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A new species of *Pluteus* sect. *Hispidoderma* (Agaricales, Basidiomycota) from the Far East, Russia

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Keywords: Agaricomycetes, ITS, phylogeny, Pluteaceae, Primorye Territory, taxonomy.

Summary. *Pluteus svetlanae* collected from birch wood in broadleaved mountain forest in Sikhote-Alin State Nature Biosphere Reserve, the Russian Far East, is described as a new species, based on its distinctive morphology and phylogeny. The fungus is characterized by small basidiomata having pale brown or beige pileus with striate and granulose-pruinose surface, whitish floccose stipe with subbulbose base, pileipellis as trichohymeniderm, and broadly fusiform or lageniform pleurocystidia with subcapitate apex and lateral projections. A phylogenetic analysis based on molecular data (ITS1-5.8S-ITS2 region) was conducted to explore the relationship of the new species. Results place the new species in sect. *Hispidoderma*. Detailed morphological description, field photograph, original drawing of microstructures, and comparison of the new species with other morphologically and phylogenetically closely related species are provided.

Новый вид *Pluteus* секции *Hispidoderma* (Agaricales, Basidiomycota) с российского Дальнего Востока

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Ключевые слова: Приморский край, таксономия, филогения, Agaricomycetes, ITS, Pluteaceae.

Аннотация. *Pluteus svetlanae*, собранный с древесины берёзы в широколиственном горном лесу в Сихотэ-Алинском государственном природном биосферном заповеднике на Дальнем Востоке России, описан как новый вид на основании его отличительной морфологии и филогении. Гриб характеризуется мелкими базидиомами с бледно-коричневыми или бежевыми шляпками с полосатой и зернистой поверхностью, беловатой хлопьевидно-опушённой ножкой с булавовидным основанием, трихогименидермным пилеипеллисом и широко веретеновидными или бутылковидными плевроцистидами с головчатой вершиной и латеральными выростами. Для выяснения эволюционной позиции нового вида был проведен филогенетический анализ, основанный на молекулярных данных (регион ITS1-5.8S-ITS2). Результаты позволяют отнести новый вид к секции *Hispidoderma*. Приведены подробное морфологическое описание, фотография плодового тела из природы, оригинальный рисунок микроструктур и сравнение нового вида с другими морфологически и филогенетически близкими таксонами.

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Introduction

Pluteus Fr., type genus of the family Pluteaceae Kotl. et Pouzar, is distributed worldwide and composed of about 500 species with saprotrophic lifestyle, predominantly growing on wood or soil (Singer, 1956; Vellinga, 1990; Justo et al., 2011a, b). It is characterized by basidiomata without volva, free lamellae, pink or pinkish brown spore print, smooth, colourless and inamyloid basidiospores, and inverse hymenophoral trama. As a result of recent phylogenetic studies (Justo et al., 2011a, b) this genus has been restricted to monophyletic lineages corresponding to three morphological sections previously recognized by Singer (1986): sect. *Pluteus*, sect. *Celluloderma* Fayod and sect. *Hispidoderma* Fayod, which are accepted by the majority of modern mycologists. The section *Hispidoderma* in the modern sense accommodates species with non-metuloid pleurocystidia and with pileipellis composed of long, elongated elements, very variable in shape and size, organized as a hymeniderm or trichoderm. Three infrasectional lineages can be recognized within sect. *Hispidoderma*: one includes *Pluteus leoninus* (Schaeff.) P. Kumm. and allied taxa, a second with *P. plautus* (Weinm.) Gillet, *P. longistriatus* (Peck) Peck, and related species, and a third clade with *P. umbrosus* (Pers.) P. Kumm. and *P. granularis* Peck (Justo et al., 2011b).

A preliminary revision of representatives of the sect. *Hispidoderma* distributed in the Holarctic region, using polyphasic taxonomy approach, revealed a much greater diversity of the group than previously known, with a large number of undescribed taxa (Justo et al., 2011a; Ševčíková et al., 2020).

During our investigations of *Pluteus* in Primorye Territory in 2024, two noteworthy specimens have been collected in oak-birch mountain forest. Detailed morphological and molecular studies have shown that they both belong to a new undescribed species. In this article, the species is described, its morphological characters based on studied specimens are illustrated, and its phylogenetic position in *Pluteus* sect. *Hispidoderma* in the /plautus-longistriatus clade is discussed.

Materials and methods

Morphological examination

Macroscopic description is based on fresh basidiomata and photos taken at the site. Colour terms are according to codes from the RAL Design System plus D2 (RAL D2 Colour fan, edition 2018, Germany). Microscopic observations were made from dried material mounted in 5 % KOH and Congo Red using an Axiolab 5 light microscope equipped with AxioCam MRc5 camera (CarlZeiss, Germany). Basidiospore size is based on the measurement of 100 randomly selected basidiospores from two basidiomata from two collections. Basidiospore dimensions are given following the form (a)b–c(d), with b–c containing at least 90 % of all values and the extremes (a, d) enclosed in parentheses, and L_{av} and W_{av} are the mean value of length and width of the total basidiospores measured. Q indicates the basidiospore length/width ratio, Q_{av} represents the mean length/width quotient of the total basidiospores measured. All morphological terms were used following Vellinga (1988). The studied specimens were deposited in the Mycological Herbarium of the Komarov Botanical Institute, Saint Petersburg (LE F).

Molecular techniques

For DNA extraction, small fragments of dried basidiomata were used. The procedure of DNA extraction completely corresponded to the manufacturer's protocol of the Phytosorb Kit (ZAO Syntol, Russia). The primer pair ITS1F-ITS4B (White et al., 1990; Gardes, Bruns, 1993) was employed both for amplification and for sequencing of the ITS1-5.8S-ITS2 (nuclear ribosomal internal transcribed spacer, ITS) region; and EF1-983F and EF1-1567R for approximately 500bp of *tef1* (translation-elongation factor 1-alpha) (Rehner, Buckley, 2005). PCR products were purified applying the CleanMag DNA PCR (Evrogen, Russia). Sequencing was performed with an ABI model 3500 Genetic Analyzer (Applied Biosystems, CA, USA). Raw data was edited and assembled in MEGA 11 (Tamura et al., 2021).

All microscopic and molecular studies of specimens were carried out at the Center for collective use of scientific equipment "Cellular and molecular technology of studying plants and fungi" (Komarov Botanical Institute of the Russian Academy of Sciences, St. Petersburg).

Phylogenetic analyses

For this study, 2 ITS and 1 *tef1* sequences were generated. However, phylogenetic analyses were performed based on only ITS marker. In addition, 50 ITS sequences of taxa from *Pluteus* sect. *Hispididerma* including *P. leoninus* as an outgroup, were retrieved from the GenBank database (www.ncbi.nlm.nih.gov/genbank/) for molecular analyses.

The sequences were aligned using MAFFT vs. 7 (MAFFT version 7. URL: https://mafft.cbrc.jp/alignment/ server/index.html) with the FFT-NS-i option and improved where necessary using MEGA11.

Phylogenetic analyses were performed with Maximum Likelihood (ML) and Bayesian Inference (BI) methods for ITS dataset. The most likely model of evolution was GTR+G determined through Akaike information criterion (AIC) function in FindModel web server (FindModel. URL: http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html). ML analysis was run on IQ-Tree web server (IQ-TREE. URL: http://iqtree.cibiv.univie.ac.at/) with 1000 rapid bootstrap replicates. BI analysis was performed with MrBayes 3.2.7 software (Ronquist et al., 2012), for two independent runs, each with 7 million generations under described model and four chains with sampling every 100 generations. To check for convergence of MCMC analyses and to get estimates of the posterior distribution of parameter values, Tracer v1.7.1 was used (Rambaut et al., 2018). We accepted the result where the ESS (Effective Sample Size) was above 200 and the PSRF (Potential Scale Reduction Factor) was close to 1. Branches with bootstrap support (BS) and posterior probabilities (PP) values greater than or equal to 70 % and 0.90, respectively, were considered significantly supported (Hillis, Bull, 1993; Alfaro et al., 2003). Tree topologies were then edited and visualized in iTOL (Letunic, Bork, 2019). Newly generated sequences were deposited in GenBank with corresponding accession numbers.

Results

Molecular phylogeny

The ITS dataset for some members of sect. *Hispidoderma*, with most species from the /plautus-longistriatus group, contained 651 characters, including gaps. Both Maximum Likelihood and Bayesian analyses produced the same topology. Therefore, we present only the ML tree with both BS and PP values (Fig. 1).

According to the phylogenetic analysis, *P. svetlanae* forms independent and highly supported (BS = 100 % and PP = 1) monophyletic clade, which is sister to unidentified *Pluteus* taxon represented by GenBank sequence “*Pluteus* sp.” (OR203581). However, *Pluteus* sp. differs significantly from our new species based on its morphology (see “Discussion” part). The whole clade included both taxa is closely related to a group of species comprising *P. lauracearum*, *P. hubregtseorum*, and *P. semibulbosus* s. l. with significant statistical support (BS = 83 % and PP = 0.92). *Pluteus svetlanae* is also the most similar to these species from the *P. plautus* group in its morphological characteristics.

Taxonomy

Pluteus svetlanae E. F. Malysheva, **sp. nov.**
MycoBank: MB 857599

Diagnosis. The species is characterized by small basidiomata with pale brown or beige pileus with sulcate and granulose-pruinose surface, whitish floccose stipe with subbulbose base, pileipellis as trichohymeniderm, broadly ellipsoid basidiospores, 5.5–7.2 × 4.5–6.0 µm, pleurocystidia with subcapitate apex and lateral projections, cheilocystidia variable in shape and numerous clavate caulocystidia across the entire stipe.

Holotype: “RUSSIA. Primorye Territory, Sikhote-Alin State Nature Biosphere Reserve, vicinity of Blagodatnoye field reserve station, N44.98104, E136.52516, oak-birch forest on the top of Mt. Lysaya, near the Sukhaya hut, on fallen branch of *Betula lanata*. 25 VIII 2024. V. Dudka” (LE F-354561) (Fig. 2). GenBank ITS: PV037629; *tef1*: PV037662.

Etymology. The species is named in honour of Svetlana Bondarchuk, a researcher at the Sikhote-Alin State Nature Biosphere Reserve, for her contribution and active participation in mycological research in this territory.

Description. Pileus 15–20 mm in diam., when young hemispherical or convex, expanding to plano-convex, with indistinct low umbo; not hygrophanous, striate to sulcate to the half of the radius; surface entirely granulose-pruinose or slightly velvety, slightly wrinkled in centre, light ash brown (RAL 070 70 20) or golden beige (RAL 070 70 30), darker at centre – ash gold (RAL 070 50 20) and pale at margin – off white (RAL 070 90 05). **Lamellae** free, crowded, slightly ventricose, whitish to pink, with concolorous even edges. **Stipe** 25–30 × 2–3 mm, cylindrical, with subbulbous base, anemone white (RAL 070 93 05) or silver thistle beige (RAL 070 85 10), entirely floccose. **Smell** indistinct, taste not recorded.

Basidiospores 5.5–7.2 × 4.5–6.0 µm (L_{av} = 6.2, W_{av} = 5.3, Q = (1.0)1.1–1.3; Q_{av} = 1.2), broadly ellipsoid or subglobose, thick-walled. **Basidia** 18–28 × 7.5–10.0 µm, 4-spored, occasionally 2-spored, clavate. **Pleurocystidia** (57)60.0–72.0(81.5) × 11.5–17.0(23.0) µm, rare, narrowly to broadly

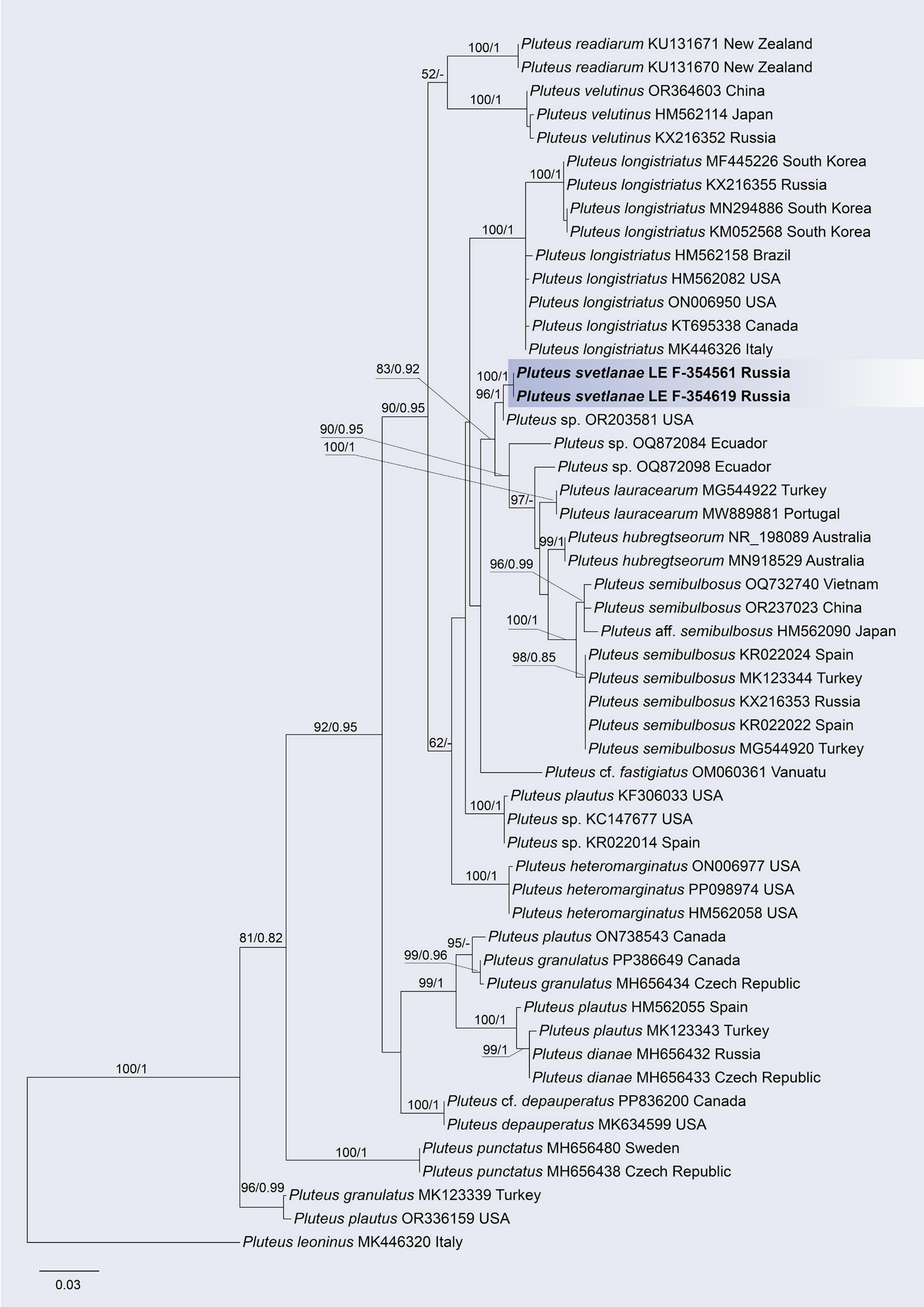

Fig. 1. Phylogenetic tree inferred from a Maximum Likelihood analysis of the ITS dataset for species *Pluteus* sect. *Hispidoderma*, with *Pluteus leoninus* as outgroup. Bootstrap support values (BS) and Posterior probabilities (PP) are given above the branches. All tips are labelled with taxon name, GenBank accession number and geographic origin of the specimen. For taxon in bold the sequences are generated in present study.

fusiform or lageniform, with rounded subcapitate apex, often with lateral excrescences, hyaline or yellowish-brown at apex in KOH, thin-walled. **Lamellar edge** heterogeneous, with cheilocystidia, basidia, and basidioles, with cheilocystidia crowded in places in clusters but not forming a continuous strip. **Cheilocystidia** 39.5–85.5(104.0) × (11.0)15.5–24.5(32.5) µm, rather numerous, strongly variable in shape, broadly lageniform or fusiform, often with subcapitate or papillate apex, utriform, sometimes spatuliform or lanceolate, colourless or yellowish in KOH, thin- or slightly thick-walled. **Pileipellis** a trichohymeniderm, with terminal elements, 48.0–98.5 × 12.0–30.0(38.5) µm, narrowly to broadly clavate or cylindrical, with brown intracellular pigment, thin- or slightly thick-walled. **Stipitipellis** consists of cylindrical, slightly thick-walled, 9–10 µm wide hyaline hyphae. **Caulocystidia** numerous, in clusters, 40.0–68.0(78.5) × 19.0–30.0 µm, broadly clavate, thick-walled, colourless or yellowish in KOH. Clamp-connections absent in all tissues.

Habitat and distribution. Solitary, on wood of deciduous trees. Currently, only known in the Primorye Territory, Russia.

Additional collection examined: "RUSSIA. Primorye Territory, Sikhote-Alin State Nature Biosphere Reserve, vicinity of Blagodatnoye field reserve station, N44.98430, E136.52116, oak-birch

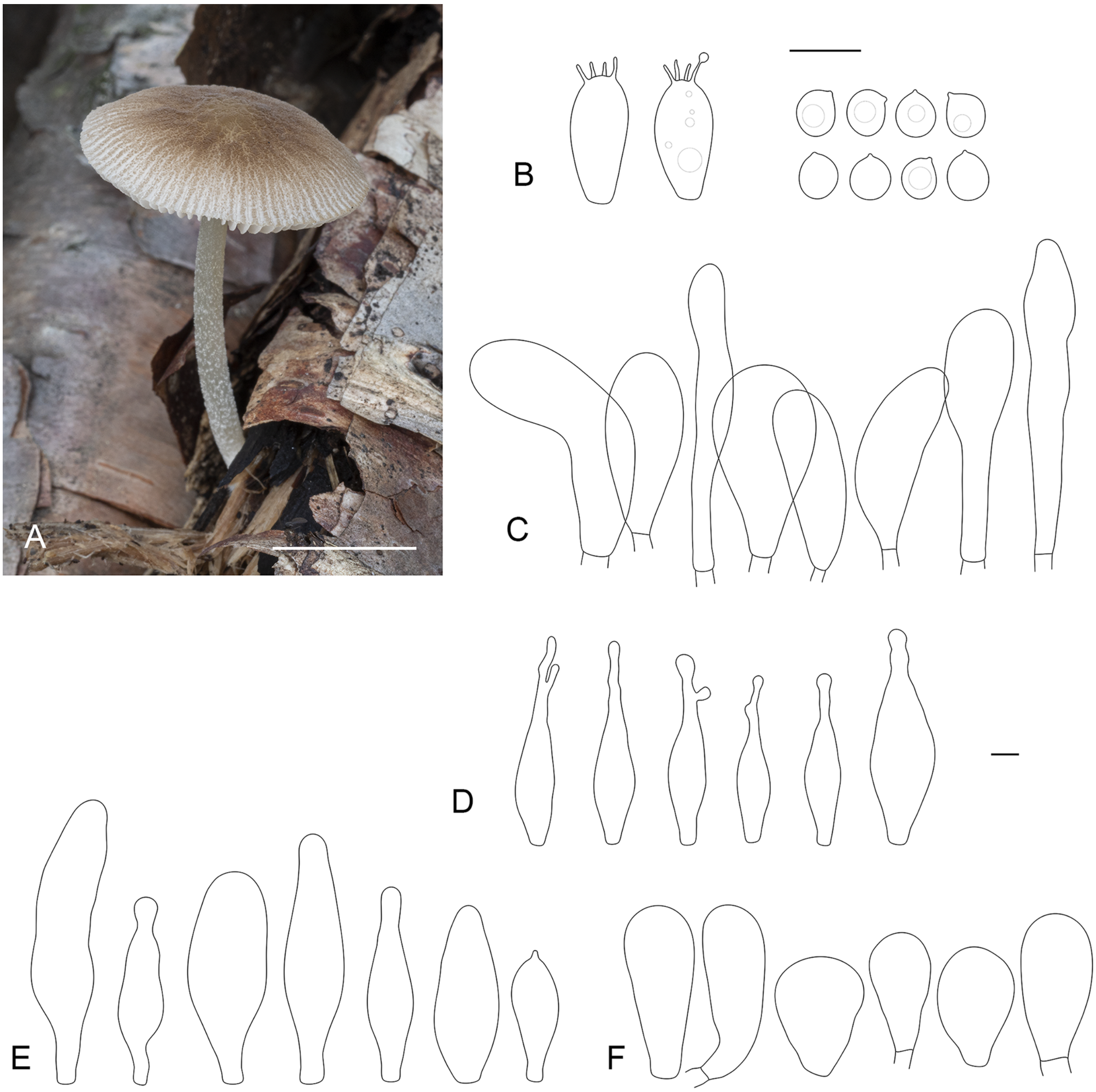

Fig. 2. Holotype specimen of *Pluteus sveltanae* (LE F-354561): A – basidioma *in situ*; B – basidia and basidiospores; C – elements of pileipellis; D – pleurocystidia; E – cheilocystidia; F – caulocystidia. Scale bars: = 1 cm (for A), = 10 µm (for B) and = 10 µm (for C–F).

forest on the top of Mt. Lysaya, *Quercus mongolica* forest, on wood of *Betula*. 25 VIII 2024. V. Malysheva" (LE F 354619). GenBank ITS: PV037630.

Discussion

Pluteus svetlanae can be distinguished by the small size of its basidiomata with pale colouration of pileus and stipe, trichohymeniderm pileipellis, and fusiform or lageniform pleurocystidia with subcapitate apex and lateral projections. Based on a combination of all morphological features as well as phylogenetic analyses *P. svetlanae* belongs to *P. plautus* species complex in sect. *Hispidoderma*. This complex includes a group of closely related species with similar morphotypes, the taxonomic interpretation of which differed greatly in different historical periods and in the treatment of different authors (Vellinga, Schreurs, 1985; Orton, 1986; Vellinga, 1990; Citérin, Eyssartier, 1998). The use of a phylogenetic approach in the systematics of the group has shown that the wide morphological concept of *P. plautus* proposed by Vellinga and Schreurs (1985) is not supported by molecular data. Recent studies (Ševčíková et al., 2020) involving a sufficient set of specimens have demonstrated that presumably at least 12 taxa can be recognised in this group based on both morphological and molecular data, most of which remain as yet undescribed species. The species we describe in this paper is one of them.

The key species of the complex, *P. plautus*, is interpreted very differently by different authors, and a wide range of morphological characters is applied to it. However, the most important diagnostic feature of the species in Weinmann's original description (1836) is the very dark colour of the pileus and stipe, which distinguishes it from both the other species of the group and our new species.

The small size of the basidiomata and their light colouration makes *P. svetlanae* similar to *P. semibulbous* (Lasch) Quel. in the broad concept of the latter (Kühner, Romagnesi, 1953; Moser, 1983; Orton, 1986). However, *P. svetlanae* can be distinguished by longer terminal pileipellis elements and pleurocystidia (and some cheilocystidia) with subcapitate apex.

Another species from *P. plautus* complex, *Pluteus granulatus* Bresadola in its original description is characterized by brown-reddish granular pileus and growth on coniferous wood, which distinguishes it from *P. svetlanae* and other species of the complex. Although the taxonomic identity of *P. granulatus* and its morphological delimitation are the subject for further research, recent data showed that the nomenclatural name of the taxon is applicable to a distinct monophyletic clade and is supported by molecular data (Ševčíková et al., 2020).

Pluteus dianae Pilát has large stout basidiomata with greyish-brown or brown pileus, predominantly clavate cheilocystidia and longer and generally narrower pileipellis elements (Ševčíková et al., 2020).

Pluteus punctatus Wichanský, differs in generally larger basidiomata, larger basidiospores, (5.0)7.0–8.5(9.5) × 5.0–7.0(7.5) μm, lageniform pleurocystidia with long obtuse apex, mostly clavate cheilocystidia, and significantly longer (up to 380 μm) pileipellis elements (Ševčíková et al., 2020).

Pluteus depauperatus Romagn. and *P. punctipes* Orton are separated by larger basidiomata with darker colour of pileus and stipe and pleurocystidia lacking lateral excrescences (Orton, 1986).

Pluteus velutinus C. K. Pradeep, Justo et K. B. Vrinda originally described from India but characterized by wide geographical distribution has similar hymenial cystidia with subcapitate apex and apical projections but differs from *P. svetlanae* in dark brown pileus and stipe and pigmented caulocystidia (Pradeep et al., 2012; Malysheva et al., 2016).

Pluteus longistriatus distributed in North and South America differs mainly in larger basidiomata, darker brown pileus and clavate or utriform pleuro- and cheilocystidia (Menolli, Capelari, 2010).

Pluteus hubregtseorum G. M. Gates, Ševčíková et Borovička currently known from Australia, New Zealand, and Tasmania differs from *P. svetlanae* in the presence of a distinct yellow tinge in the colour of the pileus and stipe, sterile lamellae edges, non-capitate pleurocystidia, and growing on the wood of the eucalyptus tree (Ševčíková et al., 2021).

Pluteus lauracearum Kaygusuz, Ševčíková et Justo recently described species from Turkey and Madeira Islands (Kaygusuz et al., 2021) is very similar to *P. svetlanae* in terms of small size of basidiomata, pruinose-granulose pale brown pileus with striate margin, pruinose whitish stipe and pleurocystidia with subcapitate apex. However, it differs in slightly larger broadly ellipsoid spores, (6.4)6.7–7.8(8.5) × (5.1)5.6–6.5(6.9) μm, mostly clavate cheilocystidia, lageniform or fusiform caulocystidia and growing on *Laurus* wood.

The phylogenetic analyses showed that the ITS sequence (GenBank OR203581) of unidentified *Pluteus* sp. from the USA is close to *P. svetlanae* clade indicating their high similarity (Fig. 1). However, according to the observed morphological features, the American specimen cannot belong to *P. svetlanae* (Mushroom Observer MO502242 and personal information from specimen's collector Teresa Clements). It differs greatly from our new species in macroscopic features by having larger stout basidiomata with velvety and darker pileus (amber grey or caramel brown with lighter soft orange spots and dark brown margin), longitudinally striate and fibrillose, golden or apricot yellow stipe. The ecology of the American specimen, growing on coniferous wood (Douglas fir), is also a significant difference from *P. svetlanae*, which found on wood of *Betula*.

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