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# Species recognition in *Pluteus* and *Volvopluteus* (Pluteaceae, Agaricales): morphology, geography and phylogeny

Alfredo Justo, Andrew M. Minnis, Stefano Ghignone, Nelson Menolli Jr., Marina Capelari, Olivia Rodríguez, Ekaterina Malysheva, Marco Contu, Alfredo Vizzini

## Abstract

The phylogeny of several species-complexes of the genera *Pluteus* and *Volvopluteus* (Agaricales, Basidiomycota) was investigated using molecular data (ITS) and the consequences for taxonomy, nomenclature and morphological species recognition in these groups were evaluated. Conflicts between morphological and molecular delimitation were detected in sect. *Pluteus*, especially for taxa in the *cervinus-petasatus* clade with clamp-connections or white basidiocarps. Some species of sect. *Celluloderma* are apparently widely distributed in Europe, North America and Asia, either with (*P. aurantiorugosus*, *P. chrysophlebius*, *P. fenzlii*, *P. phlebophorus*) or without (*P. romellii*) molecular differentiation in collections from different continents. A lectotype and a supporting epitype are designated for *Pluteus cervinus*, the type species of the genus. The name *Pluteus chrysophlebius* is accepted as the correct name for the species in sect. *Celluloderma*, also known under the names *P. admirabilis* and *P. chrysophaeus*. A lectotype is designated for the latter. *Pluteus saupeii* and *Pluteus heteromarginatus*, from the USA, *P. castri*, from Russia and Japan, and *Volvopluteus asiaticus*, from Japan, are described as new. A complete description and a new name, *Pluteus losulus*, are given for the African *P. cervinus* var. *ealaensis*. The American *Volvopluteus michiganensis* is described in detail. Taxonomic comments and a morphology-based key to all known species of *Volvopluteus* are provided.

## Keywords

Biodiversity ITS Phylogeny *Pluteus* Species delimitation *Volvopluteus*

## Introduction

The status of the genera traditionally classified in the *Pluteaceae* Kotl. & Pouzar (Agaricales, Basidiomycota) has been reassessed using molecular data, and two genera, *Pluteus* Fr. and *Volvopluteus* Vizzini et al., are now recognized and classified in the family, while *Volvariella* Speg. has been placed outside the Pluteoid clade (Justo et al. 2010). The traditional subdivision of *Pluteus* into three sections (*Pluteus*, *Celluloderma* Fayod and *Hispidoderma* Fayod) is essentially supported by molecular data, allowing the inclusion of taxa with partial veil and/or pileipellis as a cutis in sect. *Celluloderma*. The genus *Volvopluteus* has been erected to accommodate species formerly placed in *Volvariella* characterized by a gelatinous pileipellis and average basidiospore size over 11 µm (Justo et al. 2010). During the study of the phylogeny of this group we have gained some insights into several species-complexes of *Pluteus* and *Volvopluteus* and discovered new taxa that are described here.

*Pluteus cervinus* is the type species of the genus, but the limits of the morphological concept for this taxon have been a matter of debate for several decades (Banerjee and Sundberg 1995; Singer 1956; Smith 1968; Vellinga 1990). Around a dozen morphologically similar species or infraspecific taxa have been described. These differ from *P. cervinus* mostly by general aspect of the basidiocarps,

habitat, presence of clamp-connections and shape and size of cheilocystidia. A similar situation involves the closely related species *P. petasatus* and taxa in this group with white basidiocarps such as *P. pellitus* and *P. nothopellitus*. Morphological delimitation of *Pluteus* species is also controversial for many taxa in sect. *Celluloderma* and sect. *Hispidoderma*, and the boundaries between European and North American taxa in these groups are unclear.

The conflicts between morphological, biological and phylogenetic species recognition in Fungi have been addressed in the literature (Taylor et al. 2000). In the case of *Pluteus*, the practical application of a biological species recognition is even more challenging than for other groups of Agaricales, due to the hardship of growing members of the genus in culture (Banerjee and Sundberg 1993a). Here we focus on the comparison between the classical morphological/biogeographic delimitation of species and the results of the phylogenetic analyses of molecular data. The nuclear ribosomal ITS region (including ITS1, 5.8S and ITS2) has been repeatedly used as a species rank genetic marker for different groups of Agaricales (Frøslev et al. 2006; Hughes et al. 2007; Vellinga 2003, 2007), and despite some limitations (Hughes et al. 2009; Nilsson et al. 2008), it offers a good level of resolution for the species rank.

The present paper addresses the following topics in detail:

1. (i)

Species delimitation and conflicts between morphological and phylogenetic species recognition in several lineages of the genus *Pluteus*: *cervinus/petasatus* clade, *salicinus/albostipitatus* clade, *atromarginatus/losulus* clade, *chrysophlebius/phlebophorus* clade, *fenzlii/mammillatus* clade, *romellii/aurantiorugosus* clade, *plautus/longistriatus* clade and *leoninus* clade.

2. (ii)

Description of three new species of *Pluteus*, *P. saupei*, *P. heteromarginatus* and *P. castri*, found during the molecular study of *Pluteus*. A detailed description and a new name, *P. losulus*, are also given for the African *P. cervinus* var. *ealaensis*.

3. (iii)

Description of the new species *Volvopluteus asiaticus* and the type collection of *Volvopluteus michiganensis*. Additional comments on the morphological variability of *Volvopluteus gloiocephalus* and *Volvopluteus earlei* and a morphology-based key of the known species of *Volvopluteus* are also provided.

For these purposes phylogenetic analyses were performed on separate ITS datasets for each section of *Pluteus* (*Pluteus*, *Celluloderma* and *Hispidoderma*) and *Volvopluteus*.

## Materials and methods

### Fungal collections

A total of 185 fresh or dried specimens of *Pluteus* and *Volvopluteus* were selected for molecular sampling (Table 1). Additional sequences retrieved from GenBank are listed in Table 2. The geographic origin of the collections includes Africa, Asia, Europe, North and South America. In many cases, morphological study and identification of the collections was done previously to this work (Justo and Castro 2007a, 2010a; Menolli et al. 2010, Minnis and Sundberg 2010), but most specimens were re-examined during the course of this study. Newly collected materials and all the Asian and African herbarium collections were studied using standard procedures for morphological examination of the *Pluteaceae* (e.g. Justo and Castro 2007b; Minnis and Sundberg 2010). Descriptive terms for morphological features follow Vellinga (1988). The notation [60, 3, 2] indicates that measurements were made on 60 basidiospores from 3 basidiocarps in 2 collections. Color codes are from Munsell Soil-Color Charts (Munsell Color 2009). The following 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).

Table 1

New sequences generated for this study with GenBank accession numbers. P = *Pluteus*, V = *Volvopluteus*. An asterisk (\*) indicates names being published concurrently with the present article. The symbol "!" indicates a type collection (holotypus, epitypus or paratypus)

Taxon	ITS	Collection (Herbarium)	Geographic origin
P. aff. cervinus	HM562151	REG13658	Germany
P. aff. cervinus	HM562168	REG13664	Germany
P. aff. cervinus	HM562128	TNSF897	Japan
P. aff. cervinus	HM562103	TNSF12370	Japan
P. aff. cervinus	HM562102	TNSF12352	Japan
P. aff. cervinus	HM562126	TNSF16091	Japan
P. aff. cervinus	HM562100	TNSF12361	Japan
P. aff. cervinus	HM562101	TNSF12351	Japan
P. aff. cervinus	HM562034	AJ78 (LOU)	Spain
P. aff. cervinus	HM562085	AJ148 (MICH)	USA (FL)
P. aff. cervinus	HM562098	AJ181 (MICH)	USA (MA)
P. aff. cervinus (white basidiocarp)	HM562178	SF7 (BPI)	USA (IL)
P. aff. cinereofuscus	HM562115	TNSF12400	Japan
P. aff. ephebeus	HM562080	Shaffer4673 (MICH)	France
P. aff. ephebeus	HM562198	Pearson s.n. (MICH, as <i>P. plautus</i> )	UK (England)
P. aff. leoninus I	HM562188	SF19 (BPI)	USA (IL)
P. aff. leoninus I	HM562190	SF21(BPI)	USA (MO)
P. aff. leoninus II	HM562139	TNSF11908	Japan
P. aff. nothopellitus	HM562060	AHS42452 (MICH)	USA (MI)
P. aff. nothopellitus	HM562177	SF5 (BPI)	USA (MI)

<b>Taxon</b>	<b>ITS</b>	<b>Collection (Herbarium)</b>	<b>Geographic origin</b>
<i>P. aff. phlebophorus</i>	HM562186	SF16 (SIU)	USA (IL)
<i>P. aff. podospileus</i>	HM562196	Lundell2541 (MICH)	Sweden
<i>P. aff. pouzarianus</i>	HM562167	REG13620	Germany
<i>P. aff. pouzarianus</i>	HM562156	REG13683	Germany
<i>P. aff. semibulbosus</i>	HM562090	TNSF12393	Japan
<i>P. allostipitatus</i> (Dennis) Singer	HM562130	GF5374 (BR)	Democratic Rep. of Congo
<i>P. allostipitatus</i> (Dennis) Singer	HM562106	AJ187 (MICH)	USA (FL)
<i>P. allostipitatus</i> (Dennis) Singer	HM562057	AJ154 (MICH)	USA (FL)
<i>P. atromarginatus</i> (Singer) Kühner	HM562061	AJ75 (LOU)	Spain
<i>P. atromarginatus</i> (Singer) Kühner	HM562040	AJ76 (LOU)	Spain
<i>P. atromarginatus</i> (Singer) Kühner	HM562083	Sundberg3657 (SIU)	USA (OR)
<i>P. atropungens</i> A.H. Sm. & Bartelli	HM562059	A.H.Smith62033!(MICH)	USA (MI)
<i>P. aurantiorugosus</i> (Trog) Sacc.	HM562041	AJ219 (LOU)	Spain
<i>P. aurantiorugosus</i> (Trog) Sacc.	HM562081	ILLS42433	USA (IL)
<i>P. aurantiorugosus</i> (Trog) Sacc.	HM562072	Bigelow19232 (NY)	USA (MA)
<i>P. aurantiorugosus</i> (Trog) Sacc.	HM562074	Hoseny1740 (MICH)	USA (MI)
<i>P. aurantiorugosus</i> (Trog) Sacc.	HM562121	TNSF12391	Japan
<i>P. aureovenatus</i> Menolli & Capelari	HM562160	SP394388	Brazil
<i>P. brunneidiscus</i> Murrill	HM562042	AJ61 (MA)	Spain
<i>P. castri</i> Justo & E.F. Malysheva	HM562092	TNSF17602!	Japan
<i>P. castri</i> Justo & E.F. Malysheva	HM562099	TNSF17081	Japan
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562165	REG13652	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562166	REG13662	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562155	REG13616	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562150	REG16651	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562152	REG13641!	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562171	REG13661	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562169	REG13622	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562153	REG13618	Germany
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562104	TNSF12347	Japan
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562134	AJ192 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562135	AJ79 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562133	AJ191 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562035	AJ82 (LOU)	Spain
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562136	AJ77 (LOU)	Spain

<b>Taxon</b>	<b>ITS</b>	<b>Collection (Herbarium)</b>	<b>Geographic origin</b>
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562043	AJ106 (MICH)	USA (MA)
<i>P. cervinus</i> (Schaeff.) P. Kumm.	HM562176	SF4 (BPI)	USA (MI)
<i>P. cervinus</i> (Schaeff.) P. Kumm. (white basidiocarp)	HM562200	SF1 (BPI)	USA (IL)
<i>P. cervinus</i> var. <i>scaber</i> J.E. Lange	HM562075	A.H.Smith 39846 (MICH)	USA (WA)
<i>P. cf. eugraptus</i>	HM562116	TNSF12042	Japan
<i>P. cf. nanus</i>	HM562046	AJ216 (LOU)	Spain
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.	HM562125	TNSF12383	Japan
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.	HM562088	TNSF12388	Japan
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.	HM562064	AJ45 (MA)	Spain
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.	HM562180	SF10 (BPI)	USA (IL)
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.	HM562182	SF12 (BPI)	USA (IL)
<i>P. chrysophlebius</i> (Berk. & Ravenel) Sacc.	HM562181	SF11 (SIU)	USA (IL)
<i>P. cinereofuscus</i> J.E. Lange	HM562108	AJ229 (LOU)	Portugal
<i>P. cinereofuscus</i> J.E. Lange	HM562124	AJ34 (MA)	Spain
<i>P. conizatus</i> var. <i>africanus</i> E. Horak	HM562142	Thoen5250	Democratic Rep. of Congo
<i>P. densifibrillosus</i> Menolli & Capelari	HM562159	SP393696!	Brazil
<i>P. dietrichii</i> Bres.	HM562143	JLS1624 (J.L. Siquier)	Spain
<i>P. eliae</i> Singer	HM562076	Isaacs2460 (MICH)	USA (FL)
<i>P. eludens</i> E.F. Malysheva, Minnis & Justo*	HM562118	MA50497!	Portugal (Madeira)
<i>P. eludens</i> E.F. Malysheva, Minnis & Justo*	HM562185	SF15 (BPI)	USA (IL)
<i>P. ephebeus</i> (Fr.) Gillet	HM562044	AJ234 (LOU)	Spain
<i>P. fenzlii</i> (Schulzer) Corriol & P.-A. Moreau	HM562091	TNSF12376	Japan
<i>P. fenzlii</i> (Schulzer) Corriol & P.-A. Moreau	HM562111	Kotlaba F1020647 (F)	Slovakia
<i>P. glaucotinctus</i> E. Horak	HM562131	GF5274! (BR)	Democratic Rep. of Congo
<i>P. glaucotinctus</i> E. Horak	HM562147	SP394380	Brazil
<i>P. glaucotinctus</i> E. Horak	HM562157	SP394384	Brazil
<i>P. glaucotinctus</i> E. Horak	HM562132	Thoen5546 (BR)	Democratic Rep. of Congo
<i>P. granularis</i> Peck	HM562189	SF20 (BPI)	USA (IL)

<b>Taxon</b>	<b>ITS</b>	<b>Collection (Herbarium)</b>	<b>Geographic origin</b>
<i>P. granularis</i> Peck	HM562069	Strack7 (SIU)	USA (IL)
<i>P. granulatus</i> Bres.	HM562048	AJ203 (LOU)	Spain
<i>P. heteromarginatus</i> Justo	HM562058	AJ172! (MICH)	USA (FL)
<i>P. leoninus</i> (Schaeff.) P. Kumm.	HM562077	Josserand s.n (MICH, as <i>P. luteomarginatus</i> )	France
<i>P. leoninus</i> (Schaeff.) P. Kumm.	HM562071	Halling6546 (NY)	USA (NY)
<i>P. leoninus</i> (Schaeff.) P. Kumm.	HM562045	AJ212 (LOU)	Spain
<i>P. leoninus</i> (Schaeff.) P. Kumm.	HM562187	SF17 (BPI)	USA (MI)
<i>P. leoninus</i> (Schaeff.) P. Kumm.	HM562215	DrewH (BPI)	USA(ID)
<i>P. longistriatus</i> (Peck) Peck	HM562149	SP394004	Brazil
<i>P. longistriatus</i> (Peck) Peck	HM562172	SP394386	Brazil
<i>P. longistriatus</i> (Peck) Peck	HM562158	SP393700	Brazil
<i>P. longistriatus</i> (Peck) Peck	HM562082	Minnis309203 (SIU)	USA (MO)
<i>P. losulus</i> Justo(≡ <i>P. cervinus</i> var. <i>calaensis</i> Beeli)	HM562129	GF5273 (BR)	Democratic Rep. of Congo
<i>P. magnus</i> McClatchie	HM562087	CBM36790	Japan
<i>P. mammillatus</i> (Longyear) Minnis, Sundb. & Methven	HM562120	Singer244A (F)	USA (FL)
<i>P. mammillatus</i> (Longyear) Minnis, Sundb. & Methven	HM562086	Minnis309202 (SIU)	USA (MO)
<i>P. mammillatus</i> (Longyear) Minnis, Sundb. & Methven	HM562119	ASM7916! (EIU)	USA (MO)
<i>P. multiformis</i> Justo, A. Caball. & G. Muñoz*	HM562201	AC4249! (AH)	Spain
<i>P. nothopellitus</i> Justo & M.L. Castro	HM562063	AJ58!(MA)	Spain
<i>P. pallescens</i> P.D. Orton	HM562056	AJ214 (LOU)	Spain
<i>P. pallidus</i> Homola	HM562193	A.H.Smith62487! (MICH)	USA (MI)
<i>P. pantherinus</i> Courtec. & M. Uchida	HM562089	TNSF12882	Japan
<i>P. pellitus</i> (Pers.) P. Kumm.	HM562036	AJ72 (LOU)	Italy
<i>P. pellitus</i> (Pers.) P. Kumm.	HM562047	AJ74 (LOU)	Spain
<i>P. pellitus</i> (Pers.) P. Kumm.	HM562037	AJ202 (LOU)	Spain
<i>P. petasatus</i> (Fr.) Gillet	HM562084	Ammirati3103 (MICH)	USA (MI)
<i>P. petasatus</i> (Fr.) Gillet	HM562065	AJ143 (MICH)	USA (MA)
<i>P. petasatus</i> (Fr.) Gillet	HM562038	AJ201 (LOU)	Spain
<i>P. petasatus</i> (Fr.) Gillet	HM562109	AJ145 (MICH)	USA (FL)
<i>P. petasatus</i> (Fr.) Gillet	HM562175	SF3 (BPI)	USA (IL)
<i>P. petasatus</i> (Fr.) Gillet	HM562070	Mueller-Strack1 (SIU)	USA (IL)
<i>P. petasatus</i> (Fr.) Gillet	HM562073	Harrison10325 (MICH)	USA (MI)
<i>P. petasatus</i> (Fr.) Gillet	HM562179	SF9 (BPI)	USA (MO)



<b>Taxon</b>	<b>ITS</b>	<b>Collection (Herbarium)</b>	<b>Geographic origin</b>
<i>P. phaeoleucus</i> E. Horak	HM562141	GF5102! (BR)	Democratic Rep. of Congo
<i>P. phlebophorus</i> (Ditmar) Kumm.	<i>P.</i> HM562112	Homola1849 (MICH)	USA (MI)
<i>P. phlebophorus</i> (Ditmar) Kumm.	<i>P.</i> HM562039	AJ81 (MA)	Spain
<i>P. phlebophorus</i> (Ditmar) Kumm.	<i>P.</i> HM562117	TNSF12394	Japan
<i>P. phlebophorus</i> (Ditmar) Kumm.	<i>P.</i> HM562138	AJ228 (LOU)	Spain
<i>P. phlebophorus</i> (Ditmar) Kumm.	<i>P.</i> HM562137	AJ194 (LOU)	Spain
<i>P. phlebophorus</i> (Ditmar) Kumm.	<i>P.</i> HM562144	AJ193 (LOU)	Spain
<i>P. phlebophorus</i> (Ditmar) Kumm.	<i>P.</i> HM562184	SF14 (SIU)	USA (MI)
<i>P. plautus</i> (Weinm.) Gillet	HM562055	AJ209 (LOU)	Spain
<i>P. podospileus</i> Sacc. & Cub.	HM562122	TNSF12398	Japan
<i>P. podospileus</i> Sacc. & Cub.	HM562049	AJ204 (LOU)	Spain
<i>P. pouzarianus</i> Singer	HM562170	REG13619	Germany
<i>P. pouzarianus</i> Singer	HM562154	REG13626	Germany
<i>P. pouzarianus</i> Singer	HM562096	TNSF12371	Japan
<i>P. pouzarianus</i> Singer	HM562050	AJ208 (LOU)	Spain
<i>P. puttemansii</i> Menolli & Capelari	HM562164	SP393698!	Brazil
<i>P. riberalensis</i> var. <i>conquistensis</i> Singer	HM562162	SP393704	Brazil
<i>P. romellii</i> (Britzelm.) Sacc.	HM562123	TNSF12387	Japan
<i>P. romellii</i> (Britzelm.) Sacc.	HM562054	AJ215 (LOU)	Spain
<i>P. romellii</i> (Britzelm.) Sacc.	HM562183	SF13 (SIU)	USA (IL)
<i>P. romellii</i> (Britzelm.) Sacc.	HM562078	Shaffer3715 (MICH)	USA (MI)
<i>P. romellii</i> (Britzelm.) Sacc.	HM562105	Sundberg24198112 (SIU)	USA (MO)
<i>P. romellii</i> (Britzelm.) Sacc.	HM562062	AJ232 (LOU)	Spain
<i>P. rugosidiscus</i> Murrill	HM562079	Homola109 (MICH)	USA (MI)
<i>P. salicinus</i> (Pers.) P. Kumm	HM562051	MA67874	Spain
<i>P. salicinus</i> (Pers.) P. Kumm	HM562174	SF2 (BPI)	USA (MI)
<i>P. sandalioticus</i> Contu & Arras	HM562052	AJ200 (LOU)	Spain
<i>P. sandalioticus</i> Contu & Arras	HM562107	AJ60 (MA)	Spain
<i>P. saupe</i> Justo & Minnis	HM562113	ILLS42441!	USA (IL)
<i>P. seticeps</i> (G.F. Atk.) Singer	HM562199	Shaffer798 (MICH)	USA (IL)
<i>P. seticeps</i> (G.F. Atk.) Singer	HM562192	SF24 (SIU)	USA (WI)
<i>P. seticeps</i> (G.F. Atk.) Singer	HM562191	SF23 (SIU)	USA (MO)

Taxon	ITS	Collection (Herbarium)	Geographic origin
P. sp. I (sect. Hispidoderma)	HM562127	TNSF12372	Japan
P. sp. I (sect. Hispidoderma)	HM562114	TNSF12365	Japan
P. sp. II (sect. Celluloderma)	HM562148	SP394382	Brazil
P. sp. II (sect. Celluloderma)	HM562146	SP394387	Brazil
P. sp. III (sect. Celluloderma)	HM562145	SP394379	Brazil
P. sp. IV (sect. Celluloderma)	HM562173	SP394383	Brazil
P. sp. V (sect. Celluloderma)	HM562161	SP394389	Brazil
P. sp. VI (sect. Celluloderma)	HM562216	DrewU (BPI)	USA (IL)
P. spegazzinanus Singer	HM562194	SingerM3377 (MICH)	Argentina
P. stirps subcervinus	HM562093	TNSF12349	Japan
P. stirps subcervinus	HM562094	TNSF12348	Japan
P. stirps subcervinus	HM562097	TNSF12356	Japan
P. stirps subcervinus	HM562095	TNSF12360	Japan
P. stirps subcervinus	HM562217	DrewP (BPI)	USA (ID)
P. stirps subcervinus (white basidiocarp)	HM562068	Thiers39341 (SIU)	USA (CA)
P. thomsonii (Berk. & Broome) Dennis	HM562053	AJ206 (LOU)	Spain
P. thomsonii (Berk. & Broome) Dennis	HM562066	MA54629	Spain
P. thomsonii (Berk. & Broome) Dennis	HM562067	AMD120 (SIU)	USA (IL)
P. thomsonii (Berk. & Broome) Dennis	HM562197	Homola930 (MICH)	USA (MI)
P. umbrosus (Pers.) P. Kumm.	HM562140	AJ213 (MA)	Spain
P. viscidulus Singer	HM562110	SingerT797! (MICH)	Argentina
P. xylophilus (Speg.) Singer	HM562163	SP393707	Brazil
V. asiaticus Justo & Minnis	HM562206	TNSF15191!	Japan
V. earlei (Murrill) Vizzini, Contu & Justo	HM562205	Mamet 7 (BR)	Democratic Rep. of Congo
V. earlei (Murrill) Vizzini, Contu & Justo	HM562204	MA22816	Spain
V. earlei (Murrill) Vizzini, Contu & Justo	HM246496	TOAV133 (as Volvariella cookei)	Italy
V. earlei (Murrill) Vizzini, Contu & Justo	HM246498	TOHG2001	Italy
V. earlei (Murrill) Vizzini, Contu & Justo	HM246499	TOHG1973 (as Volvariella acystidiata)	Italy
V. earlei (Murrill) Vizzini, Contu & Justo	HM246497	TOAV134	Italy
V. gloiocephalus (DC.) Vizzini, Contu & Justo	HM562207	LOU18619	Portugal

<b>Taxon</b>	<b>ITS</b>	<b>Collection (Herbarium)</b>	<b>Geographic origin</b>
V. gloiocephalus (DC.) Vizzini, Contu & Justo	HM562209	LOU18247	Spain
V. gloiocephalus (DC.) Vizzini, Contu & Justo	HM562208	LOU13710	Spain
V. gloiocephalus (DC.) Vizzini, Contu & Justo	HM562202	AJ239 (LOU)	Spain
V. gloiocephalus (DC.) Vizzini, Contu & Justo	HM562203	PBM2272	USA (CA)
V. gloiocephalus (DC.) Vizzini, Contu & Justo	HM246495	TOAV136	Italy
V. gloiocephalus (DC.) Vizzini, Contu & Justo	HM246490	TOAV135	Italy
V. michiganensis (A.H. Sm.) Justo & Minnis	HM562195	A.H.Smith32-590! (MICH)	USA (MI)

Table 2

Sequences retrieved from GenBank. Original names for misidentified sequences are indicated in parentheses. P = *Pluteus*. An asterisk (\*) indicates names being published concurrently with the present article. The symbol "!" indicates a type collection (holotypus, epitypus or paratypus)

<b>Taxon</b>	<b>ITS</b>	<b>Collection</b>	<b>Geographic origin</b>
P. aff. nothopellitus (as "P. pellitus")	FJ774078	LE 217548	Russia
P. aff. romellii (as "P. romellii")	AY854065	ECV3201	USA (CA)
P. albstipitatus	FJ816656	SP393713	Brazil
P. albstipitatus	FJ816661	SP 393714	Brazil
P. atromarginatus	EF530926	UBC F16254	Canada
P. atromarginatus	FJ774075	LE 246081	Russia
P. atromarginatus	DQ494687	HKAS 31573	China
P. aureovenatus	FJ816663	SP 393697	Brazil
P. castri (as "P. aurantiorugosus")	FJ774077	LE 216873	Russia
P. cervinus	EU486448	UBC F16293	Canada
P. cf. nanus	FJ774081	LE 213093	Russia
P. dominicanus var. hyalinus Menolli & Capelari	FJ816665	SP 393695!	Brazil
P. eludens* (as "P. podospileus")	FJ774085	LE 213015	Russia
P. fenlzii	FJ774082	LE 246083	Russia
P. fluminensis Singer	FJ816664	SP 393711	Brazil
P. fluminensis Singer	FJ816655	SP 393710	Brazil
P. fuligineovenosus E. Horak	FJ816662	SP 393705	Brazil
P. granulatus	FJ774086	LE 212990	Russia
P. harrisii Murrill	FJ816666	SP 393709	Brazil
P. harrisii Murrill	FJ816654	SP 393708	Brazil

<b>Taxon</b>	<b>ITS</b>	<b>Collection</b>	<b>Geographic origin</b>
<i>P. jamaicensis</i> Murrill	FJ816657	SP 393706	Brazil
<i>P. petasatus</i>	AF085495	CBS441.85	Czech. Rep.
<i>P. plautus</i>	FJ774076	LE 213024	Russia
<i>P. romellii</i>	FJ774073	LE 217944	Russia
<i>P. salicinus</i>	FJ774087	LE 215427	Russia
<i>P. semibulbosus</i> (Lasch) Gillet	FJ774080	LE 227534	Russia
<i>P. sp. VII</i> (as " <i>P. exiguus</i> ")	FJ774083	LE 226543	Russia
<i>P. sublaevigatus</i> (Singer) Menolli & Capelari	FJ816667	SP 393694	Brazil
<i>P. thomsonii</i>	FJ774084	LE 234787	Russia
<i>P. xylophilus</i>	FJ816659	SP 393701	Brazil
<i>P. xylophilus</i>	FJ816660	NMJ147 (SP)	Brazil
Uncultured Basidiomycete	DQ672275	Environmental sample (soil)	Australia
Uncultured Basidiomycete	AM901879	Environmental sample (house dust)	Finland
Uncultured Basidiomycete	AY969369	Environmental sample (hardwood litter)	USA(NC)
Uncultured fungus	FM999644	Environmental sample (sporocarp)	USA(OH)
Uncultured fungus	FM999650	Environmental sample (sporocarp)	USA(OH)
Uncultured fungus	FM999562	Environmental sample (sporocarp)	USA(OH)
Uncultured fungus	FM999557	Environmental sample (sporocarp)	USA(OH)

Information about the morphology and ecology of the taxa here discussed is based not only on the specimens sampled for molecular analyses, but also on the study of many European and American collections of *Pluteus* and *Volvopluteus* (Justo and Castro 2007a, 2010a; Menolli and Capelari 2010; Menolli et al. 2010; Minnis and Sundberg 2010; Rodríguez and Guzmán-Dávalos 2007).

#### DNA extraction, PCR, sequencing and alignments

Approximately 0.05–0.10 g of tissue (preferably gills) from each collection were ground directly in a 1.5 ml eppendorf tube, using plastic pestles, or in a mortar with liquid nitrogen. DNA was extracted using 3% SDS extraction buffer and was then isolated by the sequential addition of phenol chloroform and chloroform-isoamyl alcohol; finally, isopropyl alcohol and 3 M sodium acetate were added to precipitate the DNA, which was washed with 70% ethanol and resuspended in sterile water.

Primer pairs ITS1F–ITS4 were used to amplify the ITS region (Gardes and Bruns 1993; White et al. 1990). The amplification products were sequenced using 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 using Sequencher 4.7 (GeneCodes, Ann Arbor, Michigan).

Sequences were aligned using MAFFT (<http://align.bmr.kyushu-u.ac.jp/mafft/online/server/>). The alignments were then examined and manually corrected using MacClade 4.05 (Maddison and Maddison 2002). Alignments have been deposited in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S10654>).

Sequence divergence was calculated using MatGAT (Campanella et al. 2003).

### Phylogenetic analyses

Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Analysis (BA) were performed with the following parameters: (i) MP: Equally weighted parsimony analysis was performed using 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 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; (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 one hundred rapid bootstrap replicates; (iii) BA: The analysis was run using MrBayes 3.1 (Ronquist and Huelsenbeck 2003) for ten million generations, under a GTR model, with four chains, and trees sampled every 100 generations; after examining the graphic representation of the likelihood scores, using Tracer (<http://tree.bio.ed.ac.uk/software/tracer/>), the burn-in period was set to 1.5 million generations for all datasets. The three analyses were performed in all the datasets presented here. The following abbreviations are used: Most Parsimonious Trees (MPT), Bootstrap (BS) and Posterior Probability (PP).

## Results

Analyses of section *Pluteus* (Fig. 1)

The dataset includes 98 *Pluteus* sect. *Pluteus* ITS sequences and 2 of sect. *Celluloderma* (*P. cinereofuscus*, *P. chrysophlebius*) that were used as an outgroup. The final dataset consists of 649 characters (gaps included), of which 229 are parsimony informative. In the MP analysis, 1840 MPT were recovered (Length = 699; CI = 0.58; RI = 0.90). One of the 1840 MPT is shown in Fig. 1.

Fig. 1 One of the 1840 MPT from the analysis of sect. *Pluteus*. Branch lengths are shown on the branches

Three major clades are recovered, with moderate to high support, in all the analyses.

*cervinus/petasatus* clade. Nine major lineages (Fig. 1, clades I to IX) can be recognized: *P. cervinus*, *P. aff. cervinus*, *P. pouzarianus* (including *P. pouzarianus*, *P. brunneidiscus*, *P. spegazzinianus* and *P. aff. pouzarianus*), *P. stirps subcervinus*, *P. petasatus* (including *P. petasatus*, *P. viscidulus*, *P. magnus*), *P. xylophilus*, *P. pellitus*, *sandalioticus* and *P. aff. nothopellitus*. All these clades are present in the strict consensus tree of MP analysis, the best tree from the ML analysis and the 50 percent majority rule consensus tree from the BA analysis. The topological relations between these groups are usually the same in the three different analyses (Fig. 1), with the following exceptions:

In the ML and BA trees, *P. aff. pouzarianus* is sister to *P. spegazzinianus* and *P. pouzarianus*, while in the strict consensus tree of the MP analysis the relations among these three clades are unresolved (Fig. 1).

In the ML and BA analyses *P. aff. nothopellitus* is placed as the sister clade to *P. petasatus*, while in the strict consensus tree of the MP analysis it is basal to *P. petasatus*, *P. xylophilus*, *P. pellitus* and *P. sandalioticus* (Fig. 1).

The internal topology of *P. cervinus*, *P. aff. cervinus* and *P. petasatus* is poorly resolved in all the analyses, though some relationships are consistently recovered:

In *P. cervinus*, the collection identified as *P. cervinus* var. *scaber* and the GenBank sequences of *P. cervinus* and an environmental sample from housedust are always grouped together and separately from the other sequences in this clade. The Japanese sequence of *P. cervinus* always clusters separately from all other sequences. The remaining sequences always cluster together, though with many possible alternative topologies in the 1840 MPT.

In *P. aff. cervinus*, only two pairs of sequences are constantly grouped together: *P. nothopellitus* AJ58 & *P. aff. cervinus* AJ78 and *P. aff. cervinus* TNSF 12352 & TNSF 12370.

In *P. petasatus*, the sequence of *P. magnus* is placed as sister to the remaining sequences in this clade in the ML tree and in the 50 percent consensus tree of the MP analysis but this relationship is not recovered in the BA tree or the strict consensus tree of the MP analysis.

*salicinus/albostipitatus* clade. Five distinct lineages (clades X to XIV in Fig. 1) are recognized: *P. albostipitatus* (including *P. albostipitatus*, *P. phaeoleucus*, *P. densifibrillous*), *P. salicinus*, *P. harrisii* (including *P. harrisii*, *P. puttemansii*), *P. saupe*, *P. glaucotinctus*. Except for the internal topology of the *P. albostipitatus*, *P. harrisii* and *P. glaucotinctus* clades, all topological relations between and inside these clades are the same in all the analyses (Fig. 1).

In *P. albostipitatus*, the two collections from Florida (AJ154, AJ187) cluster together in all the analyses, but the general internal topology of this clade changes among the different analyses and the 1840 MPT.

In *P. harrisii*, the sequence of *P. harrisii* FJ816654 alternatively clusters with the other sequence of *P. harrisii* or with the sequence of *P. puttemansii*.

In *P. glaucotinctus*, the sequence from the collection Thoen5546 alternatively clusters with the Brazilian collections (SP394380, SP394384) or with the holotype of *P. glaucotinctus* from Africa (GF 5274). In the 50 percent majority rule consensus tree from the MP analysis the clustering of Thoen5546 and the Brazilian collections is recovered.

*atromarginatus/losulus* clade. This clade (lineages XV and XVI in Fig. 1) receives 91% BS in the ML analysis, but much lower support in the MP (79% BS) and the BA (0.78 PP) analyses. In all analyses *P. losulus* ( $\equiv$  *P. cervinus* var. *ealaensis*, see Taxonomy section) is placed as the sister group of the *P. atromarginatus* clade. The sequence from the holotype of *P. atropungens* is placed as basal to all sequences of *P. atromarginatus* (Fig. 1).

Analyses of section *Celluloderma* (Fig. 2)

The dataset includes 79 *Pluteus* sect. *Celluloderma* ITS sequences and 2 of sect. *Pluteus* (*P. glaucotinctus*, *P. losulus*) that were used as an outgroup. The final dataset consists of 675 characters (gaps included) of which, 270 are parsimony informative. In the MP analysis, 259 MPT were recovered (Length = 1294; CI = 0.42; RI = 0.82). One of the 259 MPT is shown in Fig. 2.



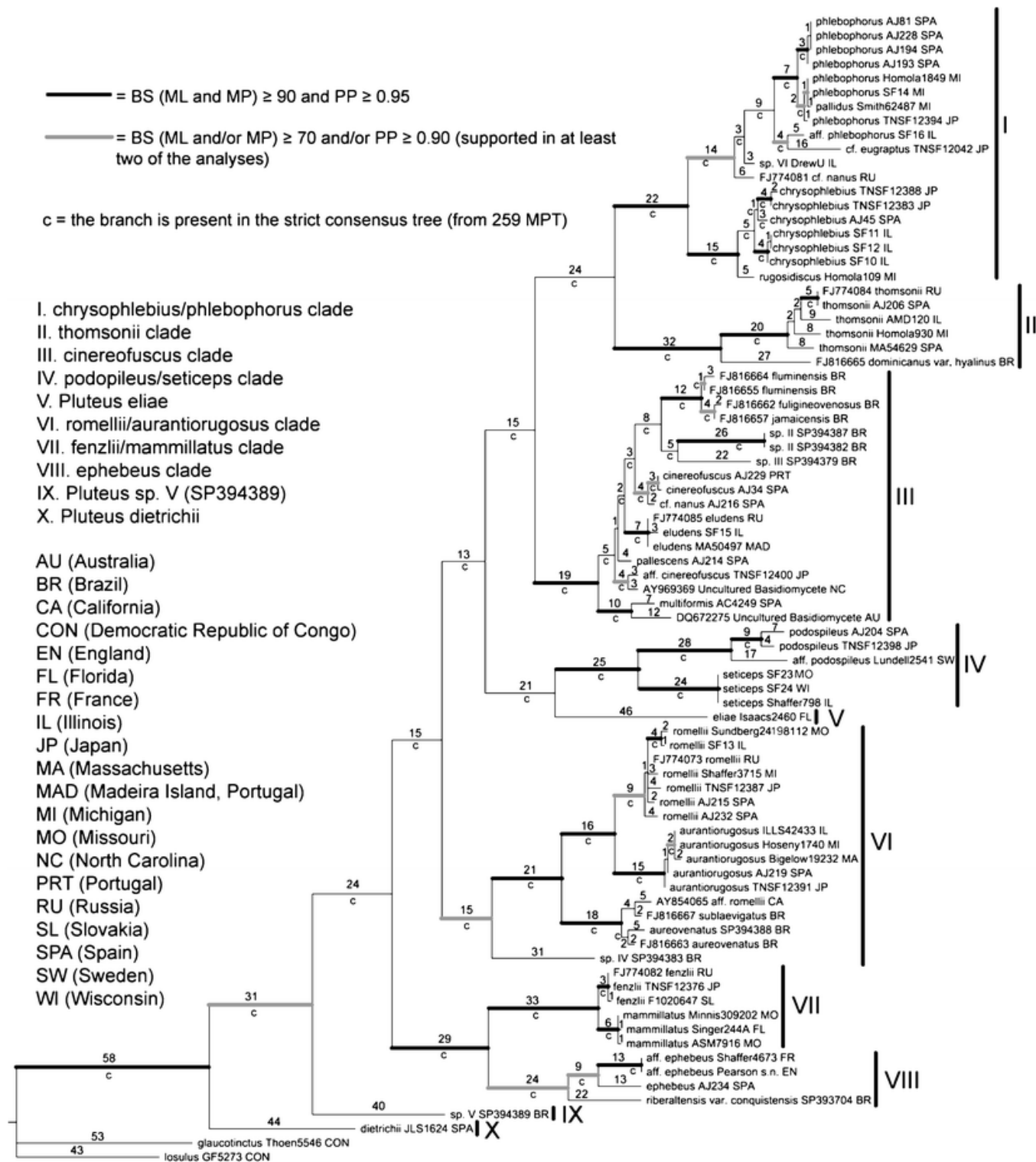


Fig. 2 One of the 259 MPT from the analysis of sect. *Celluloderma*. Branch lengths are shown on the branches

Seven well supported clades are recognized in all the analyses: *chrysophlebius/phlebophorus* clade, *thomsonii* clade, *cinereofuscus* clade, *podospileus/seticeps* clade, *romellii/aurantiorugosus* clade, *fenzlii/mammillatus* clade and *ephebeus* clade. Three species are placed outside these major lineages: (i) *P. dietrichii* is placed as the sister taxon to all other species in sect. *Celluloderma* in all the analyses. (ii) *P. eliae* is placed as the sister taxon to the *podospileus/seticeps* clade in the ML and MP analyses but with no statistical support. In the BA analysis it is placed in an unresolved position together with the *podospileus/seticeps*, *ephebeus*, *fenzlii/mammillatus*, *romellii/aurantiorugosus* and *cinereofuscus* clades. (iii) *Pluteus* sp. V (SP394389) is placed as the sister taxon of the *romellii/aurantiorugosus*

clade in the ML and BA analyses, but this relationship does not get statistical support. In all 259 MPT, this taxon is placed as sister to all taxa in sect. *Celluloderma* except *P. dietrichii* (Fig. 2).

*chrysophlebius/phlebophorus* clade (Fig. 2, clade I). Two major clades corresponding to *P. chrysophlebius* (including *P. chrysophaeus* sensu Vellinga (1990), see Discussion section) and *P. phlebophorus* (including *P. pallidus* and *P. luctuosus*) are recovered in all the analyses with essentially the same topology depicted in Fig. 2. In all analyses *P. chrysophlebius* and *P. phlebophorus* collections are grouped according to their geographic origin (see Discussion section). *P. rugosidiscus* is placed as sister to the *P. chrysophlebius* clade. Four additional taxa are placed in the same subclade as *P. phlebophorus*: *P. cf. eugraptus*, *P. aff. phlebophorus*, *Pluteus* sp. VI and *Pluteus* cf. *nanus*.

*thomsonii* clade (Fig. 2, clade II). *P. dominicanus* var. *hyalinus* is placed as sister to the sequences of *P. thomsonii* in all analyses. Except for the clustering of *P. thomsonii* LE234787 from Russia and *P. thomsonii* AJ206 from Spain, the internal topology of *P. thomsonii* varies among the different analyses.

*cinereofuscus* clade (Fig. 2, clade III). It includes *P. cinereofuscus*, *P. eludens*, *P. fluminensis*, *P. fuligineovenous*, *P. jamaicensis*, *P. multiformis* and *P. pallescens*. Several potentially undescribed taxa are also included here: *P. aff. cinereofuscus*, *Pluteus* sp. II (SP394382, SP394387), *Pluteus* sp. III (SP 394379). One of the two collections included in this study morphologically assignable to *P. nanus* (AJ216) and two environmental sequences are also placed in this clade.

*podospileus/seticeps* clade (Fig. 2, clade IV). *P. podospileus*, a closely related taxon (*Pluteus* aff. *podospileus* Lundell 2541) and *P. seticeps* are placed here. The internal topology of this clade is the same in all the analyses.

*romellii/aurantiorugosus* clade (Fig. 2, clade VI). It includes three well-supported clades, corresponding to *P. romellii*, *P. aurantiorugosus* and a third, more diverse, clade that contains *P. sublaevigatus*, *P. aureovenatus* and the Californian sequence previously identified as *P. romellii*. The Brazilian collection SP394383 is also included in this clade, and it is placed as sister to all other taxa.

*fenzlii/mammillatus* clade (Fig. 2, clade VII). It contains *P. fenzlii* and *P. mammillatus* (three sequences each). In all the analyses the internal topology for each species is unresolved as the sequences are almost 100% identical (Fig. 2).

*ephebeus* clade (Fig. 2, clade VIII). It contains two distinct clades of *P. ephebeus* sensu lato and *P. riberalensis* var. *conquistensis*. The internal topology of this clade is the same in all the analyses.

Analyses of section *Hispidoderma* (Fig. 3)

The dataset includes 29 *Pluteus* sect. *Hispidoderma* ITS sequences and 2 of sect. *Celluloderma* (*P. seticeps*, *P. dietrichii*) that were used as an outgroup. The final dataset consists of 682 characters (gaps included) of which, 222 are parsimony informative. In the MP analysis, only 1 equally most parsimonious tree (MPT) was recovered (Length = 666; CI = 0.66; RI = 0.86). The MPT is shown in Fig. 3.

**Legend:**

- Thick black line** = BS (ML and MP) ≥ 90 and PP ≥ 0.95
- Thin grey line** = BS (ML and/or MP) ≥ 70 and/or PP ≥ 0.90 (supported in at least two of the analyses)

**Clades:**

- I. leoninus clade
- II. umbrosus/granularis clade
- III. plautus/longistriatus clade
- IV. Pluteus pantherinus

**Geographical Locations:**

- BR (Brazil)
- CON (Democratic Republic of Congo)
- FL (Florida)
- FR (France)
- ID (Idaho)
- IL (Illinois)
- JP (Japan)
- MI (Michigan)
- MO (Missouri)
- NY (New York)
- RU (Russia)
- SPA (Spain)

**Species and Bootstrap Values:**

- leoninus Halling6546 NY (BS=1, PP=1)
- leoninus SF17 MI (BS=1, PP=1)
- leoninus DrewH ID (BS=2, PP=2)
- leoninus Josserand s.n. FR (BS=1, PP=1)
- leoninus AJ 212 SPA (BS=1, PP=1)
- aff. leoninus I SF19 IL (BS=7, PP=7)
- aff. leoninus I SF21 MO (BS=8, PP=8)
- aff. leoninus II TNSF11908 JP (BS=9, PP=9)
- FJ774077 castri RU (BS=24, PP=24)
- castri TNSF17081 JP (BS=1, PP=1)
- castri TNSF17602 JP (BS=13, PP=13)
- conizatus var. africanus Thoen5250 CON (BS=25, PP=25)
- granularis Strack7 IL (BS=12, PP=12)
- granularis SF20 IL (BS=13, PP=13)
- umbrosus AJ213 SPA (BS=23, PP=23)
- longistriatus SP394004 BR (BS=2, PP=2)
- longistriatus SP393700 BR (BS=13, PP=13)
- longistriatus SP394386 BR (BS=1, PP=1)
- longistriatus Minnis309203 MO (BS=8, PP=8)
- heteromarginatus AJ172 FL (BS=11, PP=11)
- FJ774080 semibulbosus RU (BS=1, PP=1)
- aff. semibulbosus TNSF12393 JP (BS=7, PP=7)
- sp. I TNSF12365 JP (BS=14, PP=14)
- sp. I TNSF12372 JP (BS=21, PP=21)
- FJ774076 plautus RU (BS=4, PP=4)
- plautus AJ209 SPA (BS=23, PP=23)
- FJ774086 granulosus RU (BS=24, PP=24)
- granulosus AJ203 SPA (BS=46, PP=46)
- pantherinus TNSF12882 JP (BS=71, PP=71)
- seticeps Shaffer798 IL (BS=53, PP=53)
- dietrichii JLS1624 SPA (BS=66, PP=66)

#### Analyses of *Volvopluteus* (Fig. 4)

The dataset includes 15 *Volvopluteus* ITS sequences and 2 of *Pluteus* sect. *Hispidoderma* (*P. longistriatus*, *P. heteromarginatus*) that were used as an outgroup. The final dataset consists of 689 characters (gaps included), of which 125 are parsimony informative. In the MP analysis, 4 MPT were recovered (Length = 219; CI = 0.89; RI = 0.94). One of the MPT is shown in Fig. 4.

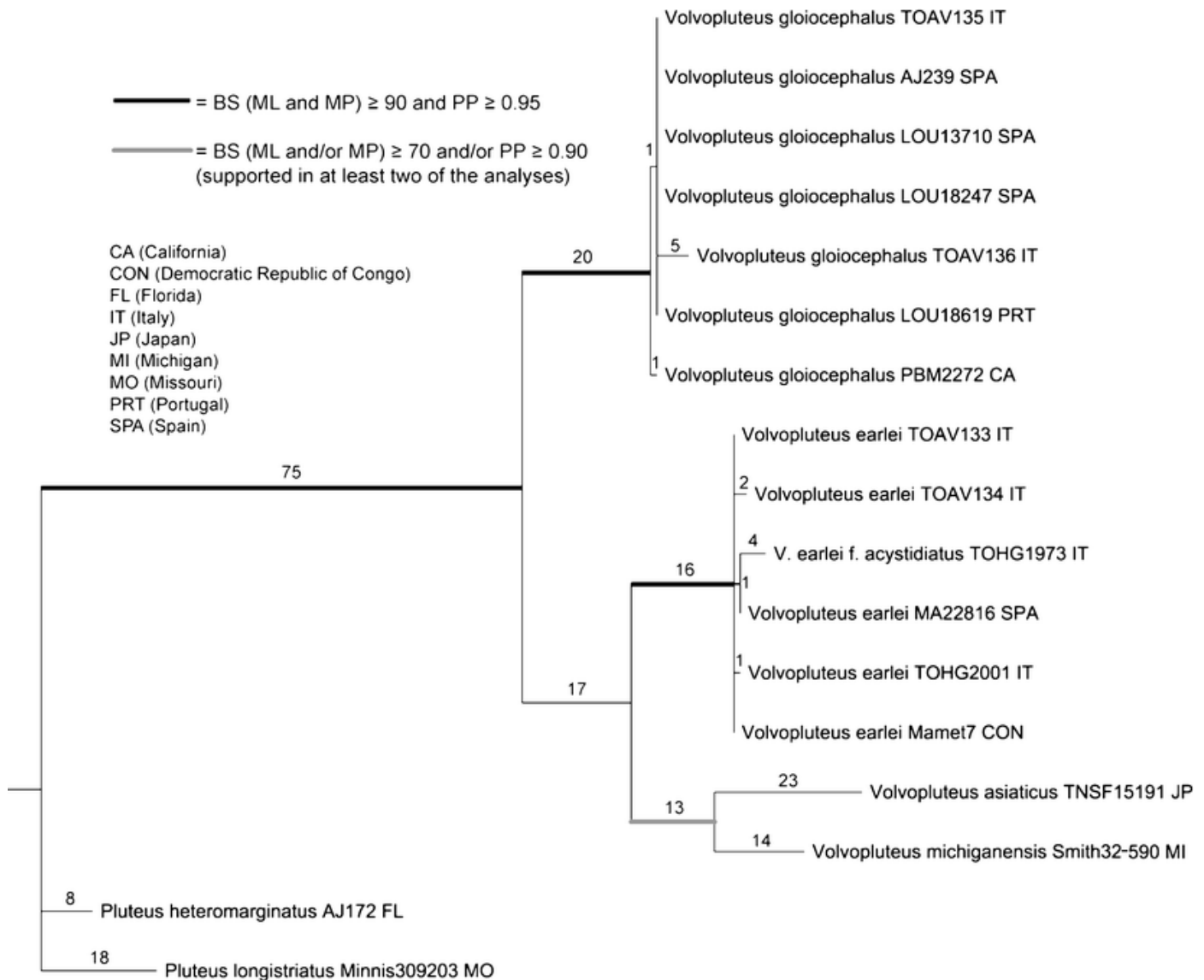


Fig. 4

One of the 4 MPT from the analysis of *Volvopluteus*. Branch lengths are shown on the branches

Three clades are recognized in the analyses:

- *V. gloiocephalus* clade. The European and Californian sequences of *V. gloiocephalus* are placed together in all the analyses. The seven sequences are 99.1-100% identical.
- *V. earlei* clade. Sequences of this species from Spain, Italy and the Democratic Republic of Congo are included here. Collections originally identified under the names *Volvariella cookei* Contu (TOAV133) and *Volvariella acystidiata* N.C. Pathak (TOHG1973) fall within the molecular variation of *V. earlei* (see Taxonomic descriptions below). The six sequences are 99.2-100% identical.

- *V. michiganensis/V. asiaticus* clade. The North American *V. michiganensis* and the Japanese *V. asiaticus* are placed together in all the analyses.

Each one of these clades is well-supported in the analyses but the relationships between them do not receive statistical support. In the all MPT (Fig. 4) *V. gloiocephalus*. is the the most early diverging clade and *V. earlei* is sister to *V. michiganensis/V.asiaticus*. In the ML tree *V. earlei* is the earliest diverging clade and *V. michiganensis/V. asiaticus* is sister to *V. gloiocephalus*. In the BA analysis relationships between the three clades are unresolved.

## Discussion

The correspondence between the lineages recovered in the ITS analyses with morphological species and conflicts between morphological/molecular delimitation are discussed below.

Section *Pluteus* (Fig. 1)

### ***cervinus-petasatus* clade (clades I-IX in Fig. 1)**

#### 1. (I)

##### *Pluteus cervinus*

Collections included here correspond to the most widely accepted morphological concept of *P. cervinus* (Schaeff.) P. Kumm. (Vellinga 1990): a taxon with brown to dark brown pileus; growing on wood of angiosperms or more rarely terrestrially; metuloids with 2-4(6) hooks at apices, not or only rarely bifid; cheilocystidia forming a well-developed strip, clavate or sphaeropedunculate, less than 70 µm long; clamp-connections absent in all parts of the basidiocarp. The only exceptions to this pattern are the collections SF1 (ILL, USA) with white basidiocarps and Smith 39846 identified as *P. cervinus* var. *scaber* J.E. Lange (WA, USA), which is characterized by the very dark (almost black) pileus.

The highest percentage of sequence divergence (2.3%) occurs between the GenBank sequence of *P. cervinus* (EU486448, Canada) and some of the European collections. All other sequences have less than 2% sequence divergence and, almost identical sequences exist in distant geographic areas (e.g. AJ191 from Spain and AJ106 from Massachusetts are 99.7% identical).

All collections sampled in this study grew on angiospermous wood or terrestrially (associated with organic debris), though *P. cervinus* has also been reported to occur on coniferous wood (Justo and Castro 2007a, Vellinga 1990).

*P. cervinus* was originally described from the state of Bavaria (SE Germany) from material growing on decayed wood (Schaeffer 1774). No type collection exists for *Pluteus cervinus*. Fourteen modern collections from Bavaria were examined and sampled for molecular analyses. Eight collections represent *P. cervinus* in both morphological and molecular characters while the remaining six collections represent different taxa (*P. aff. cervinus*, *P. pouzarianus*, *P. aff. pouzarianus*). Since these taxa are distinguishable only by using microscopic and/or molecular characters, there is no way to know to which clade the original *Agaricus cervinus* (Schaeffer 1774) should be assigned.

To avoid greater and continued confusion about the identity of *P. cervinus*, the species is lectotypified below by an illustration included in the original work and epitypified by a supporting modern collection, found near the topotype, from which DNA data were obtained.

*Pluteus cervinus* (Schaeff.) P. Kumm., Führ. Pilzk.: 99. 1871.

≡ *Agaricus cervinus* Schaeff., Fung. Bavar. Palat. 4: 6. 1774 (basionym).

Lectotypus of *Agaricus cervinus* Schaeff. (hic designatus): icon. in Schaeffer, Fung. Bavar. Palat. 1: Tab. X. 1762.

Epitypus of *Agaricus cervinus* Schaeff. (hic designatus): Germany: Bavaria, near Bodenwöhr, railroad embankment in direction Schwandorf, on wood chips, under *Frangula alnus*, 7.IX.1995, A. Bresinsky, REG 13641.

A full morphological description of *Pluteus cervinus*, in the sense accepted here, can be found in Vellinga (1990).

## 2. (II)

*Pluteus* aff. *cervinus*

Collections in this clade are not distinguishable from *P. cervinus* based on macroscopic characters, ecology or geographic distribution, though they show a greater variation on microscopical characters. The pleurocystidia are commonly provided with bifid or coralloid hooks at the apices in some collections (TNSF 12361, 12351, 12370, 12352, 12347, AJ148), but they have non-divided hooks in the remaining collections. The cheilocystidia are scarce in some collections (TNSF 12351, 12352, REG 13664, 13658) not forming a well-developed strip that completely covers the lamellar edges like in *P. cervinus*, but in other collections they are frequent. The holotype of *P. nothopellitus* Justo & M.L. Castro (AJ58), a taxon characterized by white basidiocarps, and an additional collection with the same characteristics (SF7) are included here, but the remaining collections have pigmented basidiocarps.

The maximum percentage of sequence divergence in this clade is 1.2%. The separation of *P. cervinus* and *P. aff. cervinus* is recovered in all the analyses with high statistical support. The sequence divergence between both clades varies between 2 and 4.2%. Representatives of *P. cervinus* and *P. aff. cervinus* may occur in the same locality, e.g. *P. cervinus* AJ106 and *P. aff. cervinus* AJ181 from Harvard Forest (MA, USA). Though some individual collections of *P. aff. cervinus* may be separated from *P. cervinus* based on the characteristics of the cystidia, separation of both clades is not possible when all morphological and geographic variation of each clade is compared. Therefore, *P. aff. cervinus* is considered to represent a cryptic phylogenetic lineage related to *P. cervinus*.

## 3. (III)

*Pluteus pouzarianus* and (IV) *Pluteus stirps subcervinus*

Collections in both clades differ morphologically from *P. cervinus* and *P. aff. cervinus* mainly by the presence of clamp-connections, especially at the base of cheilocystidia and in the pileipellis.

By applying very strict criteria for lineage recognition, three groups can be distinguished in the *pouzarianus* clade:

*P. pouzarianus*/*P. brunneidiscus*. Includes four collections that fit the morphological/ecological concept of *P. pouzarianus* Singer (Vellinga 1990) and one that corresponds to *P. brunneidiscus* Murrill (Justo and Castro 2007b). The main difference between these two taxa is the habitat, on conifers (*P. pouzarianus*) vs. angiosperms (*P. brunneidiscus*).

*P. spegazzinianus* Singer. This taxon is characterized by the partially pigmented cheilocystidia (Singer 1958).

*P. aff. pouzarianus*. These collections have no morphological or ecological differences with the other *pouzarianus*/*brunneidiscus* collections, though they are placed separately in all the analyses.

Sequence divergence in the *pouzarianus* clade varies between 0-3%.

Collections in the stirps *subcervinus* clade fit the morphological/ecological concept of either *P. subcervinus* (Berk. & Broome) Sacc., *P. brunneidiscus* and/or *P. pouzarianus*. The collection TNSF12356 has predominantly hyaline cheilocystidia, intermixed with some cystidia provided with pigment, and would fit the morphological concept of *P. spegazzinianus*. Sequence divergence in the stirps *subcervinus* clade varies between 0-1.3%.

Whether the three lineages in the *pouzarianus* clade are considered to represent three different taxa or all the sequences are considered to represent one single taxon, separation of the phylogenetic lineages recognized in the analyses is not possible using morphological or ecological characters. Collections growing on coniferous wood may have identical sequences to collections growing on angiospermous wood (AJ61, AJ208). Collections with pigmented cheilocystidia occur in different clades (Singer M3377, TNSF 12356). On the other hand, the presence of clamp-connections is indeed a good morphological character to separate these taxa from *P. cervinus* and *P. aff. cervinus*.

With the current sampling, it is not possible to decide which one of the over ten validly published species names in this group (Justo and Castro 2007b; Singer 1986) should be applied to each of the phylogenetic lineages. Additional collections, especially from regions of the world underrepresented in the present study (Australia, tropical Asia), should be sampled before a final taxonomic decision is made.

#### 4. (V)

##### *Pluteus petasatus*

*Pluteus petasatus* (Fr.) Gillet is accepted here in the sense of Vellinga (1990), including collections with a viscid, smooth pileus and collections with a dry, squamulose pileus, which

are recognized by others as a separate species, viz. *Pluteus patricius* (Schulzer) Boud. (Banerjee and Sundberg 1995; Singer 1986). Both characters have a wide range of variation and intergrade to a great extent in the collections studied here. Microscopically, *P. petasatus* is characterized by the (usually) poorly developed strip of cheilocystidia and the predominantly magnus-type metuloid pleurocystidia (i.e. fusiform and unhooked) near the lamellar edge (Vellinga 1990; Justo and Castro 2007c). The isotype of *P. viscidulus* Singer, a similar looking Argentinian species, with slightly smaller basidiospores and abundant cheilocystidia (Singer 1958; Justo and Castro 2007c), falls within the molecular variation of *P. petasatus*.

The Japanese collection of *Pluteus magnus* McClatchie (Takehashi and Kasuya 2009a), a species characterized by magnus-type metuloid pleurocystidia and abundant, up to 80 µm long cheilocystidia, is placed either as sister to all *P. petasatus* sequences (in the ML tree) or falls within the *petasatus* group (BA, MP strict consensus tree). There is 0.9-1.5% sequence divergence between *P. magnus* and the *P. petasatus* sequences. More collections of *P. magnus*, especially near the topotype in California, should be sampled for molecular data, to make a final decision about its possible synonymy with *P. petasatus*.

5. (VI)

*Pluteus pellitus*,

6. (VII)

*Pluteus sandalioticus* and

7. (VIII)

*Pluteus xylophilus*.

*Pluteus xylophilus* (Speg.) Singer is a South American species, similar to *P. petasatus* in the presence of magnus-type pleurocystidia near lamella edge and the scarcity of cheilocystidia. It differs from *P. petasatus* mainly by the brown to dark-brown pileus (Menolli et al. 2010). *Pluteus pellitus* (Pers.) P. Kumm. is accepted here as a white species with clamp-connections and basidiospores  $5-7.5 \times 3.5-5$  µm (Justo and Castro 2007c). *Pluteus sandalioticus* Contu & Arras is a South European species mainly associated with *Quercus* wood characterized by the long (>70 µm) cheilocystidia and presence of clamp-connections (Contu 2001, Justo et al. 2006).

The degree of sequence divergence between *P. pellitus* and *P. sandalioticus* is only 0.9-1.5% and both clades are placed as sister taxa in the MP trees. Therefore, they could be considered as one single, morphologically variable species. On the other hand, the sister taxa relationship of these clades is not recovered in the ML and BA trees, and both clades can be differentiated according to the coloration of the basidiocarps and shape and size of the cheilocystidia. For the time being, both taxa are considered to represent two closely related, but different, species.



8. (IX)

*Pluteus* aff. *nothopellitus*

This clade includes collections morphologically assignable to *P. nothopellitus*, a taxon characterized by white basidiocarps and the absence of clamp-connections (Justo and Castro 2007c). However, the holotype of *P. nothopellitus* (AJ58) is, in fact, a white variant of the normally pigmented taxon here named as *P. aff. cervinus*. The fact that usually pigmented taxa (*P. cervinus*, *P. aff. cervinus*) may exhibit albinistic phenotypes greatly complicates the recognition of *P. aff. nothopellitus* as a morphological species, though it is indeed a distinct phylogenetic lineage.

***salicinus-albostipitatus* clade (clades X-XIV in Fig. 1)**

1. (X)

*Pluteus albostipitatus* (Dennis) Singer

Molecular data support the treatment of the later described *P. phaeoleucus* E. Horak and *P. densifibrillosus* Menolli & Capelari as synonyms of *P. albostipitatus*. In the present concept, *P. albostipitatus* is a variable species regarding presence of fibrils on the pileus (from densely fibrillose to completely smooth) and size and shape of basidiospores. Modern descriptions of this species can be found in the works by Horak and Heinemann (1978), Menolli and Capelari (2010) as "*P. densifibrillosus*", Menolli et al. (2010) and Pegler (1983). *Pluteus phaeoleucus* supposedly differs from *P. albostipitatus* by the absence of pleurocystidia in the former (Horak and Heinemann 1978). However examination of the holotype of *P. phaeoleucus* (Goossens-Fontana 5102 at BR) revealed the presence of frequent pleurocystidia, morphologically similar to those found in *P. albostipitatus*.

The internal topology of this clade varies among the different analyses, except for the grouping of the two collections from Florida, USA (AJ154, AJ187). These collections show the highest level of sequence divergence between any pair of sequences in this clade with 2.6% between AJ154 and FJ816661 from Brazil. However, there are no morphological differences between the Florida collections and the rest of the collections studied here. In view of the relatively wide, and non-correlated, morphological and molecular variation, this clade is considered to represent one single morphological species that spans across the paleo and neotropical regions, though it is possible that it harbors different, but morphologically cryptic, phylogenetic species.

2. (XI)

*Pluteus salicinus* (Pers.) P. Kumm.

The combination of blue-green tinges on the basidiocarp, habitat on angiospermous wood, metuloids with distinct hooks at apex, clavate cheilocystidia and presence of clamp-connections define this species (Vellinga 1990). This taxon occurs in both Europe and North America. One of the American collections identified as *P. salicinus* represent a different species, described below as *Pluteus saupei*, which is placed in the analyses as the sister taxon to *P. harrisii*.

3. (XII)

*Pluteus harrisii* Murrill

*P. harrisii* is usually described as possessing metuloids with poorly developed hooks (Banerjee and Sundberg 1995; Pegler 1983, 1997), and the two Brazilian collections studied here do fit this morphological concept. Examination of the holotypus (Jamaica, Troy and Tyre, on dead wood, 12-14.I.1909, WA Murrill & W Harris 956, NY) and the paratypus (Cuba, El Yunque, III.1903, Underwood & Earle 425, NY) revealed the presence of metuloids with coralloid hooks in the holotypus and metuloids with poorly developed hooks and with a thinner wall in the paratypus (Rodríguez 2010). The type collection of *P. puttemansii* Menolli & Capelari, which has metuloids with well-developed hooks, shows an ITS sequence almost identical to *P. harrisii* (0.4-1.1% divergence). Therefore, *P. harrisii* is considered here as a very variable species regarding the morphology of the pleurocystidia. See Menolli and Capelari (2010) "as *P. puttemansii*" and Menolli et al. (2010) for a full morphological comparison.

4. (XIII)

*Pluteus saupei* (see Taxonomic descriptions)

5. (XIV)

*Pluteus glaucotinctus* E. Horak

This species is characterized by the thin-walled pleurocystidia and the blue-green tinges at the base of the stipe. The molecular variation in the ITS sequences, up to 4.3% sequence divergence between the holotypus (GF5274, Dem. Rep. of Congo) and the Brazilian collections (SP394380, 394384), is not correlated with the morphological variability of the collections. In the molecular analyses, the other African collection (Thoen 5546) alternatively clusters with the holotypus or with the Brazilian collections. As with *P. allostipitatus*, all collections studied here are considered to represent one morphological species, though cryptic phylogenetic lineages may exist inside this morphological concept.

***atromarginatus-losulus* clade (clades XV and XVI in Fig. 1).**

1. (XV)

*Pluteus atromarginatus* / *Pluteus atropungens*

*P. atromarginatus* (Konrad) Kühner is accepted here as a species with dark brown pileus, habitat on coniferous wood, brown pigmented lamellar edges, hooked metuloid pleurocystidia, pigmented cheilocystidia and clamp-connections in all tissues (Vellinga 1990). *P. atromarginatus* has also been reported as growing on angiospermous wood in North America (Banerjee and Sundberg 1995). *P. atropungens* A.H. Sm. & Bartellii is characterized by the unhooked pleurocystidia and the habitat on sawdust or angiospermous wood (Smith and Bartelli 1965; Justo and Castro 2007a). In all analyses the sequence from the holotype of *P. atropungens* is placed in a sister relationship to all the *P. atromarginatus* sequences, but more sampling is needed to make a taxonomic conclusion about the separation or synonymy of both species.

2. (XVI)

*Pluteus losulus* (see Taxonomic descriptions)

Section *Celluloderma* (Fig. 2)

Many taxa in this group (e.g. *P. nanus*, *P. cinereofuscus*, *P. ephebeus*, *P. thomsonii*) need significantly more collection sampling to address the correspondence between morphological species and molecular lineages, but some observations can be made for several taxa in this group.

**Differentiation of *Pluteus phlebophorus*, *P. chrysophlebius* and *P. nanus***

The morphological delimitation, and putative synonymy, of these three species has been discussed at length in the literature for over 50 years (Homola 1972; Kühner and Romagnesi 1956; Minnis and Sundberg 2010; Orton 1986; Singer 1956; Vellinga 1990). Molecular data support both the separation and the morphological species concepts of *P. chrysophlebius* (Berk. & Ravenel) Sacc. (as "*Pluteus chrysophaeus* (Schaeff.) Quél.", see Discussion below) and *P. phlebophorus* (Ditmar) P. Kumm. proposed by Vellinga (1990). The situation of *P. nanus* (Pers.) P. Kumm. needs further study since collections fitting the broad morphological concept proposed by Vellinga (1990) are placed separately from each other in the phylogenetic analyses (Fig. 2).

The morphological concept of *P. phlebophorus* proposed by Vellinga (1990) needs to be slightly expanded in view of the molecular data. *Pluteus luctuosus* Boud. is separated from other taxa in this group by the dark brown lamellar edges with pigmented cheilocystidia. A collection with this characteristic (AJ81) shows an identical ITS sequence with the Spanish collections of *P. phlebophorus* with colorless cheilocystidia. *P. pallidus* Homola, characterized by the almost pigmentless basidiocarps, also falls within the molecular variation of *P. phlebophorus*.

North American collections, that are here considered to represent *P. chrysophlebius* (SF10, SF11, SF12, Fig. 2), are usually identified in North America under the names *P. chrysophlebius* or *P. admirabilis* (Peck) Peck. (Minnis and Sundberg 2010). However, Asian and European collections are typically identified in recent times as *P. chrysophaeus* sensu Vellinga (1990) by authors including Takehashi and Kasuya (2009b) and Justo and Castro (2007a). Morphological separations of collections from Asia, Europe and North America identified under those three names is not possible. *P. chrysophaeus* has been variously applied, by European workers, as representing a species with a brown or a yellow pileus, a key morphological character in this group. The absence of a type collection or illustration has caused considerable confusion, summarized by Singer (1956) and Orton (1986). We have reexamined the protologue of *Agaricus chrysophaeus* (Schaeffer 1774) and the accompanying illustration (Schaeffer 1771). The illustration depicting this species clearly indicates a brown colored pileus with yellowish tints in the stipe, which contrasts with the diagnosis that suggests the possibility of yellow hues in the pileus. This illustration, which is selected below as the lectotype of *Agaricus chrysophaeus*, does not represent the yellow-capped species known in the literature under the names *P. chrysophaeus*, *P. chryshophlebius* or *P. admirabilis*. It may represent *P. phlebophorus* or *P. romellii*, as circumscribed by Vellinga (1990), but in our opinion it is better to definitely abandon the epithet "*chrysophaeus*", as was done by Singer (1956) and Orton (1986). The name *Pluteus chrysophlebius* should be used for *P. chrysophaeus* in the sense of Vellinga (1990). Additional data on *P. chrysophlebius*, its synonymy and type study can be found in Minnis and Sundberg (2010).

*Pluteus chrysophaeus* (Schaeff.) Quél., Mém. Soc. Émul. Montbéliard, Sér. 2, 5: 116. 1872.

≡ *Agaricus chrysophaeus* Schaeff., Fung. Bavar. Palat. 4: 67. 1774 (basionym).

Lectotypus of *Agaricus chrysophaeus* Schaeff. (hic designatus): icon. in Schaeffer, Fung. Bavar. Palat. III: Tab. CCLIII. 1771, '1770'.

*P. phlebophorus* and *P. chrysophlebius* collections are usually grouped according to their geographic origin. In both cases intercontinental sequence divergence is less than 2%. No morphological differences were found between collections from different continents. In the absence of biological intercompatibility tests, the decision to consider these geographic clades as different populations of the same species or different species that are distinguishable only by geographic distribution is, ultimately, arbitrary.

*Pluteus rugosidiscus* Murrill is considered to differ from *P. chrysophlebius* by the presence of greenish colors on the pileus (Homola 1972). In all analyses the sequence of *P. rugosidiscus* is placed in a sister relationship to all *P. chrysophlebius* sequences, with a percentage sequence divergence between 4.8-5.5%. This collection may represent an independent taxon, but more intensive sampling is needed to check the stability of the pileus color as a reliable diagnostic character to separate both taxa.

### ***Pluteus mammillatus* vs. *Pluteus fenzlii***

Both taxa are separated mainly by the color of the lamellar edge, yellow in *P. fenzlii* (Schulzer) Corriol & P.-A. Moreau, and white to greyish-red in *P. mammillatus* (Longyear) Minnis, Sundb. & Methven (Corriol and Moreau 2007; Malysheva et al. 2007, Minnis et al. 2006). Separation based on this character is not as straightforward as it seems, since collections of *P. fenzlii* may have white lamellar edges (Corriol and Moreau 2007; Malysheva et al. 2007). Other characters mentioned by Corriol and Moreau (2007) such as basidiospore size, morphology of pleurocystidia and color of the annulus, show a wide range of overlapping variation between both species.

Eurasian collections of *P. fenzlii* and North American collections of *P. mammillatus* cluster separately in all the analyses. The maximum percentage of sequence divergence between both clades is 2.5%. If they are considered as one taxon, the name *Pluteus fenzlii* has priority over *P. mammillatus*. If they are considered as separate species, geographic distribution is the only reliable character to tell them apart.

#### ***romellii/aurantiorugosus* clade**

Two clades corresponding to the morphological concepts of *P. romellii* (Britzelm.) Sacc. and *P. aurantiorugosus* (Trog) Sacc. (Minnis and Sundberg 2010, Vellinga 1990) are recovered in all the analyses. Collections of *P. romellii* from Europe, Asia and North America are not grouped according to their geographic origin. Morphological variation between *P. romellii* and collections identified by Minnis and Sundberg (2010) as *P. fulvibadius* Murrill is not correlated with species rank differences in the phylogenetic analyses. North American collections of *P. aurantiorugosus* cluster separately from the European and Asian collections sampled here, though the maximum percentage of sequence divergence between any pair of sequences (Bigelow 19232 from Massachusetts and TNSF 19391 from Japan) is 1.4%. Both *P. romellii* and *P. aurantiorugosus* are considered to be widely distributed, at least, in the Northern Hemisphere (Minnis and Sundberg 2010; Vellinga 1990).

The Californian collection identified as *P. romellii* ("AY854065 aff. romellii", Fig. 2) does not represent this species, but a different taxon, related, or perhaps identical, to the South American *P. sublaevigatus* (Menolli et al. 2010).

Many taxa in this group share the conspicuous yellow, orange or red colors in the basidiocarps, though this characteristic also occurs in other lineages of *Pluteus*.

Sect. *Hispidoderma* (Fig. 3)

#### ***plautus/longistriatus* clade**

The wide morphological concept of *P. plautus* proposed by Vellinga and Schreurs (1985) is not supported by molecular data, as taxa considered synonymous by those authors (*P. semibulbosus* (Lasch) Gillet and *P. granulatus* Bres.) represent different species. More sampling in this group is needed to establish how many taxa can be recognized based on both morphological and molecular data.

#### ***leoninus* clade**

*P. leoninus* (Schaeff.) P. Kumm. is accepted here in the sense of Vellinga (1990), which includes small to large variants, with smooth or rugose pileus surfaces, yellow or yellow-brown pilei, and white to yellow lamellar edges. The North American *P. flavofuliginus* G.F. Atk. usually has darker brown colors in the pileus, but collections morphologically corresponding to *P. flavofuliginus* sampled in this study (Halling 6546, SF17) do not show molecular differentiation with European specimens of *P. leoninus*.

Two North American collections (*P. aff. leoninus* I, SF19, SF21) represent a distinct molecular lineage, but they do not show morphological differentiation from *P. leoninus*. A collection from Japan (*P. aff. leoninus* II, TNSF11908) is also molecularly distinct from *P. leoninus*. This collection differs from *P. leoninus* in the presence of caulocystidia and probably represents a distinct species, but it is not formally described here due to the incomplete knowledge of its morphological characteristics.

## *Volvopluteus* (Fig. 4)

Four species are recognized in all the analyses: *V. asiaticus*, *V. earlei*, *V. gloiocephalus* and *V. michiganensis*. The four taxa of *Volvopluteus* are clearly distinct on molecular grounds. Morphological separation of the species, based on colors of the basidiocarp, basidiospore size and shape, and morphology of the pleuro- and cheilocystidia, is usually possible but there is some degree of overlapping variation, especially between *V. gloiocephalus* and *V. earlei* (see Taxonomic descriptions and key below).

## Taxonomic descriptions

### *Pluteus castri* Justo & E.F. Malysheva, sp. nov. (Fig. 5)

MycoBank 518595

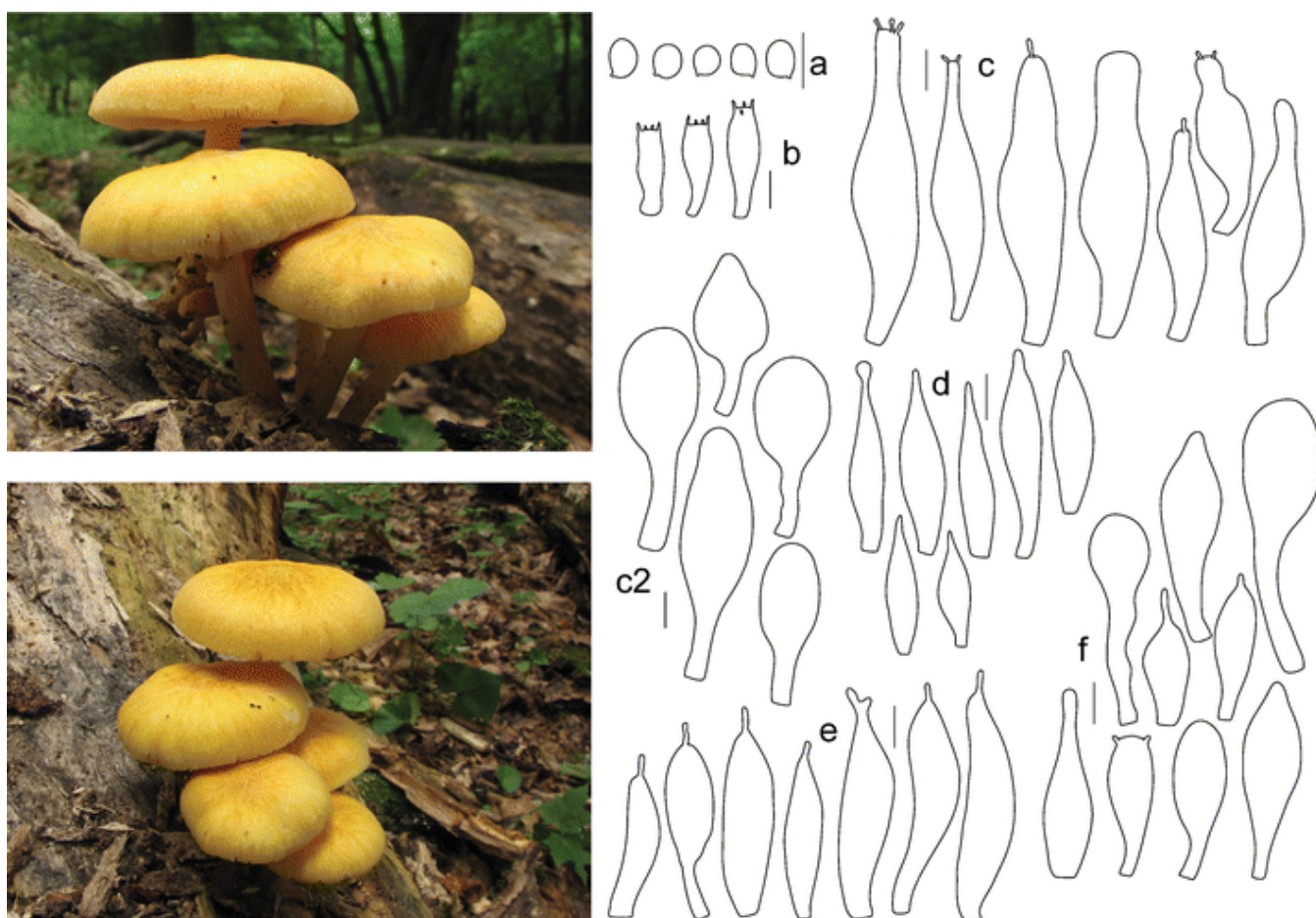


Fig. 5

*Pluteus castri*. **a.** Basidiospores; **b.** Basidia; **c.** Pleurocystidia; **c2;** Pleurocystidia near lamellar edges. **d.** Cheilocystidia; **e.** Caulocystidia; **f.** Pileipellis elements. All from holotype (TNSF 17602). Photographs from LE212090. All scale bars = 10  $\mu$ m

Latin Diagnosis. *Pluteo leonino* similis sed differt in cellulae in epicute pilei minoribus, caulocystidiis praesentibus et in spatii interne transcripti sequentia ("ITS").

Etymology. Named after Dr. Marisa Castro in honor of her outstanding contribution to the knowledge of the *Pluteaceae* and her dedication, spanning over three decades, to training new mycologists.

Pileus 10–50 mm, obtusely conical to campanulate-convex or plano-convex, with a low, broad umbo; surface smooth or covered with small squamules, at center venose and/or densely squamose, bright yellow to yellow-orange (approx. Mu. 10YR 8/8, 2.5Y 8/8) weakly hygrophanous; margin translucently striate. Lamellae crowded, free, ventricose, up to 10 mm broad, white-cream when young, later pink, with concolorous or whitish, flocculose, edges. Stipe 20–70 × 2–5 mm, cylindrical, broadened towards base (up to 7 mm); surface white or white-cream in the upper part, yellowish towards base (approx. Mu. 2.5Y 8/6), smooth or slightly pruinose at apex, longitudinally fibrillose towards base, with distinct squamules and white basal mycelium in the lower part. Context, in stipe and pileus, white to yellowish, yellow-orange under pileipellis. Smell and taste indistinct. Spore print not recorded.

Basidiospores [60, 4, 2] 5.2–7.0 (8.0) × 4.5–5.5 (6.0) μm,  $avl \times avw = 6.1 \times 5.0$  μm,  $Q = 1.0–1.40$ ,  $avQ = 1.21$ , mostly broadly ellipsoid, more rarely globose or ellipsoid. Basidia 20–35 × 6–10 μm, tetrasterigmate, clavate. Pleurocystidia, 40–85 × 12–22 μm, fusiform, narrowly lageniform or narrowly utriform, commonly provided with 1–3 finger-like excrescences at apices, near lamellar edges mostly obovoid or clavate and without excrescences, hyaline, thin-walled, frequent all over lamella faces. Lamellar edges sterile. Cheilocystidia 20–45 × 6–15 μm, fusiform or lageniform, with elongated apex or apical excrescence, hyaline, thin-walled, with thin, smooth walls, forming a well-developed strip. A second type of cheilocystidia, similar in shape and size to the pleurocystidia occurring near the lamellar edges, can be observed intermixed with the predominant fusiform-lageniform cheilocystidia. Pileipellis an euhymeniderm composed of elements 25–65 × 11–22 μm, (narrowly) clavate, conical, fusiform, spheropedunculate, sometimes with elongated apex or with apical excrescences, filled with yellow or yellow-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 (15)30–68 × 7–17 μm, fusiform or lageniform, commonly with mucronate or rostrate apices, with brown intracellular pigment, thin-walled, mostly fasciculate. Clamp-connections absent in all tissues.

Habit, habitat and distribution: Gregarious, sometimes in fascicles of up to six basidiocarps, on decayed wood of hardwoods (including *Populus*), also on piled wood chips. Known from Central Russia (Moscow and Samara Regions) and Japan (Kyushu and Honshu). Collected in July–August (Russia), May (Japan) and October (Japan).

Collections examined: JAPAN, Kyushu, Fukuoka prefecture, on piled wood chips, 5.V.2007, *M. Shintani & S. Takehashi*, TNSF 17602 (holotypus); Honshu, Ibaraki prefecture, Tsukuba, 25.X.2007, *K. Osaku*, TNSF 17081; RUSSIA: Central Russia, Moscow Region, Prioksko-Terrasny State Reserve, on decaying wood of *Populus tremula*, 15.VIII.1991, *G.E. Levitskaya*, LE 216873; Samara Region, near Pribrezhny, on decaying wood of deciduous tree, 1.VII.2007, *E.F. Malysheva*, LE 212090.

*Pluteus castri* closely resembles *P. leoninus* (Schaeff.) P. Kumm. in its external appearance and the pleurocystidia provided with small apical excrescences. Both taxa can be separated by the structure of the pileipellis: an euhymeniderm made up to clavate elements, 25–65 μm long, in *P. castri*, and a trichohymeniderm made up of narrowly fusiform elements, 60–230 μm long in *P. leoninus* (Vellinga 1990; pers. obs.). *P. castri* has caulocystidia, which are absent in *P. leoninus*. Molecular data clearly support the separation of *P. castri* from the *P. leoninus* complex (Fig. 3).



*P. conizatus* var. *africanus* E. Horak appears as sister to *P. castri* in all the analyses. Both taxa are characterized by the short elements in the pileipellis, in comparison to other members of sect. *Hispidoderma*. *P. conizatus* var. *africanus* has even shorter pileipellis elements, 16.5–40 µm long, than *P. castri*, the pleurocystidia are not provided with apical excrescences, and the cheilocystidia are slightly thick-walled (Horak and Heinemann 1978; pers. obs.). It is not known if *P. conizatus* var. *africanus* has caulocystidia as the stipitipellis has not been described for this taxon (Horak and Heinemann 1978). No caulocystidia were observed in the holotype and an additional collection of this taxon studied by us (GF 623, Thoen 5250, both at BR) but newly collected specimens should be checked to verify these observations.

***Pluteus heteromarginatus* Justo, sp. nov. (Fig. 6)**

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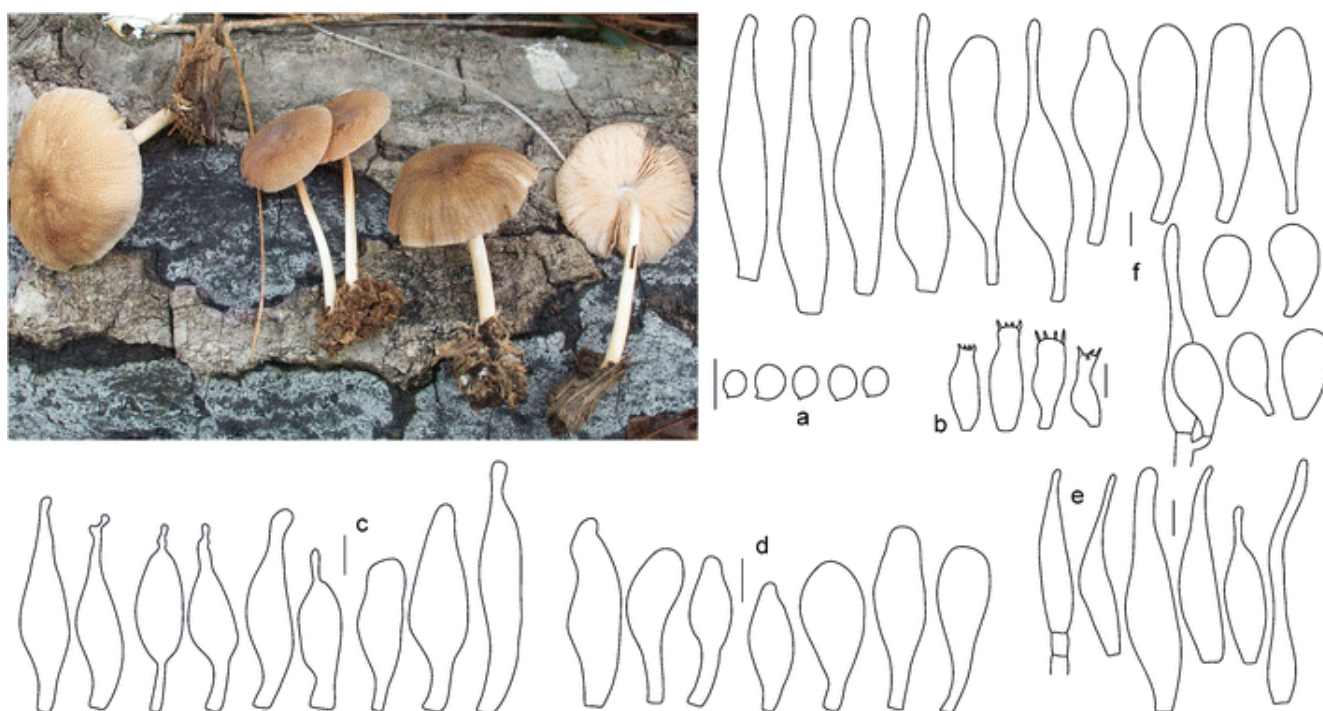


Fig. 6

*Pluteus heteromarginatus*. **a.** Basidiospores; **b.** Basidia; **c.** Pleurocystidia; **d.** Cheilocystidia; **e.** Caulocystidia; **f.** Pileipellis elements. All from Holotype (AJ172, MICH). All scale bars = 10 µm

**Latin Diagnosis.** *Pluteo longistriato similis sed differt in pileo brunneo, cheilocystidiis raris, et in spatii interne transcripti sequentia ("ITS").*

**Etymology.** The epithet makes reference to the heterogeneous nature of the lamellar edges, provided with sparse cheilocystidia, basidioles and fertile basidia.

Pileus 20–36 mm, convex to plano-convex, with or without a low, broad umbo; surface radially sulcate almost up to the center, smooth or only slightly rugose-squamulose at center, without conspicuous fibrils, brown (in the range of Mu. 7.5YR 6/6-6/8, 5/6-5/8, 4/6), darker at center and paler towards margin, hygrophanous, pallescent on drying, especially at center. Lamellae crowded, free, ventricose, up to 8 mm broad, white when young, later pink, with concolorous or whitish edges. Stipe 35–50 × 2–4 mm, cylindrical, slightly tapering towards apex; surface white or white-cream (approx. Mu. White Page 2.5Y 9.5/2), glabrous, longitudinally striate, under lens with brown dots all



over. Context in stipe and pileus white, slightly cream colored at stipe base. Smell and taste indistinct. Spore print pink (Mu. 5YR 7/4-8/4).

Basidiospores [60, 3, 1]  $5.3\text{--}7.2$  ( $8.0$ )  $\times$   $4.5\text{--}6.0$  ( $7.0$ )  $\mu\text{m}$ ,  $\text{avl} \times \text{avw} = 6.2 \times 5.3$   $\mu\text{m}$ ,  $Q = (1.0)$   $1.15\text{--}1.30$  ( $1.40$ ),  $\text{av}Q = 1.18$ , broadly ellipsoid, rarely globose or ellipsoid. Basidia  $24\text{--}34 \times 9.5\text{--}11$   $\mu\text{m}$ , 4-spored, narrowly utriform or clavate. Pleurocystidia  $40\text{--}65 \times 10\text{--}17$   $\mu\text{m}$ , mostly fusiform or narrowly lageniform, with elongated apex or an apical flexuous excrescence  $5\text{--}10$   $\mu\text{m}$  long, more rarely ovoid or (narrowly) utriform, with evenly dissolved pale brown intracellular pigment or colorless, with thin, smooth walls, common all over Lamellar sides. Lamellar edge heterogeneous, with scattered cheilocystidia, basidioles and fertile basidia near the sides. Cheilocystidia  $20\text{--}45 \times 9.5\text{--}15$   $\mu\text{m}$ , (narrowly) clavate, narrowly utriform, more rarely obovoid with long peduncle or broadly fusiform, without apical appendages, colorless, with thin, smooth walls, scattered along lamellar edge, sometimes in clusters, not forming a well-developed strip. Pileipellis a trichohymeniderm mostly composed of elements  $56\text{--}100 \times 14\text{--}20$   $\mu\text{m}$ : individual elements very variable in shape, fusiform, narrowly clavate, lageniform, obovoid, utriform, filled with brown intracellular pigment, with thin, smooth walls. At the base of that elements, a second morphological type occurs:  $24\text{--}28 \times 14\text{--}16$   $\mu\text{m}$  clavate elements, also filled with brown intracellular pigment. Stipitipellis a cutis; hyphae  $5\text{--}15$   $\mu\text{m}$  wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. Caulocystidia  $45\text{--}75 \times 7\text{--}17$   $\mu\text{m}$ , fusiform or lageniform, sometimes with elongated apex, with brown intracellular pigment, with thin, smooth walls, scattered or in clusters. Clamp-connections absent in all tissues.

Habit, habitat and distribution. Gregarious, on decayed, unidentified (probably coniferous) wood. Collected in Northern Florida (USA) during summer (August).

Collection examined: USA Florida, St. Marks Wildlife Refuge, 26.VIII.2009, *D. Floudas*, AJ172 (MICH, holotypus).

*P. heteromarginatus* is morphologically similar to *P. longistriatus* (Peck) Peck. Both taxa share the hygrophanous, sulcate pileus, colored pleurocystidia and presence of short clavate elements in the pileipellis. *P. longistriatus* has a grey-brown, fibrillose, pileus, differently shaped pleuro-, cheilo- and caulocystidia, and the lamellar edge is not heterogeneous (Menolli and Capelari 2010). *P. longistriatus* has been reported from North and South America and molecular data confirm that this taxon occurs in both hemispheres. Morphological and molecular differences between the two support the recognition of *P. heteromarginatus* as a separate taxon.

All taxa placed by Singer (1986) in stirps *longistriatus*, mainly because of the sulcate-pectinate margin of pileus, differ from *P. heteromarginatus* in the hyaline pleurocystidia and crowded cheilocystidia. Other morphological differences are: (i) *P. neophlebophorus* Singer has strongly venose cap and longer (up to  $145$   $\mu\text{m}$ ) elements of pileipellis; (ii) *P. sanctixaverii* Singer has a pileipellis composed mostly of short sphaeropedunculate or clavate elements (up to  $28.5$   $\mu\text{m}$  long), intermixed with dermatocystidia up to  $72$   $\mu\text{m}$  long; (iii) *P. oligocystis* Singer has scarce pleurocystidia and a pileipellis mostly of short sphaeropedunculate or clavate elements (up to  $41$   $\mu\text{m}$  long), intermixed with dermatocystidia up to  $150$   $\mu\text{m}$  long (Singer 1958).

Other taxa of section *Hispidoderma*, that may superficially resemble *P. heteromarginatus*, in the non-fibrillose, striate-sulcate and hygrophanous pileus, with predominantly brown colors (viz. *P. atriavellaneus* Murrill, *P. compressipes* Murrill, *P. sulcatus* Singer) are morphologically distinct from *P. heteromarginatus*, among other characters, in the crowded cheilocystidia and longer pileipellis elements, up to  $150$   $\mu\text{m}$  long (Pegler 1983, Singer 1958).

***Pluteus losulus* Justo, nom. nov. (Fig. 7a)**

≡ *Pluteus cervinus* var. *ealaensis* Beeli, Bull. Soc. Royal Bot. Belg. 61: 81. 1928 (replaced synonym); non *Pluteus ealaensis* Beeli Bull. Soc. Royal Bot. Belg. 61: 80. 1928 (= *Pluteus congolensis* Beeli).

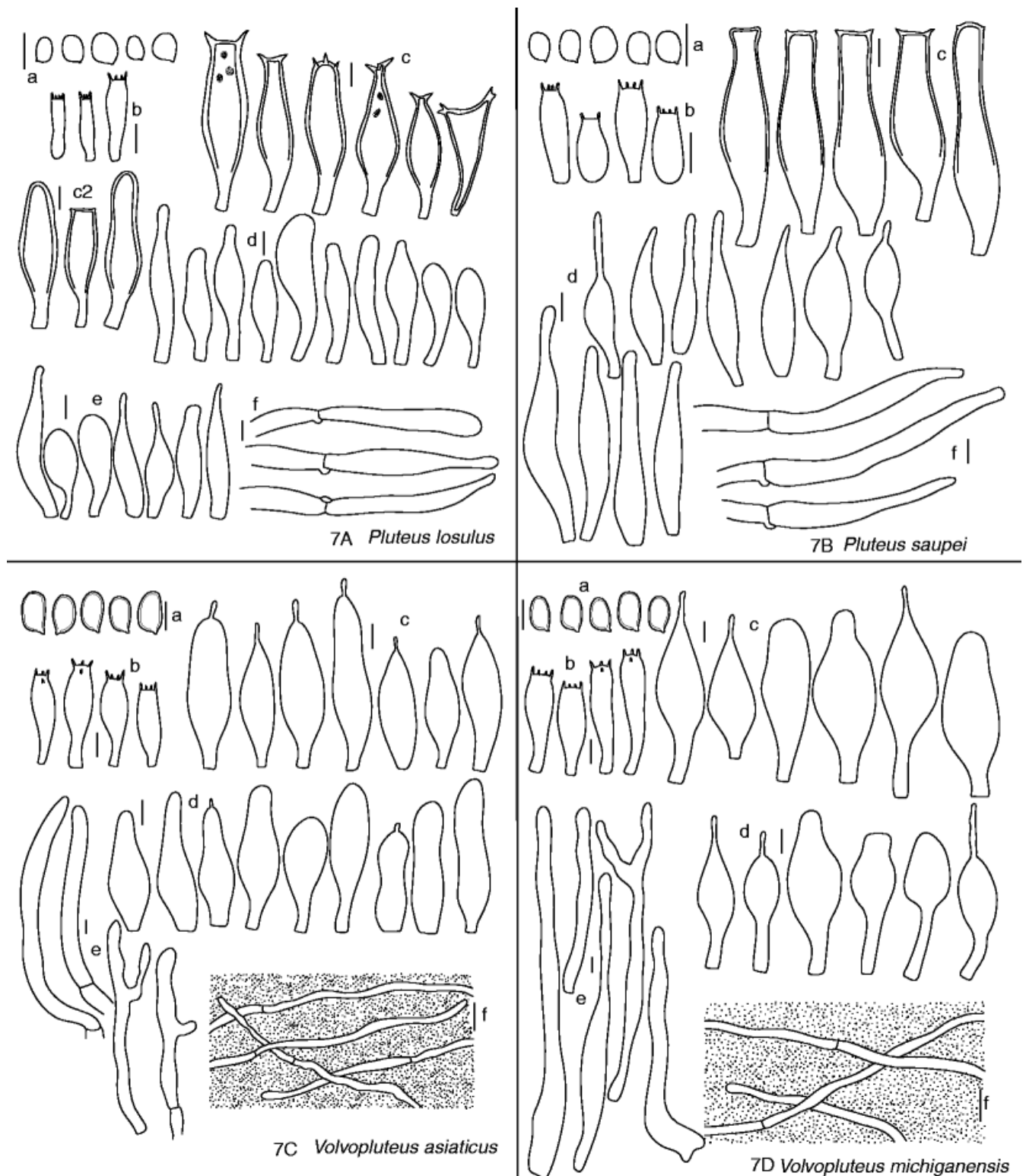


Fig. 7

**a.** *Pluteus losulus* (GF5273, BR); **b.** *Pluteus saupei* (ILLS42441); **c.** *Volvopluteus asiaticus* (TNSF 15191); **d.** *Volvopluteus michiganensis* (Smith32-590, MICH). **a.** Basidiospores; **b.** Basidia; **c.** Pleurocystidia; **c2.** Pleurocystidia near lamellar edges. **d.** Cheilocystidia; **e.** Caulocystidia; **f.** Pileipellis elements. All scale bars = 10  $\mu$ m

Etymology. The epithet makes reference to one of the common names ("losulu") that this species receives in the Democratic Republic of Congo.

Pileus 30–90 mm, obtusely conical to convex or plano-convex, with a low, broad umbo; surface covered with radially arranged fibrils, glabrescent in older specimens, usually with small squamules at center, brown, redish-brown or greyish-brown, slightly pallescent with age; margin entire, not striate. Lamellae crowded, free, ventricose, approx. 10 mm broad, whitish when young, later pink, with whitish, flocculose, edges. Stipe 35–100 × 3–10 mm, cylindrical, broadened towards base; surface whitish, covered with longitudinal fibrils, concolorous with pileus. Context white. Smell acrid, taste very bitter. Spore print pinkish-brown.

Basidiospores [30, 1, 1] 6.0–9.0 (10.0) × 5.0–8.5 (9.0)  $\mu\text{m}$ ,  $\text{avl} \times \text{avw} = 7.5 \times 6.6 \mu\text{m}$ ,  $Q = 1.0\text{--}1.45$ ,  $\text{av}Q = 1.16$ , globose to ellipsoid. Basidia 20–30 × 6–8  $\mu\text{m}$ , tetrasterigmate, clavate, some with median constriction. Pleurocystidia metuloid, 50–87 × 15–25  $\mu\text{m}$ , fusiform or narrowly utriform, provided with 2–4 hooks at apices, a few irregularly shaped and with bifid apices, near lamellar edges many metuloids without distinct hooks and with rounded apices, hyaline or with vacuolar brown pigment, with up to 4  $\mu\text{m}$  thick wall, frequent all over lamellar faces. Lamellar edge sterile. Cheilocystidia 40–70 × 10–20  $\mu\text{m}$ , (narrowly) clavate, lageniform, narrowly utriform or subcylindrical, hyaline, thin-walled, crowded, forming a well-developed strip. Pileipellis a cutis, with terminal elements 90–120 × 10–17  $\mu\text{m}$ : individual elements cylindrical, some strongly tapering towards apex, mostly filled with brown intracellular pigment, with thin, smooth walls. Stipitipellis a cutis; hyphae 5–15  $\mu\text{m}$  wide, cylindrical, colorless or with brown pigment, with thin, smooth walls. Caulocystidia 40–73 × 9–15 (–20)  $\mu\text{m}$ , fusiform, lageniform, narrowly utriform or clavate, some with elongated apices, with brown intracellular pigment, thin-walled, scattered. Clamp-connections present in all tissues, common at the base of cheilocystidia, caulocystidia and in pileipellis.

Habit, habitat and distribution: Gregarious, on decayed wood, in forests and coffee plantations. Common in the Democratic Rep. of Congo (Horak and Heinemann 1978).

Collections examined: DEMOCRATIC REP. OF CONGO. Panzi-Kivu, May 1953, Goosens-Fontana 5273 (BR).

The macroscopic description is adapted from Horak and Heinemann (1978), and the microscopic data are from our observations on the collection Goosens-Fontana 5273. Three additional collections of this species, including the holotype, are deposited at BR but they are very poorly preserved and no further observations could be made.

*P. losulus* was originally described as a variety of *P. cervinus*, but both molecular and morphological data indicate that it represents a distinct species, not closely related to *P. cervinus* or any of the clamp-bearing species in that group. *P. losulus* is characterized by the hooked and partially pigmented metuloid pleurocystidia; morphologically variable cheilocystidia and the presence of clamp-connections and caulocystidia. The latter character is rather unusual in sect. *Pluteus* as it has been described for only three species in the section. *P. amphicystis* Singer has unhooked metuloid pleurocystidia with crystals at the apices, no clamp-connections and metuloid caulocystidia. It has been reported from Bolivia, Martinique, Mexico and India (Rodríguez and Guzmán-Dávalos 2007; Pegler 1983; Pradeep et al. 2002; Singer 1958). *P. martinicensis* Singer & Fiard has predominately unhooked metuloid pleurocystidia and pigmented cheilocystidia. It is known from México, Martinique and India (Rodríguez and Guzmán-Dávalos 2007; Pegler 1983; Pradeep et al. 2002).

*Pluteus triplocystis* Singer has smaller basidiospores ( $5.5\text{--}6 \times 5\text{--}5.5\text{ }\mu\text{m}$ ) and differently shaped cheilocystidia and caulocystidia. It is known only from Mexico (Rodríguez et al. 2004).

***Pluteus saupei* Justo & Minnis, sp. nov. (Fig. 7b)**

MycoBank 518598

Latin Diagnosis. *Pluteo salicino similis sed differt in pleurocystidiis sine grandis cornus, cheilocystidiis lageniformibus, et in spatii interne transcripti sequentia ("ITS")*.

Etymology. Named after Dr. Stephen G. Saupe, the collector of the holotype, in honor of his discovery of the presence of psilocybin in *Pluteus*.

Pileus 10–35 mm, convex or plano-convex, with shallow depression at center; surface innately radially fibrillose, with blackish, spinulose squamules at center, grey to grey-brown, bruised regions become blue, with olive-green tinges in older specimens; margin translucently striate in older specimens. Lamellae crowded, free, ventricose, approx. 5 mm broad, pink, with slightly paler edges. Stipe 10–50  $\times$  3–5 mm, cylindrical, broadened towards base; surface white, with some longitudinal fibrils near the base, turning blue when bruised, with olive-green tinges in older specimens. Context color not recorded, probably whitish. Smell and taste not recorded. Spore print pinkish-brown.

Basidiospores [30, 1, 1]  $6.5\text{--}9.0$  ( $10.0$ )  $\times$   $5.0\text{--}6.5$  ( $7.0$ )  $\mu\text{m}$ ,  $avl \times avw = 7.7 \times 5.8\text{ }\mu\text{m}$ ,  $Q = 1.15\text{--}1.60$  ( $1.70$ ),  $avQ = 1.33$ , (broadly) ellipsoid, rarely oblong. Basidia  $17.5\text{--}26 \times 7.5\text{--}10\text{ }\mu\text{m}$ , tetrasterigmate, rarely bisterigmate, clavate, oblong or ellipsoid, some with median constriction. Pleurocystidia metuloid,  $70\text{--}85 \times 17\text{--}22\text{ }\mu\text{m}$ , fusiform or narrowly utriform, with 2–4 hooks at apices, but hooks usually poorly developed, hyaline, with up to  $2\text{ }\mu\text{m}$  thick walls, frequent all over lamellar faces. Lamellar edges sterile. Cheilocystidia  $45\text{--}85 \times 9.5\text{--}19.5\text{ }\mu\text{m}$ , mostly lageniform with elongated apices, some fusiform or ovoid, hyaline, thin-walled, crowded, forming a well-developed strip. Pileipellis a cutis, with terminal elements  $80\text{--}135 \times 10\text{--}16\text{ }\mu\text{m}$ : individual elements cylindrical, some strongly tapering towards apex, mostly filled with brown intracellular pigment, with thin, smooth walls. Stipitipellis a cutis; hyphae  $5\text{--}15\text{ }\mu\text{m}$  wide, cylindrical, colorless or with brown pigment, with thin, smooth walls. Caulocystidia absent. Clamp-connections present in all tissues, more abundant at the base of cheilocystidia and on pileipellis.

Habit, habitat and distribution: Solitary, on wood in mixed mesophytic upland forest. Collected in central Illinois during summer (August).

Collections examined. USA Illinois, Urbana, Brownfield Woods, on decaying log, 5.VIII.1980, SG Saupe 230 (ILLS 42441, holotypus); *idem*, 7.VIII.1980, SG Saupe 264 (ILLS 42442).

The macroscopic description is based on the dried herbarium material and Saupe's notes, especially on staining reactions. These collections were used to demonstrate the presence of psilocybin in *P. salicinus* (Saupe 1981). However, both molecular data and microscopical examination revealed that they represent a different, undescribed, species in sect. *Pluteus* (*salicinus/albostipitatus* clade).

*Pluteus saupei* differs from *P. salicinus* in the poorly developed hooks on the pleurocystidia and the morphology of the cheilocystidia. *P. salicinus* var. *achloes* Singer lacks blue-green tinges in the basidiocarps, has pleurocystidia with well-developed hooks at the apices and shorter (up to  $55\text{ }\mu\text{m}$ ), predominantly clavate, cheilocystidia (Banerjee and Sundberg 1995; Singer 1956). *P. salicinus* var. *americanus* P. Banerjee & Sundb. has pleurocystidia provided with coralloid hooks at the apices and shorter (up to  $45\text{ }\mu\text{m}$ ) predominantly clavate cheilocystidia (Banerjee and Sundberg 1993b). *P. saupei*

appears in all analyses as sister to *P. harrisii*. Both taxa have similar pleurocystidia but *P. harrisii* lacks clamp-connections and has differently shaped cheilocystidia.

***Volvopluteus asiaticus* Justo & Minnis, sp. nov. (Fig. 7c)**

MycoBank 518599

Latin Diagnosis. *Volvopluteo gloiocephalo similis sed differt in pleurocystidiis rostratis, cheilocystidiis lageniformibus et in spatii interne transcripti sequentia ("ITS")*.

Etymology. The epithet makes reference to the geographic origin of the type collection.

Pileus 72–89 mm, broadly conical in young specimens, later applanate, with umbonate center; surface rough, rugose-striate, with some powdery, minute, whitish scales, glutinous when wet, shiny on drying, greyish brown to brown, dark brown at center; margin entire. Lamellae crowded, free, (broadly) ventricose, up to 8.6 mm broad; dirty pinkish, with ciliate edges. Stipe 85–99 × 9–21 mm, cylindrical, enlarged towards the base, whitish in the upper part, with yellowish tints towards the base, longitudinally fibrillose-striate. Volva white, membranous, 38 mm high, separated from the stipe in the upper part, with abundant rizomorphs. Context white in pileus and stipe, blackish under pileipellis. Smell, taste and spore print not recorded.

Basidiospores [30, 1, 1] 12.0–14.5 × 7.0–8.5 (9.0) µm, avl × avw = 13.2 × 8.0 µm, Q = 1.50–1.80, avQ = 1.66, ellipsoid to oblong. Basidia 29–45 × 10–15 µm, tetrasterigmate, clavate. Pleurocystidia 45–85 × 12–30 µm, fusiform, narrowly utriform, commonly rostrate, provided with an apical excrescence up to 10 µm long, hyaline, thin-walled, frequent all over lamellar faces. Lamellar edges sterile. Cheilocystidia 45–70 × 14–25 µm, mostly lageniform, but also clavate, ovoid or narrowly utriform, some with an apical papilla or excrescence up to 10 µm long, hyaline, thin-walled, crowded, forming a well-developed strip. Pileipellis an ixocutis, composed of hyphae 3–7 µm wide; hyphae cylindrical or irregular in outline, embedded in a 250–450 µm thick gelatinous matrix, hyaline or with pale intracellular brown pigment, with thin, smooth walls. Stipitipellis a cutis; hyphae 4–12 µm wide, cylindrical, colorless or with brown pigment, with thin, smooth walls. Caulocystidia 100–250 × 10–15 µm, cylindrical or flexuose, some with internal septa or bifurcate apices. Volva composed of densely interwoven cylindrical hyphae, 10–15 µm wide. Clamp-connections absent in all tissues.

Habit, habitat and distribution. Gregarious, on the ground, near dead trunks of broad-leaved trees. Collected in northern Japan during Autumn (October)

Collections examined. JAPAN. Hokkaido, Iwamizawa-shi, Midorigaoka, Tonebetsu Nature Park, 5.X.2005, S. Takehashi, TNSF 15191 (holotypus).

The macroscopic description is adapted from Takehashi et al. (2010), as "*Volvariella gloiocephala*", supplemented with our observations on the type collection. The microscopic characters are from our observations on the type. This collection was originally identified as *Volvariella gloiocephala*, though molecular data indicate that it represents a distinct species. See below for a morphology-based key of the four species of *Volvopluteus*.

***Volvopluteus michiganensis* (A.H. Sm.) Justo & Minnis, Fungal Biology (in press) (Fig. 7d)**

Pileus 70–90 mm, broadly conical to plano-convex, with or without a low, broad umbo; surface covered with radially arranged fibrils, viscid; margin entire, rimose; ash grey, similar to the color of *Tricholoma terreum* (Schaeff.) P. Kumm. Lamellae crowded, free, (broadly) ventricose; up to 15 mm

broad; whitish when young, later pink, with whitish, flocculose or slightly eroded, edges. Stipe 80–110 × 10–15 mm, clavate with bulbous base (up to 30 mm broad); whitish, minutely tomentose all over. Volva white, membranous. Context white. Smell earthy, fragrant, taste not recorded. Spore print not recorded.

Basidiospores [30, 1, 1] 10.5–13.5 × 6.5–8 µm,  $avl \times avw = 11.9 \times 7.1$  µm,  $Q = 1.4–1.9$ ,  $avQ = 1.70$ , ellipsoid to oblong. Basidia 35–55 × 10–15 µm, tetrasterigmate, (narrowly) clavate. Pleurocystidia 70–110 × 25–45 µm, fusiform, narrowly utriform, or lageniform, many provided with an apical excrescence up to 15 µm long; hyaline, thin-walled, frequent all over lamellar faces. Lamellar edges sterile. Cheilocystidia 60–75 × 15–27 µm, fusiform, ovoid with long peduncle, narrowly utriform, many with an apical excrescence up to 15 (–20) µm long; hyaline, thin-walled; crowded, forming a well-developed strip. Pileipellis an ixocutis, composed of hyphae 2–10 µm wide; hyphae cylindrical or irregular in outline, embedded in a 150–300 µm thick gelatinous matrix, hyaline or with pale intracellular brown pigment, with thin, smooth walls. Stipitipellis a cutis; hyphae 3–15 µm wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. Caulocystidia (80–) 100–360 × 10–20 µm, cylindrical or flexuose, some with bifurcate apices. Volva composed of densely interwoven cylindrical hyphae, 5–15 µm wide. Clamp-connections absent in all tissues.

Habit, habitat and distribution. Gregarious, on sawdust. Collected in Michigan (USA), during the Autumn (October).

Collections examined: USA Michigan, Washtenaw Co., Ann Arbor, Jackson Rd., 17.X.1932, A.H. Smith 32-590 (holotypus, MICH).

The macroscopic description is adapted from Smith (1934) and supplemented with our observations of the type collection. This taxon was originally described as a *Pluteus*, and no mention of a volva was given in the original description, though a conspicuous membranous, white volva is present in the holotype. Since its original description, it was never reported again in the literature, and Smith did not include his own species in the revision of the North American types of *Pluteus* (Smith and Stuntz 1958). Both molecular and morphological data confirm that this collection represents a species of *Volvopluteus*. North American collections of *V. gloiocephalus* should be carefully re-examined as some may represent *V. michiganensis*.

### **Morphological variation in *Volvopluteus earlei* (Murrill) Vizzini, Contu & Justo**

*V. earlei* is usually depicted as a species with small basidiocarps (pileus < 5 cm in diameter), relatively wide basidiospores ( $avQ < 1.60$ ), absent or scarce pleurocystidia and rostrate cheilocystidia (Contu 2007, Justo and Castro 2010b, Shaffer 1957). It has overall white colors but greyish and ochraceous tinges may be present in the pileus. Variation in color led Contu (1998) to describe *Volvariella cookei*, characterized by pure white basidiocarps, as a separate species. However, *Volvopluteus earlei* was originally described as having a "white, discoloring with age" pileus (Murrill 1911: 282) and basidiocarps with pure white or slightly grey-ochre pileus may occur in the same collection (Justo and Castro 2010b, Shaffer 1957). The collection originally identified as *V. cookei* sampled for molecular data (TOAV133) shows no molecular differentiation from other collections with grey or ochraceous tinges in the basidiocarps. Therefore, *Volvariella cookei* is considered a synonym of *Volvopluteus earlei*.

*Volvariella acystidiata* N.C. Pathak is macroscopically very similar to *V. earlei*, differing by the absence of hymenial cystidia (Heinemann 1975). This species has been recently collected in Italy (Vizzini and Contu 2010) and sampled for molecular data. The results from the phylogenetic analyses

(Fig. 4, collection TOHG1973) show that it is a morphological variant of *V. earlei* without cystidia. Therefore this taxon is reduced here to a form of *V. earlei*:

*Volvopluteus earlei* f. *acystidiatus* (N.C. Pathak) Vizzini & Contu, comb. & stat. nov.

Mycobank 518901

Basionym: *Volvariella acystidiata* N.C. Pathak, Bull. Jar. Bot. Natl. Belg. 45: 195. 1975

An additional collection from Italy (TOHG2001) with spores  $11\text{--}17 \times 7\text{--}8\text{ }\mu\text{m}$  and non-rostrate cheilocystidia falls also within the molecular variation of *V. earlei* (Fig. 4). Morphological differentiation of this collections of *V. earlei* from white forms of *V. gloiocephalus* can be challenging, though usually *V. gloiocephalus* has larger basidiocarps (pileus  $> 5\text{ cm}$  in diameter).

### **Morphological variation in *Volvopluteus gloiocephalus* (DC. : Fr.) Vizzini, Contu & Justo**

*V. gloiocephalus* is accepted here in the sense of Boekhout (1990) including specimens with grey-brown colors and also white variants recognized by others (Shaffer 1957; Singer 1986) as a separate taxon (viz. *Volvariella speciosa* (Fr.: Fr.) Singer).

Both *V. michiganensis* and *V. asiaticus* share with *V. gloiocephalus* the pileus  $> 5\text{ cm}$  in diameter with grey-brown colors. *V. michiganensis* can be separated from *V. gloiocephalus* by the rimose pileus and average basidiospore length  $< 12.5\text{ }\mu\text{m}$ . In *V. asiaticus* the majority of pleurocystidia are rostrate and the cheilocystidia are lageniform (Fig. 7c).

White forms of *V. gloiocephalus* can be confused with *V. earlei*. The latter has usually smaller basidiocarps (pileus  $< 5\text{ cm}$ ), basidiospores with  $\text{avQ} < 1.6$ , absent or very scarce pleurocystidia and rostrate cheilocystidia. However, there is a great variation on the basidiospores and cystidial characteristics of *V. earlei* (see comments above) that may complicate the morphological separation of both species.

Examination of additional collections of all taxa of *Volvopluteus* is still needed to establish more precisely the morphological limits of the species. Here we provide a tentative key based on our current knowledge of the genus.

## Key to the species of *Volvopluteus*

1. 1.

Hymenial cystidia absent *V. earlei* f. *acystidiatus*

2. 1.

Hymenial cystidia (at least cheilocystidia) present

1. 2.

Average basidiospore length  $< 12.5 \mu\text{m}$ . Margin of pileus rimose. *V. michiganensis*

2. 2.

Average basidiospore length  $> 12.5 \mu\text{m}$ . Margin of pileus not rimose

1. 3.

Pileus with predominantly white colors

1. 4.

Cheilocystidia rostrate, usually each one provided with a flexuous apical appendage up to  $40 \mu\text{m}$  long. Pleurocystidia absent or scarce and then similar to the cheilocystidia *V. earlei*

2. 4.

Cheilocystidia not rostrate. Pleurocystidia common, not rostrate

1. 5.

Pileus  $< 5 \text{ cm}$  in diameter *V. earlei*

2. 5.

Pileus  $> 5 \text{ cm}$  in diameter *V. gloiocephalus*



2. 3.

Pileus with predominantly grey or grey-brown colors (Mu. 10YR 4/1-4/3, 2.5Y 5/1-5/6, 7/1-7/4)

1. 6.

The majority of pleurocystidia provided with an apical excrescence up to 15  $\mu$ m long. Most of the cheilocystidia lageniform. *V. asiaticus*

2. 6.

The majority of pleurocystidia without apical excrescences, a few with apical papilla. Most of the cheilocystidia fusiform to clavate. *V. gloiocephalus*

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