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***Pluteus* section *Hispidoderma* in Brazil with new records based on morphological and molecular data**

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Abstract – A revision of *Pluteus* section *Hispidoderma* occurring in Brazil is presented showing the occurrence of eight species considered certainly known in the country: *P. fibrillosus*, *P. chusqueae*, *P. longistriatus*, *P. maculosipes*, *P. neochrysaegis*, *P. rimosellus*, *P. varzeicola* and *P. velutinus*. Molecular analyses using ITS sequences showed the phylogenetic position of *P. cf. fernandezianus*, *P. fibrillosus*, *P. longistriatus* and *P. velutinus*. *Pluteus fibrillosus*, *P. maculosipes* and *P. velutinus* represent new records from Brazil. A type revision of *P. varzeicola* is also presented.

Agaricales / biodiversity / ITS / Pluteaceae / taxonomy

INTRODUCTION

Singer (1959, 1986) considered the infrageneric classification of *Pluteus* Fr. with three sections (*Pluteus*, *Hispidoderma* Fayod and *Celluloderma* Fayod) based on morphological features such as the structure of the pileipellis and characteristics of the pleurocystidia. *Pluteus* sect. *Hispidoderma* was recognized by Singer (1959, 1986) to include species with non-metuloid pleurocystidia and pileipellis composed of elongated elements forming a cutis, a hymeniderm or a trichoderm.

Vellinga & Schreurs (1985) proposed a variation of this classification subdividing *Pluteus* sect. *Hispidoderma* into two taxonomic units according to the structure of the pileipellis. A new section, viz. *Pluteus* sect. *Villoso* Schreurs & Vellinga, was introduced to accommodate the species with a cutis-like pileipellis,

and the remaining species were transferred to *Pluteus* sect. *Celluloderma* subsect. *Hispidodermini* (Fayod) Vellinga & Schreurs that includes species with a trichodermic pileipellis or a hymeniderm with cylindrical to fusiform elements.

Singer's classification is the most accepted for infrageneric organization of *Pluteus* and it has been supported by molecular data (Menolli *et al.*, 2010; Justo *et al.*, 2011a, b) with some rearrangements. Justo *et al.* (2011a, b) showed in the molecular analyses that the species with non-metuloid cystidia and a cutis-like pileipellis should be classified in *Pluteus* sect. *Celluloderma* instead of *Pluteus* sect. *Hispidoderma* (Singer 1959, 1986) or sect. *Villosi* (Vellinga & Schreurs 1985). Thus, Justo *et al.* (2011a, b) characterized *Pluteus* sect. *Hispidoderma* as having hymenidermal or trichodermal pileipellis composed of long and elongated elements, which are very variable in shape and size.

Based on this circumscription, 15 taxa names of *Pluteus* sect. *Hispidoderma* have been reported from Brazil by Rick (1919, 1930, 1938, 1961), Singer (1954, 1956, 1959, 1989), Pegler (1997), Meijer (2006), Wartchow *et al.* (2006), Rosa & Capelari (2009), Menolli & Capelari (2010), Justo *et al.* (2011a, b) and Menolli *et al.* (2015). The purpose of this study is to improve the knowledge of *Pluteus* sect. *Hispidoderma* in Brazil providing new records based on morphological and molecular data and presenting a list of all species previously published in the literature with an update of the taxonomic status of the Brazilian collections.

MATERIALS AND METHODS

The materials studied in the morphological examination include specimens recently collected in Brazil or previously published. When necessary, type collections or additional non-Brazilian specimens were also studied. The herbarium acronyms follow Thiers (2015) and the "Rede Brasileira de Herbários" (http://www.botanica.org.br/rede_herbarios.php) for the Herbarium of the Passo Fundo University (RSPF).

Species characterized as having non-metuloid pleurocystidia and a pileipellis as a cutis were excluded because we accept the section delimitation as complemented by Justo *et al.* (2011a, b) that considered these species in *Pluteus* sect. *Celluloderma*. Some species with pleurocystidia of thin- to slightly thick-walled and a cutis-like pileipellis were also not studied here because they were considered members of sect. *Pluteus*, viz. *P. albstipitatus* (Dennis) Singer, *P. glaucotinctus* E. Horak, *P. meridionalis* Menolli & Capelari and *P. nigrolineatus* Murril (Wartchow *et al.*, 2006; Menolli *et al.*, 2010, 2014, 2015; Justo *et al.*, 2011a, b).

The macroscopic description was based on fresh specimens. Colour codes refer to Küppers (1979). For microscopic analyses, the dried material was wetted with 70% ethanol and then rehydrated in 5% KOH or stained with Melzer's reagent to determine the amiloidity reaction of the basidiospores. Descriptive terms for micromorphological features follow Vellinga (1988). The notation [a/b/c] at the beginning of a set of basidiospore data is to be read as "(a) basidiospores were measured from (b) basidiomata taken from (c) collections". Q represents the range of the length/width ratio for all of the measured spores, Qm represents the average of all calculated Q values for all of the measured basidiospores and Lm (Wm) represents the average of all of the lengths (widths) of the measured basidiospores. At least 20 basidiospores from each basidioma were measured in lateral view, and the terms denoting basidiospore shape follow Bas (1969).

Methods for DNA isolation, PCR and sequencing follow Justo *et al.* (2011b). The ITS region was amplified using the primer pair ITS1-F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993). The molecular analyses were conducted with existing DNA sequences of *Pluteus* sect. *Hispidoderma* used by Justo *et al.* (2011a, b), Pradeep *et al.* (2012) and from newly collected sequences of materials representing species of the *plautus/logistriatus* clade (clade names follow Justo *et al.*, 2011a). The tree was rooted using sequences of the *leoninus* clade as outgroup. Newly obtained sequences were deposited in GenBank and accession numbers are given in Fig. 1. Sequences were aligned using MAFFT version 6 (<http://mafft.cbrc.jp/alignment/server/>; Katoh & Toh, 2008) with the FFT-NS-i option. Then, the alignment was visually examined and manually corrected using MacClade 4.05 (Maddison & Maddison, 2002). A Maximum Likelihood analysis was performed with the parameters specified in Justo *et al.* (2011b). Sequence divergence was calculated using MatGAT (Campanella *et al.*, 2003).

RESULTS

Phylogeny

The final dataset consists of 45 ingroup sequences with a total of 727 characters (gaps included). The phylogenetic position of *P. cf. fernandezianus*, *P. fibrillosus*, *P. longistriatus* Murrill and *P. velutinus* is highlighted in Fig. 1 and discussed in detail below while providing complete descriptions for these taxa (except for *P. longistriatus* that was fully described by Menolli & Capelari, 2010). The general phylogeny (Fig. 1) presents species of the *plautus/logistriatus* clade (Justo *et al.*, 2011a) organized in two major non-supported clades. All Brazilian sequences of the taxa previously mentioned are placed on the same major clade of *P. semibulbosus* (Lasch) Gillet and unnamed members of the complex around *P. plautus* (Weinm.) Gillet. Two sequences from undetermined Brazilian samples (NMJ193 and TSBF1_52) appear external to the *P. semibulbosus* clade. *Pluteus fibrillosus* is related to *P. heteromarginatus* Justo but with a not well-resolved relationship with the other clades. The clade formed by *P. longistriatus* (99% Bootstrap support) include sequences from Brazil and the U.S.A., as previously pointed by Justo *et al.* (2011a, b) and Pradeep *et al.* (2012), and an additional record from South Korea. *Pluteus velutinus* also represent a well-supported clade (100% Bootstrap support) including samples from different geographic origins (Brazil, India and Japan), which is linked to the *P. longistriatus* clade, although with no statistical support. Finally, *Pluteus cf. fernandezianus* represent an external branch to all sampled sequences of this major clade that is formed by at least nine taxa from four global ecozones (Indomalaya, Nearctic, Neotropic and Palearctic).

Taxonomy

Pluteus cf. fernandezianus Singer, Lloydia 21: 220, 1959

Figs 2-3

Basidiospores [20/1/1] $6.2\text{--}8.7 \times 5.0\text{--}7.5 \mu\text{m}$ ($Q = 1.00\text{--}1.34$; $Q_m = 1.18$; $L_m = 7.3 \mu\text{m}$; $W_m = 6.3 \mu\text{m}$), globose to ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. **Basidia** $30\text{--}36 \times 10\text{--}12.5 \mu\text{m}$, clavate, thin-walled, four-



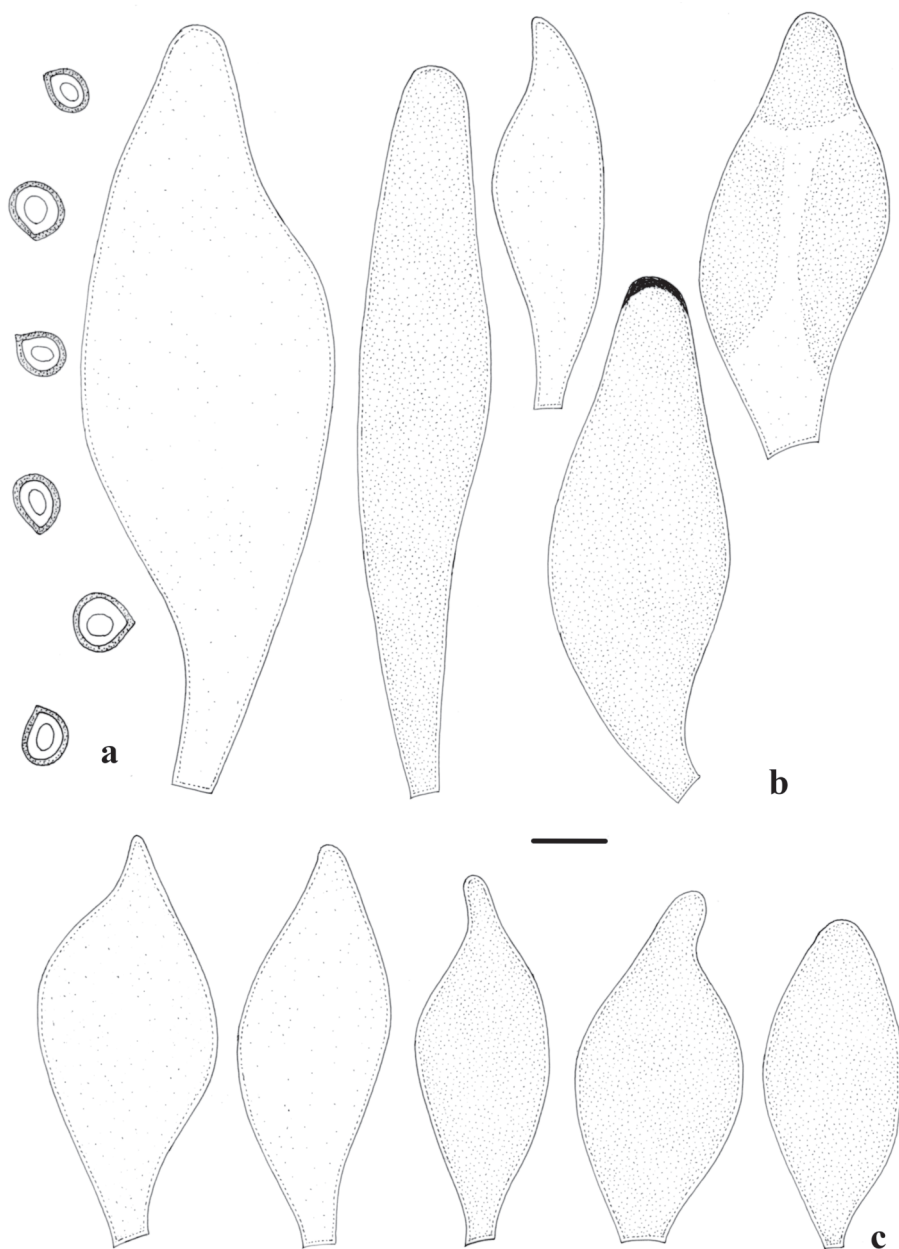


Fig. 2. *Pluteus* cf. *fernandezianus* (RSPF330). **a.** basidiospores; **b.** pleurocystidia; **c.** cheilocystidia. Scale bar: 10 μ m.

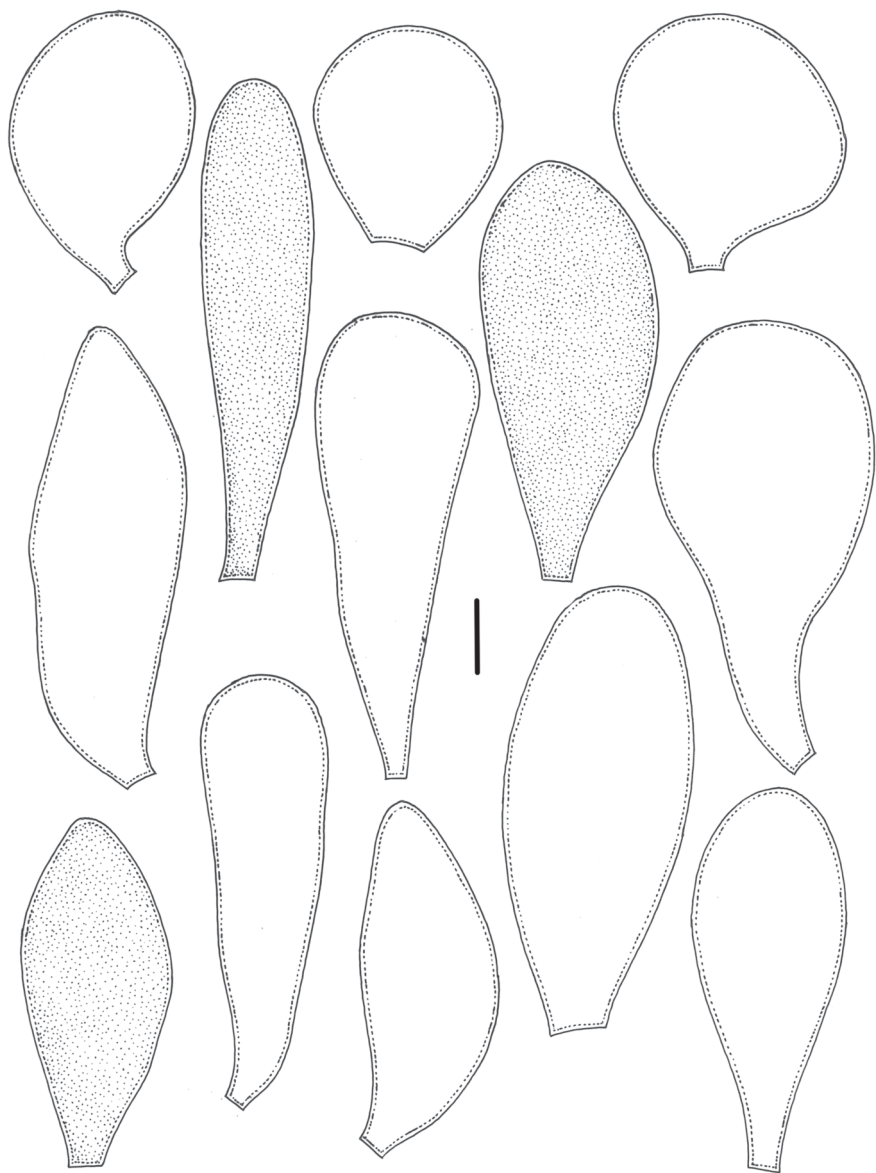


Fig. 3. Pileipellis elements of *Pluteus* cf. *fernandezianus* (RSPF330). Scale bar: 10 μ m.

spored. **Pleurocystidia** 52-104 \times 15.0-35 μ m, utriform or narrowly clavate to broadly fusiform, sometimes with some encrusting parietal pigment at apex, usually filled with pale brownish content, thin-walled, numerous. **Cheilocystidia** 44-54 \times 18.7-25 μ m, fusiform or broadly utriform, filled with pale brownish content, thin-walled, not numerous. **Lamellar edge** heteromorphous. **Pileipellis** an euhymeniderm composed of one layer of versiform elements, terminal cells 29-67 \times 13.7-29, vesiculose, clavate to fusiform cells (approx. 46-67 \times 13.7-29 μ m) or in few number intermixed by sphaeropedunculate cells (approx. 29-38 \times 24-29 μ m), with rounded apex, thin-walled, mostly hyaline and colourless but some filled with pale straw dissolved content. **Stipitipellis** a cutis of thin-walled hyphae, 2.5-8.7 μ m wide, colourless and mostly hyaline. **Clamp connections** absent in all parts examined.

Habit and habitat. – Solitary on decayed wood.

Revised specimen. – BRAZIL: Rio Grande do Sul, Passo Fundo, Jaboticabal, 13 September 2003, M.S. Rother & B.M.A. Severo *s.n.* (RSPF330).

Remarks. – The collection RSPF330 was deposited at RSPF herbarium under the name of *P. beniensis* Singer, a species of sect. *Celluloderma* characterized by an epithelial pileipellis with uniform elements. However, our molecular analyses together with the morphological data confirm this mistake and its position in *Pluteus* sect. *Hispidoderma* as part of the *plautus* clade (Fig. 1).

Despite the lack of macroscopic data for RSPF330, we tentatively related it to *P. fernandezianus* described from Chile (Singer, 1959) mainly due to the shape and color of the pleuro- and cheilocystidia. However, according to the protologue (Singer, 1959), *P. fernandezianus* as some differences when compared to RSPF330, such as smaller pleurocystidia (46-65 \times 8.7-23.3 μ m), ellipsoid to short ellipsoid but non-globose basidiospores [7-8(-9.3) \times 6-6.7(-8) μ m], and the lack of sphaeropedunculate cells in the pileipellis.

Singer (1969) considered *P. brunneoolivaceus* E. Horak as synonym of *P. fernandezianus* but without any discussion of the reasons for this. Horak (1964) described *P. brunneoolivaceus* from Argentina and reported the presence of small horns and finger-like protuberances in the pleurocystidia, which are probably similar to those described for *P. fernandezianus* by Singer (1959) as having “the tip sometimes short apiculate in the center, sometimes sinuate or nodulose at apex”. The pleurocystidia of our collection usually have apical encrustation (Fig. 2b) but they are different from the apical ornamentation described and illustrated by Horak (1964) for *P. brunneoolivaceus*. An accurate morphological comparison including type studies will be necessary to state the identity of RSPF330 and to establish the real relationship between *P. brunneoolivaceus* and *P. fernandezianus*.

The characters observed in RSPF330 are close to those described to taxa of the stirps *umbrosus* (Singer, 1986) such as *P. compressipes* Murrill, *P. espeletiae* Singer, *P. jaffuelii* (Speg.) Singer, *P. multistriatus* Murrill, *P. rimosellus* Singer and *P. umbrosus* (Pers.) P. Kumm. *Pluteus compressipes* was described by Singer (1956) and Smith & Stuntz (1959) as having narrower basidiospores (6-7 \times 5.5-6.5 μ m and 6-7 \times 5.5-6.5 μ m, respectively) and pileipellis hyphae with clamp connections (Smith & Stuntz, 1959). *Pluteus espeletiae* (Singer, 1962) has some characters close to those observed in RSPF330 such as large basidiospores (8.0-8.8 \times 6.8-7.5 μ m) and cystidia (40-90 \times 10-24 μ m, although not differentiated in pleuro- and cheilocystidia), but *P. espeletiae* has a pileipellis described with hyphal elements typical of a cutis: “epicutis of pileus consisting of parallel hyphae with uppermost members appressed to ascendant in bunches, even suberect in the region of the central scaliness”. *Pluteus jaffuelii* (Singer, 1959) has smaller pleurocystidia [(30)-48-68 \times 10-24 μ m], narrower basidiospores (6.2-7.5 \times 4.8-7 μ m) and much longer

pileipellis elements ($66\text{--}150 \times 13.8\text{--}30.8\ \mu\text{m}$). *Pluteus multistriatus*, although having pleurocystidia up to $110\ \mu\text{m}$ long (Singer, 1956), was described by Singer (1956) and Smith & Stuntz (1959) as having narrower basidiospores ($6.5\text{--}7 \times 4.8\text{--}5\ \mu\text{m}$ and $5.4\text{--}6.5 \times 4.7\text{--}5.5\ \mu\text{m}$, respectively) and hyaline cheilocystidia (Smith & Stuntz, 1959). *Pluteus rimosellus* (= *P. subfibrillosus* Singer) has non-globose and slightly narrower basidiospores [$(5.6)\text{--}6.2\text{--}7.5 \times 5.0\text{--}6.2\ \mu\text{m}$; $Q = 1.11\text{--}1.24(-1.30)$; $Q_m = 1.18$], smaller pleurocystidia ($27\text{--}50 \times 7.5\text{--}13.7\ \mu\text{m}$), and preponderantly clavate cheilocystidia (Menolli *et al.*, 2015). Finally, *P. umbrosus* represents a separate clade in *Pluteus* sect. *Hispidoderma* (Justo *et al.*, 2011a) and is a species commonly reported from Europe (Orton, 1986) with narrower basidiospores ($5\text{--}7.5 \times 4.5\text{--}6\ \mu\text{m}$) and much longer pileipellis elements ($90\text{--}250 \times 12\text{--}30\ \mu\text{m}$).

Considering the lack of data for RSPF330 and the morphological differences between the related taxa, we maintain this material as inconclusive identification until more new collections have become available for an accurate morphological and molecular comparison with our Brazilian specimen.

Pluteus fibrillosus Murrill, N. Amer. Flora 10: 134, 1917

Figs 4a-c, 5-6

Pileus 30-52 mm diam., conic to plane-convex with a distinct umbo or almost papillate, surface pale brown ($N_{80}A_{50}M_{50}$) with darker centre ($N_{90}A_{90}M_{50}$) and paler towards the margin, covered by appressed and radially arranged fibrils, sometimes exposing at the margin the pale to translucent background between the fibrils, not translucently striate or only so at extreme margin. **Lamellae** free, pinkish ($N_{10}A_{30}M_{30}$), crowded, ventricose, with concolourous edges and lamellulae of different lengths, 1-3 for each lamellae. **Stipe** 30-70 \times 2-3 (apex)-5-7 (base) mm, slightly tapering towards the apex, central, cylindrical, surface white to pale cream with greyish to brownish base, longitudinally striate and sometimes with brownish fibrils ($N_{30}A_{40}M_{20}$) at the base, with or without scanty basal mycelium. **Odour, taste and context colour** not recorded.

Basidiospores [60/3/3] $5.0\text{--}6.2 \times (4.3\text{--})5.0\text{--}5.6\ \mu\text{m}$ ($Q = 1.00\text{--}1.30$; $Q_m = 1.20$; $L_m = 6.0\ \mu\text{m}$; $W_m = 5.0\ \mu\text{m}$), globose to broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. **Basidia** 26-36 \times 6.2-7.5 μm , narrowly clavate, thin-walled, four-spored. **Pleurocystidia** 41-67(-75) \times 12.5-29 μm , broadly utriform to narrowly fusiform or rarely clavate, colourless and hyaline, thin-walled, sparse and not abundant. **Cheilocystidia** 30-65 \times (8.7-)-12.5-22(-27) μm , fusiform, utriform or clavate, sometimes subcapitate and with some encrusting parietal pigment at apex, colourless and hyaline, thin-walled, moderately abundant. **Lamellar trama** inverse, up to 31 μm wide, composed of thin-walled hyphae, 2.5-12.5 μm diam., interwoven by oleiferous up to 2.5 μm diam., hyaline. **Pileus context** undifferentiated, approx. 50 μm thick, composed of thin-walled hyphae, 2.5-15.0 μm diam., hyaline. **Pileipellis** an euhymeniderm up to 125 μm thick, composed of fusoid or narrowly clavate cells, individual terminal elements (74-)-104-162 \times (10-)-20-30 μm , with acute to subacute apex (up to 4.0 μm diam.) or few with broadly rounded apex (up to 10.0 μm diam.), sometimes with points of apical encrustation, with evenly dissolved or sometimes concentrated brown intracellular pigment. **Caulocystidia** 37-64(-81) \times 12.5-16.2 μm , fusiform to broadly utriform or mucronate, with rounded or gradually attenuated apex (2.5-6.2 μm diam.), thin-walled, filled with dissolved brownish content. **Clamp connections** absent in all parts examined.

Habit and habitat. – Subgregarious to disperse (up to three basidiomata near), on decayed wood or on soil.

Revised specimens. – BRAZIL: Minas Gerais, Marliéria, Parque Estadual do Rio Doce, 16 February 2000, L.H. Rosa & R.O. Marais PERD76 (SP307730);



Fig. 4. **a-c.** *Pluteus fibrillosus* (FK1903); **d-f.** *P. maculosipes* (FK1651); **g-i.** *P. velutinus* (FK1889). Scale bars: 1 cm.

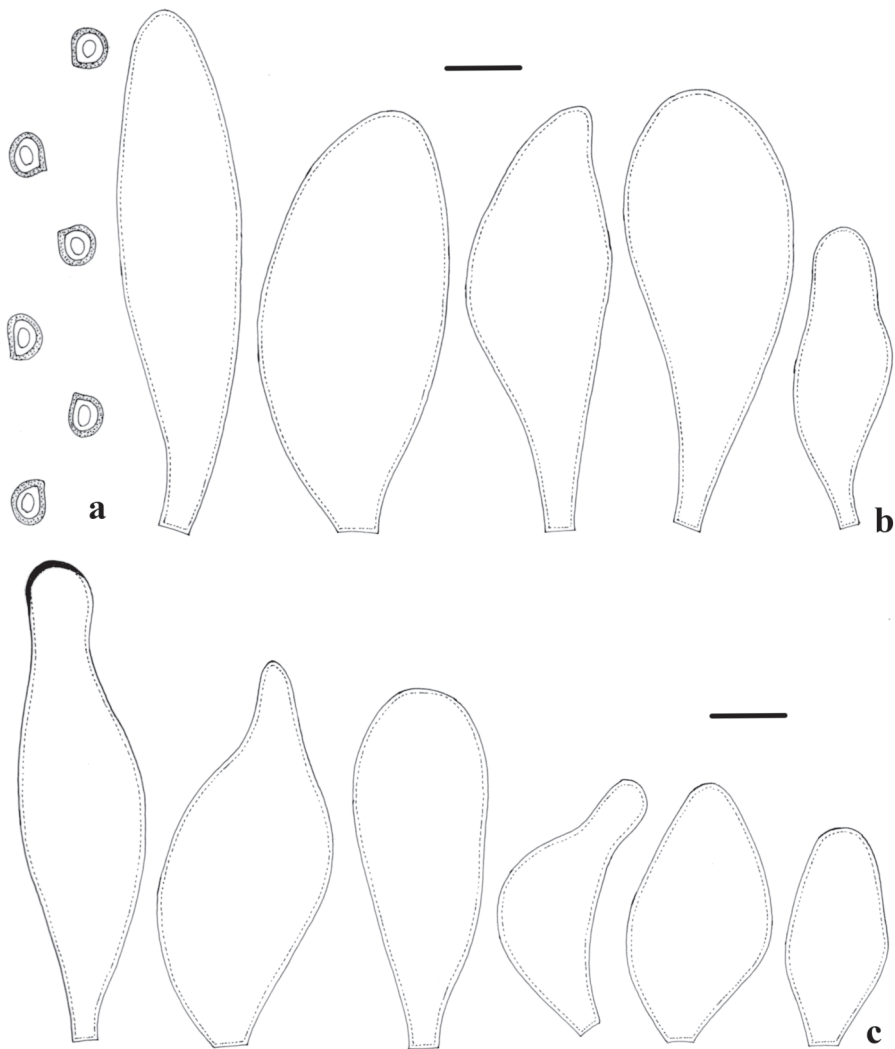


Fig. 5. *Pluteus fibrillosus* (FK1903). **a.** basidiospores; **b.** pleurocystidia; **c.** cheilocystidia. Scale bars: 10 μ m.

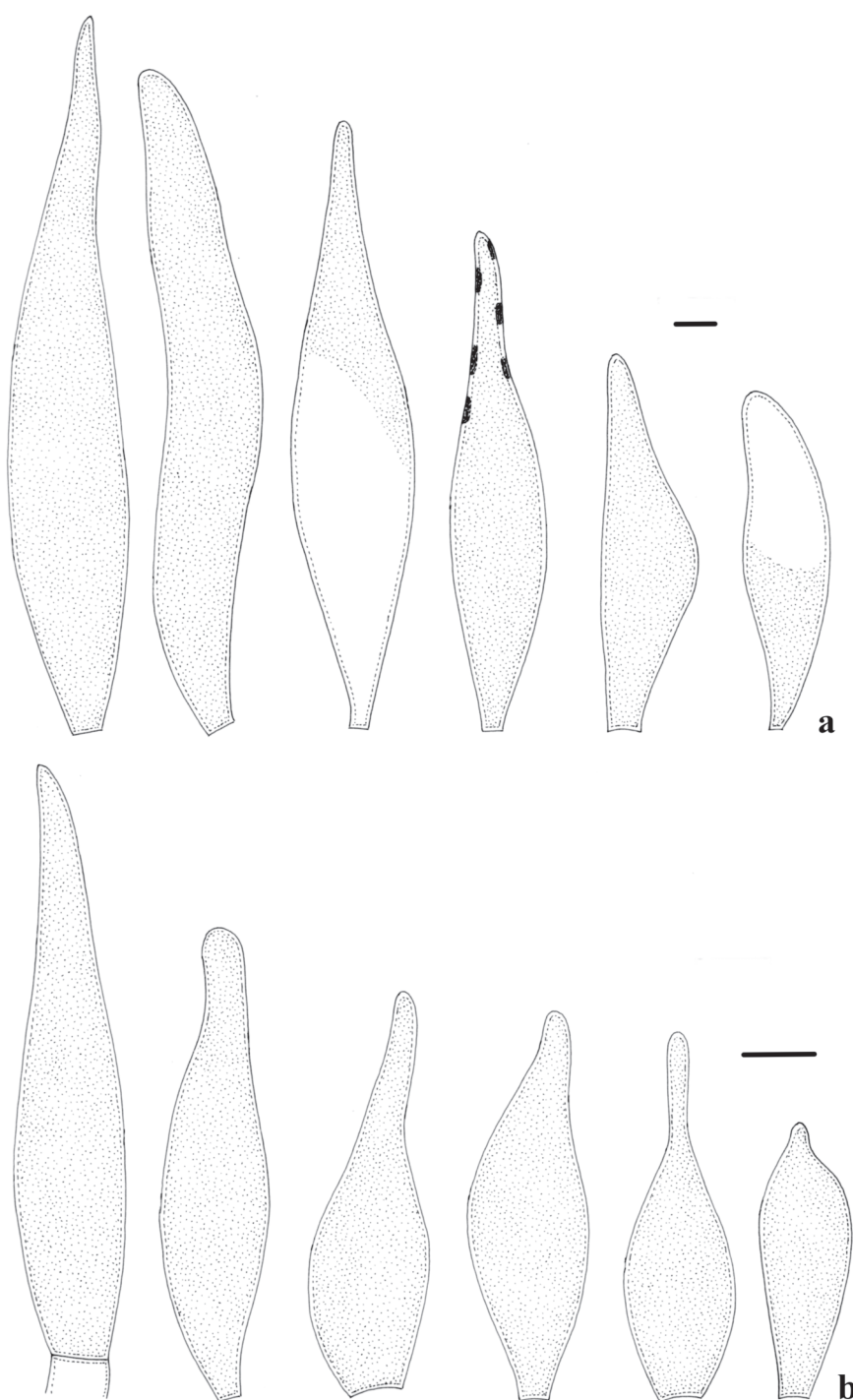


Fig. 6. *Pluteus fibrillosus* (FK1903). **a.** pileipellis elements; **b.** caulocystidia. Scale bars: 10 μ m.

São Paulo, São Paulo, Parque Estadual das Fontes do Ipiranga, 1 March 2011, F. Karstedt & J.J.S. Oliveira FK1903 (SP417462); *ibid.* 3 March 2011, F. Karstedt & J.J.S. Oliveira FK1915 (SP417457).

Remarks. – *Pluteus fibrillosus* represents a barely reported species described from the U.S.A. – Louisiana (Murrill, 1917) and with additional type studies (Singer, 1956; Smith & Stuntz, 1959; Banerjee & Sundberg, 1993; Menolli *et al.*, 2015). The terrestrial habitat allied to the presence of a brownish fibrillose pileus, globose to broadly ellipsoid basidiospores, hymenidermal pilepellis of versiform elements with apical encrustation, and cheilocystidia similar in shape and size to the pleurocystidia are useful features to recognize *P. fibrillosus* (Murrill, 1917; Singer, 1956; Smith & Stuntz, 1959; Banerjee & Sundberg, 1993; Menolli *et al.*, 2015).

From our collections it was observed some additional features that could be also related to *P. fibrillosus*: the presence of a distinctive umbo on pileus, a greyish to brownish base on stipe, some encrusting parietal pigment at cheilocystidia apex, and the occurrence of caulocystidia. In addition, the basidiospores of the Brazilian collections are slightly narrower than those described by Singer (1956; $6.6\text{--}6.8 \times 5.5\text{--}6.2\text{ }\mu\text{m}$), Smith & Stuntz (1959; $5.8\text{--}7 \times 5.5\text{--}6.8\text{ }\mu\text{m}$), and those measured ($6.2\text{--}7.5 \times 5.0\text{--}6.2\text{ }\mu\text{m}$, $Q = 1.00\text{--}1.24$; $Q_m = 1.19$; $L_m = 6.5\text{ }\mu\text{m}$; $W_m = 5.5$) by us in our type revision (F.S. Earle 129 – NY!).

Although Murrill (1917) described *P. fibrillosus* as growing on soil, we found collections morphologically identical growing on soil (FK1915) and on decayed wood (FK1903 and PERD76). Unfortunately, we were able to obtain ITS sequence only from FK1903 and therefore, until it is possible the molecular comparison between specimens from different habitats, *P. fibrillosus* is here considered a species that grows on soil and on wood.

Rick (1938) published a homonym, viz. *P. fibrillosus* Rick (nom. illegit., non Murrill), but he did not indicate a type collection and according to Menolli & Capelari (2014) it is also a *nomen dubium* because it was not found any sample under this name collected by Rick.

Based on our phylogenetic analyses (Fig. 1), *P. fibrillosus* is related to *P. heteromarginatus* described from the U.S.A. – Florida (Justo *et al.*, 2011b). Both taxa appear in a more inclusive clade with *P. semibulbosus*, a widespread but yet unnamed member of the *P. plautus* complex, and two additional sequences from undetermined Brazilian samples.

Pluteus maculosipes Singer, Sydowia 15: 122, 1962

Figs 4d-f, 7

Pileus 25 mm diam., conic-campanulate at first and then expanding, surface greyish-brown, fibrillose-pruinose to almost pulverulent, fully covered by minute squamules, margin not sulcate or striate. **Lamellae** free, at first white and then finally cream-pinkish, crowded, ventricose, with concolourous edges and lamellulae of different lengths, 1-3 for each lamellae. **Stipe** 40×3 (apex)-5(base) mm, tapering towards the apex, central, cylindrical, surface white to pale cream and minutely dotted brownish towards the base. **Odour**, **taste** and **context colour** not recorded.

Basidiospores [20/1/1] $6.2\text{--}7.5 \times (5.0\text{--})5.6\text{--}6.2\text{ }\mu\text{m}$ [$Q = 1.10\text{--}1.24(1.40)$; $Q_m = 1.17$; $L_m = 6.9\text{ }\mu\text{m}$; $W_m = 5.9\text{ }\mu\text{m}$], subglobose to broadly ellipsoid, rarely ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. **Basidia** $22.5\text{--}27.5 \times 8.7\text{--}10.0\text{ }\mu\text{m}$, clavate, thin-walled, four-spored. **Pleurocystidia** absent. **Cheilocystidia** $39\text{--}56(74) \times (6.2\text{--})8.7\text{--}11.2(24)\text{ }\mu\text{m}$, fusiform to narrowly fusiform or mucronate, usually elongated to slender, apex rounded and usually with a narrow and short papilla, rarely longer ($71\text{--}74 \times 18.7\text{--}24\text{ }\mu\text{m}$) and broadly lageniform or slightly constricted at the mid point, colourless and hyaline, thin-walled, very abundant in

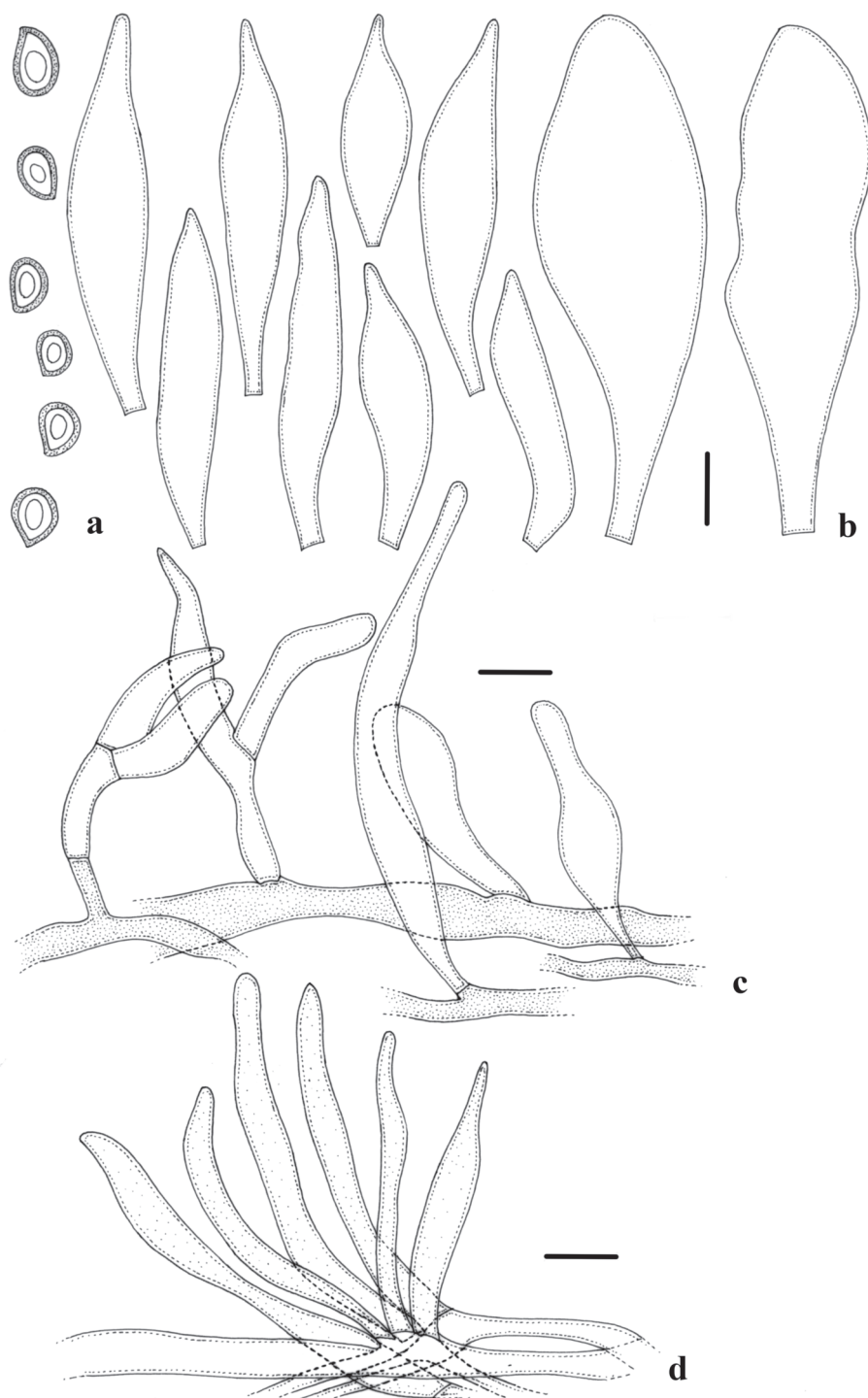


Fig. 7. *Pluteus maculosipes* (FK1651). **a.** basidiospores; **b.** cheilocystidia; **c.** pileipellis elements; **d.** caulocystidia. Scale bars: 10 µm.

fascicles. **Lamellar edge** sterile. **Lamellar trama** inverse, up to 38 μm wide, composed of thin-walled hyphae up to 6.2 μm diam., hyaline. **Pileus context** undifferentiated, approx. 50 μm thick, composed of thin-walled hyphae up to 6.2 μm diam., hyaline. **Pileipellis** a irregular trichoderm or a trichohymeniderm, with tufts of ascendant to erect elements from a subpellis of more or less parallel hyphae, individual terminal elements $20\text{--}69 \times 5\text{--}8.7 \mu\text{m}$, with broadly rounded apex or sometimes tapering towards the apex, with evenly dissolved brown intracellular pigment, mainly the elements of the subpellis, or sometimes colourless and hyaline mainly the suberect terminal elements. **Stipitipellis** a cutis of thin-walled hyphae, up to 6.2 μm diam., with many erect to suberect cystidioid elements organized in fascicles, individual terminal elements $(18.7\text{--})29\text{--}56 \times 3.7\text{--}5.0 \mu\text{m}$, gradually attenuated forming an obtuse to subacute apex (up to 2.5 μm diam.), filled with evenly dissolved pale straw pigment. **Clamp connections** absent in all parts examined.

Habit and habitat. – Solitary, on decayed wood.

Revised specimen. – BRAZIL: Paraná, Foz do Iguaçu, Parque Nacional do Iguaçu, Trilha do Poço Preto, 6 November 2010, F. Karstedt FK1651 (SP417463).

Remarks. – *Pluteus maculosipes* is a species described from Venezuela (Singer, 1962) and characterized by the absence of pleurocystidia, a distinct pileipellis and a stipitipellis with accumulations of cystidioid elements organized in fascicles. Singer (1962) reported and illustrated the presence in lamellar sides of “pseudoparaphysis-like bodies which are vesiculose-clavate, (...) $16.5 \times 9.3 \mu\text{m}$ ”, but these structures most likely represent basidioles in the hymenium. In our material we observed the presence of a second type of cheilocystidia that are slightly longer and different in shape from those commonly observed, but they are rare and hence of doubtful taxonomic value to distinguish our material from the type of *P. maculosipes*.

Pluteus maculosipes is different from the *Pluteus* species with very rare to absent pleurocystidia because most of them have a pileipellis characteristic of the species currently classified in sect. *Celluloderma*, as observed in *P. diettrichii* (= *P. rimulosus* Kühner & Romagn.), *P. insidiosus* Vellinga & Schreurs, *P. poliocnemis* Kühner, *P. seticeps* (G.F. Atk.) Singer and *P. thomsonii* (Berk. & Broome) Dennis. *Pluteus exiguus* (Pat.) Sacc. and *P. pusillulus* Romagn. are the few species lacking pleurocystidia and with a hymenidermal pileipellis of narrowly fusiform elements or as a palisade or a trichoderm (Vellinga & Schreurs, 1985; Orton, 1986). However, *P. pusillulus* has subcapitate cheilocystidia, pileipellis organized in a true hymeniderm and stipitipellis without cystidioid elements (Vellinga & Schreurs, 1985), while *P. exiguus* has as a distinctly squamulose pileus and clavate cheilocystidia with a distinct narrow apex or apical projections (Kühner & Romagnesi, 1956; Orton, 1986).

Unfortunately, we were not able to obtain ITS sequence from FK1651, but based on the morphological data we confirm the occurrence of *P. maculosipes* in Brazil and this represents the first record after its description.

Pluteus velutinus Pradeep, Justo & Vrinda, Mycol. Progress 11: 871, 2012

Figs 4g-i, 8-9

Pileus 35 mm diam., convex, slightly depressed at centre, surface uniformly beige to light brown ($\text{N}_{50}\text{A}_{50}\text{M}_{40}$), velvety to pruinose-fibrillose all over, sometimes detaching and exposing the white translucent flesh, margin pellucid striate. **Lamellae** free, pinkish ($\text{N}_{20}\text{A}_{20}\text{M}_{20}$), moderately crowded, ventricose, up to 8 mm, with concolourous edges and lamellulae of different lengths, 1-3 for each lamellae. **Stipe** $52 \times 3\text{--}4$ mm, slightly tapering towards the apex, central, cylindrical, surface white-cream with brownish points on base, fibrous, hollow, with strigose mycelium at extreme base. **Odour, taste and context colour** not recorded.

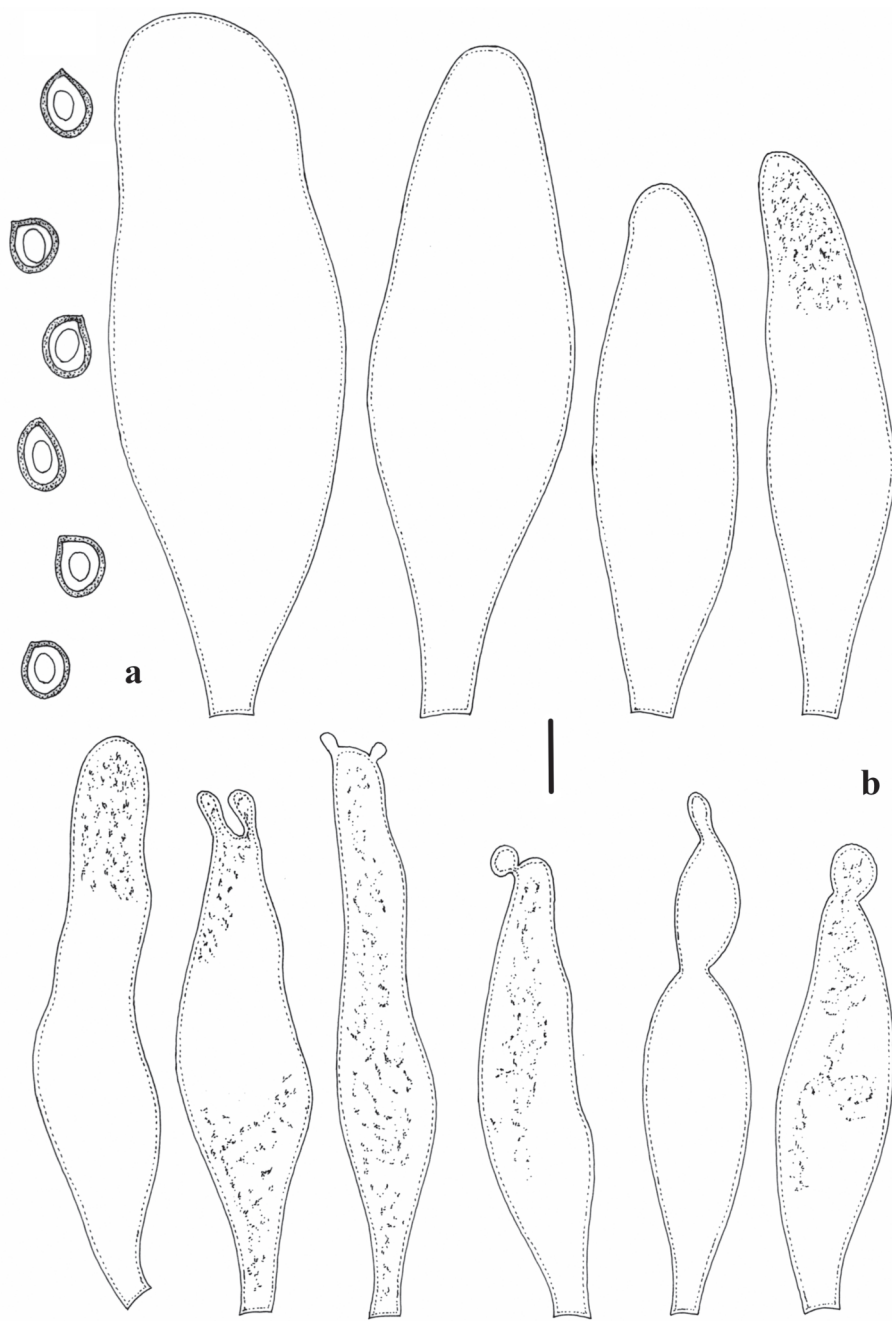


Fig. 8. *Pluteus velutinus* (FK1889). **a.** basidiospores; **b.** pleurocystidia. Scale bar: 10 μ m.

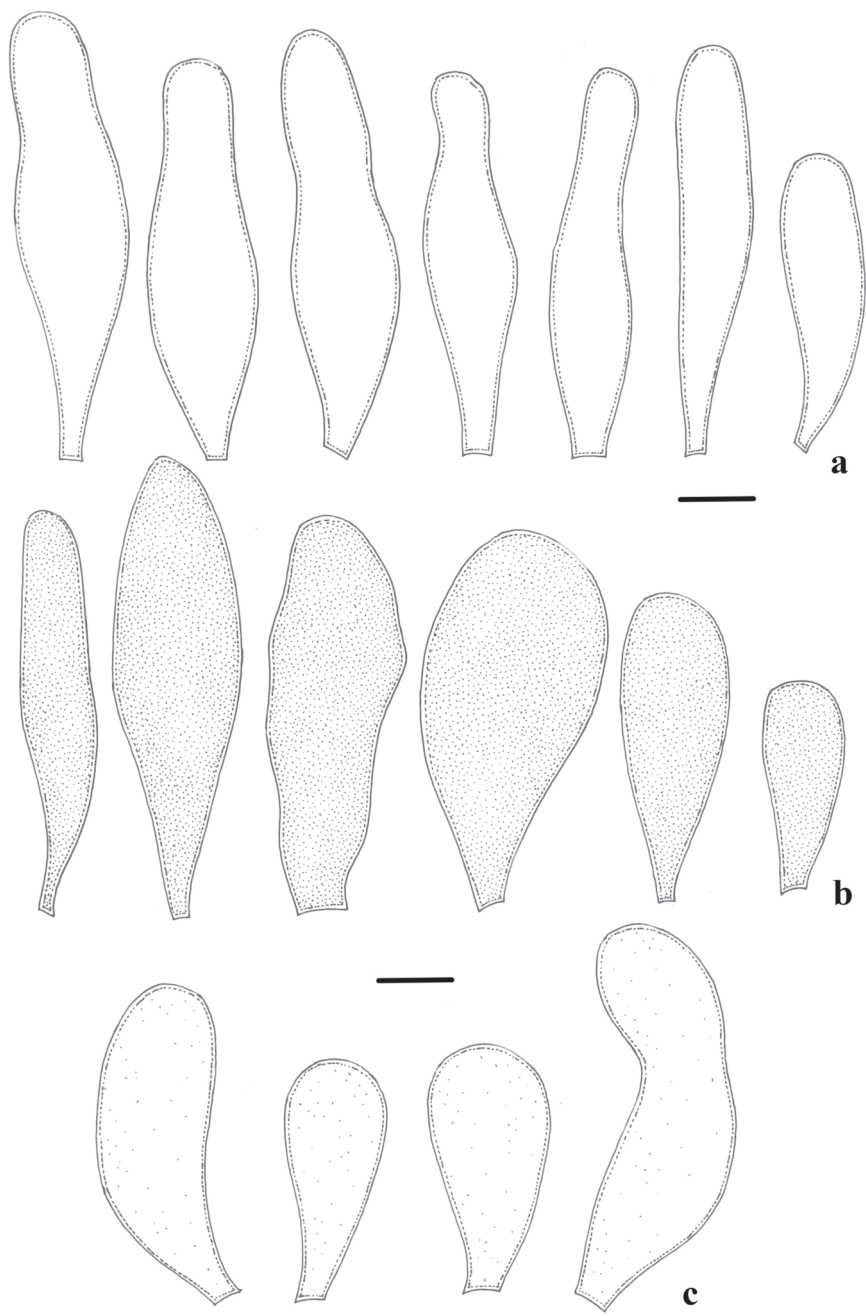


Fig. 9. *Pluteus velutinus* (FK1889). **a.** cheilocystidia; **b.** pileipellis elements; **c.** caulocystidia. Scale bars: 10 μ m.

Basidiospores [20/1/1] (6.8-)7.5-8.7 \times 5.6-6.8(-7.5) μm (Q = 1.16-1.45; Qm = 1.26; Lm = 7.9 μm ; Wm = 6.2 μm), broadly ellipsoid to ellipsoid, inamyloid, hyaline, smooth, thick-walled, guttulate. **Basidia** 20-26 \times 6.2-7.5 μm , clavate or versiform, thin-walled, four-spored, with small scattered guttules. **Pleurocystidia** 62-81(-92) \times (12.5-)15.0-18.7(-31) μm , broadly lageniform to utriform or narrowly fusiform, usually with one or two apical or lateral projections like a knob-shaped appendix, rarely with a median constriction, mostly with pale straw content, pigmentation usually concentrated at apex or sometimes evenly dissolved, rarely colourless, thin-walled, scattered and abundant. **Cheilocystidia** (38-)50-61 \times 10.0-15.0 μm , broadly utriform or rarely clavate, with a rounded apex, colourless and hyaline, thin-walled, abundant. **Lamellar edge** sterile. **Lamellar trama** inverse, up to 31 μm wide, composed of thin-walled hyphae, 2.5-16.2 μm diam., interwoven by oleiferous and inflated hyphae, hyaline. **Pileus context** undifferentiated, composed of thin-walled hyphae, 2.5-16.2 μm diam., interwoven by oleiferous and inflated hyphae, hyaline. **Pileipellis** a trichohymeniderm up to 75 μm thick, composed of narrowly clavate, clavate or vesiculose cells, (28-)41-61 \times (22-)29-34 μm , thin-walled, with brown dissolved content. **Caulocystidia** 31-51 \times 13.7-16.2 μm , mostly clavate or sometimes with a median constriction, thin-walled, with pale straw to brownish content. **Clamp connections** absent in all parts examined.

Habit and habitat. – Subgregarious (two basidiomata near), apparently on dead leaf sheath of palm tree.

Revised specimen. – BRAZIL: Pará, Belém, Ilha do Combu, 20 February 2011, F. Karstedt & C. Castro FK1889 (SP417670).

Remarks. – *Pluteus velutinus* was recently described from India with additional collections from Japan (Pradeed *et al.*, 2012) and appears in the phylogenetic analyses as sister to *P. longistriatus* (Fig. 1). The ITS sequence of *P. velutinus* from Brazil is up to 99.47% identical (three base pair differences) with the sequence of the holotype and up to 99.65% identical (two base pair differences) with the Japanese sequences.

Comparing the Brazilian collection to the Asian materials, some minor morphological differences were observed, mainly regarding the shape and size of the microstructures and the most evident difference that is the pigmentation and the apical shape of pleurocystidia. The majority pleurocystidia of the Brazilian collection has pale yellowish content and apical or lateral projections like a knob-shape, while those studied by Pradeed *et al.* (2012) are colourless and commonly with an apical digitate projection at the apex.

Also, the cheilocystidia herein studied are more slender than those described in the protologue (up to 30 μm width) and preponderantly with a rounded apex, like those illustrated from the tropical collection (holotype from India) and different from those with a small apical projection that are also characteristic for *P. velutinus*, but apparently restrict to the Japanese collections as illustrated by Pradeep *et al.* (2012). Finally, the pileipellis cells of the Brazilian collection are much smaller in length and the caulocystidia are slightly more slender than those described on the protologue that are up to 140 μm long for the pileipellis cells and up to 30 μm broad for the caulocystidia.

Despite these micromorphological differences, which in part can be also observed between Indian and Japanese collections according to illustration from Pradeep *et al.* (2012), based on molecular results (Fig. 1) it was possible to recognize them as the same species with a relatively wide range of morphological variation and mainly a considerable habitat divergence over the widespread geographic distribution.

The occurrence of *P. velutinus* in Brazil and India shows an apparent pantropical distribution for this species. However, its occurrence in a temperate area in Japan (Hokkaido) is an exception for this pattern. Data from the nearest climatological station (Iwamizawa Station – Japan Meteorological Agency) indicated that during the period of fructification (July and September 2005) of the collections studied by Pradeep *et al.* (2012), the monthly mean temperature was respectively 19.2°C (daily mean min. 15.7°C and max. 24°C) and 17.5°C (daily min. 12.5°C and max. 22.7°C), while the monthly mean relative humidity was 79 and 77%, respectively. These data show a considerable difference between the climatic conditions observed during the preceding period of collecting in Brazil. Data from a climatological station installed in the Brazilian collecting area (Ilha do Combu) indicated that during the beginning of the rainy season (mid-December 2010 and mid-January 2011) the mean temperature was 24.9°C (daily min. 22.0°C and max. 30.9°C) while the mean relative humidity was 90.62% (Freire, 2011 and pers. com.).

As previously pointed out by Pradeep *et al.* (2012), it is difficult to understand the distribution pattern of *P. velutinus* in both temperate and tropical zones with different climatic conditions and habitat characteristics and whether its dispersal is natural or anthropic. On the other hand, the coexistence of *P. velutinus* in similar but distant tropical areas such as Brazil and India could be better understood if we consider the general climatic conditions between both localities. However, the collecting area of *P. velutinus* in Brazil represent a very interesting habitat that, although it being part of a tropical region, is located in a particular area of the Amazonian forest that is extremely peculiar to fungal adaptation and fructification due to the condition of periodical inundations. This type of Amazonian forest is known as *várzea* (swamp) and it is characterized by a seasonal variation of flooding caused by white-water rivers (Prance, 1979).

The area occupied by the Ilha do Combu can be considered a specific type of *várzea* called Tidal *várzea* forest, which is flooded and drained twice daily by the tidal movements, because high tides temporarily block the flow of rivers in the estuarine region and cause them to flood towards the adjacent forest (Prance, 1979). The periodical *várzea* inundation causes deficiency of the O₂ level that affects directly the fungal development and also prevents the basidiospores discharge at ground level (Singer, 1984). In addition, according to Singer (1984), the *várzea* forest is clearly anectotrophic (free of ectomycorrhiza) what precludes an alternative mycelial survival strategy restricted to ectomycorrhizal and root parasitic fungi. So that, Singer (1984) proposed four different ways to understand how the saprophytic and parasitic basidiomycetes adapt themselves to a long and deep immersion under *várzea* conditions.

According to the period and pattern of fructification observed for *P. velutinus* from Brazil and considering the proposition of Singer (1984), probably the adaptation of *P. velutinus* is related to “*some of the litter and alluvium inhabiting, mostly smaller Agaricales form large numbers of carpophores in the relatively short period between the beginning of the rainy season in December and the time when inundation cover the ground level. It is remarkable that many of these produce ± thick-walled spores or mycelia capable of remaining viable during several months of immersion with the current lack of oxygen*”. Considering that *P. velutinus* occurs in so distant tropical areas (Brazil and India) and also that it is able to develop something like this survival strategy, maybe a similar adaptation pattern could be used to explain its occurrence in temperate conditions and lower temperatures.

Within this same adaptation pattern, Singer (1984) reported a species apparently endemic to *várzea*, which was later described (Singer, 1989) as *P. varzeicola* from Amazonas State, Brazil. *Pluteus velutinus* and *P. varzeicola*

apparently share the same adaptation pattern for resistance to *várzea* conditions and the re-examination of the holotype of *P. varzeicola* (BRAZIL: Amazonas, Ilha Marchantaria, 9 March 1983, Singer B 12397, F!) indicated that they are close in the micro-morphological features.

The holotype of *P. varzeicola* (Figs 10, 11) has broadly ellipsoid to ellipsoid or rarely subglobose basidiospores [20/1/1] $6.2\text{--}7.5(-8.1) \times 5.0\text{--}6.2(-6.8) \mu\text{m}$ ($Q = 1.10\text{--}1.50$; $Q_m = 1.30$; $L_m = 7.2 \mu\text{m}$; $W_m = 5.5 \mu\text{m}$); narrowly clavate to broadly utriform narrowly fusiform pleurocystidia, $45\text{--}62 \times 13.7\text{--}21 \mu\text{m}$, which are without any kind of projections and mostly with concentrated pale straw content or rarely colourless; clavate to vesiculose and colourless cheilocystidia, $32\text{--}57 \times 15.0\text{--}27 \mu\text{m}$; trichohymenidermic pileipellis composed of vesiculose, clavate to fusoid cells with dissolved or commonly condensed brown content, $(43\text{--})60\text{--}90(-112) \times 18.7\text{--}34 \mu\text{m}$; and clavate to fusoid caulocystidia, $44\text{--}87 \times 13.7\text{--}20 \mu\text{m}$, which are colourless and rarely with narrow papillate apex.

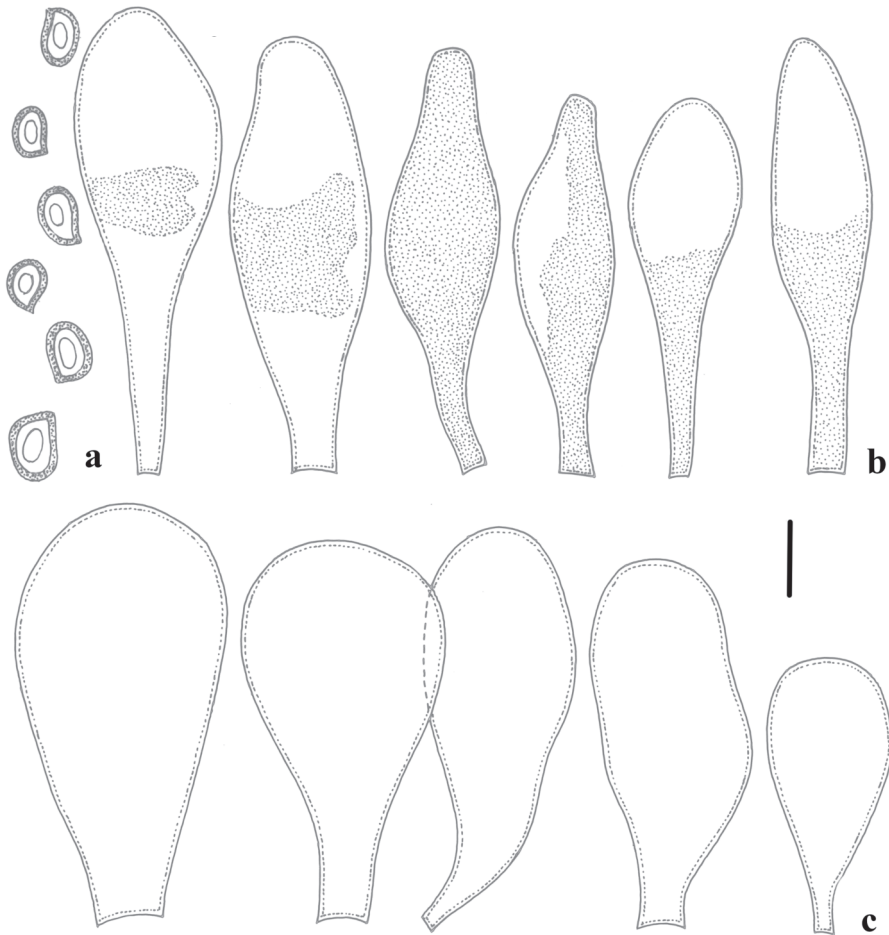


Fig. 10. *Pluteus varzeicola* (Singer B 12397 – holotype). a. basidiospores; b. pleurocystidia; c. cheilocystidia. Scale bar: 10 μm .

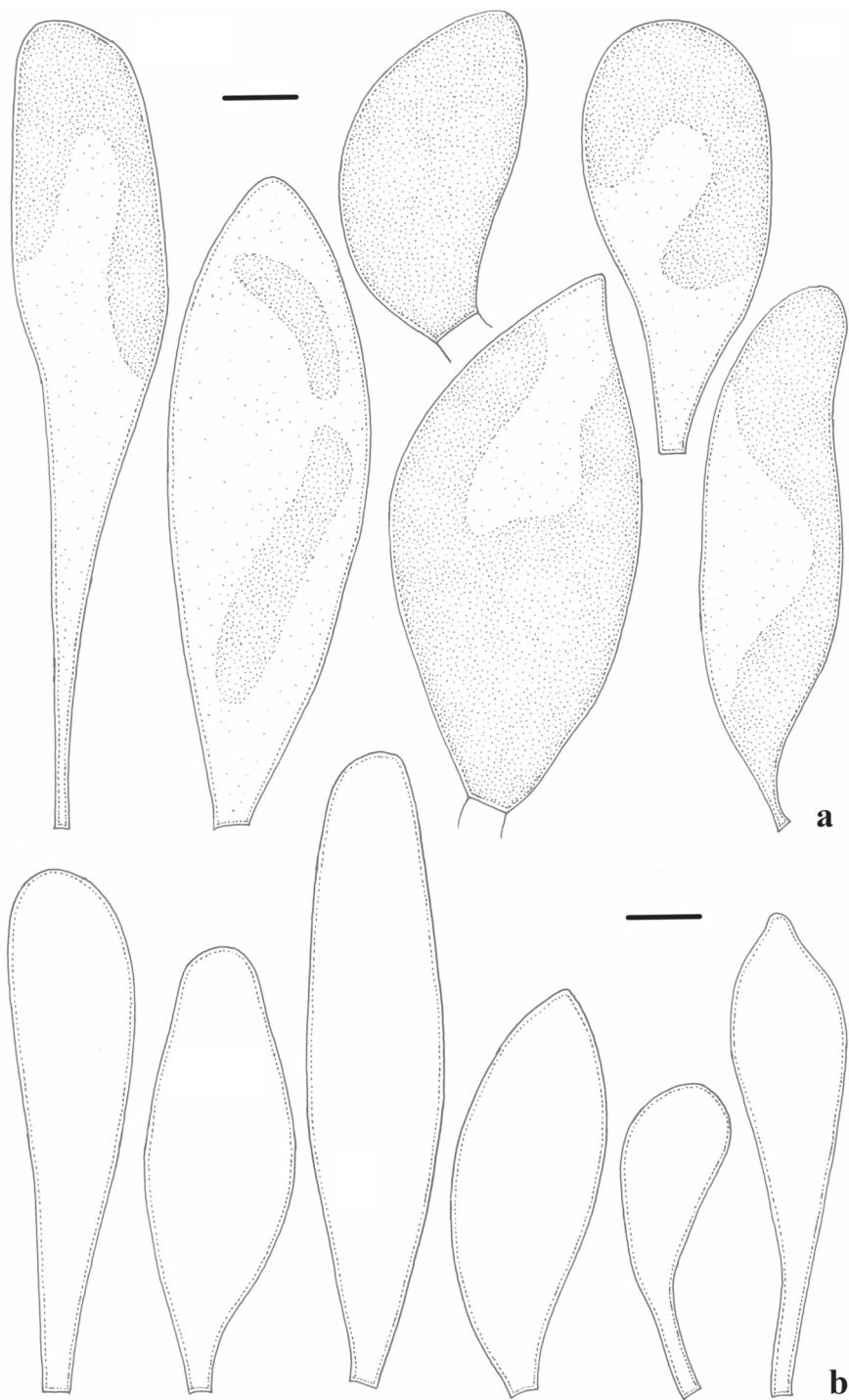


Fig. 11. *Phuteus varzeicola* (Singer B 12397 – holotype). **a.** pileipellis elements; **b.** caulocystidia. Scale bars: 10 μ m.

According to the description of Singer (1989) for *P. varzeicola* and Pradeep *et al.* (2012) for *P. velutinus*, the main morphological characters that distinguish them are regarding the macroscopic appearance of the pileus. *Pluteus varzeicola* has a pileus with a distinctly sulcate pileus margin and the slightly venose-rugulose centre, while in *P. velutinus* the margin and centre of the pileus are straight and smooth.

Unfortunately, we were not able to obtain ITS sequence from the holotype of *P. varzeicola*, but considering the data discussed above, both, *P. varzeicola* and *P. velutinus*, are considered as species certainly known from Brazil and it represent the first record of *P. velutinus* in the Neotropical region.

DISCUSSION

The species concepts of many non-Brazilian taxa are still in flux, and many names of the sequences included in the tree need to be reconsidered, but this falls outside the scope of our paper.

Based on the morphological data we recognize the occurrence of *P. fibrillosus*, *P. maculosipes*, *P. varzeicola* and *P. velutinus* in Brazil. Searches of the bibliographical and herbarium records led to a total of 19 taxa names of *Pluteus* sect. *Hispiderma* that were linked to specimens collected in Brazil (Table 1). However, including the four species previously mentioned, only eight taxa are here considered to occur with certainty in Brazil. Data of recent studies (Menolli & Capelari, 2014; Menolli *et al.*, 2015) that re-examined some Brazilian collections of *Pluteus* sect. *Hispiderma* was also considered to confirm or disregard the occurrence of certain taxa in Brazil. The record of *P. cf. fernandezianus* is maintained as uncertain determination due to the lack of macroscopic data for the specimen studied and due to the fact that a few micromorphological differences could be found between the protologue (Singer, 1959) and our collection. Additionally, the record of *P. aquosus* needs new collections to certify its existence in Brazil (Table 1). The occurrence of the other nine taxa was not confirmed because the collections associated to these names were not located, are too insufficient for study, or represent species of other sections or synonyms of other taxa.

The infrageneric classification of some species listed in Table 1 should be regarded with caution. The exact position of *P. aquosus*, *P. polycystis* and *P. sergii* is not clear to us, but based on characteristics of the pileipellis described by Singer (1956, 1959) we prefer to maintain them as belonging to *Pluteus* sect. *Hispiderma*. Nevertheless, the occurrence *P. polycystis* and *P. sergii* could not be confirmed in Brazil (Table 1). The same applies to *P. exiguus* and its variety because we considered its pileipellis structure as a palisade or a trichoderm (Vellinga & Schreurs, 1985; Orton, 1986) to classify them in *Pluteus* sect. *Hispiderma*.

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Table 1: Species of *Pluteus* sect. *Hispidoderma* recorded from Brazil and notes about the identification of the Brazilian collections

<i>Species</i>	<i>Reference</i>	<i>Notes about identification of the Brazilian collections</i>
<i>P. aquosus</i> Singer ^{a, b}	Wartchow <i>et al.</i> (2006)	too insufficient for study (pers. obs.)
<i>P. chusqueae</i> (E. Horak) Menolli ^c	Menolli <i>et al.</i> (2015)	
<i>P. exiguus</i> (Pat.) Sacc. ^a	Rick (1938, 1961)	not found (Menolli & Capelari, 2014)
<i>P. exiguus</i> var. <i>venosus</i> Rick, nom. inv. ^a	Rick (1961)	most likely <i>P. jamaicensis</i> Murrill (Menolli & Capelari, 2014)
<i>P. cf. fernandezianus</i>	this paper	see comments in Taxonomy part
<i>P. fibrillosus</i>	this paper	
<i>P. granulatus</i> Bres.	Rick (1930, 1938, 1961)	most likely <i>P. glaucotinctus</i> (Menolli & Capelari, 2014)
<i>P. haywardii</i> Singer ^b	Pegler (1997)	too insufficient for study (pers. obs.)
	Rosa & Capelari (2009)	too insufficient for study (pers. obs.)
<i>P. cf. haywardii</i>	Meijer (2006)	not found (Menolli <i>et al.</i> , 2015)
<i>P. leoninus</i> (Schaeff.) P. Kumm.	Rick (1938, 1961)	most likely <i>P. conizatus</i> (Berk. & Broome) Sacc. (Menolli & Capelari, 2014)
<i>P. longistriatus</i>	Menolli & Capelari (2010), Justo <i>et al.</i> (2011a, b), this paper (Fig. 1)	
<i>P. maculosipes</i>	this paper	
<i>P. neochrysaegis</i> Menolli & de Meijer ^c	Menolli <i>et al.</i> (2015)	
<i>P. cf. polycystis</i> Singer ^{a, b}	Meijer (2006)	not found / too insufficient for study (Menolli <i>et al.</i> , 2015)
<i>P. rimosellus</i> Singer ^c	Singer (1954), Menolli <i>et al.</i> (2015)	
<i>P. sergii</i> Singer ^a	Pegler (1997)	<i>P. albstipitatus</i> (pers. obs.)
<i>P. subfibrillosus</i> Singer	Singer (1956, 1959)	synonym of <i>P. rimosellus</i> (Menolli <i>et al.</i> , 2015)
<i>P. umbrosus</i> (Pers.) P. Kumm.	Rick (1919, 1938, 1961)	most likely <i>P. angustisporus</i> Singer (Menolli & Capelari, 2014)
<i>P. varzeicola</i> Singer	Singer (1989), this paper	
<i>P. velutinus</i>	this paper	
<i>Pluteus</i> sp.	this paper (Fig. 1)	too insufficient for study (NMJ193) ^d

Names in bold are considered species certainly known in Brazil. ^aSee discussion on text about the infrageneric classification of these species. ^bSpecies that need new collections to certify their existence in Brazil. ^cSpecies not studied in this work but that are considered as occurring in Brazil due to the well-preserved collections and the complete description presented by the authors referenced. ^dCollection too insufficient for morphological identification, but included in the current molecular analyses.

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