

Phylogenetic and morphological comparison of *Pluteus variabilicolor* and *P. castri* (Basidiomycota, Agaricales)

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Abstract: Based on several collections from Italy, a detailed description (including also macrophotographs, microphotographs, and drawings) of the morphological characters of the poorly known *Pluteus variabilicolor*, originally described from Hungary, is provided. The analysis of the ITS sequences placed this species within the *P. leoninus* clade of sect. *Hispidoderma*, in spite of the presence of clavate elements in the pileipellis. According to the molecular comparison of the type collections, *Pluteus castri*, a species recently described on the basis of material collected in Japan and Central Russia, is reduced to a synonym of *P. variabilicolor*.

Key words:

Agaricomycetes
Italy
ITS sequences
pluteoid clade
Pluteus sect. *Celluloderma*
sect. *Hispidoderma*
subsect. *Mixtini*
taxonomy

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INTRODUCTION

The genus *Pluteus*, typified by *P. cervinus*, includes saprobic agaricoid fungi characterized by free lamellae, no universal veil (a partial veil may be present in a few species), pinkish spore-print, inverse hymenophoral trama, and inamyloid, cyanophilic spores (Vellinga & Schreurs 1985, Orton 1986, Singer 1986, Vellinga 1990, Heilmann-Clausen 2012). It belongs to the pluteoid clade as circumscribed by several authors on the basis of molecular data (Moncalvo *et al.* 2000, 2002, Bodensteiner *et al.* 2004, Binder *et al.* 2006, Matheny *et al.* 2006, Justo *et al.* 2011a, b, Vizzini *et al.* 2011). The infrageneric taxonomy is based primarily on the characteristics of the hymenial cystidia and pileipellis. Historically, three sections based on morphological features are widely accepted (Homola 1975, Horak & Heinemann 1978, Orton 1986, Singer 1986, Citérin & Eyssartier 1998, Malysheva *et al.* 2009, Minnis & Sundberg 2009, 2010): namely sect. *Pluteus*, with thick-walled (metuloid) pleurocystidia, usually with apical hooks, and pileipellis as a cutis; sect. *Hispidoderma* with non-metuloid pleurocystidia, and a pileipellis consisting of elongated, filamentous elements organized either as a cutis, a hymeniderm or a trichoderm; and sect. *Celluloderma* with non-metuloid pleurocystidia and a pileipellis composed of swollen clavate or spheropedunculate elements organized in a hymeniderm, with transitions to an epithelium. Singer (1956, 1958, 1986) sometimes also recognized two subsections in section *Celluloderma*: *Mixtini* and *Eucellulodermini*, differing respectively in the presence or absence of elongated cystidium-

like elements in the pileipellis. Finally, Vellinga & Schreurs (1985) proposed an infrageneric classification for *Pluteus* differing from Singer's classificatory scheme only with respect to the placement of species with non-metuloid cystidia and a filamentous pileipellis: they reduced sect. *Hispidoderma* to a subsection of *Celluloderma*, including only species with a hymenodermal or trichodermal pileipellis, and established sect. *Villosi* for species with a pileipellis which was a differentiated cutis. This system has not been widely accepted by later authors (e.g. Banerjee 1992, Banerjee & Sundberg 1993, Citérin & Eyssartier 1998, Rodríguez & Guzmán-Dávalos 2001).

Justo *et al.* (2011a, b) showed a general agreement between the morphological subdivision of *Pluteus* and the molecular phylogeny they obtained, even though: (1) some species with non-metuloid pleurocystidia and a pileipellis which was a differentiated cutis (formerly sect. *Villosi*) were placed either in sect. *Celluloderma*, together with the species characterized by a hymenidermal pileipellis, or in sect. *Pluteus* with metuloid bearing species; and (2) the division of sect. *Celluloderma* into subsections *Mixtini* and *Eucellulodermini* was not supported by the phylogenetic analyses of the molecular data; species with a mixture of vesiculose-clavate and elongate elements in the pileipellis were not monophyletic and were distributed over sections *Celluloderma* and *Hispidoderma*.

Here we fully describe, based on recent Italian collections, the rarely reported *Pluteus variabilicolor*, which was originally assigned to subsect. *Mixtini* of sect. *Celluloderma* by Babos (1978). A phylogenetic analysis of ITS sequences was performed

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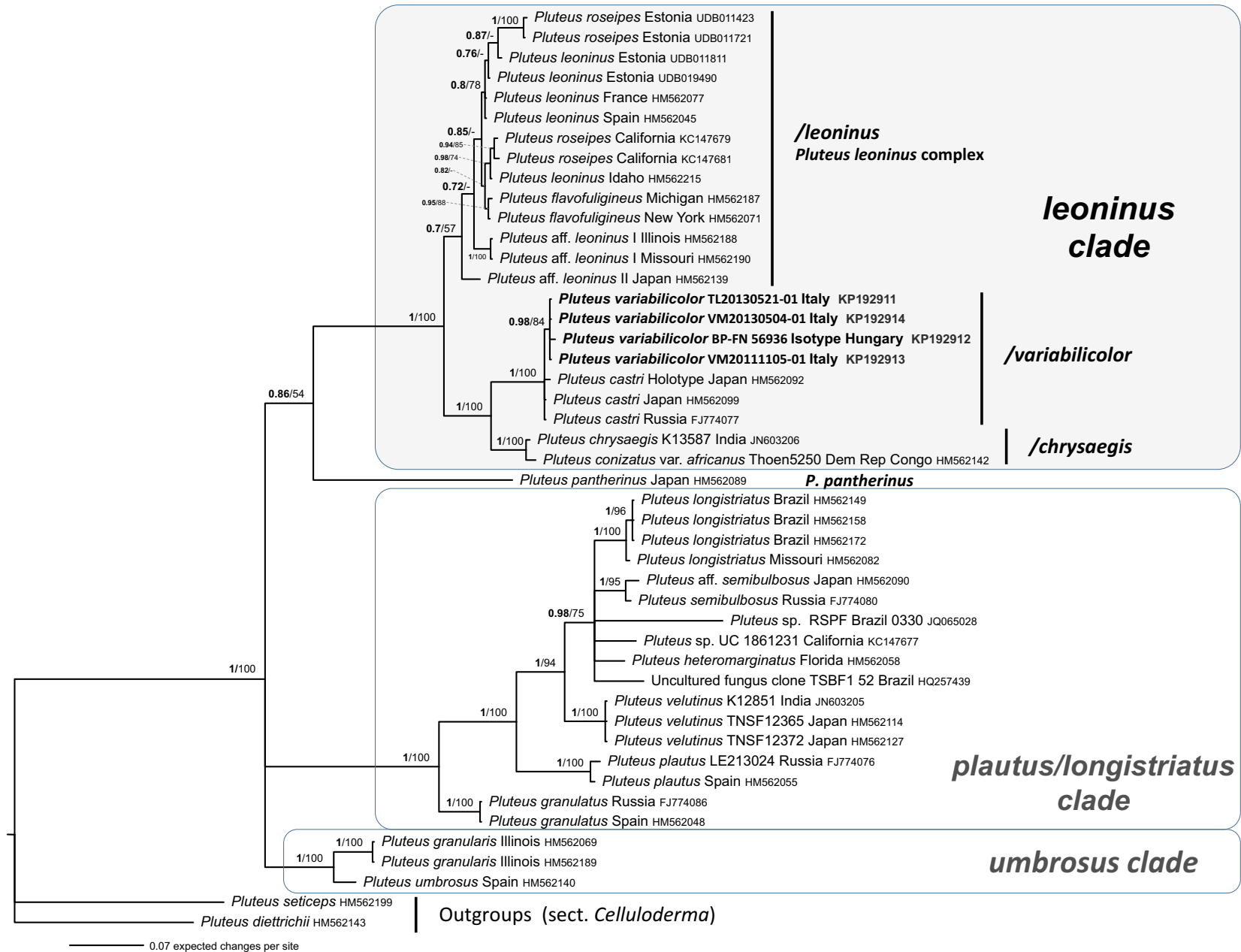


Fig. 1. Bayesian tree analysis of *Pluteus* sect. *Hispidoderma*. BPP values (in bold) ≥ 0.7 and MLB values $\geq 50\%$ are shown on the branches. Clade names follow Justo *et al.* (2011a) and Pradeep *et al.* (2012). Newly sequenced collections are in bold.

to: (1) provide molecular insights on its status and taxonomic placement within *Pluteus*; and (2) assess whether this species, with a mixture of elongated and short elements in the pileipellis, was phylogenetically closer to species in sect. *Hispidoderma* or in sect. *Celluloderma*.

The ITS sequence of the isotype collection was also obtained to confirm our identifications of the recent collections.

MATERIAL AND METHODS

Morphology

Microscopic descriptions are based on fresh and dried specimens. The optical microscope used was a Nikon Eclipse E400 trinocular with plan-achromatic objectives. Micrographs were taken with a Moticam 580 camera. The primary mounting media used were: distilled water for the observation of the pigments, and ammoniacal Congo red for the other structures. Spore measurements were based on 90 elements in ammoniacal Congo red and randomly selected from three collections. The following abbreviations are used: I = number of lamellulae between each pair of entire lamellae; and Q = the length divided by width of the spores in side view, Qm being the average quotient. Descriptive terms used in the descriptions follow Vellinga (1988), and collection acronyms follow *Index Herbariorum* except that "TL" and "VM" refer to the personal reference collections of Tomaso Lezzi and Vincenzo Migliozi.

DNA extraction, amplification and sequencing

Genomic DNA was isolated from 1 mg per specimen of four dried specimens (TM20130521-01, VM20111105-01, VM20130504-01 and BP-FN 56936) using the DNeasy Plant Mini Kit (Qiagen, Milan, Italy). Universal primers ITS1F/ITS4 were used for the ITS region amplification (White *et al.* 1990, Gardes & Bruns 1993). Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 μ L reaction mixture using the following final concentrations or total amounts: 10 ng DNA, 1 \times PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 0.4 μ M of each primer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.5 units of Taq polymerase (Promega). The PCR program was as follows: 3 min at 95 °C for one cycle; 30 s at 94 °C, 45 s at 50 °C, 2 min at 72 °C for 35 cycles, and 10 min at 72 °C for one cycle. PCR products were resolved on a 1.0 % agarose gel and visualized after staining with ethidium bromide. The PCR products were purified with the AMPure XP kit (Beckman) and sequenced by MACROGEN (Seoul, Republic of Korea). Sequence assembly and editing were performed using Geneious v. 5.3 (Drummond *et al.* 2010). The sequences are deposited in GenBank (www.ncbi.nlm.nih.gov/) under the accession numbers given in Fig. 1.

Sequence alignment and phylogenetic analysis

The sequences obtained in this study were combined with published *Pluteus* ITS rDNA sequences selected from GenBank and UNITE (<http://unite.ut.ee>) databases on the basis of the greatest similarity based on BLASTsearch, outcomes of recent phylogenetic studies focused on *Pluteus* (Justo *et al.* 2011a, b, Pradeep *et al.* 2012), and subsequent phylogenetic analysis (preliminary trees not

shown). Alignments were generated using MAFFT (Kato *et al.* 2002) with default conditions for gap openings and gap extension penalties. The sequence alignments were then imported into MEGA v. 5.10 (Tamura *et al.* 2011) for manual adjustment. *Pluteus diettrichii* (HM562143) and *Pluteus seticeps* (HM562199) were used as outgroup taxa following Justo *et al.* (2011a) and Pradeep *et al.* (2012). Best-fit models were estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest v. 0.1.1 (Posada 2008) to provide a substitution model for the alignment. Phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum likelihood (ML) approaches. The BI was performed with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run over 10 million generations, under the GTR+ Γ evolutionary model. Trees were sampled every 1 000 generations resulting in an overall sampling of 10 001 trees; the first 2 500 trees were discarded as "burn-in" (25 %). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). ML estimation was performed through RAxML v. 7.3.2 (Stamatakis 2006) with 1 000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the "-f a" option of RAxML and "-x 12345" as a random seed to invoke the novel rapid bootstrapping algorithm. Only BPP values over 0.70 and MLB over 50 % are reported in the resulting tree (Fig. 1). Branch lengths were estimated as mean values over the sampled trees. Clade names follow Justo *et al.* (2011a) and Pradeep *et al.* (2012); pairwise % identity values of ITS sequences were calculated using MEGA 5.10 (Tamura *et al.* 2011).

RESULTS

Both Bayesian and Maximum likelihood analyses produced the same topology; therefore, only the Bayesian tree with both BPP and MLB values is shown (Fig. 1). The ITS data matrix comprised 46 sequences (including 38 from GenBank and 4 from UNITE). The alignment comprised 715 characters, and contains 312 variable sites. The three Italian collections of *P. variabilicolor* and the isotype collection cluster with the three collections of *P. castri* (holotype included) forming a well-supported clade (*l*variabilicolor; BPP = 1, MLB = 100 %) within the *Pluteus leoninus* clade of sect. *Hispidoderma*. The pairwise % identity value among the sequences of the *l*variabilicolor is 98.8. The clade is in sister position to *l*chrysaegis (BPP = 1, MLB = 100 %). The clade consisting of *l*variabilicolor and *l*chrysaegis is sister (BPP = 1, MLB = 100 %) to *l*leoninus (= the *P. leoninus* complex).

The *P. leoninus* complex includes collections identified as *P. leoninus*, *P. roseipes*, *P. flavofuliginus*, and two provisionally undescribed taxa (*Pluteus* aff. *leoninus* I and *Pluteus* aff. *leoninus* II).

The three phylogenetic major clades recognized in sect. *Hispidoderma* by Justo *et al.* (2011a, b) and Pradeep *et al.*



Fig. 2. *Pluteus variabilicolor*. Macroscopic features. **A, C, E.** Basidiomata (TL20130521-01, TL20130530-01, VM20130504-01). **B.** Pileus smooth and lined up to the middle (TL20130521-01). **D.** Pileus with a rugulose-veined centre (VM20111105-01). **F.** Stipe and hymenophore (free lamellae with lamellulae) (TL20130521-01). Photos A–B, F by Tomaso Lezzi; C by Massimo Biraghi; and D–E by Vincenzo Migliozi. Bars = 10 mm.

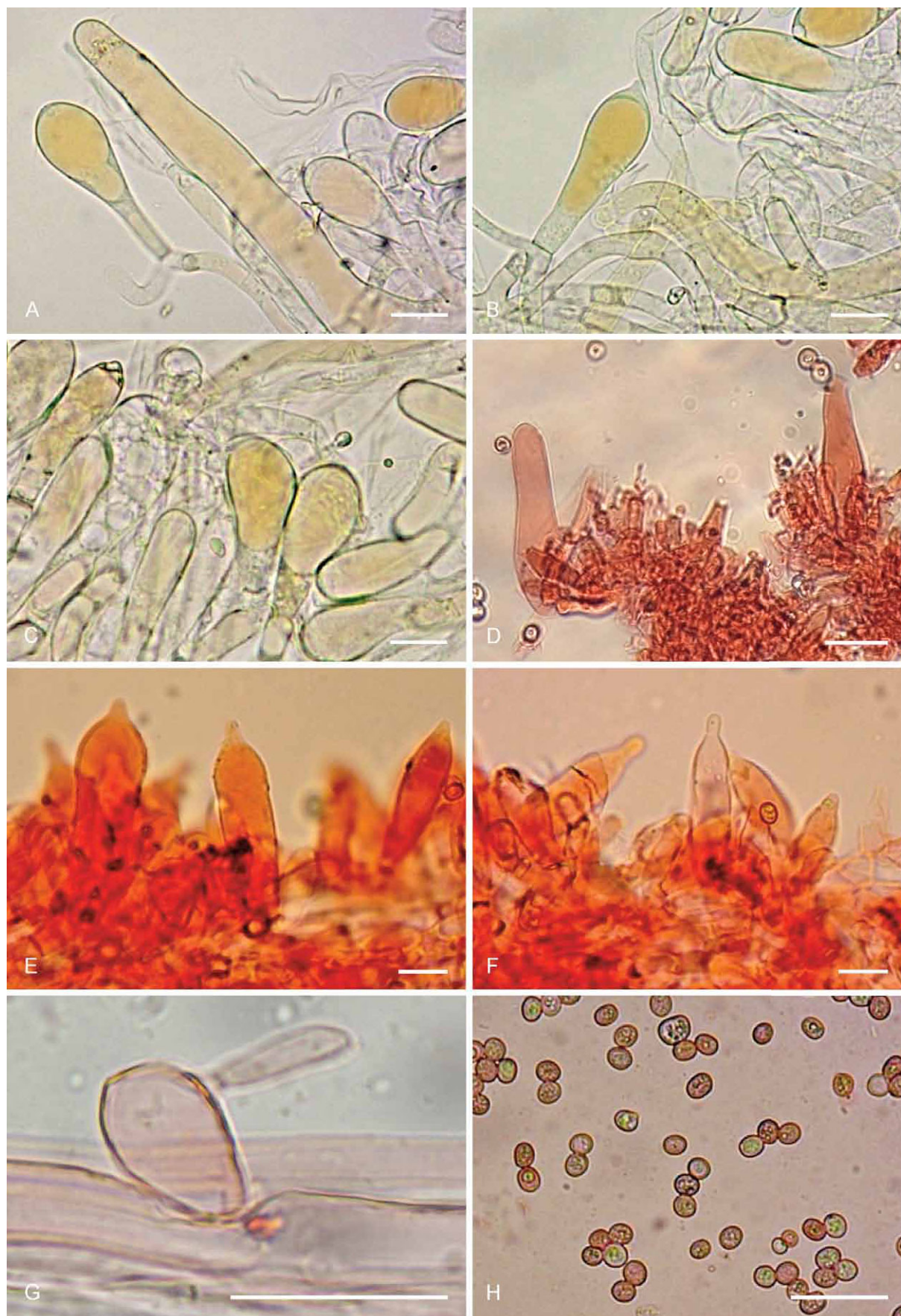


Fig. 3. *Pluteus variabilicolor*. Microscopic features. **A.** Portion of pileipellis with both short and elongated elements (in H₂O) (TL2030521-01). **B, C.** Portions of pileipellis with only short elements (in H₂O) (TL2030521-01). **D.** Pleurocystidia (in ammoniacal Congo red) (TL20130530-01). **E, F.** Cheilocystidia (in ammoniacal Congo red) (TL2030521-01). **G.** Caulocystidia (in ammoniacal Congo red) (TL20130726-01). **H.** Basidiospores (in ammoniacal Congo red) (TL2030521-01). Photos A–C, E–H by Tomaso Lezzi; and D by Massimo Biraghi. Bars = 20 μm.

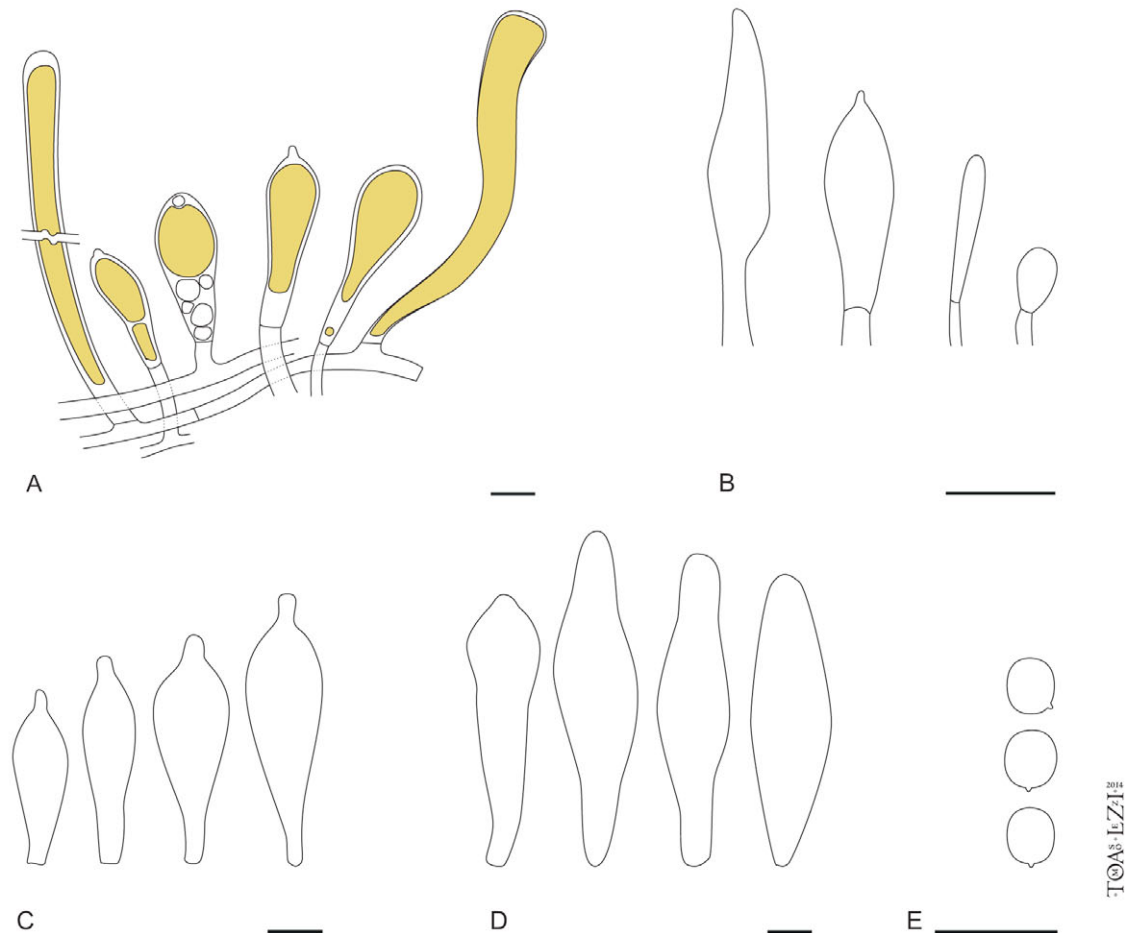


Fig. 4. *Pluteus variabilicolor*. Line drawings of microscopic features (TL20130521-01). **A.** Pileipellis. **B.** Caulocystidia. **C.** Cheilocystidia. **D.** Pleurocystidia. **E.** Basidiospores. Drawing by Tomaso Lezzi. Bars = 20 μ m.

(2012) are also recovered in our nrITS sequence analyses (Fig. 1), with high statistical support.

TAXONOMY

Pluteus variabilicolor Babos, *Annls hist.-nat. Mus. natn. hung.* **70**: 93 (1978).

(Figs 2–4)

Synonym: *Pluteus castri* Justo & E.F. Malysheva, *Mycol. Progr.* **10**: 470 (2011).

Selected descriptions: Babos (1978: 193–195); Lohmeyer *et al.* (1994: 96–98); Migliozi (2011: 4–7); Justo *et al.* (2011a: 470–471, as *P. castri*).

Selected illustrations: Lohmeyer *et al.* (1994: fig. 5, p. 101); Lanconelli *et al.* (1998: 96, 127); Albert (2011, two unnumbered figs); Migliozi (2011: 4, 8); Justo *et al.* (2011a: fig. 5, p. 471, as *P. castri*).

Description: *Basidiomata* pluteoid. *Pileus* medium-sized, 30–80 mm broad, smooth, lined up to middle, colour distinctly yellow-orange, with central darker umbo, often radially rugulose-veined, especially in mature specimens, hygrophanous, clearly striate or not at margin. *Lamellae* free, quite crowded, ventricose, first

white then pink, with the presence of lamellulae ($l = 0-2$), with concolorous or whitish, flocculose edge. *Stipe* 30–70 \times 4–12 mm, cylindrical, slightly enlarged at the base, streaked-fibrillose over the entire length, yellow, with reddish tinges at the base in mature specimens. Context white-yellowish, yellowish orange under the pileus surface, without distinctive smell and taste. *Spore print* pinkish. *Basidiospores* 5.5–7.0 \times 4.5–5.5(–6.0) μ m, on average 6.0 \times 4.9 μ m, $Q = 1.10-1.35$; $Q_m = 1.22$, broadly ellipsoid to subglobose, thin-walled. *Basidia* 25–32 \times 6–8 μ m, clavate, tetrasporic. *Cheilocystidia* fusiform to lageniform, 50–90 \times 25–30 μ m, hyaline, thin-walled, frequently with short, wide appendix at the apex (mucronate). *Pleurocystidia* not frequent, fusiform, lageniform or utriform, 60–160 \times 20–40 μ m. *Pileipellis* a hymeniderm consisting of clavate, rounded terminal elements and cylindrical, elongated cells 40–200 \times 22–40 μ m; with yellow intracellular pigment. In some parts, the hymeniderm with short cells is strongly predominant; in other parts the elongated cells are strongly predominant. Often the two types of elements are mixed independently from whether they are found: in the centre or in the margin of the pileus. *Stipitipellis* a cutis made of cylindrical, 2–3 μ m wide hyphae. *Caulocystidia* present over the whole length of the stipe, 13–70 \times 3–15 μ m, cylindrical to claviform, fusiform, sometimes mucronate, usually grouped in clusters. *Clamp-connections* not observed.

Habitat: On decaying wood of *Quercus cerris* and branches of *Castanea sativa* on the ground.

Specimens examined (of *P. variabilicolor*): **Italy:** Lazio: Manziana (RM), on decaying wood of *Quercus cerris*, 21 May 2013, T. Lezzi (TL20130521-01); *loc. cit.*, 26 July 2013, L. Perrone (TL20130726-01); Lombardia: Parco di Monza (MI), on decaying wood of *Castanea sativa*, 30 May 2013, M. Biraghi (TL20130530-01); Lazio: Manziana (RM) on decaying wood of *Quercus cerris*, 5 Nov. 2011, V. Migliozi (VM20111105-01); *loc. cit.*, 4 May 2013, V. Migliozi (VM20130504-01). – **Hungary:** near Szárliget, on a pile of sawdust, 06 July 1977, M. Babos & A. Friesz (BP-FN 56936 – isotype); Budakeszi, on a pile of sawdust, 27 July 1980, L. Babos (BP-FN 65137); Vallis Lepence, near Visegrád, on a pile of sawdust, 31 July 1981, M. Babos (BP-FN 72875); Com. Heves, Mátra-hegység, Gyöngyössolymos, on sawdust, 3 Aug. 2008, G. Vasas & C. Locsmándi (BP-FN 100005).

Specimens examined (of *P. castri*): **Japan:** Fukuoka Prefecture: Kyushu, on piled wood chips, 5 May 2007, M. Shintani & S. Takehashi (TNSF 17602 – holotype); Ibaraki Prefecture: Honshu, Tsukuba, 25 Oct. 2007, K. Osaku (TNSF 17081). – **Russia:** Central Russia, Moscow Region, Prioksko-Terrasny State Reserve, on decaying wood of *Populus tremula*, 15 Aug. 1991, G. E. Levitskaya (LE 216873); Samara Region, near Pribrezhny, on decaying wood of deciduous tree, 1 July 2007, E. F. Malysheva (LE 212090).

DISCUSSION

According to our molecular analysis (Fig. 1) the four sequenced collections of *Pluteus variabilicolor* (isotype included) and the three of *P. castri* retrieved from GenBank (holotype included) are conspecific. Consequently, we have placed *P. castri* as a later synonym of *P. variabilicolor*.

Pluteus variabilicolor was originally described from Hungary and primarily characterized by a pileus which was at first yellowish orange and then becoming chrome-yellow (hence the epithet “variabilicolor”), rugose-veined at the centre, and a pileipellis consisting of dimorphic elements, viz. a mixture of spheropedunculose-vesiculose and elongated, more or less cystidioid elements, the presence of caulocystidia, and growth on decaying sawdust (Babos 1978). Because of the pileipellis structure, it was included in subsect. *Mixtini* of sect. *Celluloderma*. Apart from Hungary, where it was found several times after the first report (e.g. Babos 1981, 1989, 1991, Rimóczi & Vetter 1990, Lukács 2010, Albert 2011), *P. variabilicolor* is reported only from Austria (Lohmeyer *et al.* 1994), Italy (Lanconelli *et al.* 1998, Migliozi 2011), Romania (Béres 2012), Slovenia (Jogan *et al.* 2012), and Moldova (E. Musumeci, pers. comm.).

Pluteus variabilicolor is mainly reported from sawdust deposits, even though our finds and those of Migliozi (2011) and Musumeci (pers. comm.) are on branches and rotten wood of *Fagaceae* (mainly *Quercus* species).

As suggested by Babos (1978, 1981) and Courtecuisse (2006), the species may not be native to Europe and could have been introduced through timber import. In support of this hypothesis, all the nations in which the species was found are geographically related. On the other hand, the species is widely distributed from Europe to Japan in relatively “natural”

habitats, so it may very well be a native Eurasian species that is just not common.

Our description, based on Italian specimens, fits quite well with the protologue, with the morphologic features reported by later authors (Lohmeyer *et al.* 1994, Lanconelli *et al.* 1998, Migliozi 2011), and the Hungarian collections we have checked (isotype included).

Pluteus castri was recently described (Justo *et al.* 2011a) based on collections from Japan (on piled wood chips, holotype) and Central Russia (on decaying wood of deciduous trees). The main difference with respect to the original description of *P. variabilicolor* and the Italian collections found was in the structure of the pileipellis. In *P. castri* we did not observe the elongated elements (to 200 µm long) found in other collections of *P. variabilicolor*. However, molecular data indicate that all collections of *P. castri* should be considered to represent *P. variabilicolor*.

In the phylogenetic analysis (Fig. 1) *P. variabilicolor*, in spite of the presence of both vesiculose-clavate and elongate elements in the pileipellis, clustered into the *leoninus* clade of sect. *Hispidoderma* as delimited by Justo *et al.* (2011a, b) and Pradeep *et al.* (2012), where it is sister to *P. chrysaegis* and very close to the *P. leoninus* complex. Most species of the *leoninus* clade are characterized by yellowish tinges on the pileus surface.

Pluteus chrysaegis (syn. *P. conizatus* var. *africanus*) resembles *P. variabilicolor* in the general structure of the pileipellis and the yellow colouring of the basidiome, the veined pileus surface, and the presence of caulocystidia (Pradeep & Vrinda 1996, Pradeep *et al.* 2012). However, it differs in the even shorter elements of the pileipellis (to 40 µm long), and the non-mucronate, slightly thick-walled cheilocystidia (Horak & Heinemann 1978, Pradeep *et al.* 2012).

The species in the *P. leoninus* complex (i.e. *P. leoninus*, *P. roseipes*, *P. flavofulgineus*, *P. aff. leoninus* I and *P. aff. leoninus* II) all have a pileipellis made up exclusively of narrowly fusiform elements to 230 µm long (Justo *et al.* 2011a) and, with the exception of *P. aff. leoninus* II, a stipitipellis without caulocystidia. The separation of the species inside this complex still requires further study.

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