



Taxonomy and phylogeny of *Pluteus glaucotinctus* sensu lato (Agaricales, Basidiomycota), a multicontinental species complex

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Abstract

In order to better understand species delimitation in the *Pluteus glaucotinctus* species complex, we present a detailed study based on morphological and DNA sequence (nrITS + *tefl*) data. *Pluteus glaucotinctus* sensu stricto is known only from the type collection (Democratic Republic of the Congo), which is re-examined here. Four other species are recognized in this complex and described herein as new: *P. izurun* from Spain, *P. meridionalis* from Brazil, *P. padanilus* from India and *P. thoenii* from Africa. Phylogenetic analyses based on nrITS + *tefl* sequence data support the recognition of all species in this complex.

Key words: nrITS, Pluteaceae, section *Pluteus*, species delimitation, *tefl*

Introduction

The genus *Pluteus* Fr. (Pluteaceae, Agaricales) comprises ca. 300 species with a circumglobal distribution (Kirk *et al.* 2008) and is very common in forested areas from boreal to tropical regions. The genus is found primarily in association with well-rotted wood and it is characterized by free lamellae, absence of a volva, pinkish spore print, inamyloid basidiospores and inverse hymenophoral trama (Singer 1986). Recent molecular and morphological studies (Minnis *et al.* 2006; Corriol & Moreau 2007; Menolli *et al.* 2010; Vizzini & Ercole 2011; Justo *et al.* 2011a, b) found *Pluteus* to include some annulate species previously placed in the genus *Chaemaeota* (W.G. Sm.) Earle and also have typically supported the traditional infrageneric subdivision into three sections: *Pluteus*, *Celluloderma* Fayod and *Hispidoderma* Fayod.

According to the infrageneric classification of *Pluteus* proposed by Singer (1959, 1986) and as clarified by DNA sequence data (Justo *et al.* 2011a, b), *Pluteus* sect. *Pluteus* includes species with a pileipellis as a cutis, with metuloid pleurocystidia, as well as species with indistinct metuloids [*Pluteus albstipitatus* (Dennis) Singer] or with non-metuloid pleurocystidia (*Pluteus glaucotinctus*).

Justo *et al.* (2011b) recognized sixteen well-supported clades in *Pluteus* sect. *Pluteus*, most of which are represented by species complexes with apparent intercontinental distributions. Many of these have been investigated (Justo *et al.* 2014) and others require further molecular and morphological studies to better understand species delimitation, diversity, and biogeography in the genus. In the present article we critically examine the widespread species complex around *P. glaucotinctus* using morphological approaches and molecular phylogenies based on two loci, the nuclear ribosomal internal transcribed spacers region (nrITS) and translation-elongation factor 1-alpha (*tefl* a single-copy, protein-coding gene).

Materials and Methods

Taxon sampling and morphological studies:—The studied collections include the materials described from Africa (Democratic Republic of the Congo) by Horak (1977) and Horak & Heinemann (1978) as *P. glaucotinctus* and other morphologically similar specimens collected in Asia (India), Europe (Spain) and South America (Brazil).

Color terms are used according to Küppers (1979) for *P. meridionalis* and Munsell Color (2009) for *P. izurun* and *P. padanilus*. The macroscopic description presented for *P. glaucotinctus* was translated and adopted from the protologue published by Horak (1977). For microscopic analyses, the dried material was wetted with 70% ethanol and then rehydrated in 5% KOH or stained with Melzer's reagent. The notation [a/b/c] at the beginning of the 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 quotient for all the measured basidiospores, Qm represents the average of all computed Q values for all the measured basidiospores and Lm (Wm) represents the average of all lengths (widths) of the measured basidiospores. At least 20 basidiospores from each basidioma were measured in profile view. Basidiospore shape terminology follows Bas (1969). Generic and infrageneric concepts follow Singer (1986) as complemented by Justo *et al.* (2011a, b). Herbarium acronyms follow Thiers (2014, continuously updated).

Molecular methods and phylogenetic analyses:—Molecular datasets were composed of existing DNA sequences used by Justo *et al.* (2011a, b, 2014) and from newly generated sequences including those of materials representing at least one collection of each taxa discussed herein. Methods for DNA isolation, PCR and sequencing follow Justo *et al.* (2011a, b). The ITS region was amplified using the primer pair ITS1-F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993). The *tefl* gene was amplified using the primer pair *efl*-983F and *efl*-1567R (Rehner & Buckley 2005). Newly obtained sequences were deposited in GenBank. Molecular analyses were conducted using sequences of taxa from *Pluteus* sect. *Pluteus*, including those from the *salicinus/albostipitatus* clade, and the tree was rooted using sequences of the *cervinus/petasatus* clade as outgroup (clade names follow Justo *et al.* 2011a). GenBank accession numbers and other collection information for taxa outside of the *P. glaucotinctus* complex are given in Fig. 1. Both nrITS and *tefl* sequences were aligned using MAFFT version 6 (<http://mafft.cbrc.jp/alignment/server/>; Katoh & Toh 2008) with the Q-INS-I option. The alignment was then visually examined and manually corrected using MacClade 4.05 (Maddison & Maddison 2002). The alignment has been deposited in TreeBASE (S13390).

Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Analysis (BA) were performed with the parameters specified in Justo *et al.* (2011b). The following abbreviations are used: Most Parsimonious Trees (MPT), Bootstrap (BS) and Posterior Probability (PP). All analyses were performed on the individual nrITS and *tefl* datasets and also on a combined dataset

Results

Phylogeny

The internal topology of the *P. glaucotinctus* complex and the relationship among the recognized species is not well resolved because different rearrangements were verified in the individual (nrITS and *tefl*) and combined (nrITS + *tefl*) dataset. However, the recognition of all studied species is supported by the molecular phylogenetic reconstructions, which can be better evidenced on the combined analyses (Fig. 1–I) with *P. meridionalis* forming a well-supported clade (100% BS in ML and MP) clustered to a sister-clade including *P. thoenii*, *P. glaucotinctus*, *P. padanilus* and *P. izurun*, but that does not receive statistical support. The topology of the *tefl* tree (Fig. 1–III) is basically the same of that from the combined analyses, except for the absence of the *P. glaucotinctus* sample (from which a *tefl* sequence was not obtained) and by the support (> 80% BS in both MP and ML, and 0.99 PP in BA) of the clade including *P. thoenii*, *P. padanilus* and *P. izurun*. The most notable differences with respect to the nrITS (Fig. 1–II) and the combined analyses are regarding topology that includes *P. padanilus*, *P. meridionalis* and *P. izurun* in a well-supported clade (> 70% BS in both MP and ML) and *P. glaucotinctus* and *P. thoenii* alternatively in a non-supported sister-clade.

Combined dataset:—The dataset includes 39 isolates in the *salicinus/albostipitatus* clade (9 in the *glaucotinctus* complex) and two outgroup taxa [*P. cervinus* (Schaeff.: Fr.) P. Kumm. and *P. hongoi* Singer]. The dataset consists of 1231 characters (gaps included): 867 constant, 35 parsimony uninformative and 329 parsimony informative. In the MP analyses 1394 MPT were recovered (Length = 656; CI = 0.75, RI = 0.94).

The best tree from the ML analysis is depicted in Fig. 1 (I). The overall topologies of the MP, ML and BA trees, with respect to the taxa in the *glaucoinctus* complex, are essentially the same.

nrITS dataset:—The dataset includes 39 isolates in the *salicinus/albostipitatus* clade (9 in the *glaucoinctus* complex) and two outgroup taxa (*P. cervinus* and *P. hongoi*). The dataset consists of 655 characters (gaps included): 470 constant, 18 parsimony uninformative 167 parsimony informative. In the MP analyses 1091 MPT were recovered (Length = 334; CI = 0.71, RI = 0.93).

The relationships of the taxa in the *P. glaucoinctus* complex, as recovered in the ML analysis, are depicted in Fig. 1 (II). The overall topologies of the MP, ML and BA trees, with respect to the taxa in the *glaucoinctus* complex, are essentially the same.

tef1 dataset:—The dataset includes 31 isolates in the *salicinus/albostipitatus* clade (8 in the *glaucoinctus* complex) and two outgroup taxa (*P. cervinus* and *P. hongoi*). The dataset consists of 576 characters (gaps included): 397 constant, 17 parsimony uninformative 162 parsimony informative. In the MP analyses 615 MPT were recovered (Length = 312; CI = 0.81, RI = 0.95).

The relationships of the taxa in the *P. glaucoinctus* complex, as recovered in the ML analysis, are depicted in Fig. 1 (III). The overall topologies of the MP, ML and BA trees, with respect to the taxa in the *glaucoinctus* complex, are essentially the same.

Taxonomy

Pluteus glaucoinctus E. Horak, *Bull. Jard. Bot. Belg.* 47: 88, 1977 (Fig. 2)

Type description:—*Pileus* up to 80 mm wide, convex, broadly umbonate; surface fibrillose or glabrous, near gray or pale brown to tan, apex turning bluish gray when young. *Lamellae* free; pinkish. *Stipe* up to 120 × 8 mm, cylindrical; surface fibrillose, concolorous with the pileus above and bluish gray or greenish blue towards the base. *Odor, taste* none. *Basidiospores* [30/1/1] 6.5–9.0 × 5.0–6.5 μm (Q = 1.15–1.55; Qm = 1.33; Lm = 7.6 μm; Wm = 5.7 μm), broadly ellipsoid to ellipsoid, inamyloid, hyaline, smooth, thick-walled. *Basidia* not observed. *Pleurocystidia* 50–70 × 15.0–22 μm; lageniform with rounded to truncate apices, colorless, thin-walled; scattered to relatively common. *Lamellar edge* sterile. *Cheilocystidia* 45–61 × 12.0–17.0 μm; lageniform with rounded or sometimes with truncate apices, colorless or (a few) with pale brown intracellular pigment, thin-walled; crowded and forming a well-developed strip. *Pileipellis* a cutis; individual terminal elements 70–90 × 12.0–17.0 μm, cylindrical or fusiform, colorless or with brown intracellular pigment, thin-walled. *Stipitipellis* a cutis; hyphae 5.0–12.0 μm wide; colorless, thin-walled. *Clamp connections* not observed.

Distribution:—Africa: Known only from Democratic Republic of the Congo.

Specimen examined:—DEMOCRATIC REPUBLIC OF THE CONGO. Lake Edward and Kivu District: Panzi, May 1953, *Goossens-Fontana* 5274 (Holotype BR!; nrITS: HM562131).

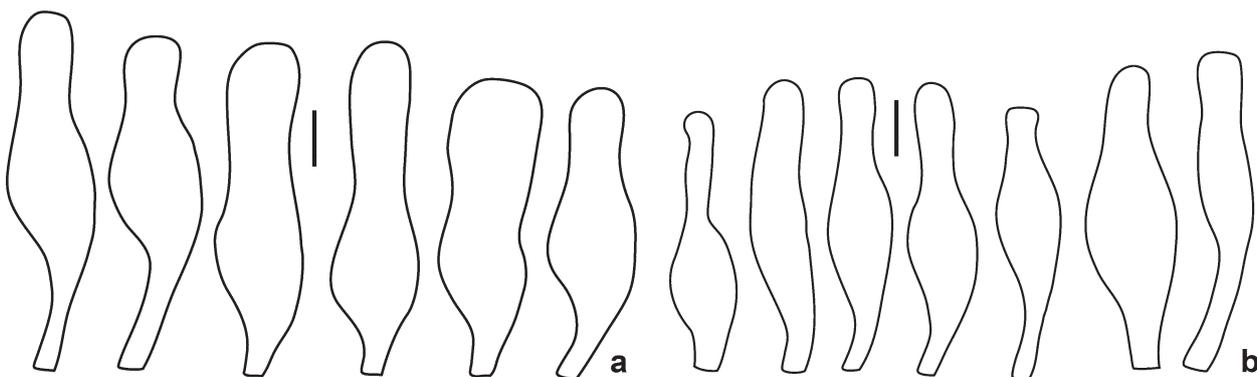


FIGURE 2. *Pluteus glaucoinctus* (holotype). a. Pleurocystidia. b. Cheilocystidia. Scale bars: 10 μm. Line drawings a–b by A. Justo.

Notes:—*Pluteus glaucoinctus* was described originally based on one African collection (Horak 1977). A second collection was added in a subsequent description of the species (Horak & Heinemann 1978). The nrITS sequence from the holotype (*Goossens-Fontana* 5274) is only 95 to 96 % identical with all other sequences in this species complex, including the other African collection studied by us (*Thoen* 5546). We could not obtain a *tef1*

sequence from the holotype, but based on nrITS data we consider the holotype to be the only collection to represent *P. glaucotinctus* sensu stricto.

Morphologically, the holotype collection is characterized by the broadly ellipsoid to ellipsoid basidiospores ($Q_m = 1.33$), lageniform pleuro- and cheilocystidia with rounded to truncate apices, and the presence of pale brown intracellular pigment in some cheilocystidia. All of these characters should be checked in future collections to evaluate their reliability for morphological identification. Cheilocystidial pigmentation has been shown to be a variable character in other species of *Pluteus*, e.g., *P. eludens* E.F. Malysheva, Minnis & Justo (Justo *et al.* 2011c) and *P. phlebophorus* (Ditmar) P. Kumm. (Justo *et al.* 2011b), and, thus, it is not reliable for species rank identification.

Pluteus izurun P. Arrill. & Justo, *sp. nov.* (Fig. 3)

Mycobank: MB809614

Diagnosis:—Similar to *P. meridionalis* but differing in the *tefl* sequence (KJ010047), presence of distinct blue-green tinges at stipe base, shape of basidiospores, pleurocystidial apex, and length of the pileipellis elements.

Type:—SPAIN. Guipúzcoa: Donostia/San Sebastián, 18 August 2011, leg. *P. Arrillaga s.n.* (Holotype ARAN-Fungi 3008424-1!; nrITS: JQ065023; *tefl*: KJ010047).

Pileus 20–52 mm in diam., campanulate when young, expanding to convex or plano-convex, with a broad obtuse umbo that becomes depressed at center with age; surface of young specimens white and smooth except at center, which is set with olivaceous (2.5Y 6/6, 6/8, 7/6, 7/8) to dark gray or bluish gray (in the range of Gley2 4/1, 5/1, 6/1) squamules and fibrils, becoming entirely fibrillose in older specimens; dry or slightly viscid when moist, slightly hygrophanous; margin translucent-striate up to 1/3 of the pileus. *Lamellae* crowded, free, ventricose, up to 7 mm broad; white when young, later pink (7.5R 8/4); with white, flocculose edges. *Stipe* 35–70 × 2–4 mm, cylindrical, with a slightly broadened base; surface white, with distinct blue or blue-green tinges at base, smooth or with longitudinal gray-brown fibrils especially near the base. *Context* in stipe and pileus white, in some places with slight blue-green tinges. *Odor* indistinct. *Taste* indistinct or slightly sweet. *Spore print* pinkish-brown (2.5YR 7/6–7/8). *Basidiospores* [84/2/2] (6.0–)6.5–9.0(–9.5) × (5.0–)5.3–7.0(–7.3) μm ($Q = 1.12–1.42$; $Q_m = 1.24$; $L_m = 7.7$ μm; $W_m = 6.2$ μm), broadly ellipsoid to ellipsoid, rarely subglobose, inamyloid, hyaline, smooth, thick-walled. *Basidia* 23–36 × 8.0–18 μm, four-spored, clavate or narrowly utriform. *Pleurocystidia* (50–)55–85 × 13.0–28 μm; ovoid, conical or narrowly utriform with rounded apices, colorless, thin-walled; scattered to relatively common all over lamellar sides. *Lamellar edge* sterile. *Cheilocystidia* 35–60(–70) × 12.0–25 μm; narrowly lageniform to narrowly utriform, sometimes mucronate, apices usually with a short and narrow papilla, colorless, thin-walled; crowded, forming a well-developed strip. *Pileipellis* a cutis; individual terminal elements 55–225(–380) × 5.0–18.0(–23) μm, cylindrical or fusiform, colorless or with blue-green intracellular pigment in fresh specimens, turning brown on drying, additional parietal pigment sometimes present; thin-walled. *Stipitipellis* a cutis; hyphae 3.5–7.5(–10.0) μm wide; apex with distinct terminal elements 75–138 × 12.0–16.0 μm; narrowly clavate to fusiform, colorless or with flexuous hyphae filled with blue-green or greyish pigment at base, thin-walled. *Clamp connections* absent.

Etymology:—*izurun* is the earliest recorded name given to the place that would later become Donostia/San Sebastián. We choose to use the more euphonic noun form “izurun” instead of the adjectival “izurunensis”.

Habit, habitat and distribution:—Gregarious, in pairs or subgregarious up to three basidiomata. A total of 10 basidiomata was collected in four independent consecutive fruitings on the same well-decayed (brown-rot) log of *Quercus* sp. between mid-August and mid-September in an urban park. Europe: Known only from Northern Spain.

Additional specimens examined:—SPAIN. Guipúzcoa: Donostia/San Sebastián, 1 September 2011, leg. *P. Arrillaga s.n.* (ARAN-Fungi 3008424-2!; nrITS: JQ065024, *tefl*: KJ010048); *ibid.* 3 September 2011, leg. *P. Arrillaga s.n.* (ARAN-Fungi 3008424-3!); *ibid.* 11 September 2011, leg. *P. Arrillaga s.n.* (ARAN-Fungi 3008424-4!).

Notes:—*Pluteus izurun* is characterized by a stipe with distinct blue or blue-green tinges at base, broadly ellipsoid to ellipsoid or rarely subglobose basidiospores ($Q_m = 1.24$), pleurocystidia with rounded apices, cheilocystidia usually with a short and narrow papilla at the apices, and long pileipellis elements (up to 380 μm long). *Pluteus glaucotinctus* differs in the slightly more elongate basidiospores ($Q_m = 1.33$), the slightly pigmented cheilocystidia with no apical appendage, and in the significantly shorter pileipellis elements (up to 90 μm long). *Pluteus meridionalis* lacks distinct blue-green tinges on the stipe, has more elongate basidiospores ($Q_m = 1.40$), and their pleurocystidia have a truncate apex or minor apical and lateral projections. *Pluteus padanilus* differs in the pleurocystidia that have truncate to subcapitate apices or indistinct 2–3 short apical projections and that sometimes are provided with internal septa and/or incrusting parietal pigment at the apex. *Pluteus thoenii* has predominantly subglobose basidiospores ($Q_m = 1.15$) and longer cheilocystidia (up to 94 μm).

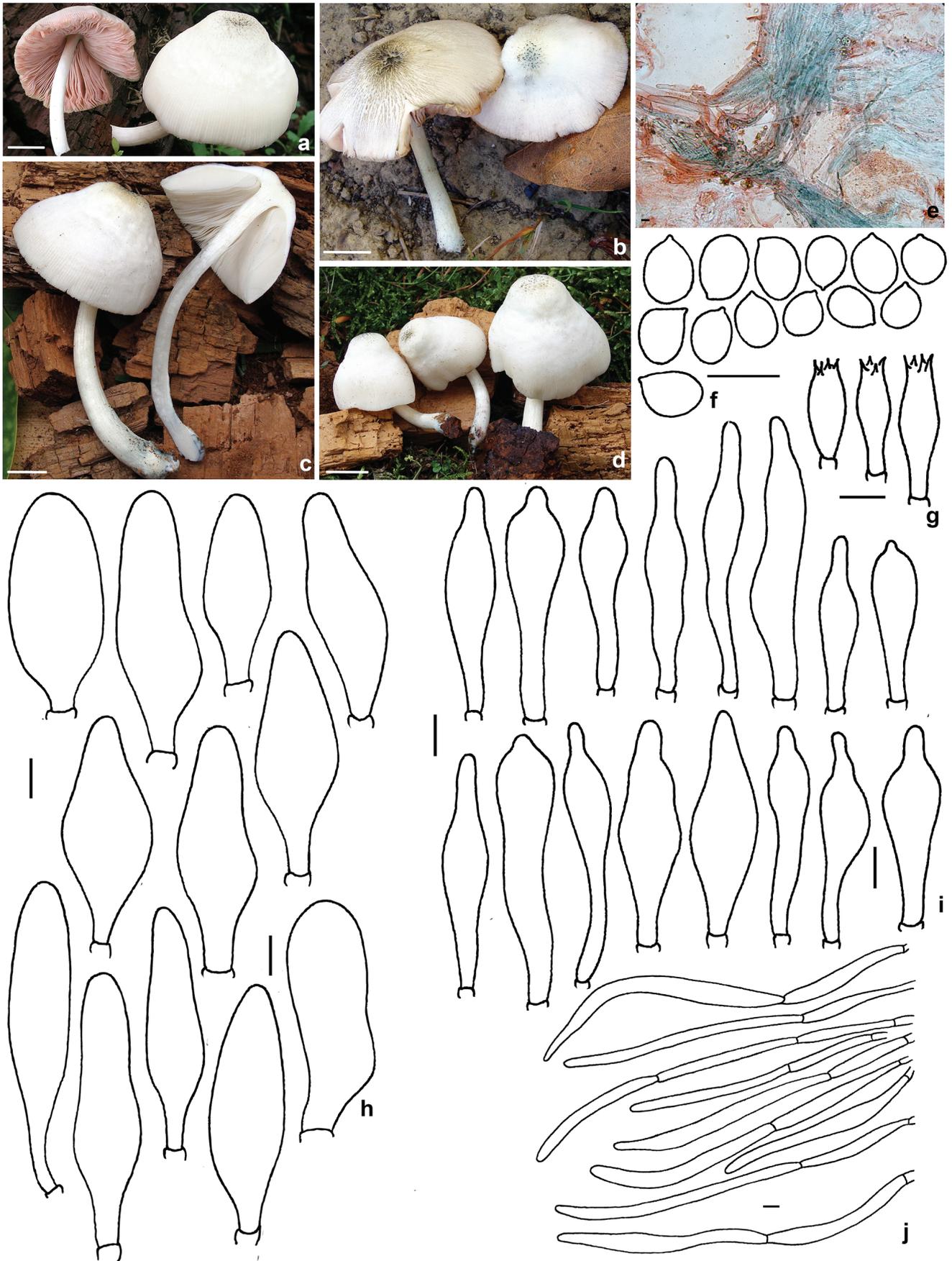


FIGURE 3. *Pluteus izurun*. a–d. Basidiomata. e. Pileipellis elements in Congo Red. f. Basidiospores. g. Basidia. h. Pleurocystidia. i. Cheilocystidia. j. Pileipellis elements. a. ARAN-Fungi 3008424-3. b. ARAN-Fungi 3008424-4. c, e, f–j. ARAN-Fungi 3008424-1 (holotype). d. ARAN-Fungi 3008424-2. Scale bars: a–d = 1 cm. e–j = 10 μ m. Photos a–e by P. Arrillaga, line drawings f–j by A. Justo.

Considering the tropical distribution of *P. glaucotinctus*, *P. meridionalis*, *P. padanilus* and *P. thoenii*, the occurrence of *P. izurun* in the Iberian Peninsula seems to be exceptional. When compared to these species, the fruiting pattern of *P. izurun* in Spain could be explained tentatively by the climatological condition during the collecting period. Data from the nearest climatologic station (Oiartzun Observatory, located less than 10 km away from the collecting site) indicate that between mid-August and mid-September 2011 the daily average temperatures were between 17°C and 26°C and the relative humidity was between 70% and 97%, which are similar to the conditions found in tropical areas.

The habitat observed in *P. izurun* is also worthy of comment. The fruiting on a brown-rotted log is particularly noteworthy as the habitat is atypical for *Pluteus*, which is usually found on white-rotted wood or more rarely on other organic debris. Wood that has undergone brown-rot decay is deprived of most of its cellulosic components while the lignin remains mostly intact, thus providing limited nutrients for fungal species (Yelle *et al.* 2008; Martinez *et al.* 2009). While it is very unlikely that *P. izurun* was responsible for the brown-rot decay observed in the substrate, it is interesting that this fungus is not only able to survive in such a nutrient-poor environment but that it is also able to produce basidiomata many times in less than a month. No other fungal basidiomata were observed on the same log where *P. izurun* was growing, but that does not exclude the fact that other fungi were present at the same time or before the arrival of *P. izurun*.

Pluteus meridionalis Menolli & Capelari, *sp. nov.* (Fig. 4)

Mycobank: MB809615

Diagnosis:—Similar to *P. izurun* but differing in the *tefl* sequence (KJ010052), absence of distinctly blue-green tinges at stipe base, narrower basidiospores, pleurocystidial apex, and shorter pileipellis elements.

Type:—BRAZIL. São Paulo: São Paulo, Parque Estadual das Fontes do Ipiranga, 2 December 2008, *M. Capelari & U.C. Peixoto MC4412* (Holotype SP!; nrITS: HM562157; *tefl*: KJ010052).

Pileus 35–65 mm in diam., conic when young, expanding to plane, and becoming slightly depressed at center; surface apparently smooth but slightly fibrillose under lens when young, becoming entirely fibrillose to densely fibrillose, fibrils more concentrated at center, with olive green to greenish gray ($N_{80}A_{00}M_{40}$) shades, becoming paler outward, whitish at least one-half the radius towards margin in young specimens, sometimes developing reddish brown tones in age; dry to viscid when moist, slightly hygrophanous; margin translucent-striate mainly on insertion points of the lamellae. *Lamellae* crowded, free; white-cream when young, later pinkish. *Stipe* 30–75 × 4–10 mm, cylindrical or compressed (but probably due to development on the substrate), with slightly broad base; surface longitudinally striate, white to pale cream with light brown to slightly greenish punctations or longitudinal fibrils at base, lacking any blue-green tinges; with scanty basal mycelium. *Context*, *odor*, *taste* and *spore print color* not recorded. *Basidiospores* [80/4/4] (6.2–)7.5–10.0 × 5.0–6.2(–7.5) μm ($Q = 1.21–1.74$; $Q_m = 1.40$; $L_m = 8.1$ μm; $W_m = 5.9$ μm), broadly ellipsoid to elongate, inamyloid, hyaline, smooth, thick-walled. *Basidia* 17.5–31 × 6.2–11.2 μm, four-spored, clavate or narrowly utriform. *Pleurocystidia* (31–)40–65(–75) × (8.7–)10.0–21(–25) μm; narrowly lageniform, narrowly utriform or fusoid, rarely clavate, with a rounded apex or some with an almost truncate apex with minor apical and lateral projections, colorless, thin-walled; scattered, rare to relatively common. *Lamellar edge* sterile. *Cheilocystidia* 37–67 × 6.2–20 μm; narrowly lageniform to narrowly utriform, sometimes clavate, fusiform or mucronate, apices usually with a short and narrow papilla, colorless, thin-walled; crowded, forming a well-developed strip. *Pileipellis* a cutis; individual terminal elements 98–140 × 8.0–16.0 μm; cylindrical or fusiform; mostly with evenly dissolved brownish intracellular pigment; thin-walled. *Stipitipellis* a cutis; hyphae 6.2–33 μm wide; cylindrical; colorless or with intracellular brown pigment; thin-walled. *Clamp connections* absent.

Etymology:—*meridionalis*, belonging to the south, in this case the southeast Brazil. *Pluteus meridionalis* is also the species with the most meridional distribution in this complex.

Habit, habitat and distribution:—Gregarious or in pairs. On dead wood or on standing trees with fruitbodies of polyporoid fungi. October to February. South America: Known only from southeast Brazil.

Additional specimens examined:—BRAZIL. São Paulo: Santo André, Reserva Biológica do Alto da Serra de Paranapiacaba, 21 October 1990, *L.K. Okino & A.M. Gugliotta 3630* (SP! as *P. atriavellaneus* Murrill); São Paulo, Parque Estadual da Cantareira, 19 February 2008, *F. Karstedt et al. FK1084* (SP!; nrITS: KJ009767; *tefl*: KJ010054); *ibid.* Parque Estadual das Fontes do Ipiranga, 27 October 2009, *L.A.S. Ramos LASR64* (SP!; nrITS: HM562147, *tefl*: KJ010052).

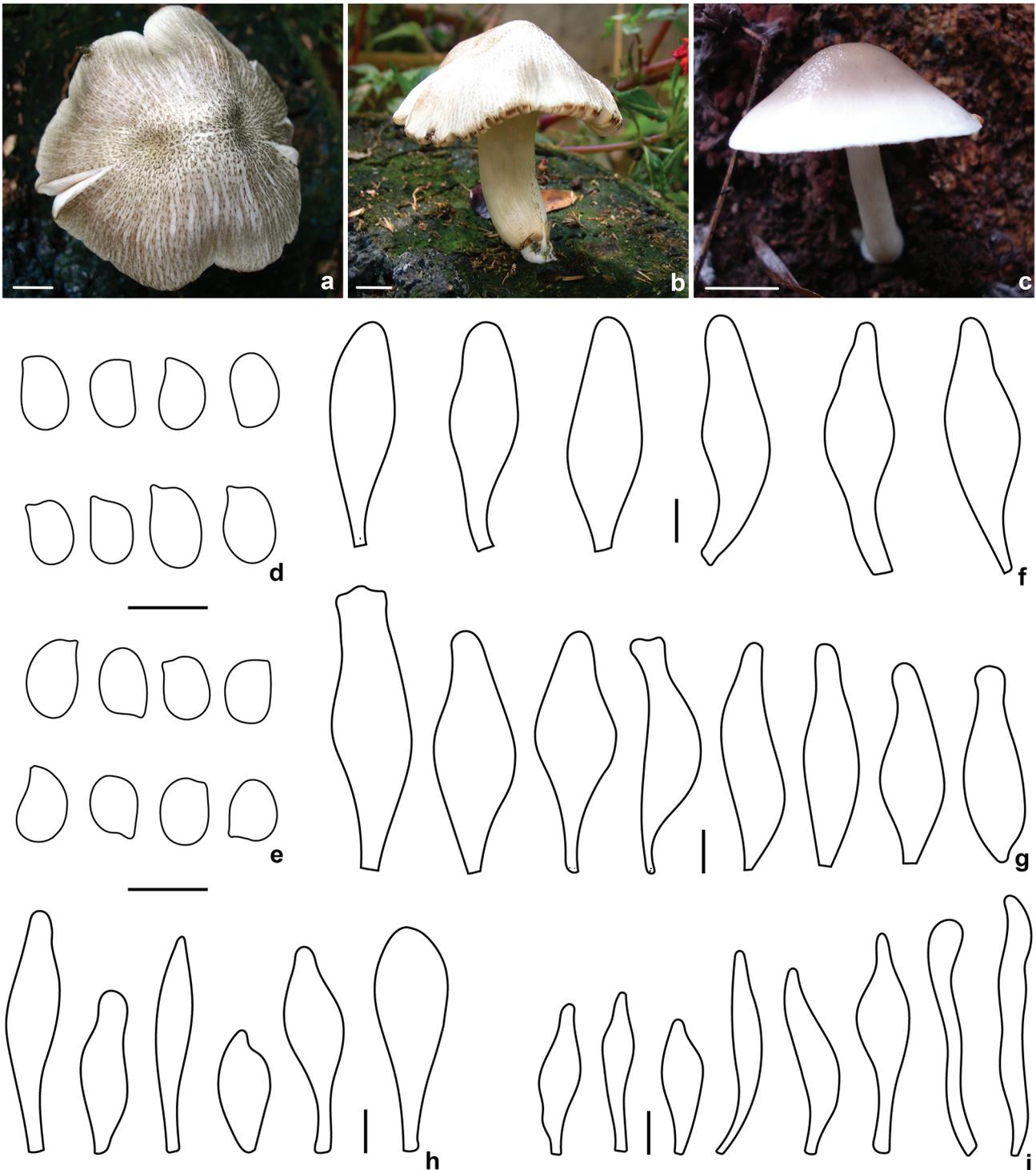


FIGURE 4. *Pluteus meridionalis*. a–c. Basidiomata. d, e. Basidiospores. f, g. Pleurocystidia. h, i. Cheilocystidia. a, b, d, f, h. *MC4412* (holotype). c, e, g, i. *LASR64*. Scale bars: a–c = 1 cm. d–i = 10 μ m. Photos a–c by M. Capelari, line drawings d–i by N. Menolli Jr. and Luiz Antonio S. Ramos.

Notes:—*Pluteus meridionalis* is characterized by broadly ellipsoid to elongate basidiospores ($Q_m = 1.40$), pleurocystidia with truncate apices and minor apical or lateral projections, and cheilocystidia usually with a short and narrow apical papilla. Besides the difference in geographical distribution, *P. meridionalis* can be separated from the other species in this complex by the comparatively more elongated basidiospores and the absence of distinctly blue-green tinges at the stipe base. *Pluteus glaucotinctus* lacks any apical or lateral projections on the pleurocystidia, their cheilocystidia do not have an apical papilla, and it has shorter pileipellis elements (up to 90 μ m long). *Pluteus izurun* has pleurocystidia with rounded apices and longer pileipellis elements. *Pluteus padanilus* has incrusting parietal

pigment at the apices of some pleuro- and cheilocystidia. *Pluteus thoenii* has predominantly subglobose basidiospores ($Q_m = 1.15$), longer cheilocystidia (up to 94 μm long) and pleuro- and cheilocystidia with a different shaped apex. The occurrence of one of the Brazilian specimens (*LASR64*) on a standing tree (apparently a living tree) also is an anomalous habit for *Pluteus*. However, the presence of a polyporoid fungus likely indicates that this species was responsible for the death of the plant tissue instead of *P. meridionalis*.

Although the presence of distinctly blue-green tinges was not observed in *P. meridionalis*, the occurrence of slightly greenish punctations or longitudinal fibrils at stipe base could correlate to the presence of psilocybin, which has been recorded from other taxa in this clade. Besides *P. glaucotinctus*, *P. izurun*, *P. padanilus*, and *P. thoenii* (considering the macro-morphological data described for *P. glaucotinctus* by Horak & Heinemann 1978) and among the species sampled in the *salicinus/albostipitatus* clade, the presence of blue-green tinges or psilocybin has been previously reported for *P. salicinus* (Pers.: Fr.) P. Kumm. (Saupe 1981; Stijve & Bonnard 1986; Justo *et al.* 2014), *P. americanus* (P. Banerjee & Sundb.) Justo, E.F. Malysheva & Minnis (Justo *et al.* 2014) and *P. saupei* Justo & Minnis (Saupe 1981; Justo *et al.* 2011b; Justo *et al.* 2014). Additionally, *P. nigrolineatus* Murrill is also frequently reported with blue-green pigments at the stipe base (Singer 1962; 1969; Rodríguez & Guzmán-Dávalos 2001) and recently Menolli *et al.* (2010) showed by molecular data that this species is also positioned with other ones of the *salicinus/albostipitatus* clade. Stijve & de Meijer (1993) also recorded psilocybin for materials previously identified as *P. glaucus* Singer, but which most likely represent *P. glaucotinctus* sensu lato (Menolli *et al.* 2014—see comments under *P. padanilus*).

Considering these records, the presence of blue-green tinges or psilocybin within *Pluteus* sect. *Pluteus* seems to be restricted to species of the *salicinus/albostipitatus* clade, although blue-green tinges were not reported for *P. sepiicolor* E.F. Malysheva (Justo *et al.* 2014), *P. oreibatus* Justo (Justo *et al.* 2014), *P. albostipitatus* (Dennis) Singer (Menolli *et al.* 2010), *P. harrisii* Murrill (Menolli *et al.* 2010), and *P. meridionalis*. To confirm this statement, the phylogenetic position of *P. nigroviridis* Babos within *Pluteus* sect. *Pluteus* needs to be investigated because Stijve & Bonnard (1986) also mentioned the presence of psilocybin for this species. Furthermore, *Pluteus* species with blue-green tinges are also present in *Pluteus* sect. *Celluloderma*, viz. *P. cyanopus* Quél (Homola 1972) and *P. phaeocyanopus* Minnis & Sundb. (Minnis & Sundberg 2010), which could indicate that this character has originated at least twice during the evolution of the genus.

Pluteus padanilus Justo & C.K. Pradeep, *sp. nov.* (Fig. 5)

MycoBank: MB809616

Diagnosis:—Similar to *P. izurun* but differing in the *tefl* sequence (KF010049), differentiation of the cystidial apices and shorter pileipellis elements.

Type:—INDIA. Kerala: Trivandrum District, Enikkara, 1 November 2011, leg. C.K. Pradeep 13844 (Holotype CUW!; nrITS: JQ801374; *tefl*: KJ010049).

Pileus 20–35 mm in diam., hemispherical when young, expanding to convex or plano-convex, with a broad obtuse umbo; surface strongly radially fibrillose around center, becoming smooth towards margin, brown (5YR 3/4, 4/4–4/6,) or gray (10YR 5/1–5/2, 6/1–6/2), darker at center and paler towards margin; dry; margin translucent-striate. *Lamellae* crowded, free, ventricose, up to 3 mm broad; white when young, later pink; with white, flocculose edges. *Stipe* 30–60 \times 2–5 mm, cylindrical; surface white, with distinct blue or blue-green tinges at base, smooth or with longitudinal gray-brown or bluish fibrils, sometimes grouped forming squamules, especially near the base. *Context* in stipe and pileus white, in some places on the stipe context with slight blue-green tinges. *Odor* indistinct. *Taste* indistinct. *Spore print* pinkish brown (2.5YR 7/6–7/8). *Basidiospores* [60/2/2] (6.0–)6.5–8.0 \times (4.5–)5.0–6.0 μm ($Q = 1.15$ –1.42; $Q_m = 1.28$; $L_m = 7.0$ μm ; $W_m = 5.6$ μm), broadly ellipsoid to ellipsoid, inamyloid, hyaline, smooth, thick-walled. *Basidia* 20–35 \times 7.0–14.0 μm , four-spored, rarely one-spored (only a single one was observed), clavate or narrowly utriform. *Pleurocystidia* 50–85 \times 15.0–27 μm ; predominantly narrowly utriform or lageniform, some with truncate to subcapitate apices or with 2–3 indistinct short projections at the apex, a very few with an internal septa; colorless but a few with some incrusting parietal pigment at the apices, thin-walled; common. *Lamellar edge* sterile. *Cheilocystidia* 40–65 \times 10.0–20(–25) μm ; predominantly (narrowly) clavate or narrowly utriform, a few with a short apical appendage, colorless but a few with some incrusting parietal pigment at apex, thin-walled; crowded, forming a well-developed strip. *Pileipellis* a cutis with repent and slightly ascending elements towards the center of the pileus; individual terminal elements 90–160(–190) \times 8.0–15 μm ; cylindrical or fusiform; mostly with brown intracellular pigment; thin-walled. *Stipitipellis* a cutis; hyphae 5.0–20 μm wide; cylindrical; colorless or with brown pigments; thin-walled. *Clamp connections* absent on pileipellis hyphae; present, but scarce, on some hyphae of the hymenophoral trama.

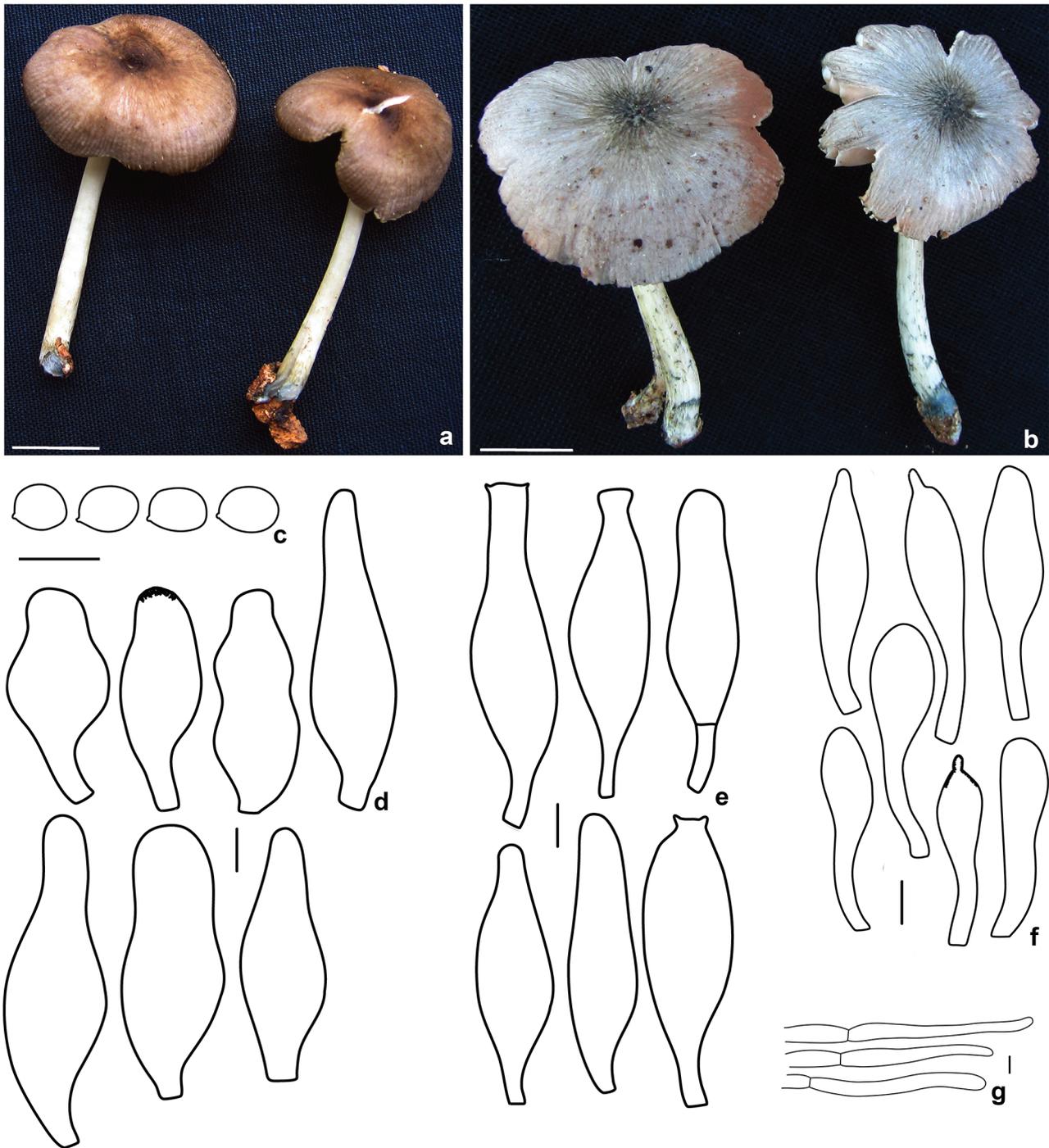


FIGURE 5. *Pluteus padanilus*. a–b. Basidiomata. c. Basidiospores. d, e. Pleurocystidia. f. Cheilocystidia. g. Pileipellis elements. a, c, d, f, g. *C.K. Pradeep 13844* (holotype) b, e. *C.K. Pradeep 14418*. Scale bars: a, b = 1 cm. c–g = 10 μ m. Photos a–b by C.K. Pradeep, line drawings c–g by A. Justo.

Etymology:—*padanilus* is the transliterated and Latinized form of two Sanskrit words “pada” (foot) and “neela” (blue), making reference to the blue colors on the stipe.

Habit, habitat and distribution:—Fruiting in pairs. On decayed angiospermous wood. November. Asia: Known only from Kerala state (India)

Additional specimen examined:—INDIA. Kerala: Trivandrum District, Enikkara, 6 November 2012, leg. *C.K. Pradeep 14418* (CUW!; nrITS: KJ009768; *tefl*: KJ010050).

Notes:—*Pluteus padanilus* is characterized by a stipe base with distinct blue or blue-green tinges, broadly ellipsoid to ellipsoid basidiospores ($Q_m = 1.28$), pleurocystidia with truncate to subcapitate apices or with 2–3 indistinct short apical projections and the presence of incrusting parietal pigment at the apex in some pleuro- and cheilocystidia. All species

discussed herein differ from *P. padanilus* in the lack of incrusting parietal pigment at apex of the cystidia. Additionally, *P. glaucotinctus* has few cheilocystidia with brownish pigment and a majority of pleuro- and cheilocystidia with rounded apices and with no apical projections; *P. izurun* has longer pileipellis elements (up to 380 μm); *P. meridionalis* has a stipe with no distinct blue-green tinges and narrower basidiospores ($Q_m = 1.40$); and *P. thoenii* differs in having preponderantly subglobose basidiospores ($Q_m = 1.15$) and longer cheilocystidia (up to 94 μm long).

An additional morphological character that separates *P. padanilus* from the other species in this complex is the presence of clamp-connections at some septa of the hymenophoral trama hyphae. The presence/absence of clamp-connections in the pileipellis hyphae is a useful character for the species-level taxonomy of *Pluteus* sect. *Pluteus*, but its presence in other parts of the basidioma is not constant enough to be used as a reliable character (Justo *et al.* 2014). Horak & Heinemann (1978) also reported the presence of a few clamp-connections on the pileipellis elements in the African collections named as *P. glaucotinctus*, but they were not observed during our re-examination. One should not disregard the fact that clamp connections are often more difficult to find in older, dried herbarium material.

Recently, Menolli *et al.* (2014) reported the presence of cheilocystidia with incrusting parietal pigment at the apex in one Brazilian material (coll. ANc-4214) under the name of *P. glaucotinctus*. According to their description it could fit also within the concept of *P. padanilus*, however, further molecular investigation will be necessary to elucidate the identity of the material studied by the authors and to certify the real occurrence of *P. glaucotinctus* or *P. padanilus* in Brazil.

Pluteus thoenii Menolli & Minnis, *sp. nov.* (Fig. 6)

Mycobank: MB809617

Diagnosis:—Similar to *P. glaucotinctus* but differing in the nrITS sequence (HM562132) and in having predominantly subglobose basidiospores ($Q_m = 1.15$) and longer cheilocystidia (up to 94 μm long).

Type:—DEMOCRATIC REPUBLIC OF THE CONGO. Haut-Katanga: Kipopo, 19 December 1972, *Thoen 5546* (Holotype BR!; nrITS: HM562132; tef1: KJ010051).

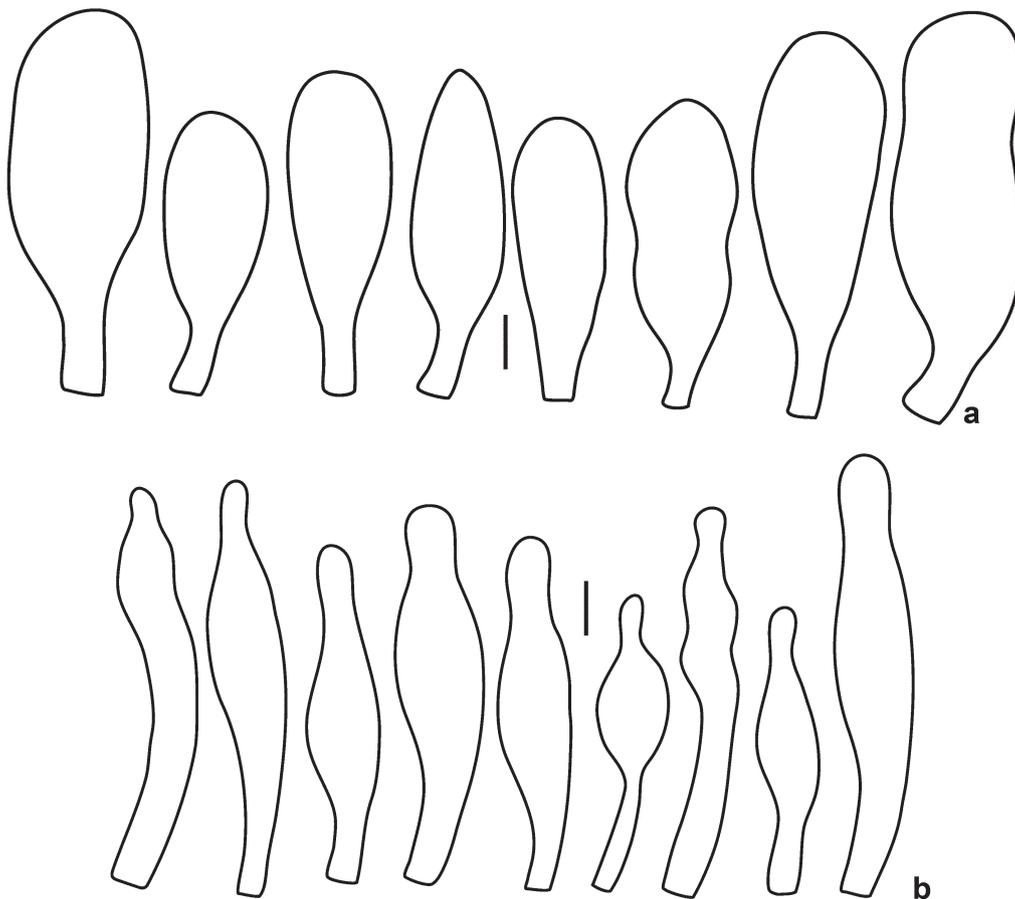


FIGURE 6. *Pluteus thoenii* (holotype). a. Pleurocystidia. b. Cheilocystidia. Scale bars: 10 μm . Line drawings a–b by A. Justo.

Basidiospores [30/1/1] 6.5–8.5(–9.0) × 5.5–7.5 µm (Q = 1.10–1.17; Q_m = 1.15; L_m = 7.7 µm; W_m = 6.7 µm), subglobose to broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled. *Pleurocystidia* 55–85 × 18.0–32 µm; ovoid, clavate or oblong, with rounded apices, colorless, thin-walled. *Cheilocystidia* 53–94 × 12.0–15.0 µm; narrowly utriform, cylindrical, lageniform, flexuous, colorless, thin-walled. *Pileipellis* a cutis; terminal elements 70–105 × 10.0–20 µm; cylindrical or tapering towards apex; colorless or with brown pigments; thin-walled. *Stipitipellis* a cutis; hyphae 5.0–15.0 µm wide; colorless; thin-walled. *Clamp-connections* not observed.

Etymology:—*thoenii* in honor of the collector of the holotype, Daniel Thoen, and for his contribution to the knowledge of fungi from tropical Africa.

Distribution:—Africa: Known only from Democratic Republic of the Congo.

Specimen examined:—DEMOCRATIC REPUBLIC OF THE CONGO. Haut-Katanga: Kipopo, 19 December 1972, *Thoen 5546* (BR!; nrITS: HM562132; *tefl*: KJ010051).

Notes:—Despite the lack of macro-morphological data from fresh collections of *P. thoenii*, we consider the DNA sequences, the shape of the basidiospores (Q_m = 1.15) and the size of the cheilocystidia (up to 94 µm long) as sufficiently distinctive characters to propose this new species. *Pluteus thoenii* has been up until now only represented by the holotype, which represents a single herbarium collection from more than 50 years ago that was previously identified by Horak & Heinemann (1978) as *P. glaucotinctus*.

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References

- Bas, C. (1969) Morphology and subdivision of *Amanita* and a monograph on its section *Lepidella*. *Persoonia* 5: 285–579.
- Corriol, G. & Moreau, P.A. (2007) *Agaricus (Annularia) fenzi* redécouvert dans les Pyrénées. Notes sur le genre *Chamaeota* en Europe. *Persoonia* 19: 233–250.
- Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
<http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Homola, R.L. (1972) Section *Celluloderma* of the genus *Pluteus* in North America. *Mycologia* 64: 1211–1247.
<http://dx.doi.org/10.2307/3757960>
- Horak, E. (1977) Neue zairische Arten aus der Gattung *Pluteus* Fr. *Bulletin du Jardin Botanique National de Belgique* 47: 87–89.
<http://dx.doi.org/10.2307/3667984>
- Horak, E. & Heinemann, P. (1978) *Flore illustrée des champignons d’Afrique centrale 6: Pluteus & Volvariella (compléments)*. National Botanical Garden of Belgium, Meise.
- Justo, A., Vizzini, A., Minnis, A.M., Menolli Jr, N., Capelari, M., Rodríguez, O., Malysheva, E., Contu, M., Ghignone, S. & Hibbett, D. S. (2011a) Phylogeny of *Pluteaceae (Agaricales, Basidiomycota)*: taxonomy and character evolution. *Fungal Biology* 115: 1–20.
<http://dx.doi.org/10.1016/j.funbio.2010.09.012>
- Justo, A., Minnis, A.M., Ghignone, S., Menolli Jr, N., Capelari, M., Rodríguez, O., Malysheva, E., Contu, M. & Vizzini, A. (2011b) Species recognition in *Pluteus* and *Volvopluteus (Pluteaceae, Agaricales)*: morphology, geography and phylogeny. *Mycological Progress* 10: 453–479.
<http://dx.doi.org/10.1007/s11557-010-0716-z>
- Justo, A., Caballero, A., Muñoz, G., Minnis, A.M. & Malysheva, E. (2011c) Taxonomy of *Pluteus eugraptus* and morphologically similar taxa. *Mycologia* 103: 646–655.
<http://dx.doi.org/10.3852/10-280>
- Justo, A., Malysheva, E., Bulyonkova, T., Vellinga, E.C., Cobian, G., Nguyen, N., Minnis, A.M. & Hibbett, D.S. (2014) Molecular

- phylogeny and phylogeography of Holarctic species of *Pluteus* section *Pluteus* (Agaricales: Pluteaceae), with description of twelve new species. *Phytotaxa* 180: 1–85.
<http://dx.doi.org/10.11646/phytotaxa.180.1.1>
- Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.
<http://dx.doi.org/10.1093/bib/bbn013>
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J. A. (Eds.) (2008) *Ainsworth & Bisby's dictionary of the fungi, 10th edn.* CAB International, Wallingford.
- Küppers, H. (1979) *Atlas de los colores.* Editorial Blume, Barcelona.
- Maddison, D.R. & Maddison, W.P. (2002) *MacClade4: analysis of phylogeny and character evolution.* Sinauer Associates, Sunderland.
- Martinez, D., Challacombe, J., Morgenstern, I., Hibbett, D., Schmoll, M., Kubicek, C. P., Ferreira, P., Ruiz-Duenas, F. J., Martinez, A.T., Kersten, P., Hammel, K.E., Vanden Wymelenberg, A., Gaskell, J., Lindquist, E., Sabat, G., Splinter BonDurant, S., Larrondo, L.F., Canessa, P., Vicuna, R., Yadav, J., Doddapaneni, H., Subramanian, V., Pisabarro, A.G., Lavín, J.L., Oguiza, J.A., Master, E., Henrissat, B., Coutinho, P.M., Harris, P., Magnuson, J.K., Baker, S.E., Bruno, K., Kenealy, W., Hoegger, P.J., Kues, U., Ramiaiya, P., Lucas, S., Salamov, A., Shapiro, H., Tu, H., Chee, C.L., Misra, M., Xie, G., Teter, S., Yaver, D., James, T., Mokrejs, M., Pospisek, M., Grigoriev, I.V., Brettin, T., Rokhsar, D., Berka, R. & Cullen, D. (2009) Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *Proceedings of the National Academy of Sciences* 106: 1954–1959.
<http://dx.doi.org/10.1073/pnas.0809575106>
- Menolli Jr, N., Asai, T. & Capelari, M. (2010) Records and new species of *Pluteus* from Brazil based on morphological and molecular data. *Mycology* 1: 130–153.
<http://dx.doi.org/10.1080/21501203.2010.493531>
- Menolli Jr, N., de Meijer, A.A.R. & Capelari, M. (2014) The genus *Pluteus* (Pluteaceae, Agaricales) from the state of Paraná, Brazil. *Nova Hedwigia* (in press).
- Minnis, A.M., Sundberg, W.J., Methven, A.S., Sipes, S.D. & Nickrent, D.L. (2006) Annulate *Pluteus* species: a study of the genus *Chamaeota* in the United States. *Mycotaxon* 96: 31–39.
- Minnis, A.M. & Sundberg, W.J. (2010) *Pluteus* section *Celluloderma* in the U.S.A. *North American Fungi* 5: 1–107.
<http://dx.doi.org/10.2509/naf2010.005.001>
- Munsell Color (Ed.) (2009) *Munsell Soil-Color Charts.* X-Rite, Grand Rapids.
- Rehner, S.A. & Buckley, E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence from cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98, 2005.
<http://dx.doi.org/10.3852/mycologia.97.1.84>
- Rodríguez, O., & Guzmán-Dávalos, L. (2001) Clave dicotômica de las especies del género *Pluteus* Fr. (Pluteaceae) conocidas de La región de Nueva Galicia y algunas áreas aledañas, Mexico. *Acta Botanica Mexicana* 57: 23–36.
- Saupe, S.G. (1981) Occurrence of psilocibin/psilocin in *Pluteus salicinus* (Pluteaceae). *Mycologia* 73: 781–784.
<http://dx.doi.org/10.2307/3759505>
- Singer, R. (1959) Monographs of South American Basidiomycetes, especially those of the east slope of the Andes and Brazil. 1. The genus *Pluteus* in South America. *Lloydia* 21: 195–299.
- Singer, R. (1962) Monographs of South American Basidiomycetes, especially those of the east slope of the Andes and Brazil. 4. *Inocybe* in the Amazone region, with a Supplement to part 1 (*Pluteus* in South America). *Sydowia* 15: 112–132.
- Singer, R. (1969) Mycoflora Australis. *Beiheft zur Nova Hedwigia* 29: 1–405.
- Singer, R. (1986) *The Agaricales in modern taxonomy.* 4th edn. Koeltz Scientific Books, Koenigstein.
- Stijve, T., & Bonnard, J. (1986) Psilocybine et urée dans le genre *Pluteus*. *Mycologia Helvetica* 2: 123–130.
- Stijve, T., & de Meijer, A.A.R. (1993) Macromycetes from the state of Paraná, Brazil, 4. The psychoactive species. *Arquivos de Biologia e Tecnologia* 36: 313–329.
- Thiers, B. (2014) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/ih/> (accessed July 2014).
- Vizzini, A. & Ercole, E. (2011) A new annulate *Pluteus* variety from Italy. *Mycologia* 103: 904–911.
<http://dx.doi.org/10.3852/10-382>
- White, T.J., Bruns, T., Lee, S.S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: a guide to methods and applications.* Academic Press, New York, pp. 315–322.
<http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Yelle, D.J., Ralph, J., Lu, F., & Hammel, K. (2008) Evidence for cleavage of lignin by a brown rot basidiomycete. *Environmental Microbiology* 10: 1844–1849.
<http://dx.doi.org/10.1111/j.1462-2920.2008.01605.x>