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ORIGINAL ARTICLE

Description of two new *Pluteus* species of sect. *Pluteus* (*Agaricales*, *Pluteaceae*) from the Russian Far East based on morphological and molecular data

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Abstract

Two new *Pluteus* species, *P. nigritus* and *P. fumidus*, were described based on specimens collected in the Far East, the easternmost part of Russia. *Pluteus nigritus* is characterized by rather large basidiomata with dark-coloured blackish brown pileus and greyish brown bulbose stipe, pigmented lamellae edges, metuloid pleurocystidia, presence of clamps in pileipellis, and growing on conifer wood. Another species, *P. fumidus*, also growing on conifers, has medium-sized basidiomata with greyish brown pileus and silvery-white stipe with grey base, concolorous lamellae edges, metuloid pleurocystidia, and abundant clamp-connections in all tissues. The phylogenetic placements of the two newly described species within *Pluteus* were confirmed using DNA data (nuclear ribosomal internal transcribed spacer (ITS) and translation-elongation factor 1-alpha (*TEF1*)). Detailed morphological descriptions, field photographs, and comparisons of two new species with other morphologically and phylogenetically closely related species are provided. Morphological characters and phylogenetic trees inferred from ITS and *TEF1* of nc DNA sequences showed that both of our new species belong to the section *Pluteus*.

Keywords Primorye Territory · *Basidiomycota* · ITS · *TEF1* · Molecular phylogeny · Taxonomy

Introduction

The genus *Pluteus* Fr. is the largest genus in the *Pluteaceae* and is characterized by basidiocarps without volva, free lamellae, pink or pinkish brown spore print, smooth and inamyloid basidiospores, and inverse hymenophoral trama (Justo et al. 2011b). *Pluteus* species are widely distributed and often grow on wood of deciduous and coniferous trees of various degrees of decomposition and soil. They are a group of saprophytic fungi widespread from the Arctic to tropical areas (Singer 1956; Vellinga 1990; Justo et al. 2011a, b).

According to the modern classification based on morphological and molecular studies (Justo et al. 2011a, b), three sections are distinguished in the genus *Pluteus*: sect. *Pluteus* characterized by metuloid pleurocystidia and a pileipellis organized as a cutis; sect. *Celluloderma* which includes members with non-metuloid (or absent) pleurocystidia and a pileipellis as a hymeniderm or epithelium; and sect. *Hispidoderma* which included species with non-metuloid pleurocystidia and hymeniderm or trichoderm pileipellis.

Currently, there are about 500 documented species of the genus *Pluteus* worldwide (He et al. 2019). In Russia, *Pluteus* is represented by about 60 species (Bolshakov et al. 2021). The Russian Far East is the region with the greatest species diversity of plants and fungi. Eight species of *Pluteus* sect. *Pluteus* have so far been known to occur in this region, including previously reported (Justo et al. 2014) and unpublished data.

Our investigations on the *Pluteus* collections from the mountain forests in Primorye Territory revealed two new species from sect. *Pluteus*: *P. nigritus* and *P. fumidus*. In this article the species are described, detailed descriptions and illustrations are provided, and the phylogenetic relationships of the new species and related taxa from the genus *Pluteus* are analyzed on the basis of molecular data.

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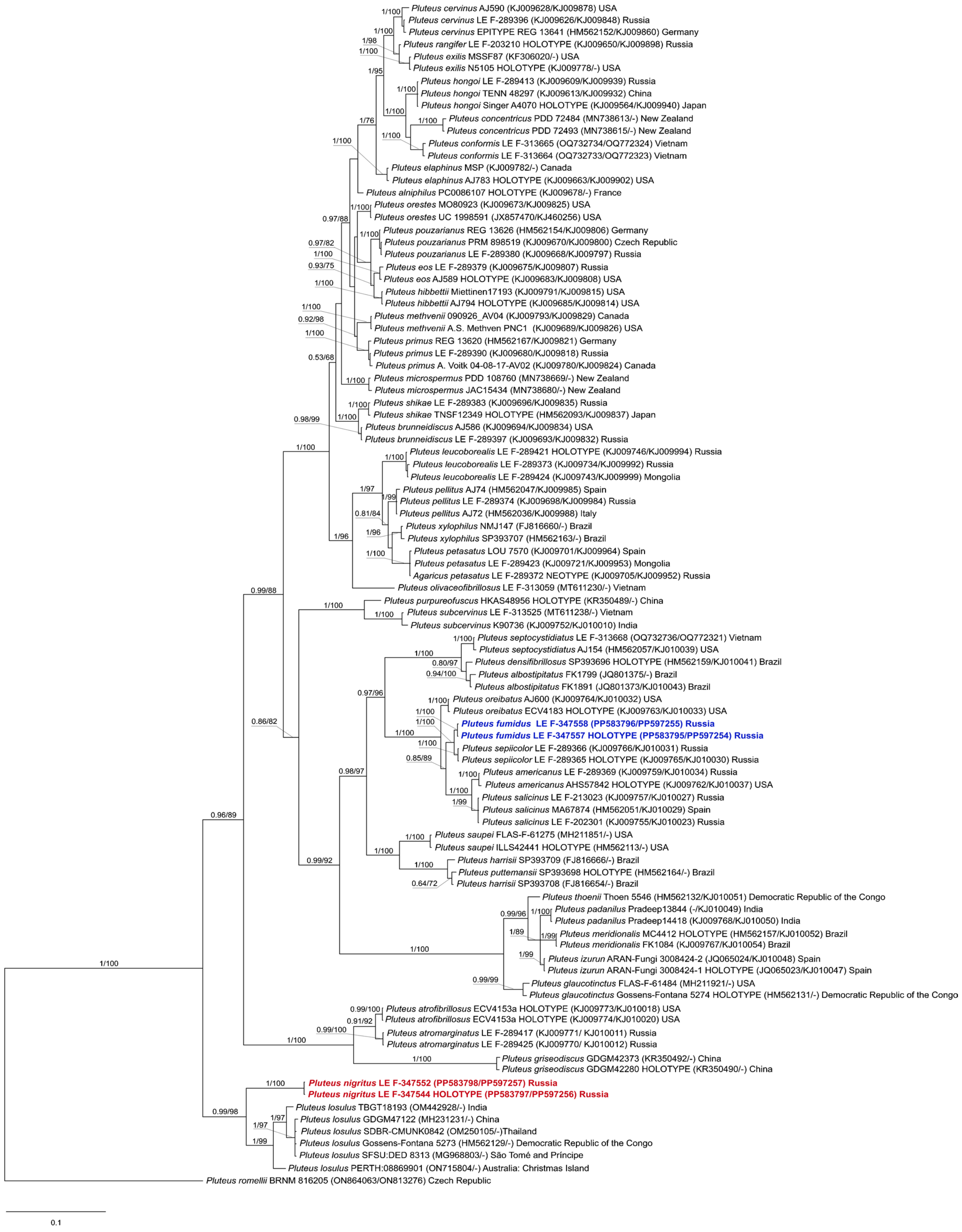
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Pluteus exilis MSSF87 (KF306020/-) USA
Pluteus exilis N5105 HOLOTYPE (KJ009778/-) USA
Pluteus hongoi LE F-289413 (KJ009609/KJ009939) Russia
Pluteus hongoi TENN 48297 (KJ009613/KJ009932) China
Pluteus hongoi Singer A4070 HOLOTYPE (KJ009564/KJ009940) Japan
Pluteus concentricus PDD 72484 (MN738613/-) New Zealand
Pluteus concentricus PDD 72493 (MN738615/-) New Zealand
Pluteus conformis LE F-313665 (OQ732734/OQ772324) Vietnam
Pluteus conformis LE F-313664 (OQ732733/OQ772323) Vietnam
Pluteus elaphinus MSP (KJ009782/-) Canada
Pluteus elaphinus AJ783 HOLOTYPE (KJ009663/KJ009902) USA
Pluteus alniphilus PC0086107 HOLOTYPE (KJ009678/-) France
Pluteus orestes MO80923 (KJ009673/KJ009825) USA
Pluteus orestes UC 1998591 (JX857470/KJ460256) USA
Pluteus pouzarianus REG 13626 (HM562154/KJ009806) Germany
Pluteus pouzarianus PRM 898519 (KJ009670/KJ009800) Czech Republic
Pluteus pouzarianus LE F-289380 (KJ009668/KJ009797) Russia
Pluteus eos LE F-289379 (KJ009675/KJ009807) Russia
Pluteus eos AJ589 HOLOTYPE (KJ009683/KJ009808) USA
Pluteus hibbettii Miettinen17193 (KJ009791/KJ009815) USA
Pluteus hibbettii AJ794 HOLOTYPE (KJ009685/KJ009814) USA
Pluteus methvenii 090926_AV04 (KJ009793/KJ009829) Canada
Pluteus methvenii A.S. Methven PNC1 (KJ009689/KJ009826) USA
Pluteus primus REG 13620 (HM562167/KJ009821) Germany
Pluteus primus LE F-289390 (KJ009680/KJ009818) Russia
Pluteus primus A. Voitk 04-08-17-AV02 (KJ009780/KJ009824) Canada
Pluteus microspermus PDD 108760 (MN738669/-) New Zealand
Pluteus microspermus JAC15434 (MN738680/-) New Zealand
Pluteus shikae LE F-289383 (KJ009696/KJ009835) Russia
Pluteus shikae TNSF12349 HOLOTYPE (HM562093/KJ009837) Japan
Pluteus brunneidiscus AJ586 (KJ009694/KJ009834) USA
Pluteus brunneidiscus LE F-289397 (KJ009693/KJ009832) Russia
Pluteus leucoborealis LE F-289421 HOLOTYPE (KJ009746/KJ009994) Russia
Pluteus leucoborealis LE F-289373 (KJ009734/KJ009992) Russia
Pluteus leucoborealis LE F-289424 (KJ009743/KJ009999) Mongolia
Pluteus pellitus AJ74 (HM562047/KJ009985) Spain
Pluteus pellitus LE F-289374 (KJ009698/KJ009984) Russia
Pluteus pellitus AJ72 (HM562036/KJ009988) Italy
Pluteus xylophilus NMJ147 (FJ816660/-) Brazil
Pluteus xylophilus SP393707 (HM562163/-) Brazil
Pluteus petasatus LOU 7570 (KJ009701/KJ009964) Spain
Pluteus petasatus LE F-289423 (KJ009721/KJ009953) Mongolia
Agaricus petasatus LE F-289372 NEOTYPE (KJ009705/KJ009952) Russia
Pluteus olivaceofibrillosus LE F-313059 (MT611230/-) Vietnam
Pluteus purpureofuscus HKAS48956 HOLOTYPE (KR350489/-) China
Pluteus subcervinus LE F-313525 (MT611238/-) Vietnam
Pluteus subcervinus K90736 (KJ009752/KJ010010) India
Pluteus septocystidiatus LE F-313668 (OQ732736/OQ772321) Vietnam
Pluteus septocystidiatus AJ154 (HM562057/KJ010039) USA
Pluteus densifibrillosus SP393696 HOLOTYPE (HM562159/KJ010041) Brazil
Pluteus albostipitatus FK1799 (JQ801375/-) Brazil
Pluteus albostipitatus FK1891 (JQ801373/KJ010043) Brazil
Pluteus oreibatus AJ600 (KJ009764/KJ010032) USA
Pluteus oreibatus ECV4183 HOLOTYPE (KJ009763/KJ010033) USA
Pluteus fumidus LE F-347558 (PP583796/PP597255) Russia
Pluteus fumidus LE F-347557 HOLOTYPE (PP583795/PP597254) Russia
Pluteus sepiicolor LE F-289366 (KJ009766/KJ010031) Russia
Pluteus sepiicolor LE F-289365 HOLOTYPE (KJ009765/KJ010030) Russia
Pluteus americanus LE F-289369 (KJ009759/KJ010034) Russia
Pluteus americanus AHS57842 HOLOTYPE (KJ009762/KJ010037) USA
Pluteus salicinus LE F-213023 (KJ009757/KJ010027) Russia
Pluteus salicinus MA67874 (HM562051/KJ010029) Spain
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Pluteus saupei FLAS-F-61275 (MH211851/-) USA
Pluteus saupei ILLS42441 HOLOTYPE (HM562113/-) USA
Pluteus harrisii SP393709 (FJ816666/-) Brazil
Pluteus puttemansii SP393698 HOLOTYPE (HM562164/-) Brazil
Pluteus harrisii SP393708 (FJ816654/-) Brazil
Pluteus thoenii Thoen 5546 (HM562132/KJ010051) Democratic Republic of the Congo
Pluteus padanilus Pradeep13844 (-/KJ010049) India
Pluteus padanilus Pradeep14418 (KJ009768/KJ010050) India
Pluteus meridionalis MC4412 HOLOTYPE (HM562157/KJ010052) Brazil
Pluteus meridionalis FK1084 (KJ009767/KJ010054) Brazil
Pluteus izurun ARAN-Fungi 3008424-2 (JQ065024/KJ010048) Spain
Pluteus izurun ARAN-Fungi 3008424-1 HOLOTYPE (JQ065023/KJ010047) Spain
Pluteus glaucotinctus FLAS-F-61484 (MH211921/-) USA
Pluteus glaucotinctus Gossens-Fontana 5274 HOLOTYPE (HM562131/-) Democratic Republic of the Congo
Pluteus atrofibrillosus ECV4153a HOLOTYPE (KJ009773/KJ010018) USA
Pluteus atrofibrillosus ECV4153a HOLOTYPE (KJ009774/KJ010020) USA
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Pluteus nigritus LE F-347552 (PP583798/PP597257) Russia
Pluteus nigritus LE F-347544 HOLOTYPE (PP583797/PP597256) Russia
Pluteus losulus TBGT18193 (OM442928/-) India
Pluteus losulus GDGM47122 (MH231231/-) China
Pluteus losulus SDBR-CMUNK0842 (OM250105/-)Thailand
Pluteus losulus Gossens-Fontana 5273 (HM562129/-) Democratic Republic of the Congo
Pluteus losulus SFSU:DED 8313 (MG968803/-) São Tomé and Príncipe
Pluteus losulus PERTH:08869901 (ON715804/-) Australia: Christmas Island
Pluteus romellii BRNM 816205 (ON864063/ON813276) Czech Republic
0.1

◂**Fig. 1** The phylogenetic relationships of *Pluteus* species in sect. *Pluteus* using Bayesian Inference and Maximum Likelihood methods based on a two-locus dataset (nrITS and *TEF1*). Posterior probability and bootstrap support values (PP/BS) are indicated on the branches. All sequences are labeled with taxon name, voucher/isolate number, and GenBank accession numbers (nrITS/*TEF1*). Sequences newly generated in this study are shown in colours

Materials and methods

Sampling sites

The specimens of both species were collected in the Far Eastern Federal District of Russia, Primorye Territory. Anisimovka village is situated in the very south of the Primorsky Territory, whereas Sikhote-Alin State Nature Biosphere Reserve is located far north. In terms of geography, both sites belong to the Sikhote-Alin mountain region. The whole area is occupied by Manchurian broadleaved and mixed forests.

The more southern territory, where *P. fumidus* was found, is close to Vladivostok and possesses the richest flora comprising about 50 woody species, representing a rather unusual combination of northern taiga elements with subtropical jungle. But in this area the prevailing mixed forests are dominated by Korean pine (*Pinus koraiensis* Siebold & Zucc.) with a wide range of broadleaved (*Quercus mongolica* Fisch. ex Turcz., *Betula costata* Trautv., *Acer mandshuricum* Maxim., *A. mono* Maxim., *Ulmus japonica* Sarg., *Fraxinus mandshurica* Rupr., *Phellodendron amurense* Rupr., *Juglans mandshurica* Maxim., *Tilia amurensis* Rupr., *T. mandshurica* Rupr., *T. taquetii* Schneid., *Carpinus cordata* Blume, etc.) species as well as coniferous trees (*Abies holophylla* Maxim., *A. nephrolepis* (Trautv.) Maxim., *Picea ajanensis* Fisch. ex Carriére, etc.).

The Sikhote-Alin State Nature Biosphere Reserve, where *P. nigritus* was collected, is located in the central part of the watershed of the Sikhote-Alin mountain range with an average elevation of 800 m. It is characterized by an impoverished woody plant community and pronounced altitudinal vegetation zones. There are maritime oak forests (*Q. mongolica*), mixed forests of Korean pine with several broadleaved (*Q. mongolica*, *T. amurensis*, *B. costata*, and *Ulmus laciniata* Mayr) or coniferous trees (*A. nephrolepis* and *P. ajanensis*), mountainous spruce-fir forests (*P. ajanensis*, *A. nephrolepis*, *Betula lanata* (Regel) V.N. Vassil.), and subalpine forests with *Betula ermanii* Cham. in this territory.

Morphological examination

All descriptions of macroscopic morphological features were based on both fresh specimens and photos taken in the field. The RAL Design System plus D2 (RAL D2 Colour fan, edition 2018, Germany) was used for the basidioma colour description. The collections were examined using standard microscopic techniques (Clémençon 2009). Microscopic structures were observed in dried material mounted in 5% KOH and Congo Red using an Axio Imager.A1 light microscope (CarlZeiss, Germany) equipped with differential interference contrast optics (DIC). For statistical evaluation of basidiospore dimensions, a minimum of 40 randomly selected basidiospores were measured for each collection; [80, 2, 2] indicates measurements based on 80 basidiospores from 2 basidiomata in 2 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 values of length and width of the total basidiospores measured respectively. 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 following primers were used for amplification and sequencing: ITS1F-ITS4B (White et al. 1990; Gardes and Bruns 1993) for the ITS1-5.8S-ITS2 (nuclear ribosomal internal transcribed spacer) fragment; EF1-983F and EF1-1567R for approximately 500 bp of *TEF1* (translation-elongation factor 1-alpha) (Rehner and 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 were 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, 4 new ITS sequences and 4 *TEF1* sequences were generated. In addition, 91 ITS sequences and 63 *TEF1* sequences of taxa from *Pluteus* sect. *Pluteus* and *P. romellii* (as an outgroup) were retrieved from the GenBank database (www.ncbi.nlm.nih.gov/genbank/) for molecular analyses. The taxonomic identities of these sequences and collection information for taxa are given in the phylogenetic tree (Fig. 1). The sequences of both genetic markers were aligned using MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/index.html; Katoh et al. 2019) with the FFT-NS-i option and improved where necessary using MEGA11. Alignment was deposited in TreeBASE: S31599 (ITS + *TEF1* dataset).

Phylogenetic reconstructions were performed with Maximum Likelihood (ML) and Bayesian Inference (BI) analyses for combined ITS + *TEF1* dataset. Before the analyses, the best-fit substitution models were estimated separately for both alignments using FindModel web server (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) under the Akaike information criterion. The GTR + G model was chosen for the nrITS dataset and K80 + G for *TEF1*. Hence, a partitioned evolution model for concatenated ITS + *TEF1* dataset in ML and Bayesian analyses to reconstruct a robust phylogenetic hypothesis was used.

Maximum likelihood analysis was run on IQ-Tree web server (http://iqtree.cibiv.univie.ac.at/; Trifinopoulos et al. 2016) 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 5 million generations under described models 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). The result where the ESS (Effective Sample Size) was above 200 and the PSRF (Potential Scale Reduction Factor) was close to 1 was accepted. 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 and Bull 1993; Alfaro et al. 2003). Tree topologies were then edited and visualized in iTOL (Letunic and Bork 2019). Pairwise distances between the nrITS sequences were calculated using p-distance methods and the maximum composite likelihood model in the MEGA 11 program. Newly generated sequences were deposited in GenBank with corresponding accession numbers.

Results and discussion

Phylogeny

The combined dataset of ITS + *TEF1* sequences for members of sect. *Pluteus* contained 1256 characters, including gaps (ITS: 1–678 and *TEF1*: 679–1256). Both Bayesian and Maximum likelihood analyses produced the same topology. Therefore, only the Bayesian tree with both PP and BS values is presented (Fig. 1). The output BI tree resulting from the phylogenetic analyses encompassed a total of 95 specimens belonging to sect. *Pluteus* and 1 specimen from sect. *Celluloderma* as an outgroup (*P. romellii* (Britzelm.) Lapl., BRNM 816205). Most of *Pluteus* species included in the analyses form strongly supported clades, in agreement with the earlier studies (Justo et al. 2011a, b, 2014; Menolli et al. 2014; Xu et al. 2015; Malysheva et al. 2016).

According to the phylogenetic analysis, *P. nigritus* formed independent and highly supported (PP = 1.0 and BS = 100%) monophyletic clade, which is sister to *P. losulus* Justo. The sequences of *P. fumidus* form their own branch with the highest support value (PP = 1.0 and BS = 100%), which is close to *P. sepiicolor* E.F. Malysheva, and both species together form a sister clade to a group of *P. americanus* (P. Banerjee & Sundb.) Justo, E.F. Malysheva & Minnis and *P. salicinus* (Pers.) P. Kumm. specimens.

Based on this phylogenetic result and morphological substantiation, two new species of *Pluteus* are described herein.

Taxonomy

Pluteus nigritus E.F. Malysheva, **sp. nov.** (Figs. 2a, b and 3).

MycoBank: MB 853433.

Diagnosis: Differs from *Pluteus atromarginatus* by smaller basidiospores, shorter pleuro- and cheilocystidia and terminal elements of pileipellis, and by distinct nrITS and *TEF1* sequences.

Holotype: Russia, Primorye Territory, Sikhote-Alin State Nature Biosphere Reserve, vicinity of Yasnyi field reserve station, 45°14′14.3″ N 136°30′37.7″ E, valley coniferous-broadleaved forest, on fallen trunk of *Abies*, 18 Aug. 2012, col. V. Malysheva (LE F-347544). GenBank ITS: PP583797; *TEF1*: PP597256.

Etymology: The specific epithet “nigritus” (blackness) emphasizes the dark colouration in all parts of the basidioma, including the lamellae.

Pileus 70−80 mm in diameter, plano-convex or applanate with subtle broad umbo; not hygrophanous, not striate, with even margins; tropical wood brown (RAL 050 30 20) with darker night brown (RAL 050 20 16) or granite brown (RAL 050 20 10) centre; surface dry, radially fibrillose and faintly squamulose at centre. Lamellae free, crowded, with lamellulae, slightly ventricose, pinkish grey, with brown edges. Stipe 75−80 × 9.5−10.5 mm, slightly broadened towards base, with distinct bulb up to 20 mm wide, solid, sometimes kinked, longitudinally fibrillose with coffee bean brown (RAL 060 40 20) or moor oak grey (RAL 060 40 10) fibrils. Smell absent, taste not recorded.

Basidiospores [80/2/2] 5.7−7.6 × 4.3−5.3 µm ($L_{av} = 6.5$, $W_{av} = 4.7$, $Q = (1.2)1.3−1.5(1.6)$; $Q_{av} = 1.4$), ellipsoid to broadly ellipsoid, some ovoid or oblong, thick-walled. Basidia 24−33 × 6.8−8.8 µm, 4-spored, clavate. Pleurocystidia metuloid, 53.3−84.0 × (14)16−21 µm, numerous, fusiform to broadly fusiform, some utriform, usually pedunculated, sometimes bifurcated at apex, with 3−5 entire,

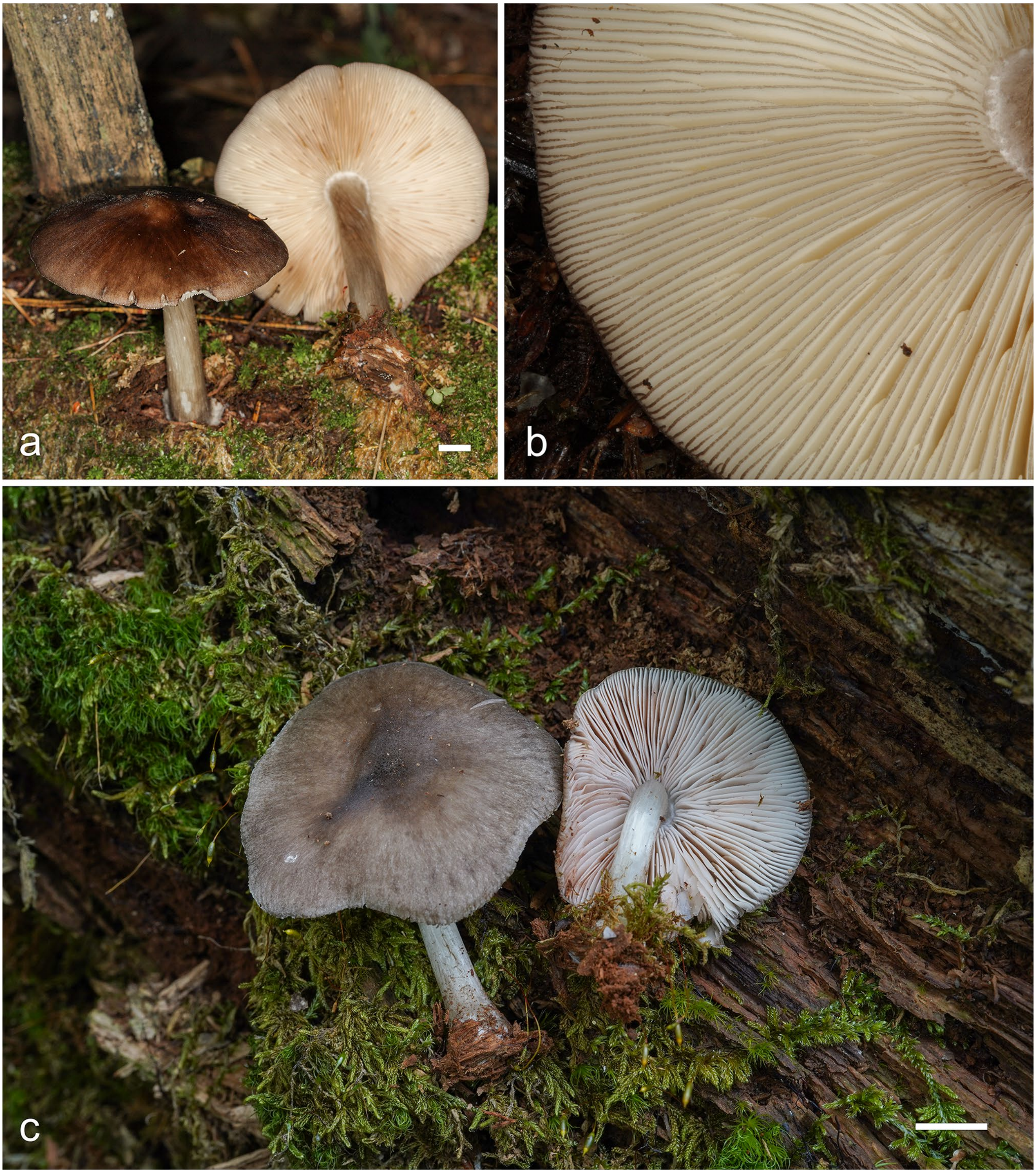

Fig. 2 Basidiomata in situ. **a**, **b** *Pluteus nigritus* (LE F-347544) (photos by E. Malysheva). **c** *Pluteus fumidus* (LE F-347557) (photo by O. Morozova). Scale bars 10 mm

short and acimunate, apical hooks, hyaline, slightly thick-walled. Intermediate cystidia similar to the pleurocystidia in shape and size, but with smaller, often indistinct 2–3 apical hooks. Cheilocystidia (35)42–54(61) × 11.5–17.5(22.3) µm, abundant, forming a sterile layer at the edge of lamellae, broadly clavate or rarely utriform, with intracellular brown pigment, thin-walled. Pileipellis a cutis, with cylindrical or narrowly fusiform, somewhat ascending terminal elements,

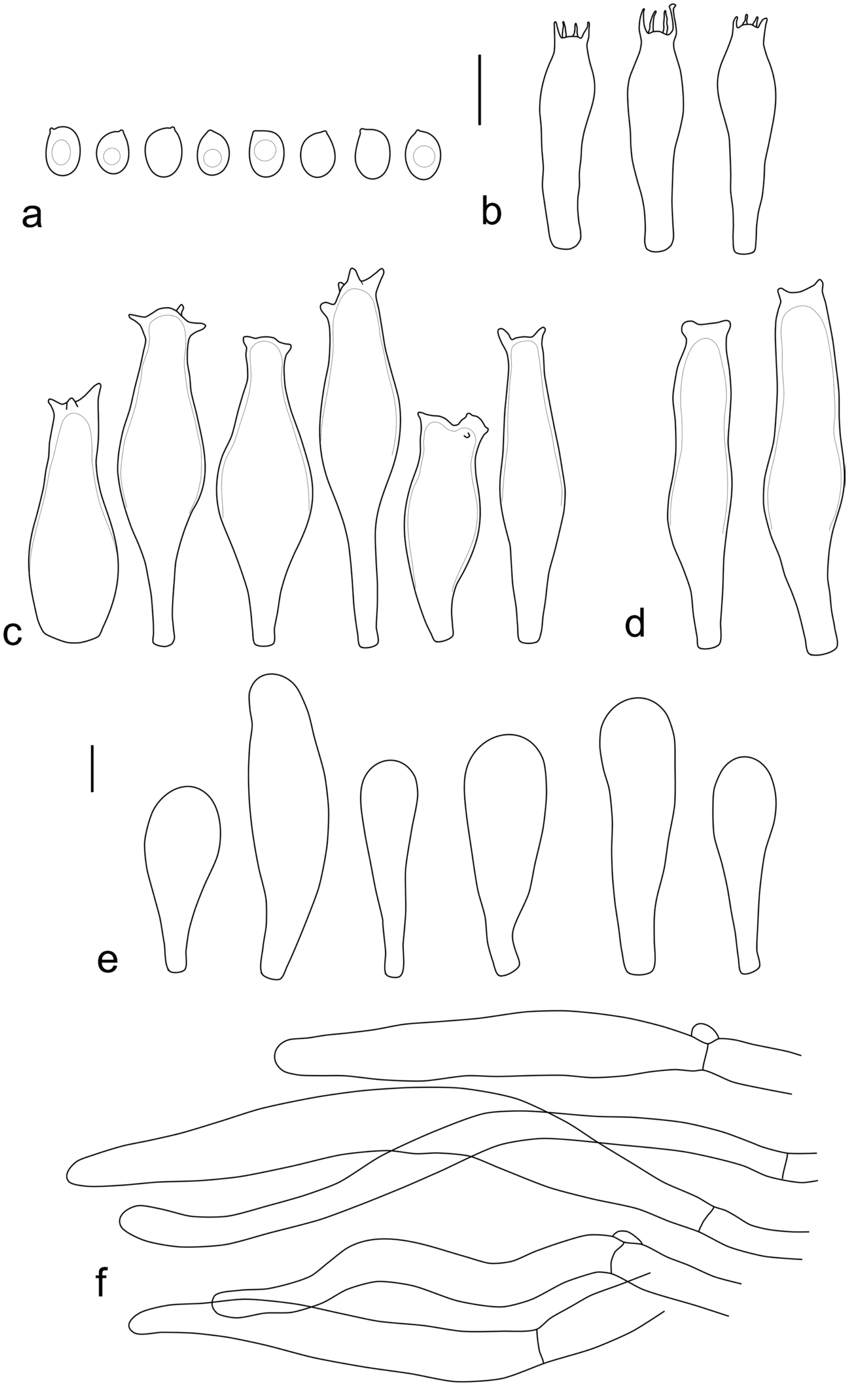

Fig. 3 Microscopic features of *Pluteus nigritus* (LE F-347544, holotype). **a** Basidiospores. **b** Basidia. **c** Pleurocystidia. **d** Intermediate cystidia. **e** Cheilocystidia. **f** Terminal elements of pileipellis. Scale bars: 10 µm (**a**, **b**) and 10 µm (**c**–**f**)

93−125(160) × (8.5)12.0−14.5 µm; with brown intracellular pigment, thin- or slightly thick-walled. Stipitipellis a cutis of cylindrical, slightly thick-walled, 9−13 µm wide hyphae, with yellow–brown intracellular pigment. Caulocystidia absent. Clamp-connections present in pileipellis but not at every septum.

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

Additional collection examined: Russia, Primorye Territory, Sikhote-Alin State Nature Biosphere Reserve, vicinity of Yasnyi field reserve station, 45°14′07.4″ N 136°30′39.8″ E, coniferous-broadleaved forest, on rotten trunk of coniferous tree, 24 Aug. 2013, col. E. Malysheva (LE F-347552). GenBank ITS: PP583798; *TEF1*: PP597257.

Notes: *Pluteus nigritus* is characterized by rather large basidiomata with dark-coloured blackish brown pileus and greyish brown bulbose stipe, pigmented lamellae edges, metuloid pleurocystidia, presence of clamps in pileipellis, and growing on conifer wood.

The most macroscopically similar species, *P. atromarginatus* (Singer) Kühner, also having large dark-coloured basidiomata, pigmented lamellae edges, clamp-connections and similar ecology, strongly differs in microscopic characters, such as larger basidiospores, (5.5)6.0–8.5(9.0) × 4.0–6.0(7.0) µm, larger pleuro- and cheilocystidia, and longer pileipellis terminal elements (Justo et al. 2014).

Microscopically *P. nigritus* is almost indistinguishable from *P. hibbettii* Justo, E.F. Malysheva & Bulyonk., but the latter has smaller basidiomata and non-coloured lamellae edges (Justo et al. 2014).

The presence of clamp-connections sets it apart from those members of *cervinus* clade that can also have dark-coloured basidiomata and/or pigmented lamellae edges. *P. rangifer* Justo, E.F. Malysheva & Bulyonkova differs in concolorous lamellae edges and growth on well-decayed wood of angiosperms. *Pluteus brunneoolivaceus* Horak, only known from Argentina (Horak 1964), is macroscopically distinguishable from *P. nigritus* in the markedly squamulose pileus, significantly smaller basidiomata, and olivaceous stipe. Two New Zealand species, *P. microspermus* Horak and *P. concentricus* Horak, also having pigmented lamellar edges, may resemble *P. nigritus* but both of them have smaller basidiospores (less than 6.5 µm long) and grow in association with *Nothofagus* wood (Horak 2008).

Pluteus nigritus also shares similar basidioma morphology and ecology with *P. pouzarianus* Singer and *P. primus* Bonnard. However, the latter two species have concolorous lamellae edges, the geographical distribution of *P. pouzarianus* does not extend to the Russia Far East, and *P. primus* is characterized by larger basidiospores, 7.0–9.5(10.0) × (4.5)5.0–7.0 µm, and long narrowly clavate cheilocystidia (Justo et al. 2014).

Three other morphologically similar species can be found in the same area, in the Primorye Territory of the Russian Far East: *P. shikae* Justo & E.F. Malysheva, *P. eos* Justo & E.F. Malysheva and *P. sepiicolor* E.F. Malysheva. However, the basidiomata of *P. shikae* are smaller and lighter in colour, whose lamellae edges are non-coloured, and they grow on angiosperm wood. Both species, *P. eos* and *P. sepiicolor*, differ in larger basidiospores and larger pleurocystidia with commonly bifid hooks (Justo et al. 2014).

According to Singer's description (Singer 1959), *P. fibulatus* Singer distributed in South America (Singer 1959; Menolli and Capelari 2013) can resemble our new species *P. nigritus* in terms of large basidioma, deep-coloured pileus, metuloid pleurocystidia of similar size, clavate cheilocystidia, and the presence of clamp-connections in pileipellis. However, *P. fibulatus* has concolorous lamellae edges, larger (7.5−8.0 × 6.6 µm according to Singer 1959 and (5.6)6.2−7.5 (8.7) × (5.0)5.6−6.8(7.5) µm following Menolli and Capelari 2013) globose or subglobose basidiospores, and growing on wood buried in the soil. *Pluteus nigropallescens* Singer, only known from Venezuela (Singer 1961), can also have dark colours in the pileus but it differs from *P. nigritus* in the terrestrial habitat. *Pluteus velutinornatus*, having pigmented lamellar edges, is distinguishable from *P. nigritus* by its pleurocystidia with bifid hooks and a more markedly fibrillose-squamulose pileus, usually wrinkled at centre, and it is only known from New Zealand (Stevenson 1962; Horak 2008).

Pluteus nigritus comes very close to *P. losulus* Justo in the molecular analyses (Fig. 1), but the morphological differences between the two species are significant. *Pluteus losulus*, originally described from Democratic Republic of Congo (Justo et al. 2011a, b), is currently distributed in subtropical and tropical forests of Republic of São Tomé and Príncipe (Desjardin and Perry 2018), India (Keerthi and Pradeep 2022), and China (Hosen et al. 2018). It differs from *P. nigritus* in lighter-coloured stipe, non-pigmented lamellae edges, larger (6.5−8.5 × 5.5−8.0 µm) globose or subglobose basidiospores, cheilocystidia of variable shapes, and the presence of caulocystidia.

Pluteus fumidus E.F. Malysheva, **sp. nov.** (Figs. 2c and 4).

MycoBank: MB 853434.

Diagnosis: Differs from *Pluteus sepiicolor* by grey tint in basidioma colouration, non-hygrophanous and non-striate pileus, smaller basidiospores, and pleurocystidia with predominantly entire hooks.

Holotype: Russia, Primorye Territory, Anisimovka village, vicinity of mounting skiing resort Gribanovka, road to Cape Mihalych, 43°06′52.8″ N 132°47′20.3″ E, by the stream, mixed forest, on rotten trunks of coniferous trees, 1 Sept. 2021, col. O. Morozova (LE F-347557). GenBank ITS: PP583795; *TEF1*: PP597254.

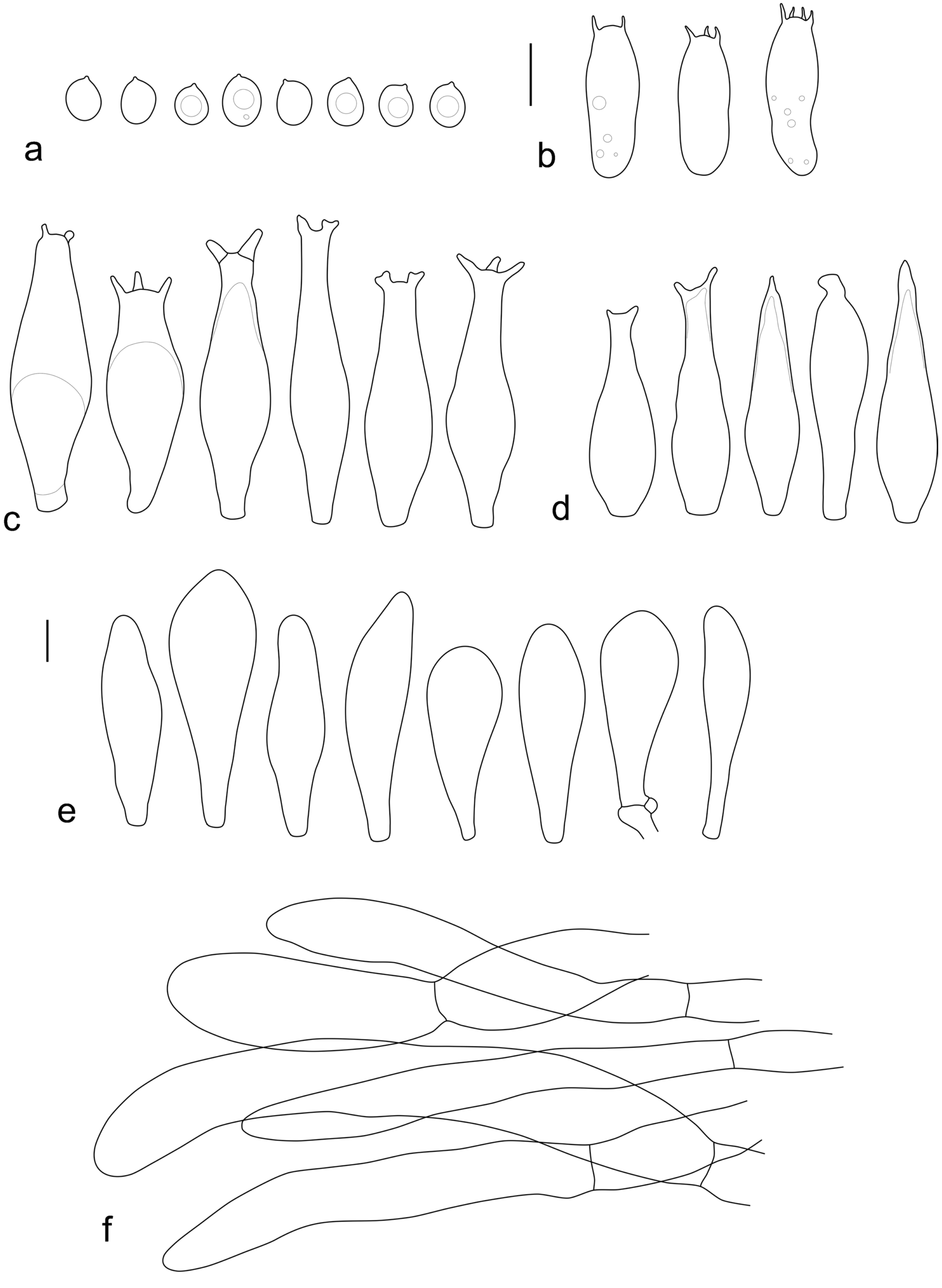

Fig. 4 Microscopic features of *Pluteus fumidus* (LE F-347557, holotype). **a** Basidiospores. **b** Basidia. **c** Pleurocystidia. **d** Intermediate cystidia. **e** Cheilocystidia. **f** Terminal elements of pileipellis. Scale bars: 10 µm (**a**, **b**) and 10 µm (**c**–**f**)

Etymology: The specific epithet "fumidus" reflects the greyish-brown colouring of the basidiomata.

Pileus 30−40 mm in diameter, initially hemispherical or broadly campanulate, then plano-convex or applanate with low broad umbo or with central depression; not hygrophanous, not striate; sandstone grey (RAL 060 50 10), mushroom brown (RAL 060 50 20), with darker centre – moor oak grey (RAL 060 40 10) to nutria fur brown (RAL 060 30 10) or industrial black (RAL 060 20 05); surface dry, radially fibrillose, minutely squamulose at only centre and slightly serrulate at margin. Lamellae free, crowded, with lamellulae, slightly ventricose, at first whitish then pale pinkish, with concolorous even edges. Stipe 35−60 × 3−7 mm, slightly broadened towards base but without distinct bulb, solid, silver thistle beige (RAL 070 85 10) or almost white, saruk grey (RAL 070 50 10) at base, longitudinally fibrillose. Smell indistinct, taste not recorded.

Basidiospores [80/2/2] 5.8−7.5(8.5) × 4.7−5.7(6.6) µm ($L_{av} = 6.8$, $W_{av} = 5.3$, $Q = 1.2−1.4(1.5)$; $Q_{av} = 1.3$), broadly ellipsoid, sometimes ovoid, thick-walled. Basidia 20−25 × 6.7−8.5 µm, 2- and 4-spored, clavate. Pleurocystidia metuloid, (47.5)56−66.5(73.5) × 12.5−21.0 µm, numerous, narrowly to broadly fusiform, with 3−5, predominantly entire, but rarely bifid, acuminate or rounded apical hooks, 3.5−8.5 × 1.5−2.8 µm, hyaline, thick-walled. Intermediate cystidia similar to the pleurocystidia in shape, but slightly smaller, 48.0−63.5 × 12.5−16.5 µm, often with 1−2 apical hooks. Cheilocystidia (42.2)45.5−56.5(59.5) × 9.5−21.5 µm, abundant, forming a sterile layer at the edge of lamellae, narrowly to broadly clavate or utriform, hyaline, thin-walled, clamped at base. Pileipellis a cutis, with cylindrical, narrowly fusiform or narrowly clavate, somewhat ascending terminal elements, 158−175 × 10−21 µm; with brown intracellular pigment, thin- or slightly thick-walled. Stipitipellis a cutis of cylindrical, slightly thick-walled, 5−8 µm wide hyphae, hyaline or with yellow–brown intracellular pigment. Caulocystidia absent. Clamp-connections numerous in all tissues.

Habitat and distribution: Solitary or in small groups, on rotten wood of conifers. Currently, only known in the Primorye Territory, Russia.

Additional collection examined: Russia, Primorye Territory, vicinity of Anisimovka village, 43°09′02.3″ N 132°47′11.0″ E, mixed forest, on very rotten wood of coniferous tree, 3 Sept. 2021, col. O. Morozova (LE F-347558). GenBank ITS: PP583796; *TEF1*: PP597255.

Notes: *Pluteus fumidus* is introduced here based on two collections, both found in the southern part of Primorye Territory in mountainous mixed forest. The species is characterized by medium-sized basidiomata with greyish brown pileus and silvery-white stipe with greyish base, concolorous lamellae edges, metuloid pleurocystidia, abundant clamp-connections in all tissues, and growing on conifer wood.

Based on a combination of macromorphological features such as greyish brown pileus and grey-coloured stipe base, this newly described species can be compared with taxa from *salicinus* clade. Significantly larger basidiospores, 7.0–10.0(12.0) × (4.5)5.0–7.5(8.0) µm (Justo et al. 2014), longer pleuro- and cheilocystidia, as well as growing on wood of deciduous trees, distinguish *P. fumidus* from *P. salicinus* (Pers.: Fr.) Kummer.

Pluteus americanus (P. Banerjee & Sundb.) Justo, E.F. Malysheva & Minnis also has larger basidiospores compared to *P. fumidus*, hygrophanous pileus, often more prominent blue-green tinges on stipe, and it dwells on decayed hardwoods.

In terms of phylogeny, *P. fumidus* is closely related to *P. sepiicolor* E.F. Malysheva, and both species cluster together in a single clade with strong statistical support (Fig. 1). These two species are distributed in the same area and are associated with coniferous wood, but there are sufficient morphological differences between them. *Pluteus sepiicolor* differs in dark brown, hygrophanous and striate pileus without greyish tint, stipe with blackish brown fibrils, larger basidiospores, 7.8–8.6 (9.0) × 5.0–6.2(6.5) µm, and pleurocystidia with predominantly bifid hooks.

Another phylogenetically close species is *P. oreibatus* Justo, whose nrITS sequences show the highest similarity to *P. fumidus* according to the BLAST search algorithm. The difference between *P. fumidus* and *P. oreibatus* is that the latter has basidiospores a little wider (7.0)7.5–9.0 × 5.0–7.0 µm, pleurocystidia with undeveloped hooks and cylindrical or lageniform cheilocystidia (Justo et al. 2014). The ITS sequence divergence between *P. oreibatus* and *P. fumidus* clades is 2.5%.

Two tropical and subtropical taxa belonging to the *salicinus* clade and somewhat similar to *P. fumidus*, *P. albostipitatus* (Dennis) Singer, Lloydia, and *P. harrisii* Murrill differ in striate or sulcate pileus, pleurocystidia with undeveloped hooks, and the absence of clamp-connections (Menolli et al. 2010, 2015; Justo et al. 2011a; Desjardin and Perry 2018).

Conclusions

Obviously, the study region of the Russian Far East remains underestimated in terms of fungal species diversity. Recent survey articles on the genus *Pluteus* have demonstrated the richness of the genus in the area (Justo et al. 2014; Ševčíková et al. 2022a, b, 2023). The present work makes an additional contribution to the study of the group. Based on data presented above, two new species within sect. *Pluteus*, *P. nigritus* and *P. fumidus*, are described, and their morphology and phylogenetic relationships are discussed in detail. Macroscopically, *P. nigritus* differs from all similar species by the combination of a large basidioma with blackish brown pileus and a dark lamellae edge, while *P. fumidus* is remarkable for its greyish brown pileus and silvery-white stipe with greyish base. Microscopically, the spore size, pleurocystidia shape, and the pileipellis structure can serve as reliable criteria for species delimitation.

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Author contribution All the authors contributed to the study conception and design. Material preparation and data collection and analysis were performed by Ekaterina Malysheva and Vera Malysheva. The first draft of the manuscript was written by Ekaterina Malysheva and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

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Data Availability The sequences generated during the current study are available in the GenBank repository.

Declarations

Competing Interests The authors declare no competing interests.

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