

## Two new species of *Tulostoma* (Agaricales, Basidiomycota) from the Neotropics

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Received: 13.03.2023 • Accepted/Published Online: 16.08.2023 • Final Version: 26.09.2023

**Abstract:** *Tulostoma catimbauense* and *T. deltaconcaevum*, both collected from Neotropical region, in the Vale do Catimbau National Park, Brazil, are proposed as new to science. Detailed macro- and micromorphological descriptions, including scanning electron microscopy of the basidiospores, are provided. ITS nrDNA sequence analyses were used to investigate the phylogenetic position of these taxa in the genus *Tulostoma*. Discussions about related species were carried out.

**Key words:** Caatinga, gasteroid fungi, ITS nrDNA, phylogeny, stalked puffballs, taxonomy

### 1. Introduction

The genus *Tulostoma* Pers. was sanctioned by Persoon (1801) and comprises the gasteroid fungi characterized by a stipe, inserted at the base of the peridium, which opens through a mouth or apical stoma (Wright, 1987) and a powdery gleba, composed of capillitium and basidiospores with different ornamentation patterns (Miller and Miller, 1988). With a wide geographic distribution, representatives of this genus are commonly found in arid and semiarid regions (Wright, 1987; Miller and Miller, 1988), although they also occur in humid environments (Wright, 1987). Wright (1987) described 139 species in his worldwide monograph, and Kalichman et al. (2020) confirmed 172 species.

To date, few molecular phylogenetic studies on *Tulostoma* have been published, and those that exist are geographically restricted to European countries (Hernández-Caffot et al., 2011; Hussain et al., 2016). In Europe, through integrative taxonomy, Jeppson et al. (2017) and Rusevska et al. (2019) pointed out the monophyly of the genus, revealing unexpectedly high diversity, and confirms the ITS region as a very useful marker for discriminating *Tulostoma* species. However, Neotropical species have been sampled very little regarding phylogenetic work.

The Neotropical region covers geographical areas with biodiversity hotspots and ecological habitats with

high potential to harbour fungal species not yet known to science (Hawksworth and Lücking, 2017). Moreover, countries in this region with great biodiversity, such as Brazil, have presented great potential for taxa of the genus *Tulostoma*: Baseia and Galvão (2002), Baseia and Milanez (2002), Silva et al. (2007), and Cortez et al. (2009), including the description of new species to science, such as *Tulostoma brasiliense* J.E. Wright, *T. dumeticola* Long, and *T. rickii* Lloyd (Wright, 1987).

This paper proposes two species of *Tulostoma* new to science, collected from Northeast Brazil in the Caatinga, which is the only exclusively Brazilian biome. Both species are described based on morphological and molecular characters.

### 2. Materials and methods

#### 2.1. Morphological analyses

The morphological analyses were based on specimens collected from the Caatinga biome in Northeast Brazil and deposited in the mycological collection UFRN-Fungos (Thiers, 2023, continuously updated). Measurements of basidiomata were made with a pachymeter. The colours of the structures followed Kornerup and Wanscher (1978). Peridium and stipe characteristics were checked using a Leica EZ4 stereomicroscope. Microstructures were mounted on slides for light microscopy (LM) with 5% potassium hydroxide (KOH). Cotton blue or Congo red

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were used for visualization of specific structures, such as crystals and connection clamps (Miller and Miller, 1988). The Nikon Eclipse NiR1 optical microscope with Nikon DS-Ri1 camera attached and the supporting program for image capture – NIS-Elements, version 4.13, was used for the measurement and photography of each of the microstructures. Thirty measurements for basidiospores and 20 measurements for other microstructures (capillitium, peridium hyphae and rhizomorphs) were performed and the amplitudes were determined. Other values were noted, namely: L = mean height, W = mean width, Q = height/width ratio (minimum and maximum values) Qm = mean height/width ratio. The scanning electron microscopy (SEM) was performed at the 'Laboratory of the Centro de Tecnologia do Gás e Energias Renováveis (CTGÁS-ER) – Natal/RN', for detailed observation of the basidiospores. The identification of the specimens was performed with specialized literature, such as Cunningham (1944), Wright (1987), Jeppson et al. (2017), and Rusevska et al. (2019). The mycological terminology was based on the nomenclature of Kirk et al. (2008).

## 2.2. Molecular methods

DNA was extracted from basidiomata dried on FTA® cards following the methodology of Telleria et al. (2014). The internal transcribed spacer region of nuclear ribosomal DNA (ITS nrDNA) was amplified and sequenced using the primers ITS1-F and ITS4 (White et al., 1990). Sequencing was conducted by the company MACROGEN (South Korea). The resulting sequences were assembled and edited in BioEdit software (Hall, 1999) and deposited in GenBank.

## 2.3. Phylogenetic analyses

The ITS sequences generated in this study were submitted to the BLASTn algorithm at NCBI (GenBank, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to retrieve similar sequences. Sequences with an identity percentage of 81.84% were admitted into the dataset. *Lycoperdon perlatum* Pers. and *Calvatia gigantea* (Batsch) Lloyd were selected as the outgroup following Jeppson et al. (2017). Sequences were aligned using MAFFT v.7 (Kuraku et al., 2013; Katoh et al., 2019) under E-INS-i criteria. Seaview v.4 (Gouy et al., 2010) was used to manually visualize and adjust the alignment. Alignment is available in Figshare (<https://doi.org/10.6084/m9.figshare.22236748.v1>). Maximum Likelihood (ML) analyses were performed under RAxML-HPC algorithm v.8.2.12 (Stamatakis, 2014). The nucleotide substitution model, TPM2uf+G, was selected with Bayesian Information Criterion (BIC) using jModelTest2 v.2.1.6 (Darriba et al., 2012). Bayesian Inferences (BI) were performed using MrBayes v.3.2.7a (Ronquist et al., 2011), performing  $2 \times 10^7$  MCMC generations, sampling one tree every 1000 generations. The software used for the ML

analyses, Bayesian Inferences and nucleotide substitution model selection were implemented on the CIPRES Science Gateway 3.3 platform (Miller et al., 2010). Trees were visualized and rooted in FigTree v.1.4.4, and the final images were done in GNU Image Manipulation Program (GIMP) v.2.10.32. A node was considered significantly supported if it received Bootstrap (BS)  $\geq 80\%$ , and/or posterior probability (PP)  $\geq 0.95$ .

## 3. Results

### 3.1. Molecular results

The dataset included 59 sequences with 765 characters (including alignment gaps), comprising 57 sequences of *Tulostoma*, in which five were generated under this study, and two from the outgroup (Table). Phylogenetic inferences from ITS nrDNA obtained by ML and Bayesian Inferences resulted in similar tree topologies. Figure 1 shows the tree generated using the ML analyses, including bootstrap (BS) values and posterior probabilities (PP) of well-supported branches.

Our analyses place *Tulostoma catimbauense* and *T. deltaconcaum* related to species of clade 6 (93% BS / 1.0 PP) and clade 11 (93% BS / 0.95 PP) of Jeppson et al. (2017), respectively. The ITS sequences of the *Tulostoma catimbauense* specimens appeared together in a highly supported clade (100% BS/ 1.0 PP), as a sister group to the cluster formed by the *Tulostoma* aff. *cretaceum* sequences. The branch of *Tulostoma deltaconcaum* sequences is also well supported (100% BS/ 1.0 PP) and appears independent.

### 3.2. Taxonomic treatment

*Tulostoma catimbauense* A.A. Lima, Accioly, Baseia & M.P. Martín sp. nov. (Figure 2)

**Mycobank:** MB847852

**Type:** BRAZIL. Pernambuco: Buíque, Vale do Catimbau National Park (Breu), (8°30'42" S, 37°14'49" W), on sand, 965 m, 10 December 2006, T.B.S. Ottoni, T3 (UFRN-Fungos 979, **holotype!**, ITS nrDNA Genbank sequence: OQ599932).

**Diagnosis:** Distinct from other species due to hyphal, persistent, light brown to greyish brown exoperidium, papery endoperidium, mouth definite, tubular, slightly projecting, peristome concolor with endoperidium. Basidiospores  $5.6\text{--}7.8 \times 5.4\text{--}7.4 \mu\text{m}$ , globose to subglobose, reticulate-spiny (under LM), guttulate; coarsely reticulate with evident short apiculus (under SEM).

**Etymology:** In reference to the Vale do Catimbau National Park, where the specimens were collected.

**Description:** Peridium globose to subglobose, 8–17 mm diam. Exoperidium hyphal, persistent, detaching in parts, remaining at base in some basidiomata, light brown to greyish brown (6D7/ 6F3) and encrusted with grains of sand. Endoperidium glabrous, papery, very thin (<1

**Table.** Samples, collection vouchers, origin, and GenBank accession numbers of ITS nrDNA sequences used in molecular analyses. Accession numbers in bold were generated for this study.

Scientific name	Voucher collection	Origin	ITS accession n°
<i>T. caespitosum</i>	ANK Akata 6329	Turkey	MT804370
<i>T. calcareum</i>	Breili S0133 (GB)	Norway	KU519081
<i>T. calcareum</i>	Finy 4 (GB)	Hungary	KU519088
<i>T. calcareum</i> (Holotype)	MJ 6965 (GB)	Sweden	KU519086
<b><i>T. catimbauense</i> sp. nov. (Paratype)</b>	<b>UFRN-Fungos 978</b>	<b>Brazil</b>	<b>OQ599933</b>
<b><i>T. catimbauense</i> sp. nov. (Holotype)</b>	<b>UFRN-Fungos 979</b>	<b>Brazil</b>	<b>OQ599932</b>
<b><i>T. catimbauense</i> sp. nov. (Paratype)</b>	<b>UFRN-Fungos 1014</b>	<b>Brazil</b>	<b>OQ599934</b>
<b><i>T. deltaconcaum</i> sp. nov. (Holotype)</b>	<b>UFRN-Fungos 1296</b>	<b>Brazil</b>	<b>OQ599935</b>
<b><i>T. deltaconcaum</i> sp. nov. (Paratype)</b>	<b>UFRN-Fungos 1298</b>	<b>Brazil</b>	<b>OQ599936</b>
<i>T. excentricum</i> (Holotype)	BPI 729284	United States	KU519055
<i>T. kotlabae</i>	Bruzek 140918 (GB)	Czech Republic	KU519028
<i>T. kotlabae</i>	MJ 5426 (GB)	Sweden	KU519021
<i>T. kotlabae</i>	MJ 7441 (GB)	Denmark	KU519026
<i>T. kotlabae</i>	MJ 7923 (GB)	Hungary	KU519027
<i>T. kotlabae</i>	MJ 9585 (GB)	France	KU519024
<i>T. kotlabae</i> (Holotype)	PRM 704203	Slovakia	KX576544
<i>T. melanocyclus</i>	AKB 050529 (GB)	Sweden	KU519103
<i>T. melanocyclus</i>	Knudsen 64727 (C)	Russia	KU519099
<i>T. melanocyclus</i>	MJ 090418 (GB)	Hungary	KU519106
<i>T. melanocyclus</i>	MJ 9596 (GB)	France	KU519101
<i>T. melanocyclus</i>	RBG Kew K(M)64453	United Kingdom	EU784435
<i>T. melanocyclus</i>	06MCF6448	Macedonia	MG768900
<i>T. pannonicum</i> (Holotype)	MJ 7764 (GB)	Hungary	KU519010
<i>T. pannonicum</i>	MJ 7803 (GB)	Hungary	KU519011
<i>T. pannonicum</i>	MJ 990617a (GB)	Hungary	KU519008
<i>T. pseudopulchellum</i>	AH 11603	Spain	KU519012
<i>T. pseudopulchellum</i> (Holotype)	AH 11605	Spain	KX513827
<i>T. punctatum</i>	MJ 10058 (GB)	Slovakia	KU518953
<i>T. punctatum</i>	05MCF4866	Macedonia	MG768882
<i>T. rufum</i> (Lectotype)	BPI 704578	United States	KU519107
<i>T. rufum</i>	MES-3805_FLAS-F-68003	United States	ON383404
<i>T. simulans</i>	Bruzek 131201 (GB)	Czech Republic	KU519043
<i>T. simulans</i>	MJ 9302 (GB)	Spain	KU519042
<i>T. simulans</i> (Paratype)	W.W. Stockberger (PC)	United States	KX576547
<i>T. squamosum</i>	CUH AM696	India	MN809136
<i>T. squamosum</i>	EL 260-06 (GB)	France	KU519097
<i>T. squamosum</i>	Mrazek 1300 (GB)	Northern Europe	DQ415732
<i>T. squamosum</i>	SNMH 13	Slovakia	MK907426
<i>T. squamosum</i>	TPK2	Pakistan	KT285883

Table. (Continued).

Scientific name	Voucher collection	Origin	ITS accession n°
<i>T. squamosum</i>	03MCF1226	Macedonia	MG768905
<i>T. subsquamosum</i>	AH 19024	Spain	KU519092
<i>T. subsquamosum</i>	ANK Akata & Altuntas 575	Turkey	MT798591
<i>T. subsquamosum</i>	MJ 6563 (GB)	Hungary	KU519093
<i>T. subsquamosum</i>	07MCF8291	Macedonia	MG768910
<i>Tulostoma</i> aff. <i>cretaceum</i>	Knudsen 01.70n (C)	Russia	KU518993
<i>Tulostoma</i> aff. <i>cretaceum</i>	MJ 7759 (GB)	Hungary	KU518996
<i>Tulostoma</i> aff. <i>cretaceum</i>	MJ 9304 (GB)	Spain	KU519000
<i>Tulostoma</i> cf. <i>cretaceum</i>	SNMH10	Kazakhstan	MK907420
<i>Tulostoma</i> sp.	Knudsen 99-337 (C)	Russia	KU519007
<i>Tulostoma</i> sp.	MJ 3830 (GB)	Hungary	KU519018
<i>Tulostoma</i> sp.	MJ 8710 (GB)	Spain	KU518980
<i>Tulostoma</i> sp.	MJ 9046 (GB)	Spain	KU519066
<i>Tulostoma</i> sp.	MLHC200 (CORD)	Argentina	HQ667595
<i>Tulostoma</i> sp.	MJ 6081 (GB)	Hungary	KU519019
<i>Tulostoma</i> sp.	02MCF1203	Macedonia	MG768880
<i>Tulostoma</i> sp.	03MCF2939	Macedonia	MG768897
<i>Tulostoma</i> sp.	03MCF10327	Macedonia	MG768899
<i>Calvatia gigantea</i> (Outgroup)	MJ3566	-	DQ112623
<i>Lycoperdon perlatum</i> (Outgroup)	MJ4684	-	DQ112630

mm), orange white (5A2). Mouth definite, tubular, slightly projecting, peristome concolor with endoperidium. Collar separated from the stipe, conspicuous, lacerate membrane. Stipe 4–17 × 1–3 mm, brown (5E8/ 6E7), hollow, smooth to striate, fragile, with a mycelial bulb in some basidiomata. Gleba orange brown (5C4/ 6C7).

Exoperidium composed of hyphae 1.6–4.7 µm, hyaline, without septa and branched. Endoperidium composed of capillitium-like hyphae, 2.4–5.7 µm, hyaline, lumen visible, branched and septate, septa swollen, hyaline, frequent, hardly disjunct. Basidiospores 5.6–7.8 × 5.4–7.4 µm; L = 6.7 µm, W = 6.4 µm, Q = 1.00–1.24, Qm = 1.05; globose to subglobose, yellowish, ornamentation (LM) 0.3–1.0 µm, reticulate to spiny, guttulate; under SEM the ornamentation appears coarsely reticulate, with evident short apiculus. Capillitium 2.2–5.5 µm, hyaline, lumen visible, branched and septate, septa swollen, slightly coloured, frequent, hardly disjunct.

**Habitat:** Sandy soil.

**Distribution:** Known only for the type locality.

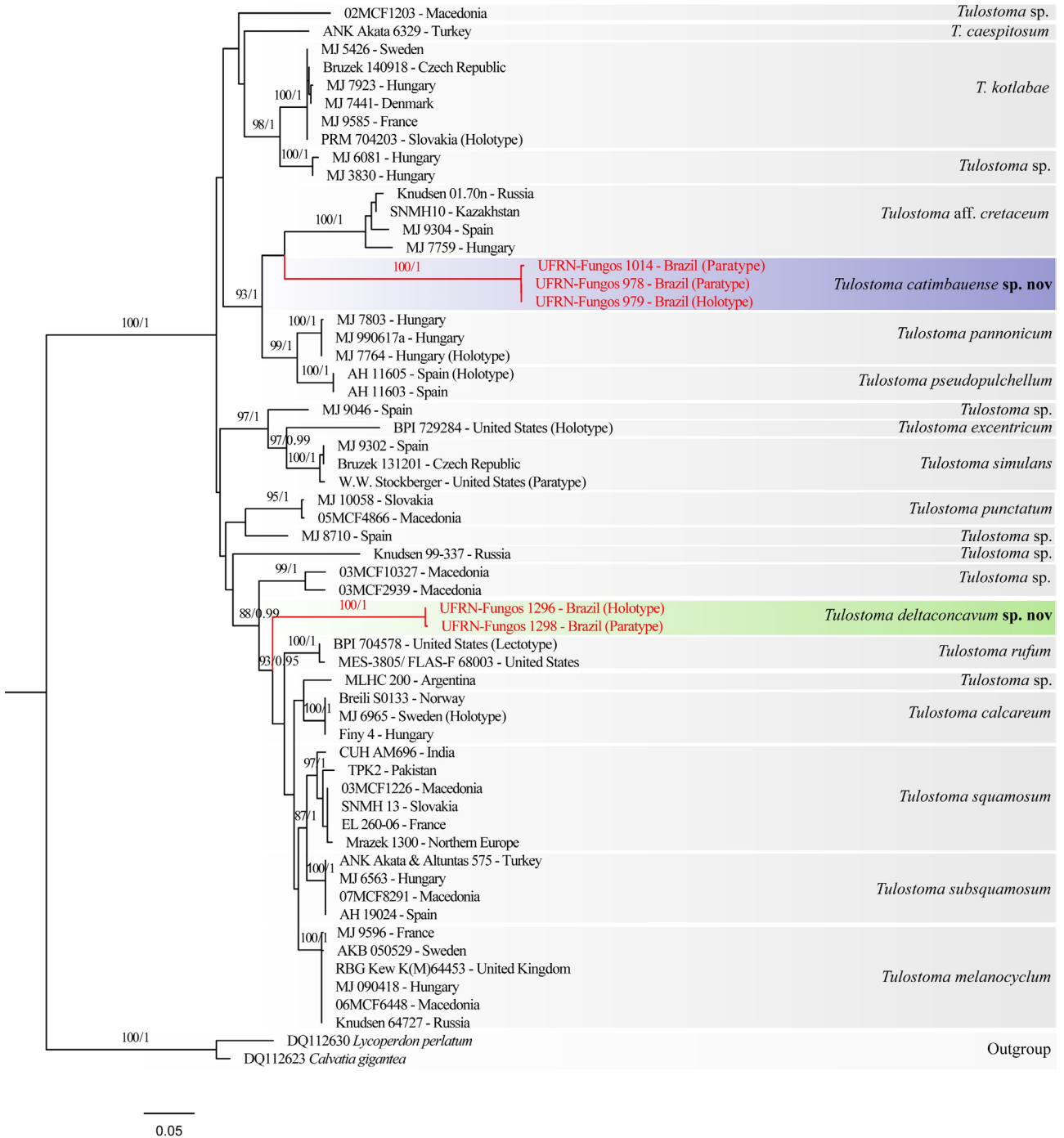
**Additional material examined:** BRAZIL. Pernambuco: Buique, Vale do Catimbau National Park (Breu), (8°30'42" S, 37°14'49" W), on sand, 10 December 2006, T.B.S. Ottoni, T4 (**paratype:** UFRN-Fungos 978, ITS nrDNA Genbank

sequence: OQ599933). Pernambuco: Tupanatinga, Vale do Catimbau National Park, (8°29'12" S, 37°19'09" W), on sand, 751 m, 09 February 2007, T.B.S. Ottoni, T5 (**paratype:** UFRN-Fungos 1014, ITS nrDNA Genbank sequence: OQ599934).

**Remarks:** *Tulostoma catimbauense* is recognized by a definite, tubular mouth, hyphal exoperidium, papery endoperidium, reticulate-spiny and guttulate basidiospores (under LM); coarsely reticulate ornamentation, with short apiculus (under SEM).

Based on ITS nrDNA, *T. catimbauense* is related to species in clade 6 of Jeppson et al. (2017): *Tulostoma* aff. *cretaceum*, *T. pannonicum* Jeppson, Altés, G. Moreno & E. Larss. and *T. pseudopulchellum* G. Moreno, Altés & J.E. Wright. These species differ from *T. catimbauense* by having an indistinct to fibrillose-fimbriate mouth and smooth or irregularly rugulose basidiospores (Jeppson et al., 2017).

Morphologically, *T. catimbauense* can be confused with *T. reticulatum* G. Cunn. because it presents a definite mouth and coarsely reticulate basidiospores. The latter differs by depressed-globose peridium up to 12 mm diam, by the slightly larger basidiospores (6–7.5 µm), without apiculus (Cunningham, 1944). *Tulostoma amnicola* Long & S. Ahmad, *T. brevistipitatum* B. Liu,

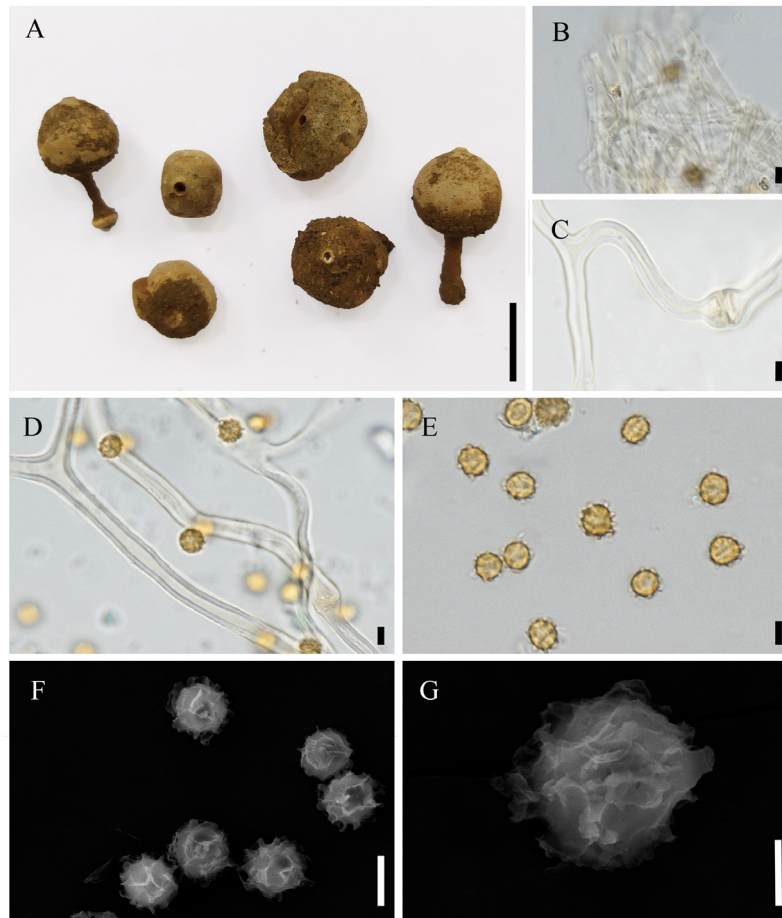


**Figure 1.** ML phylogeny based on ITS nrDNA sequences. *Tulostoma catimbauense* sp. nov. and *Tulostoma deltaconcaevum* sp. nov. are highlighted. Bootstrap values ( $\geq 80\%$ ) and posterior probabilities ( $\geq 0.95$ ) are indicated on the branches.

Z.Y. Li & Du, and *T. nanum* (Pat.) J.E. Wright also have a tubular mouth and hyphal exoperidium, but can be easily differentiated by the ornamentation of basidiospores. *Tulostoma amnicola* and *T. brevistipitatum* have smooth

or nearly smooth basidiospores under SEM, *T. nanum* has asperulate basidiospores (Wright, 1987). *Tulostoma exasperatosporum* J.E. Wright and *T. clathrosporium* J.E. Wright resemble *T. catimbauense* due to a definite





**Figure 2.** *Tulostoma catimbauense* A- basidiomata ex situ, B- exoperidium hyphae, C- endoperidium hyphae, D- capillitium, E- basidiospores (under LM), F/G- basidiospores (under SEM). Bars: A= 10 mm, B-F= 5  $\mu$ m, G= 2  $\mu$ m.

tubular mouth and reticulate basidiospores, but may be segregated by membranous exoperidium (Wright, 1987). Other similar species are *T. exasperatum* Mont., *T. opacum* Long, and *T. rickii* Lloyd. However, *T. exasperatum* differs in its fimbriate-fibrillose mouth and verrucose to spiny exoperidium (Wright, 1987). *T. opacum* and *T. rickii* can be segregated by a fibrillose mouth, membranous and smooth exoperidium (Wright, 1987). Species such as *T. cyclophorum* Lloyd, *T. fimbriatum* Fr., and *T. squamosum* (J.F. Gmel.) Pers. have subreticulate basidiospores (Esqueda et al., 2004). However, *T. cyclophorum* differs from *T. catimbauense* by having a fibrillose mouth and the presence of mycoesclereids covering the exoperidium (Esqueda et al., 2004). *Tulostoma fimbriatum* presents fimbriate mouth and *T. squamosum* presents membranous, sometimes verrucose exoperidium (Jeppson et al., 2017). ***Tulostoma deltaconcaevum*** A.A. Lima, Accioly, Baseia & M.P. Martín sp. nov. (Figure 3)

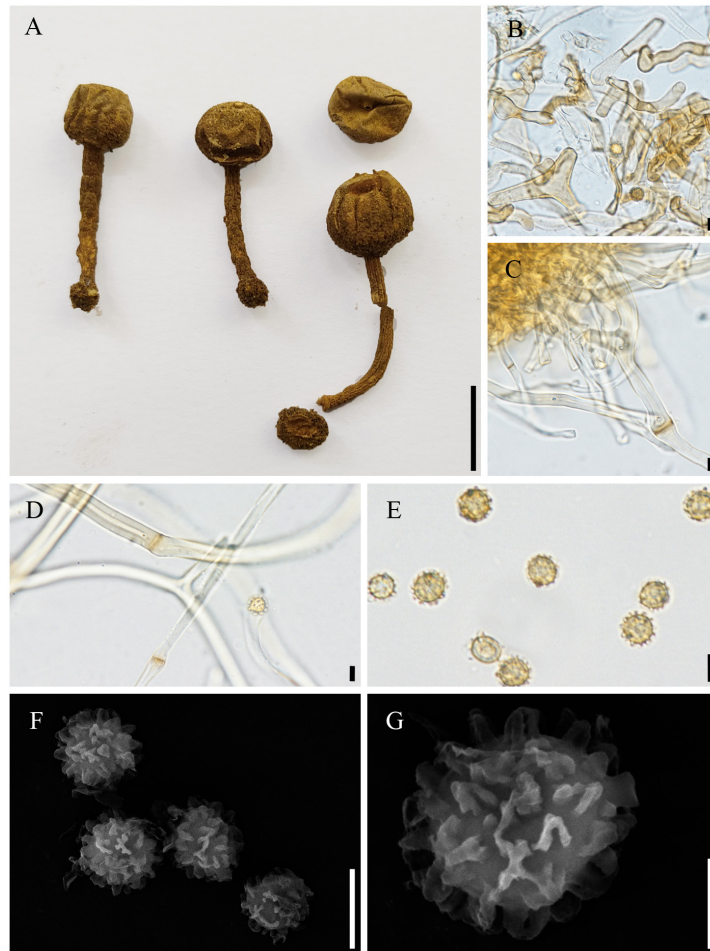
**Mycobank:** MB847853

**Type:** BRAZIL. Pernambuco: Buíque, Vale do Catimbau National Park (Breu), (8°30'47" S, 37°14'49" W), on sand, 22 July 2007, T.B.S. Ottoni (UFRN-Fungos 1296 **holotype!**, ITS nrDNA Genbank sequence: OQ599935).

**Diagnosis:** Distinct from other *Tulostoma* species by its membranous exoperidium, dark brown, falling into scales, with polymorphous hyphae, mouth definite, tubular, peristome concolor with the endoperidium. Basidiospores 5.2–7.2  $\times$  4.9–7  $\mu$ m, globose to subglobose, verrucose to spiny (under LM); triangular concave spines, some anastomosed, with short apiculus (under SEM).

**Etymology:** From the Latin *delta* (Greek letter with triangular shape) and *concaevum* (concave) in reference to the basidiospores ornamentation under SEM.

**Description:** Peridium subglobose, 6–11 mm diam. Exoperidium membranous, falling into scales, usually persistent from middle down, dark brown (6F6/ 7F4),



**Figure 3.** *Tulostoma deltaconcaevum* A- basidiomata ex situ, B- exoperidium hyphae, C- endoperidium hyphae, D- capillitium, E- basidiospores (under LM), F/G- basidiospores (under SEM). Bars: A= 10 mm, B-F= 5  $\mu$ m, G= 2  $\mu$ m.

encrusted with sand grains. Endoperidium glabrous, papery, very thin (<1 mm), greyish orange (5B3). Mouth definite, tubular, slightly projecting, peristome concolor with endoperidium. Collar separated from the stipe, conspicuous, lacerate membrane. Stipe 14–21  $\times$  2 mm, brown (6E6), hollow, striate, fragile, with a mycelial bulb at the base in some basidiomata. Gleba brownish orange (6C6).

Exoperidium composed of polymorphous hyphae, yellowish, branched and septate. Endoperidium composed of capillitium-like hyphae, 2–5  $\mu$ m, slightly yellowish, lumen visible, branched and septate, septa slightly swollen, coloured, frequent, hardly disjunct. Basidiospores 5.2–7.2  $\times$  4.9–7.0  $\mu$ m; L = 6.2  $\mu$ m, W = 5.8  $\mu$ m, Q = 1.00–1.23, Qm = 1.08; globose to subglobose, slightly yellowish, ornamentation (LM) 0.5–1.0  $\mu$ m, verrucose to spiny; under SEM the spines are concave triangular, some anastomosed, with short apiculus. Capillitium 2–6  $\mu$ m, slightly yellowish,

lumen visible, branched and septate, septa slightly swollen, coloured, frequent, moderately disjunct.

**Habitat:** Sandy soil.

**Distribution:** Known only for the type locality.

**Additional material examined:** BRAZIL. Pernambuco: Buíque, Vale do Catimbau National Park (Breu), (8°30'47" S, 37°14'49" W), on sand, 23 July 2007, T.B.S. Ottoni (**paratype:** UFRN-Fungos 1298, ITS nrDNA Genbank sequence: OQ599936).

**Remarks:** *Tulostoma deltaconcaevum* is distinguished by membranous exoperidium with brown coloration, falling into scales, circular mouth, striate stipe and basidiospores with concave triangular spines under SEM (reminiscent of cat ears) and short apiculus.

Based on ITS nrDNA, *T. deltaconcaevum* is related to species of clade 11 by Jeppson et al. (2017): *Tulostoma* sp. (HQ667595), *T. rufum* Lloyd, *T. calcareum* Jeppson, Altés, G. Moreno & E. Larss., *T. squamosum* (J.F. Gmel.) Pers., *T.*

*subsquamosum* Long & S. Ahmad, and *T. melanocyclum* Bres. These species are morphologically characterized by having rather dark colours of the stipe, endoperidia with initially brownish colours, and moderately to strongly ornamented, verrucose-echinate basidiospores (Jeppson et al., 2017). *Tulostoma deltaconcaum* differs from these species due to the exoperidia and the ornamentation of the basidiospores under SEM (Wright, 1987; Jeppson et al., 2017). *Tulostoma rufum*, *T. calcareum*, and *T. melanocyclum* have hyphal exoperidium (Wright, 1987; Jeppson et al., 2017). *Tulostoma squamosum* under SEM the ornamentation appears formed by rib-like, much anastomosed verrucae, some formed by more than one column mixed with smaller verrucae (Wright, 1987). *Tulostoma subsquamosum* under SEM the ornamentation appears as a labyrinth of anastomosed crests with similar verrucae among them (Wright, 1987).

Morphologically, it can be confused with *T. bruchii* Speg. because it shows membranous exoperidium and circular mouth, but differs in its smaller basidiospores (3.4–5.0 µm diam.) with small and large warts united into ridges under SEM (Wright, 1987). *Tulostoma dennisii* J.E. Wright, *T. dumeticola* Long, and *T. pusillum* Berk. are species that also occur in the Neotropics and are similar to *T. deltaconcaum* by the definite mouth, coarsely warty to spiny basidiospores. However, they differ in the verrucose exoperidia and ornamentations under SEM: columns anastomosed in crests; spines as pegs, much anastomosed and the whole appearing almost reticulate, very irregular or clathrate; and large spines conical or pyramidal, respectively (Wright, 1987). This species was initially identified at the UFRN-Fungos collection as *T. brumale* Pers., but the latter differs in having a mouth with

dark peristome, sparsely verrucose basidiospores, 4–6 µm diam. and abruptly swollen capillitium on the septa, brown and encrusted (Wright, 1987; Jeppson et al., 2017).

#### 4. Discussion

The morphoanatomical and phylogenetic study based on ITS nrDNA indicated that our specimens belong to two distinct species of the genus *Tulostoma* from the Neotropical Region. In the genus *Tulostoma*, the barcode region proved to be very useful to discriminate species, mainly when characteristics are very similar, corroborating Rusevska et al. (2019).

According to Hernández-Navarro et al. (2018), considering only the complete ITS region, currently fewer than 40 *Tulostoma* species have been properly sequenced, representing approximately 25% of known morphospecies. Regarding species that occur in the Neotropics, only *T. domingueziae* Hern. Caff. has been sequenced.

Balasundaram et al. (2015) emphasized the importance of sampling more specimens from wider geographical ranges to determine intraspecific species variation. It demonstrates the need for the inclusion of Neotropical taxa in existing databases, through further field research, taxonomic study and the generation of new sequences to better understand *Tulostoma* species richness and distribution in the Neotropics, potentially revealing new species.

#### Acknowledgment and/or disclaimers, if any

The authors thank the Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE-Brazil) for the PhD scholarship granted to Alexandro de A. de Lima.

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