiospores	Elongated; 3.6–4.8 × 2.1–2.7 µm; hyaline, smooth	Elliptical to cylindrical; 3.5–4.5 × 1.5–2 µm; greenish or hyaline, smooth	Elliptical; 3.9–6 × 2–2.3 µm; hyaline, smooth

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