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Psathyrella atlantica (Agaricales: Basidiomycota), a new species from Brazil

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ABSTRACT

Psathyrella atlantica is described based on material collected in an Atlantic Forest fragment in the state of Pernambuco, Northeast Brazil. It is characterized by slender, whitish and caespitose basidiomata; pileus with orange, small and floccose-squamulose veil remnants; presence of a persistent but fragile-membranous annulus; basidiospores 5–7 × 3–4 µm; and inconspicuous germ pore. A full description plus illustrations and comments on morphological and phylogenetic data are provided.

Keywords: Agaricomycetes, Agaricomycetidae, Neotropics, Psathyrellaceae, taxonomy

Introduction

Psathyrella (Psathyrellaceae, Agaricales) is a large genus of dark-spored mushrooms, with 500 (Vašutová *et al.* 2008) to 600 (Kirk *et al.* 2008) known species. This genus is considered difficult to study (Nagy *et al.* 2013), due to its fragile basidiomata and because many known species possibly represent species complexes (Hoashi 2008). According to Vašutová *et al.* (2008), the known taxa are predominantly described from the Northern hemisphere and are frequently cited in the Southern hemisphere, demonstrating the importance of studying this genus for searching undescribed species in the tropics.

In recent years, molecular phylogenetic methods are being used in the studies of *Psathyrella* and satellite genera, based on nu-rDNA sequences (Larsson & Örstadius 2008; Padamsee *et al.* 2008; Vašutová *et al.* 2008; Nagy *et al.* 2013). This has resulted in considerable advances in the understanding of the relationships within these fungi and revealed that the current infrageneric classification does not reflect the phylogeny of *Psathyrella* (Vašutová *et al.* 2008). A recent study by Örstadius *et al.* (2015) changed the systematic of this group, placing all psathyrelloid fungi in *Psathyrella sensu stricto*, *Coprinopsis*, *Cystoagaricus*, *Homophron*, *Kauffmania*, *Typhrasa* and an unnamed clade called/cordisporus. However, only six among 116 samples analyzed in this study were collected from the tropics.

For Brazil, at least 23 names are reported: *Psathyrella ampelina*, *P. annulosa*, *P. araguana*, *P. argillospora*, *P. atricastanea*, *P. atomata*, *P. candolleana*, *P. coprinoceps*, *P. euthygramma*, *P. hortulana*, *P. janauariensis*, *P. lignatilis*, *P. microcarpella*, *P. murrillii*, *P. obtusata*, *P. palmigena*, *P. piluliformis*, *P. plana*, *P. polycystidiosa*, *P. pygmae*, *P. roystoniae*, *P. typhae* and *P. varzeae* (Viégas 1945; Singer 1961; 1973; 1989; Pegler 1983; 1997; Maia *et al.* 2002; Cortez & Coelho 2005; Meijer 2006; 2008; 2010; Drechsler-Santos *et al.* 2007; Bononi *et al.* 2008; Rosa & Capelari 2009; G.C. Alves *et al.*

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2012; M.H. Alves *et al.* 2012; Lazarotto *et al.* 2014; Pereira *et al.* 2018; Wartchow & Gomes 2019).

Meijer (2006) cited 17 taxa only from Paraná State (South Brazil), out of which, only five were identified at the species level. In Northeast Brazil, where our current study was undertaken, this genus is poorly known, with only four reported species: *P. ampelina*, *P. atomata*, *P. euthygramma*, and *P. obtusata*, all from the State of Pernambuco (Maia *et al.* 2002; Wartchow & Gomes 2019). Furthermore, M.H. Alves *et al.* (2012) reported two additional unnamed *Psathyrella* from the State of Piauí and Pereira *et al.* (2018) reported another one from Bahia.

During a survey regarding agaricoid fungi in the Atlantic Rain Forest in Northeast Brazil, several macrofungi were collected, along with an undescribed psathyrelloid species. Thus, the aim of this study is to describe this new species of *Psathyrella* based on the morphological and phylogenetic data, as well as to provide comments on its systematic placement among psathyrelloid genera along with illustrations of the microstructures, and photographs of the basidiomata.

Materials and methods

Collection site

The basidiomata of were collected from the 'Reserva Biológica de Saltinho' (8°44'13" and 8°43'S, 35°10' and 35°11'W), a 475,21 ha Atlantic Forest remnant located in the municipalities of Tamandaré and Rio Formoso, Pernambuco State, Northeast Brazil (Andrade & Moura 2011). Its climate is tropical humid (As') according to Köppen's classification, with average temperature ranging from 22 °C to 26 °C. This reserve is dominated mainly by Lowland Ombrophilous Dense Forest in regeneration process (Teixeira *et al.* 2010).

Morphological study

The basidiomata were analyzed as described by Singer (1986) and Largent (1986) and basidiospore data terminology was according to Tulloss *et al.* (1992), slightly modified by Wartchow *et al.* (2012) and Wartchow (2012). Statistics are based on 20 basidiospores. The abbreviations for the biometrical values are L(W) = basidiospore length (width) average, Q = the length:width ratio range as determined from all measured basidiospores, and Qm = the Q value averaged from all the basidiospores measured.

Color names were according to Kornerup & Wanscher (1978). Drawings of the microstructures were done with the aid of a camera lucida and photographs of the fresh basidiomata were taken at the field. The exsiccatum was deposited at Herbarium URM, Departamento de Micologia, Universidade Federal de Pernambuco (Thiers 2019).

DNA study

Genomic DNA was extracted from frozen field basidiomata by using the CTAB method proposed by Góes-Neto *et al.* (2005). For the amplification of ITS and LSU rDNA regions, the following primer sets were used: ITS5/ITS4 for ITS region (White *et al.* 1990; Gardes & Bruns 1993); and LR0R/LR7 for LSU region (Vilgalys & Hester 1990). PCR products were purified using GeneJet Purification Kit (Thermo Fisher Scientific, USA), followed by sequencing using the primers ITS5, ITS4, LR0R and LR5 performed at 'Centro de Pesquisas sobre o Genoma Humano e Células-Tronco, Universidade de São Paulo' (São Paulo, Brazil).

Contig was prepared using Sequencher v. 4.1.4 (Gene Codes Corp.) and the obtained sequence was combined with previously published *Psathyrella* (Fr.) Quél. sequences (Tab. 1), including the closest matches from GenBank (Benson *et al.* 2007) and aligned in MEGA 5.0 (Tamura *et al.* 2011). Maximum likelihood (ML) analyses were conducted with 1,000 bootstrap replications and Bayesian analyses (BA) were conducted with 2.0 million replications both in TOPALi v2 (Milne *et al.* 2009) and the models of evolution GTR+I+G were used to ML and BA based on AIC value. Following Larsson & Örstadius (2008), we selected *Agrocybe pusiola* (Fr.) R. Heim, a member of Bolbitiaceae Singer, a closely related family to Psathyrellaceae Vilgalys, Moncalvo & Redhead, as outgroup. The obtained sequences have been deposited in GenBank database.

Results

Phylogenetic analyses

The final ITS+LSU rDNA alignment comprised 1,669 characters with gaps and included 27 specimens representing 22 species. The ML and BA analyses produced similar topologies, and the BA tree was chosen to represent the phylogenetic placement of *P. atlantica* (Fig. 1). The analyses revealed that *P. atlantica* has strong support (bp =100, pp = 1) in the phylogeny, clustering close to *P. leucotephra*.

Psathyrella atlantica V. Coimbra & Wartchow, sp. nov.

Figs. 2, 3

MycoBank: MB 834994

Diagnosis – *Psathyrella atlantica* is characterized by the slender, whitish and caespitose basidiomata, pileus with small orange floccose-squamulose veil remnants, presence of persistent but fragile-membranous annulus, basidiospores measuring 5–7 × 3–4 µm, lacking pleurocystidia and presenting utriform to utriform-lageniform thin walled cheilocystidia.

Type – BRAZIL, Pernambuco, Tamandaré, Reserva Biológica de Saltinho, 8°43'47"S, 35°10'37"W, 52 m alt., 12 April 2012, V.R.M. Coimbra s/n (URM 84467– holotype).

Etymology – based on the biome of occurrence of this species, the Brazilian Atlantic Forest.

Gregariously caespitose in cluster of 11 small basidiomata. Pileus: 10–28 mm diam., convex-campanulate, sometimes shallowly umbonate; white (4A1), sometimes with brown shades (5F5) due to spore-print covering; margin slightly sulcate and edge regularly toothed; context fleshy, white (4A1); veil on pileus surface as small floccose-squamulose remnants, light orange (5A5), more abundant at the centre then turning more scarce toward margin. Lamellae: adnate, close, dark brown (6F5), edge entire; lamellulae abundant, with diverse lengths. Stipe: 40–110 × 1.5–4 mm, cylindrical, central, shiny white (4A1), surface appressed squamulose-fibrillose downwards; squamules minutely fibrillose, light orange (5A5); context hollow, whitish, unchanging; base strongly strigose, presenting droplets of yellowish exudates; annulus persistent even in mature basidiomata but fragile, membranous, blackish, apical. Odor: unnoted when fresh, as dried *Boletus edulis* Bull. when dried.

Basidiospores 5–7 × 3–4 µm (L = 5.7 µm; W = 3.4 µm; Q = 1.40–2.00; Qm = 1.70), phaseoliform in profile, ovate with slightly rectangular base in face view, constricted at middle in the adaxial side; brown in KOH (5E7, 5E8), inamyloid; germ pore inconspicuous; hilar appendix very small and inconspicuous; smooth, thin-walled. Basidia 14–18 × 5–7 µm, clavate, hyaline, thin-walled, 2–4 sterigmata. Lamellae edge sterile with crowded cheilocystidia. Cheilocystidia 25–36 × 7–10 µm, utriform to subutriform, hyaline, thin-walled. Pleurocystidia absent. Lamellar trama regular, made of 17 cylindrical hyphae of 6–24 (–32) µm diam., usually inflated, hyaline, thin-walled. Pileipellis cellular, made of clavate, ovoid to subglobose and inflated cells 20–52 × 12–27 µm in diam., colorless, thin-walled. Stipitipellis with abundant caulocystidia 30–49 × 11–17.5 µm, clavate, hyaline, thin walled, more frequent at apex. Elements of veil on pileus with cylindrical hyphae 3–6 µm diam., brownish orange intracellular pigment, thin-walled. Hyphae clamped in all tissues examined.

Known distribution – So far, known only from the type locality. It grows in the litter deposited on the soil, in an area of Lowland Ombrofilous Dense Forest *sensu* Instituto Brasileiro de Geografia e Estatística - IBGE (1992) in the Atlantic Rain Forest domain.

Notes – *Psathyrella atlantica* is a noteworthy species, mainly recognized by the slender, whitish, and caespitose basidiomata, pileus with small orange floccose-squamulose veil remnants, presence of persistent but fragile-membranous annulus, and basidiospores of 5–7 × 3–4 µm size. Based on the infrageneric classification of *Psathyrella*

Table 1. Data of specimens used in the phylogenetic analysis.

Species	Voucher	Country	GenBank		References
			nLSU	nITS	
Agrocybe pusiola	LÖ304-05	Sweden	DQ389732	DQ389732	Larsson & Örstadius (2008)
Coprinellus silvaticus	LÖ172-08	Sweden	KC992943	KC992943	Örstadius *et al.* (2015)
Coprinellus christianopolitanus	LÖ141-08 type	Sweden	KC992944	KC992944	Örstadius *et al.* (2015)
Coprinopsis canoceps	LÖ148-95	Sweden	KC992964	KC992964	Örstadius *et al.* (2015)
Coprinopsis cineraria	CBM-FB-24142 type	Japan	KC992962	KC992962	Örstadius *et al.* (2015)
Coprinopsis cineraria	CBM-FB-32880	Japan	KC992963	KC992963	Örstadius *et al.* (2015)
Coprinopsis melanthina	WU19918	Portugal	KC992961	KC992961	Örstadius *et al.* (2015)
Coprinopsis submicrospora	AH27055 type/	Spain	KC992959	KC992959	Örstadius *et al.* (2015)
Coprinopsis udicola	AM1240 type	Germany	KC992967	KC992967	Örstadius *et al.* (2015)
Psathyrella atlantica	**URM 84467 type**	**Brazil**	**KC348448**	**KC348454**	**This study**
Psathyrella badhyzensis	79478 (TAA) type	Turkmenistan	KC992883	KC992883	Örstadius *et al.* (2015)
Psathyrella bivelata	MCVE29117	Italy	MF325962	MF325962	Genbank
Psathyrella candolleana	TNS-F-12192	Japan	AB306311	AB306311	Ogura-Tsujita & Yukawa (2008)
Psathyrella efflorescens	Pegler2133 (K)	Sri Lanka	KC992941	KC992941	Örstadius *et al.* (2015)
Psathyrella hymenocephala	2766	-	FJ168608	FJ168608	Slot *et al.* (2010)
Psathyrella hymenocephala	PBM2768	-	FJ168609	FJ168609	Slot *et al.* (2010)
Psathyrella leucotephra	MCVE28705	Spain	MF325979	MF325979	Genbank
Psathyrella leucotephra	LÖ138-01	-	KC992885	KC992885	Örstadius *et al.* (2015)
Psathyrella luteopallida	Sharp20863 (MICH) type	USA	KC992884	KC992884	Örstadius *et al.* (2015)
Psathyrella lutulenta	21379 (AH) type	Spain	KC992875	KC992875	Örstadius *et al.* (2015)
Psathyrella lyckebodensis	LÖ301-11 type	Sweden	KC992921	KC992921	Örstadius *et al.* (2015)
Psathyrella magnispora	24929 (AH) type	Spain	KC992863	KC992863	Örstadius *et al.* (2015)
Psathyrella magnispora	Barta, Spittelberg	Austria	KC992864	KC992864	Örstadius *et al.* (2015)
Psathyrella mesobromionis	Arnolds01-174 (L) type	Netherlands	KC992862	KC992862	Örstadius *et al.* (2015)
Psathyrella tuberculata	ADK4162 (BR)	Togo	KC992886	KC992886	Örstadius *et al.* (2015)
Psathyrella tuberculata	ADK3564	Benin	KC992934	KC992934	Örstadius *et al.* (2015)
Psathyrella trinitatensis	TL9035 (C)	Ecuador	KC992882	KC992882	Örstadius *et al.* (2015)

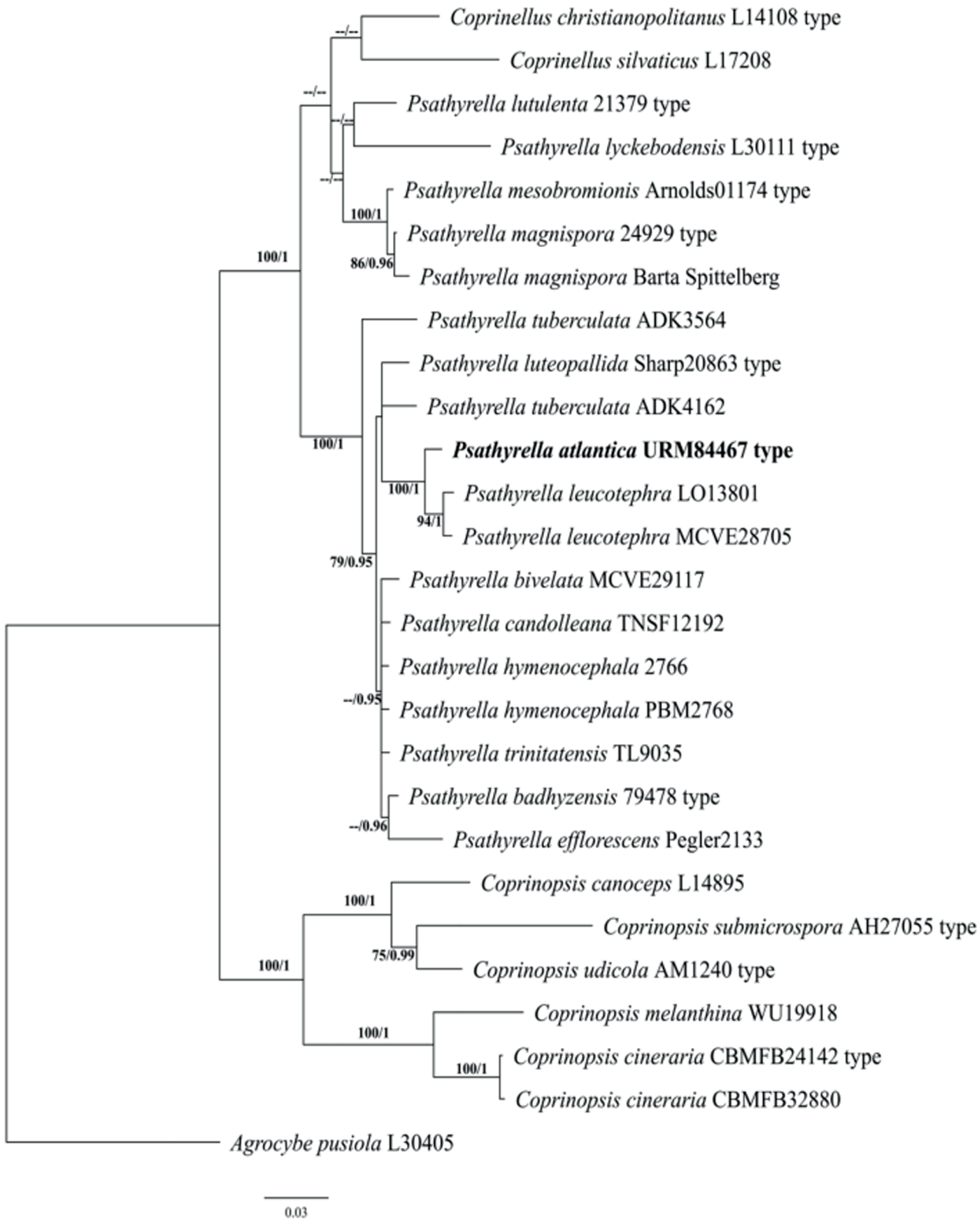

Figure 1. Phylogenetic relationship of *Psathyrella atlantica* and related taxa, inferred from ITS+LSU rDNA. Sequences generated for this study are indicated in bold. The voucher number is given for each specimen. Support values (ML/BI) are given above the branches. Scale bar shows expected changes per site.

proposed by Kits van Waveren (1985), *P. atlantica* can be placed in subgenus *Psathyra* sect. *Spintrigerae* due to its small basidiospores (up to 10 µm diam.), clavate basidia with less than 10 µm diam. and absence of pleurocystidia. Smith's (1972) circumscription included species only with annulate and species lacking pleurocystidia in the sect. *Spintrigerae*.

Discussion

Our results suggest the placement of *P. atlantica* among members of clade/candolleana *sensu* Örstadius *et al.* (2015). They also corroborate the determination of this taxon as a new species of *Psathyrella sensu stricto*. Padamsee *et al.*

Figure 2. *Psathyrella atlantica* (holotype): **A**. basidiomata in situ. **B**. Detail of the hymenium **C**. Basidiospores in 3 % KOH. Bars: A-B = 20 mm, C = 10 µm.

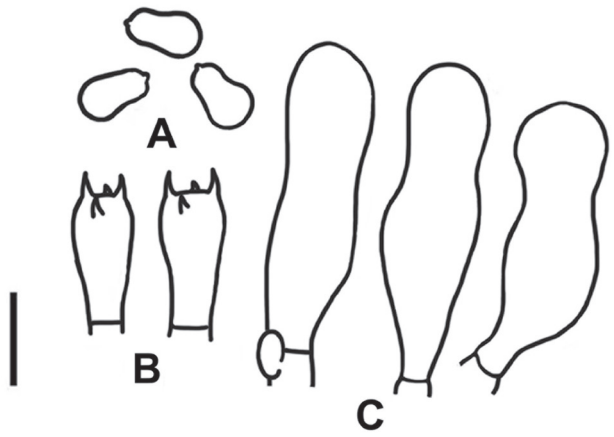

Figure 3. *Psathyrella atlantica* (holotype): **A**. Basidiospores. **B**. Basidia. **C**. Cheilocystidia. Bar = 10 µm. Drawing by V. R. M. Coimbra.

(2008) already stated that the clade/candolleana is among the ones that form a monophyletic group that better represents the limits of *Psathyrella sensu stricto*. This clade is dominated by the members of subgen. *Psathyra* sect. *Spintrigerae* (according to the classification of Kits van Waveren 1985) or subgen. *Pseudostropharia* sect. *Spintrigerae* (according to the classification of Smith 1972). Later, Nagy *et al*. (2013), Moreno *et al*. (2015), Yan & Bau (2018) and Voto *et al*. (2019) demonstrated that this clade is well-supported, characterized by species with pale colored basidiomata with fibrillose veil, and lacking pleurocystidia

Among morphologically close species that fall in the same clade, the European *P. leucotephra* is the most similar species. It also grows caespitosely in large clusters of 10 basidiomata, presents utriform to utriform-lageniform cheilocystidia, small hilar appendix and inconspicuous germ pore on basidiospores, pale colored pileus, and whitish sericeous-fibrillose stipe. Differences are focused on more robust basidiomata, larger basidiospores (usually 7–10 × 5–7 µm), and lignicolous habit of *P. leucotephra* (Berkeley & Broome 1870; Orton 1960; Kits van Waveren 1985; Breitenbach & Kränzlin 1995).

The new species *P. atlantica* also clustered with the sequences from an African specimen named as *P. tuberculata*. The protologue from Guadeloupe by Patouillard (1899) and later revisions by Morgan (1908) and Smith (1972) reported scattered squamules slightly projecting on the lower portion of the stipe; however, it clearly differs in the greenish pileus with small obtuse tubercles on pileus surface.

The decorated stipe of *P. atlantica* can be compared with two other Neotropical species reported by Smith (1972): *P. floccosa* and *P. vanhermanii*, both from Cuba. *Psathyrella floccosa* differs from *P. atlantica* in its depressed, ochraceous-brown and densely lanose-squamulose pileus, densely villose stipe, and a whitish, well developed annulus. *Psathyrella vanhermanii* shares similarities with *P. atlantica* in the pallid pileus, but differs in the adnexed lamellae, white and thick annulus, more robust stipe (5–10 mm diam.) tapered at the base, and fusoid-ventricose cheilocystidia of 26–30 × 9–15 µm (Smith 1972).

Psathyrella incerta and *P. hymenocephala*, both morphologically similar to the new species and widely distributed in North-America, differ mainly in lacking annulus, having less slender stipes and larger cheilocystidia [32–46 × 9–14 µm and 32–46 (–50) × 10–18 µm respectively] (Smith 1972).

Psathyrella candolleana is considered as the most common and variable species in North America by Smith (1972). This cosmopolitan taxon differs from *P. atlantica* in the absence of annulus, pileal veil remnants flocculose and white to yellowish-ochraceous, mainly located on the peripheral half of pileus, ellipsoid basidiospores and very crowded lamellae (Kits van Waveren 1985; Breitenbach & Kränzlin 1995).

BLASTn analysis of our ITS sequence (KC348454) showed highest similarities with *P. leucotephra* (97.17%, MCVE28705 - MF325979; 96.74%, LÖ138-01 - KC992885), while LSU sequence (KC348448) showed highest similarities (98%) with sequences of *P. candolleana* (AY207279, DQ389720, DQ110874, DQ986225, DQ986250, FN396165), *P. leucotephra* (FM160683, DQ986240), *P. typhae* (DQ389721, DQ986229), *P. multipedata* (AM712279, GQ249291), *P. huronensis* (DQ986270), *P. incerta* (DQ986246), *P. calcarea* (DQ389671), *P. prona* var. *utriformis* (FM160687), *P.* aff. *vanhermanii* (AF261487), *P. floccosa* (DQ986235) and *P. spadiceogrisea* (DQ389682).

Based on our morphological and phylogenetic analyses of the specimen found in the Brazilian Atlantic Rain Forest, it is possible to propose *P. atlantica* as a genuine member of *Psathyrella sensu stricto* as also a new taxon to science. Thus, future mycological studies in this threatened biome are strongly encouraged, as a way of aiding the scientific community to scavenge its hidden mycobiota. In addition, the future records of *P. atlantica* collections will specify a variability of its morphological characters and its ecology.

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