Mycologia, 103(3), 2011, pp. 646–655. DOI: 10.3852/10-280

Taxonomy of *Pluteus eugraptus* and morphologically similar taxa

Alfredo Justo[1]
Biology Department, Clark University, 950 Main Street, Worcester, Massachusetts 01610

Agustín Caballero
Calle Andalucía, 3, 4.° D, E-26500 Calahorra, La Rioja, Spain

Guillermo Muñoz
Avenida Valvanera A, 32, 5.° D, E-26500 Calahorra, La Rioja, Spain

Andrew M. Minnis
Systematic Mycology & Microbiology Laboratory, USDA-ARS, B011A, 10300 Baltimore Avenue, Beltsville, Maryland 20705

Ekaterina Malysheva
Komarov Botanical Institute, 2 Popov Street, St Petersburg, RUS-197376, Russia

Abstract: The status of the taxa morphologically similar to *Pluteus eugraptus* (Basidiomycota, Agaricales) was investigated with morphological and molecular (ITS region) characters. This group of species belongs in *Pluteus* sect. *Celluloderma* based on morphological and molecular characters. Two species, *Pluteus multiformis*, from Spain and *Pluteus eludens* from Madeira, Portugal, Russia and USA, are described as new. Both species share pigmented cheilocystidia and a pileipellis composed of both clavate-spheropedunculate and elongated elements with *P. eugraptus*, but they can be separated based on the characteristics of the cystidia and pileipellis. *Pluteus multiformis* is characterized by the scarce pleurocystidia, clavate cheilocystidia and caulocystidia and highly polymorphic elements of the pileipellis. *Pluteus eludens* is characterized mainly by utriform pleurocystidia. *Pluteus eugraptus* is known only with certainty from the type collection (Sri Lanka), which has been re-examined here, and it is characterized by narrowly lageniform pleurocystidia. Phylogenetic analyses based on ITS region sequence data supported the separation of *P. multiformis*, *P. eludens* and an additional collection from Japan that likely represents the true *P. eugraptus*.

Key words: biodiversity, *Celluloderma*, ITS, phylogeny, Pluteaceae

Submitted 16 Sep 2010; accepted for publication 15 Nov 2010.
[1] Corresponding author. E-mail: ajusto@clarku.edu

INTRODUCTION

Genus *Pluteus* Fr. (Basidiomycota, Agaricales) traditionally has been subdivided into three sections according to characteristics of the hymenial cystidia and pileipellis anatomy (Singer 1986). Section *Celluloderma* Fayod is characterized by non-metuloid cystidia and a pileipellis as a hymeniderm or epithelium composed of clavate or spheropedunculate elements intermixed or not with elongated elements. Two subgroups are commonly recognized in this section: (i) subsection *Mixtini* Singer that has elongated elements in the pileipellis and (ii) subsection *Eucellulodermini* Singer that lacks elongated elements (Singer 1986, Vellinga 1990). A recent phylogenetic study employing DNA data showed that these two subsections are not natural groups because elongated elements have appeared several independent times during the evolution of sect. *Celluloderma* and also that some species with the pileipellis as a cutis (*P. ephebeus* [Fr. : Fr.] Gillet, *P. fenzlii* [Schulzer] Corriol & P.-A. Moreau) are members of this section (Justo et al. 2010a). During the phylogenetic study it became evident that collections morphologically assignable to *Pluteus eugraptus* (Berk. & Broome) Sacc., one of the (apparently) more distinctive species of subsection *Mixtini*, represent several species that are discussed in detail here.

Pluteus eugraptus is characterized by the combination of dark brown lamellar edges (pigmented cheilocystidia) and a pileipellis containing both elongated and short clavate-spheropedunculate elements. It originally was described from Sri Lanka (Berkeley and Broome 1871) and has been reported from Africa (Pegler 1977), North America (Homola 1972, Minnis and Sundberg 2010) and South America (Singer 1956, 1958). Among the described species of *Pluteus*, only *Pluteus psychriophorus* var. *chusqueae* E. Horak (≡ *Pluteus eugraptus* var. *chusqueae* [E. Horak] Singer) shares the combination of uniformly darkly pigmented lamellar edges and a *Mixtini*-type pileipellis (Horak 1964, Minnis and Sundberg 2010).

After re-examining the type collection of *P. eugraptus* and in view of the results from the phylogenetic analyses two new species, *P. multiformis* and *P. eludens*, are described here. A collection from Japan, very probably representing the ''true'' *Pluteus eugraptus*, also was studied and sampled for molecular data. Morphological characteristics such as shape of cystidia and pileipellis elements and molecular data support the recognition of these as distinct taxa.

MATERIALS AND METHODS

Sequences.—Approximately 0.05–0.10 g tissue (preferably lamellae) from each collection were ground in an 1.5 mL Eppendorf tube with plastic pestles. DNA was extracted with 3% SDS extraction buffer and then isolated by the sequential addition of phenol-chloroform and chloroform-isoamyl alcohol. Isopropyl alcohol and 3 M sodium acetate were added to precipitate DNA, which was washed with 70% ethanol and resuspended in sterile water. The ITS region (ITS1, 5.8S, ITS2) was amplified with the primer pair ITS1F–ITS4 (Gardes and Bruns 1993, White et al. 1990). The amplification products were sequenced with ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction reagents with the same primer combination. Sequencing was carried out on an ABI model 3130 Genetic Analyzer. Raw data were processed with Sequencher 4.7 (Gene Codes Corp., Ann Arbor, Michigan).

Results from a broader phylogenetic study of *Pluteus* sect. *Celluloderma* (Justo et al. 2010b) were considered for selecting which sequences, in addition to those from ''*P. eugraptus*-like'' collections, would be included in the analyses. All sequences were generated during that study with the following exceptions that already were present in GenBank: *Pluteus* cf. *nanus* (FJ774081, originally as ''*Pluteus nanus*''), *Pluteus eludens* (FJ774085, originally as ''*Pluteus podospileus*''), *Pluteus fluminensis* Singer (FJ816664, FJ816665), *Pluteus fuligineovenosus* E. Horak (FJ16662), *Pluteus jamaicensis* Murrill (FJ816657), uncultured basidiomycete (AY969369, isolated from hardwood litter, North Carolina, USA), uncultured basidiomycete (DQ672275, isolated from soil, Australia). Collection numbers, herbarium acronyms (when not included in the collection number), geographic origin of each sample and GenBank accession numbers are given (FIG. 1).

Alignment.—Sequences were aligned with MAFFT (Katoh et al. 2002, http://align.bmr.kyushu-u.ac.jp/mafft/online/server/). The alignments were examined and manually corrected in MacClade 4.05 (Maddison and Maddison 2002). Alignments were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S10865).

The dataset included 37 ITS sequences of *Pluteus* sect. *Celluloderma* and two of *Pluteus* sect. *Pluteus* (*P. glaucotinctus* E. Horak and *P. losulus* Justo) that were used as outgroup. The final dataset consists of 39 sequences of 644 characters (gaps included) of which 163 are parsimony informative.

Phylogenetic analyses.—Maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis (BA) were performed with these parameters: (i) MP: Equally weighted parsimony analysis was performed with PAUP* 4.0.b10 (Swofford 2002). One thousand heuristic search replicates were performed with starting trees generated by stepwise addition with random addition sequences followed by tree bisection reconnection (TBR) branch swapping. Up to two trees were kept in each replicate. Parsimony bootstrap analysis was performed with 1000 replicates, each with 10 random taxon addition sequences and branch swapping set to subtree pruning and regrafting (SPR). (ii) ML: The analysis was run in the RAxML servers (http://phylobench.vital-it.ch/raxml-bb/index.php; which implements the search protocol of Stamatakis et al. 2008), under a GTR model with 1000 rapid bootstrap replicates. (iii) BA: The analysis was run with MrBayes 3.1 (Ronquist and Huelsenbeck 2003) 10 000 000 generations under a GTR model with four chains and trees sampled every 100 generations. After examining the graphic representation of the likelihood scores with Tracer (http://tree.bio.ed.ac.uk/software/tracer/) the burn-in period was set to 1 500 000 generations.

Morphological descriptions.—Specimens were studied with standard procedures for morphological examination of *Pluteus* (Justo and Castro 2007, Minnis and Sundberg 2010). Descriptive terms for morphological features follow Vellinga (1988). Color annotations are from Munsell Soil-Color Charts (Munsell Color 2009). The notation [60, 3, 1] indicates that measurements were made on 60 spores, from 3 basidiocarps, in 1 collection. These abbreviations are used in the descriptions: avl for average length, avw for average width, Q for quotient of length and width and avQ for average quotient. Herbarium acronyms follow Thiers (2010).

RESULTS

In the MP analysis a total of six equally most parsimonious trees (MPT) were recovered (Length = 478, CI = 0.68, RI = 0.89). One MPT is illustrated (FIG. 1). Topological differences are minor among the six MPTs, the strict consensus tree from the MP analysis, the best tree from the ML analysis and the 50% majority rule consensus tree from the Bayesian analysis. For the taxa here discussed all trees showed essentially the same evolutionary scenario (FIG. 1).

In all analyses two major clades are recognized, *chrysophlebius-phlebophorus* and *cinereofuscus*. *Pluteus multiformis* and *P. eludens* are placed in the *cinereofuscus* clade. The general internal topology of this clade is not well resolved, although an environmental sample from Australia (DQ672275) consistently clusters as sister of *P. multiformis*. *Pluteus* cf. *eugraptus* clusters in the *chrysophlebius-phlebophorus* clade, together with *P. phlebophorus* and *P.* aff. *phlebophorus*, although the relationships among these taxa are not resolved in any of the analyses.

TAXONOMY

Pluteus multiformis, Justo, A. Caball. & G. Muñoz, sp. nov. FIG. 2

MycoBank MB518902

Pluteo eugrapto similis sed differt in pleurocystidiis raris, cheilocystidiis clavatis, caulocystidiis clavatis, cellulae in epicute pilei multum variabilis.

Etymology. *multiformis* means ''with multiple shapes'', in reference to the highly variable morphology of the pileipellis cells.

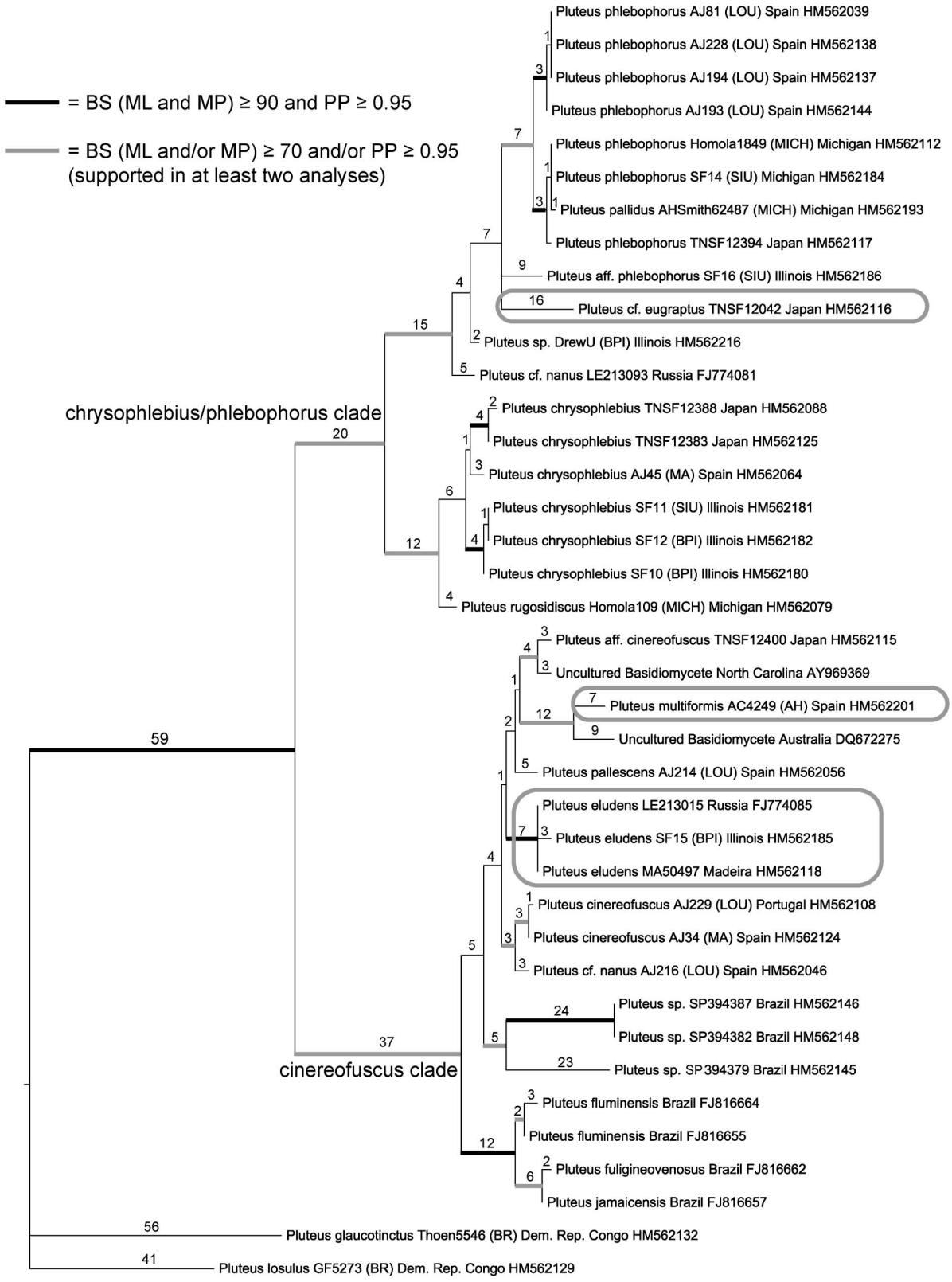

FIG. 1. One of six MPT from the ITS dataset including *Pluteus* cf. *eugraptus* and morphologically similar taxa, selected representatives of *Pluteus* sect. *Celluloderma* and *P. glaucotinctus* and *P. losulus* as outgroup taxa. Branch lengths (number of changes) are given above the branches.

FIG. 2. *Pluteus multiformis*. A. Basidiocarps. B. Basidospores. C. Pileipellis elements. D. Pleurocystidia. E. Cheilocystidia. F. Caulocystidia. All from holotype (AC 4249). Bars = 10 µm.

Pileus 15–20 mm, convex to plano-convex, without umbo; surface rugose-venose at center, smooth towards margin, very dark brown to black at center (Mu. 7.5YR 2.5/1–2.5/3), paler toward margin (Mu. 7.5YR 5/6–5/8, 6/6–6/8); margin translucently striate up to half the radius of pileus, undulate or crenulate with age. Lamellae relatively crowded, free, ventricose or broadly ventricose, up to 5 mm broad; white-cream when young, later pink (Mu. 8/3–8/4), with dark brown, undulate-crenate, edges. Stipe 18–22 × 1.5–2 mm, cylindrical; surface white with grayish tints (Mu. 10YR 8/1), longitudinally fibrillose or pruinose. Context white in pileus, white-grayish in stipe. Odor and flavor indistinct. Spore print pink.

Basidiospores [30, 1, 1] 6.0–8.0 × 5.0–7.5(–8.0) µm, avl × avw = 7.0 × 6.6 µm, Q = 1.0–1.17, avQ = 1.07, mostly globose or subglobose, a few broadly ellipsoid. Basidia 20–25 × 8.5–10 µm, tetrasterigmate, clavate or narrowly utriform. Pleurocystidia 40–71 × 10–20 µm, fusiform, narrowly lageniform or utriform, hyaline, thin-walled, scarce and scattered. Lamellar edges covered with cheilocystidia, sterile. Cheilocystidia 20–50(–60) × 10–20 µm, clavate or narrowly clavate, mostly filled with brown intracellular pigment, a few hyaline, thin-walled. Pileipellis an euhymeniderm composed of elements 20–65(–105) × (10–)15–35 µm; individual elements clavate, spheropedunculate, utriform, narrowly lageniform, ovoid, conical, fusiform, flexuose, some mucronate or rostrate, some irregularly shaped, filled with brown, intracellular or vacuolar, pigment, with thin or slightly thickened at apex, smooth walls. Stipitipellis a cutis; hyphae 5–10 µm wide, cylindrical, colorless or with brown pigment, with thin, smooth walls. Caulocystidia 35–65 × 10–15 µm, clavate or narrowly utriform, with brown intracellular pigment, thin-walled or walls up to 1.5 µm thick, scattered or in loosely arranged clusters, present all over the stipe length but more abundant toward the apex. Clamp connections absent in all tissues.

Habit, habitat and distribution. Gregarious, known only from the type locality (northern Spain). Collected in a *Quercus ilex* forest with *Cistus*, during autumn. Apparently terrestrial.

Collections examined. SPAIN. LA RIOJA. Santa Lucía, Valle de Ocón (800 m), 21-XI-2009, *A. Caballero & G. Muñoz*, AC 4249 [AH 40107, holotypus].

Observations. *Pluteus multiformis* is characterized mainly by the highly variable morphology of the pileipellis cells, scarce pleurocystidia and clavate cheilocystidia and caulocystidia. In *P. eludens*, *P. eugraptus* and *P. psychriophorus* var. *chusqueae* pleurocystidia are more abundant; cheilocystidia are variously shaped but not predominantly clavate; caulocystidia (if present) are predominantly lageniform or cylindrical; and the elongated elements in the pileipellis are less variable (mostly fusiform or lageniform).

In all phylogenetic analyses, an environmental sample from Australia was placed as sister to *P. multiformis* (FIG. 1). Based on the percentage similarity between both sequences (94.5%), it probably represents a closely related but different species. None of the described species of *Pluteus* from Australia (Grgurinovic 1997) or New Zealand (Horak 2008) share the morphological characteristic of *P. multiformis*.

The difference between the ITS sequence of *P. multiformis* and the sequences of *P. eludens* is 5.8–6%. The difference between the ITS sequence of *P. multiformis* and the sequences of *P.* cf. *eugraptus* is 17%.

Pluteus eludens, E.F. Malysheva, Minnis & Justo, sp. nov. FIG. 3

MycoBank MB518903

Pluteo eugrapto similis sed differt in pleurocystidiis utriformibus.

Etymology. *eludens* is from the latin *eludere*, meaning ''to elude or deceive, to escape discovery'', in reference to the fact that only detailed morphological and molecular analyses enabled the recognition of this species, despite its wide distribution.

Pileus 12–20 mm, convex to plano-convex, with low, broad umbo; surface strongly rugose-venose all over or only at center, brown, dark brown or gray-brown (approx. Mu. 7.5YR 4/1-4/6, 5/1-5/4), darker at center; margin rugose-venose or translucently striate. *Lamellae* crowded, free, ventricose or broadly ventricose, up to 5 mm broad, whitish when young, later pink, with dark-brown or concolorous, floccose edges. Stipe 20–35 × 2–5 mm, cylindrical; surface white, slightly brownish near base, smooth, pruinose or longitudinally fibrillose, especially at base. Context white. Odor and flavor indistinct. Spore print not recorded.

Basidiospores [60, 4, 3] (5.5–) 6.0–8.2 × 5.2–7.3 µm, avl × avw = 6.4–6.6 × 5.8–6.0 µm, Q = 1.0–1.23, avQ = 1.06–1.12, globose to broadly ellipsoid. Basidia 20–36 × 8–11 µm, tetrasterigmate, clavate or narrowly clavate. Pleurocystidia 35–75 × 12–30 µm, mostly utriform or narrowly utriform, also narrowly clavate, narrowly lageniform or ovoid with long pedicel, hyaline, thin-walled, abundant all over lamellar faces. Lamellar edges covered with cheilocystidia, sterile. Cheilocystidia 34–75 × 10–20 µm, narrowly utriform, fusiform, narrowly lageniform, narrowly clavate, filled with brown intracellular pigment or hyaline (either type predominant), thin-walled. Pileipellis an euhymeniderm composed of elements 20–75 × 10–25(–40) µm; individual ele-

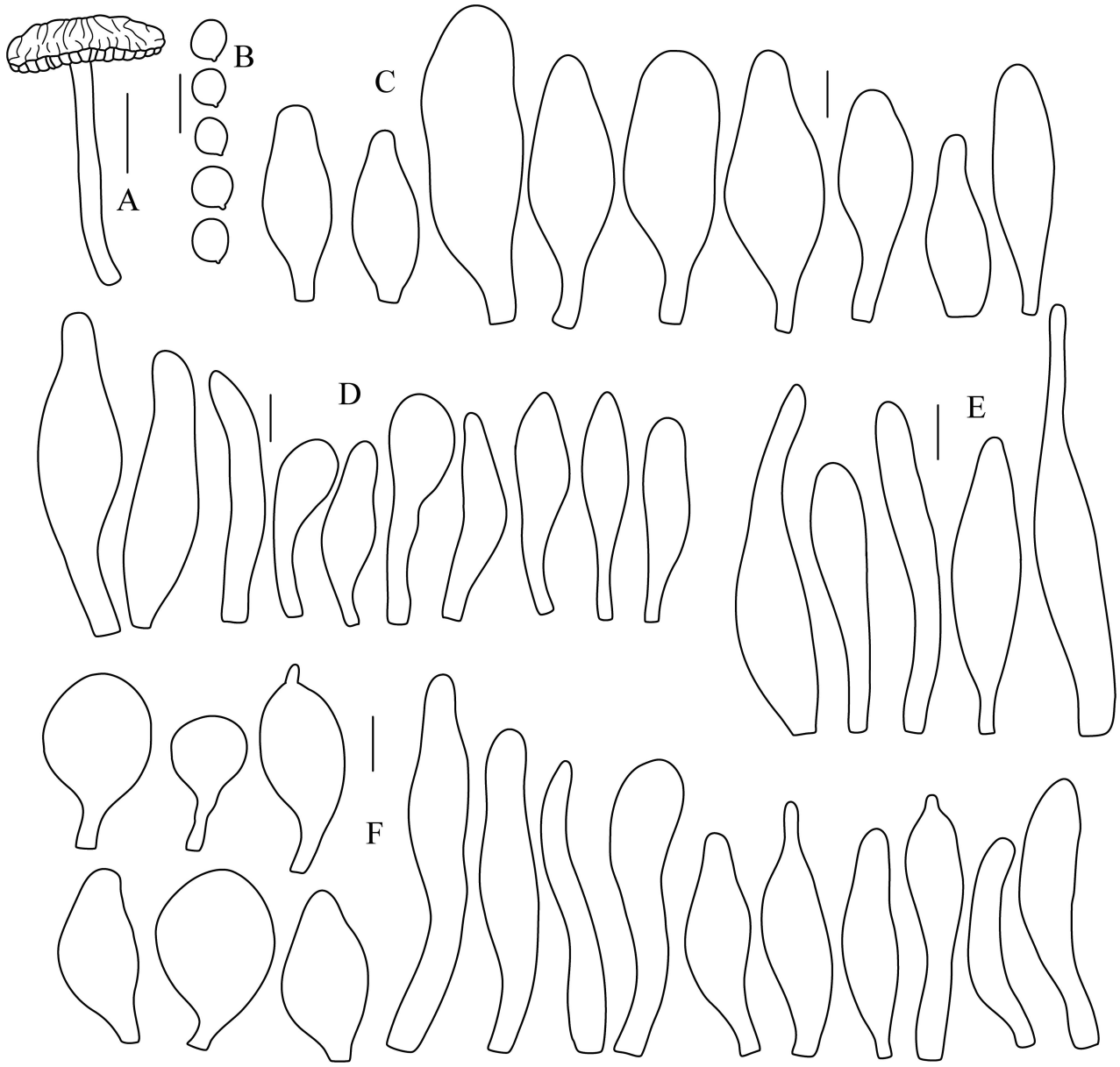

FIG. 3. *Pluteus eludens*. A. Basidiocarp. B. Basidospores. C. Pleurocystidia. D. Cheilocystidia. E. Caulocystidia. F. Pileipellis elements. All from holotype (MA 50497). Bars = 10 μm.

ments clavate, spheropedunculate, broadly fusiform, narrowly clavate, lageniform or broadly fusiform, a few mucronate, filled with brown, intracellular pigment, with thin or slightly thickened at apex, smooth walls. Stipitipellis a cutis; hyphae 5–15 μm wide, cylindrical, colorless or with brown pigment, with thin, smooth walls. Caulocystidia present or absent, 50–90 × 7–15 μm, cylindrical or lageniform, with brown intracellular pigment or hyaline, thin-walled, scattered all over the stipe length. Clamp connections absent in all tissues.

Habit, habitat and distribution. Solitary or gregarious. Known from Madeira Island (Portugal), Russia (Samara Region) and USA (Illinois). Recorded on wood remnants under *Cupressus lusitanica* (Madeira), on fallen trunk of *Tilia cordata* (Russia) and on unidentified wood (Illinois). Fruiting in January (Madeira) and September (Russia, Illinois).

Collections examined. PORTUGAL. MADEIRA. Parque Ecológico, 24-I-2001, *A. Ferro & F.D. Calonge*, (MA-Fungi 50497, holotypus). RUSSIA. CENTRAL RUSSIA. Samara Region, Zhigulevsky State Nature Reserve, 1-IX-2001, *E. F. Malysheva*, (LE 213015). U.S.A. ILLINOIS. Southern Illinois, 25-IX-2009, *A.M. Minnis SF15* (BPI 880693).

Observations. *Pluteus eludens* is variable in terms of pigmentation of the cheilocystidia: in the holotype the lamellar edges are darkly pigmented and the majority

of the cheilocystidia are pigmented; in the Russian collection the lamellar edges are not distinctly darkly pigmented, but many cheilocystidia are pigmented; and in the collection from Illinois, the lamellar edges are not darkly pigmented and hyaline cheilocystidia are predominant, although pigmented cheilocystidia are also present. Caulocystidia were found only in the holotype. ITS sequences from the three collections are 99.4–99.6% identical, and other morphological characters do not differ greatly between collections. Therefore this morphological variability is considered to represent infraspecific variation.

The difference between the ITS sequence of *P. eludens* and the sequence of *P.* cf. *eugraptus* is 16%. The ITS sequences of *Pluteus cinereofuscus* J.E. Lange, *Pluteus pallescens* P.D. Orton, *P.* aff. *cinereofuscus*, *P.* cf. *nanus* and the environmental sample AY969369 (FIG. 1) are the most similar to the sequences of *P. eludens* (3–3.5% sequence divergence). These taxa differ from *P. eludens* among other characters by the absence of elongated elements in the pileipellis. All other taxa grouped in the *cinereofuscus* clade have ITS sequences with more than 5% divergence with respect to *P. eludens*.

Judging from the morphological characteristics, the North American collection of *Pluteus eugraptus* described by Homola (1972) and Minnis and Sundberg (2010) also might represent *P. eludens*. Unfortunately attempts to obtain ITS data from this collection (A.H. Smith 6325, MICH) were unsuccessful.

Pluteus eugraptus differs from *P. eludens* in the narrowly lageniform or fusiform pleurocystidia. *P. psychriophorus* var. *chusqueae* has much longer elements in the pileipellis (up to 140 μm) and grows on herbaceous substrates (Horak 1964). *Pluteus stigmatophorus* (Berk. & Broome) Sacc. has lamellar edges ornamented with dark brown spots (pigmented cheilocystidia) but differs from *P. eludens* in the pileus and stipe covered with dark brown squamules, yellowish hues in the stipe, pigmented, clavate pleurocystidia and longer (30–195 μm) pileipellis elements (Pegler 1986). This species is known only from Sri Lanka.

Pluteus eugraptus (Berk. & Broome) Sacc., Sylloge Fungorum 5:678. 1887. FIG. 4

≡ *Agaricus eugraptus* Berk & Broome, J. Linn. Soc., Bot. 11:535. 1871.

Pileus 10–30 mm, convex to plano-convex or depressed, with low, broad umbo; surface smooth to slightly rugose-venose, glabrous; margin translucently striate; yellowish brown to tawny brown. Lamellae distant, free, ventricose or broadly ventricose; up to 4 mm broad; pinkish, with dark brown edges. Stipe 15–20 × 1–2 mm, cylindrical; whitish in the upper part, darker toward the base, smooth. Context white. Odor and flavor not recorded. Spore print not recorded.

Basidiospores [30, 1, 1] 5.4–7.8 × 4.5–5.5 μm, avl × avw = 6.6 × 5.1 μm, Q = 1.15–1.55, avQ = 1.29, broadly ellipsoid or ellipsoid. Basidia 18–25 × 6–9 μm, tetrasterigmate, clavate, narrowly clavate or cylindrical. Pleurocystidia 35–60 × 8–15 μm, mostly narrowly lageniform or narrowly fusiform, hyaline or with pale brown intracellular pigment (especially near lamellar edge), thin-walled, scattered all over lamellar faces. Lamellar edges covered with cheilocystidia, sterile. Cheilocystidia 30–45 × 10–15 μm, narrowly utriform, fusiform, narrowly lageniform, narrowly clavate, mostly filled with brown intracellular (rarely vacuolar), pigment, thin-walled. *Pileipellis* an euhymeniderm composed of elements 20–70 × 10–25 μm, clavate, spheropedunculate, fusiform, narrowly clavate or lageniform or broadly fusiform, filled with brown, intracellular pigment; with thin, smooth walls. Stipitipellis a cutis; hyphae 5–15 μm wide, cylindrical, colorless or with brown pigment, with thin, smooth walls. *Caulocystidia* absent. Clamp connections absent in all tissues.

Habit, habitat and distribution. Gregarious. Known with certainty only from the type collection (Sri Lanka). On dead wood. Fruiting in June.

Collection examined. SRI LANKA. KANDY DISTRICT. Jun 1869, *Thwaites 1148* (K, Holotype)

Observations. The above description is based on the holotype description by Pegler (1986) supplemented with our observations on the same collection. Except for the slightly larger cystidia and pileipellis elements recorded by us, our observations are in accordance with those of Pegler (1986). *Pluteus eugraptus* is mainly characterized by the narrowly lageniform or fusiform pleurocystidia. *P. multiformis* has scarce and mainly narrowly utriform pleurocystidia, clavate cheilocystidia and caulocystidia, and differently shaped pileipellis elements. *P. eludens* has predominantly utriform pleurocystidia.

Pluteus eugraptus also has been reported from Tanzania (Pegler 1977) as well as Argentina and Bolivia (Singer 1956, 1958). The African collection (Pegler 1977) does indeed have the lageniform pleurocystidia that are typical for *P. eugraptus* and otherwise fits the above description. The South American collections (Singer 1958) have comparatively broader and utriform pleurocystidia and longer elements in the pileipellis (up to 140 μm).

Pluteus psychriophorus var. *chusqueae* was described originally, and so far only known, as growing on graminoid substrates (*Chusquea couleo*) in Argentina (Horak 1964). This taxon was considered to be a variety of *P. eugraptus* by Singer (1969). Shape of the

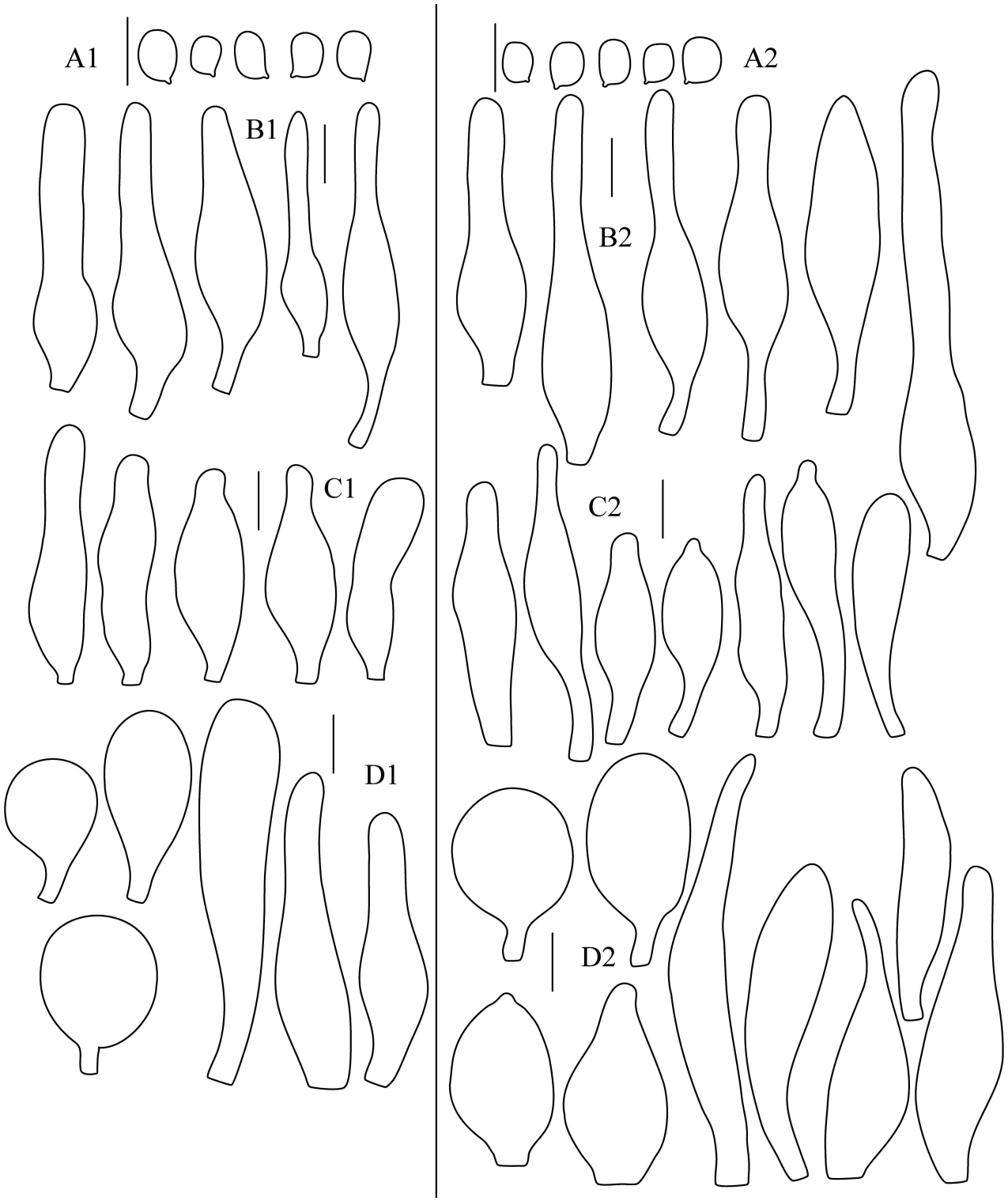

FIG. 4. *Pluteus eugraptus* and *Pluteus* cf. *eugraptus*. A1, A2. Basidospores. B1, B2. Pleurocystidia. C1, C2. Cheilocystidia. D1, D2. pileipellis elements. A1–D1 from *P. eugraptus* (Thwaites 1148, holotype), A2–D2 from *P.* cf. *eugraptus* (TNSF 12042). Bars = 10 μm.

pleurocystidia (utriform or clavate) and the size of pileipellis elements (70–140 × 20–35 µm) indicate that this is probably a different species not identical with *P. eugraptus*, *P. eludens* or *P. multiformis*, but new collections are necessary to clarify its taxonomic position.

One Japanese collection (TNSF 12042) included in the phylogenetic analyses comes close to *P. eugraptus* as described above, differing only in the slightly longer pleurocystidia (up to 95 µm) and cheilocystidia (up to 65 µm). It has been shown in other groups of Agaricales that subtle morphological differences, such as length of the cystidia, indeed may characterize different phylogenetic species, for example *Leucoagaricus decipiens* Contu, A. Vizzini & Vellinga and *Leucoagaricus erytrophaeus* Vellinga (Vellinga et al. 2010), but also that a relatively wide variation in the length of the cystidia may occur between collections of the same species, for example *Macrolepiota clelandii* Grgur. (Vellinga 2003).

More than 100 y have passed since *Agaricus eugraptus* was first described (Berkeley and Broome 1871), but the holotype specimen is still the only collection that can be undoubtedly considered to represent this species. The African collection described by Pegler (1977) and the Japanese collection studied by us likely represent *P. eugraptus*, but no additional collections of this species from Asia, especially from the topotype in Sri Lanka, are available for morphological and molecular comparison.

Given this situation, we prefer to maintain the identification of TNSF 12042 as ''*Pluteus* cf. *eugraptus*'' until modern collections of *P. eugraptus* from the Indian subcontinent are sampled for molecular analysis. With the current sampling the closest relatives of *Pluteus* cf. *eugraptus* are *P. phlebophorus* and an additional (probably undescribed) species here named *P.* aff. *phlebophorus* (FIG. 1). Both taxa differ from *P.* cf. *eugraptus* by the lack of elongated elements in the pileipellis. The difference between the ITS sequence of *P.* cf. *eugraptus* and the sequences of *P. phlebophorus* and *P.* aff. *phlebophours* is 5.2–5.8%.

The following observations of the Japanese collection were made (FIG. 4):

Basidiospores [60, 3, 1] 5.2–7.1 × 4.4–5.8 µm, avl × avw = 6.2 × 5.0 µm, Q = 1.15–1.40, avQ = 1.22, broadly ellipsoid or ellipsoid. Basidia 20–30 × 8–12 µm, tetrasterigmate, clavate or narrowly clavate. Pleurocystidia 50–95 × 12–17 µm, mostly narrowly lageniform or narrowly fusiform, hyaline, near lamella edge some with pale brown (intracellular) pigment, thin-walled, abundant all over lamellar faces. Lamella edge covered with cheilocystidia, sterile. Cheilocystidia 30–65 × 10–15 µm, narrowly utriform, fusiform, narrowly lageniform, narrowly clavate, mostly filled with brown intracellular pigment, thin-walled. Pileipellis an euhymeniderm composed of elements 30–80 × 9.5–30 µm; individual elements clavate, spheropedunculate, fusiform, narrowly clavate or lageniform, filled with brown, intracellular pigment with thin, smooth walls. Stipitipellis a cutis; hyphae 5–15 µm wide, cylindrical, colorless or with brown pigment, with thin, smooth walls. Caulocystidia absent. Clamp connections absent in all tissues.

Collection examined. JAPAN. HONSHU. Chiba Prefecture, Isumi, 4-VI-2006, *K. Osaku*, (TNSF 12042).

ACKNOWLEDGMENTS

The curators of AH, BPI, K, LE, MA, MICH and TNSF are gratefully acknowledged for managing the loans of their respective collections. Comments from two reviewers and from Dr Else Vellinga were very useful for improving the manuscript. Financial support was received from a postdoctoral grant of the Autonomous Government of Galicia (Spain) to A. Justo and from the NSF grant DEB0933081. A. Minnis acknowledges the support from the Alexander H. and Helen V. Smith Research Fund (Mycological Society of America).

LITERATURE CITED

Berkeley MJ, Broome CE. 1871. The fungi of Ceylon (Hymenomycetes from *Agaricus* to *Cantharellus*). J Linnean Soc Bot 11:494–567.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118, doi:10.1111/j.1365-294X.1993.tb00005.x

Grgurinovic AC. 1997. Larger fungi of south Australia. Australia: Botanic Gardens of Adelaide.

Homola R. 1972. Section *Celluloderma* of the genus *Pluteus* in North America. Mycologia 64:937–1210, doi:10.2307/3757960

Horak E. 1964. Fungi austroamericani II. *Pluteus* Fr. Nova Hedwig 8:163–199.

———. 2008. Agaricales of New Zealand 1: *Pluteaceae-Entolomataceae*. Hong Kong: Fungal Diversity Press.

Justo A, Castro ML. 2007. Observations in *Pluteus* section *Pluteus* in Spain: two new records for Europe. Mycotaxon 102:209–220.

———, Vizzini A, Minnis AM, Menolli Jr N, Capelari M, Rodríguez O, Malysheva E, Contu M, Ghinone S, Hibbett DS. 2010a. Phylogeny of the *Pluteaceae* (Agaricales, Basidiomycota): taxonomy and character evolution. Fungal Biol. doi: 10.1016/j.funbio.2010.09.012

———, Minnis AM, Ghinone S, Menolli Jr N, Capelari M, Rodríguez O, Malysheva E, Contu M, Vizzini A. 2010b. Species recognition in *Pluteus* and *Volvopluteus* (*Plu-*

teaceae, Agaricales): morphology, geography and phylogeny. Mycol Prog. doi: 10.1007/s11557-010-0716-z

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acid Res 30: 3059–3066, doi:10.1093/nar/gkf436

Maddison DR, Maddison WP. 2002. MacClade4: analysis of phylogeny and character evolution. Sunderland, Massachusetts: Sinauer Associates.

Minnis AM, Sundberg WJ. 2010. *Pluteus* section *Celluloderma* in the U.S.A. N Am Fungi 5:1–107, doi:10.2509/naf2009.005.001

Munsell, Color, ed. 2009. Munsell Soil-Color Charts. Grand Rapids, Michigan.

Pegler DN. 1977. A preliminary Agaric flora of east Africa. Kew Bull, Additional Ser 6:1–615.

———. 1986. Agaric flora of Sri Lanka. Kew Bull, Additional Ser 12:1–519.

Ronquist F, Huelsenbeck JP. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574, doi:10.1093/bioinformatics/btg180

Singer R. 1956. Contributions toward a monograph of the genus *Pluteus*. Trans Br Mycol Soc 39:145–232, doi:10.1016/S0007-1536(56)80001-6

———. 1958. 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.

———. 1969. Mycoflora Australis. Beihefte Nova Hedwigia 29:1–405.

———. 1986. The Agaricales in modern taxonomy. 4th ed. Koenigstein: Koeltz Scientific Books.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. Syst Biol 75:758–771, doi:10.1080/10635150802429642

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). 4.0 Beta. Sunderland, Massachusetts: Sinauer Associates.

Thiers B. 2010. (continuously updated). Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium, http://sweetgum.nybg.org/ih/

Vellinga EC. 1988. Glossary. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC, eds. Flora Agaricina Neerlandica 1:54–64.

———. 1990. *Pluteus*. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC, eds. Flora Agaricina Neerlandica 2:31–55.

———. 2003. *Chlorophyllum* and *Macrolepiota* (Agaricaceae) in Australia. Aust Syst Bot 16:361–370, doi:10.1071/SB02013

———, Contu M, Vizzini A. 2010. *Leucoagaricus decipiens* and *La. erythrophaeus*, a new species pair in sect. *Piloselli*. Mycologia 102:447–454, doi:10.3852/09-164

White TJ, Bruns T, Lee SS, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. New York: Academic Press. p 315–322.