

Pluteus ochroviridans sp. nov., belonging to *Pluteus* sect. *Celluloderma* (Pluteaceae) from Mediterranean habitats in Spain and California (USA)

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Abstract

Pluteus ochroviridans sp. nov. is described based on collections made in Spain and California (USA). This species is characterized by a strongly hygrophanous, brown, gray-brown to yellow-brown pileus that develops green tones, and by its terrestrial habitat in Mediterranean ecosystems. Phylogenetic analyses of ITS and *TEF1- α* sequences separate this species from closely related taxa: *P. californicus*, *P. olivaceus*, and *P. phaeocyanopus*. We obtained sequences from the type collections of these three closely related taxa, thus establishing DNA reference data for the modern taxonomic concepts of these species. A full description, illustrations, and phylogenetic analysis results of the new species are provided.

Key words: Agaricales, Basidiomycota, ITS, nanopore sequencing, phylogeny, taxonomy, *TEF1- α*

Introduction

The genus *Pluteus* Fries (1836: 338) (Basidiomycota, Agaricales, Pluteaceae) is characterized by free lamellae, pink spore print, smooth basidiospores mostly ranging from globose to ellipsoid, an inverse hymenophoral trama, and present hymenial cystidia, at least as cheilocystidia (Vellinga & Schreurs 1985, Singer 1986, Vellinga 1990). Within the genus, *Pluteus* section *Celluloderma* (Fayod 1889: 364) is characterized by the non-metuloid cystidia and a pileipellis organized in most species as a hymeniderm or epithelium, although exceptions in pileipellis organization do occur (Justo *et al.* 2010a, b, Polhorský *et al.* 2023).

The taxonomy of the brown, gray, and olive colored *Pluteus* species in section *Celluloderma* remains largely unresolved. This morphological group includes widely used names such as *P. cinereofuscus* J.E. Lange (1917: 9), *P. cyanopus* Quél. (1883: 391), *P. nanus* (Pers.) P. Kumm. (1871: 98), *P. pallidus* Homola (1972: 1232), *P. phaeocyanopus* Minnis & Sundb (2010: 44), and *P. phlebophorus* (Ditmar) P. Kumm. (1871: 98), but also many validly published names of unclear taxonomic status, including: *P. alachuanus* Murrill (1945: 118), *P. brunneoater* Wichanský (1966: 30), *P. californicus* McClatchie (1897: 384), *P. combustorum* Velen. (1929: 25), *P. homolae* Minnis & Sundb. (2010: 37), *P. griseoluridus* P.D. Orton (1984: 609), *P. griseopus* P.D. Orton (1960: 356), *P. jamaicensis* Murrill (1911: 278), *P. laevis* Velen. (1947: 80), *P. luctuosus* Boud. (1905: 70), *P. ludovicianus* Murrill (1917: 133), *P. mammifer* Romagn.

(1979: 374), *P. olivaceus* P.D. Orton, (1960: 359), *P. pulverulentus* Murrill (1917: 137), *P. pumilus* Murrill (1946: 326), *P. roseocandidus* G.F. Atk. (1909: 373), *P. satur* Kühner & Romagn. (1956: 182), *P. spinosae* Velen. (1940: 144) and *P. suzae* Velen. (1940: 144) (Vellinga 1990, Minnis & Sundberg 2010). The problematic taxonomy of this group, referred here as “*P. nanus* group” for simplicity, is a consequence of the lack of clarity on how to apply older names, the overlapping intra- and interspecific morphological variation, and the limited usefulness of the fungal barcode (nuclear ribosomal internal transcribed spacer region, ITS hereafter) to separate closely related taxa in many species-complexes. Previous molecular studies (Justo *et al.* 2011b, Menolli *et al.* 2015) have placed species morphologically assignable to the “*P. nanus* group” in three distinct phylogenetic lineages within *Pluteus* sect. *Celluloderma*: /cinereofuscus-nanus clade, /phlebophorus clade and /dietrichii clade.

Continuing our detailed taxonomic studies of the Holarctic species of *Pluteus* sect. *Celluloderma* (Ševčíková *et al.* 2022a, b, 2023), we have generated complementary ITS and *TEF1-α* (translation elongation factor 1-alpha) datasets for the “*P. nanus* group”, currently comprising 816 ITS and 260 *TEF1-α* sequences. Preliminary analyses of these datasets indicate that the “*P. nanus* group” is extremely species-rich across the Holarctic region, with 82 species currently recognized in molecular phylogenies and virtually no overlap in species composition between Eurasia and North America (Keerthi *et al.* 2024; Justo *et al.* unpublished data). Separate monographic studies are in preparation for each continent, but the present paper focuses on a newly described species that constitutes one of the few exceptions to this pattern, as it has been confirmed to occur in Spain and California (USA).

Here we describe a new species, *Pluteus ochroviridans*, a visually striking species closely related to other members of the *P. nanus* group with distinct green or blue basidiomes, such as *P. californicus*, *P. olivaceus*, and *P. phaeocyanopus*. Our molecular phylogenies also include newly generated ITS sequences from the type collections of these three species, which match several modern collections made as part of our ongoing monographic studies in *Pluteus*.

Material and methods

Fungal collections

Collections were made during regular mycological field work conducted in Spain and the USA. Photographs and field notes were taken in situ (Rathnayaka *et al.* 2025) for the majority of collections studied here. Collections were then dried at 40°C. Color codes are from Munsell Soil Color Charts (Munsell Color 2009). The dried collections were then studied using standard procedures for morphological examination of agaricoid fungi (Largent *et al.* 1977). Microscopic preparations were mounted in H₂O, 3% KOH, and/or 1% Congo Red. Microscopic observations were made with the following microscopes: Motic BA300, Motic BA310, and Olympus BH-2, with magnifications of 400× and 1000×. Descriptive terms for morphological characters follow Vellinga (1988). The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for the quotient of length and width, and avQ for the average quotient. The notation [x, y, z] indicates that measurements were made on x basidiospores from y samples in z collections. The average values for basidiospore length (avl), width (avw), and Q (avQ) were calculated for each collection examined and reported in the description as the full range of average values across all collections. Institutional Herbaria acronyms follow Thiers (2025), with the additional acronyms or abbreviations being used to refer to individual collections: AJ (Alfredo Justo), FS (Francisco Sánchez), GM (Guillermo Muñoz), HRL (Renée Lebeuf), iNat (iNaturalist observation), Kelkar (Saumitra Kelkar), LB (Luis Ballester), MO (Mushroom Observer observation), and SGS (Santi Gibert).

Molecular data

Protocols for DNA extraction, PCR, and sequencing are as outlined in Justo & Hibbett (2011) and Alvarado *et al.* (2012). PCR amplification and sequencing of the ITS region were performed using primers ITS1F/ITS4 (White *et al.* 1990, Gardes & Bruns 1993), and *TEF1-α* was amplified with EF1-983F/EF1-1567R primers (Rehner & Buckley, 2005). Raw sequence data were edited and assembled in Chromas Pro 2.1.9 (Technelysium, Australia).

Sequences for the type collection of *Pluteus olivaceus* were generated using Illumina sequencing, with the protocols detailed in Ševčíková *et al.* (2023).

The original collections of *P. californicus* and many of the iNaturalist collections were sequenced through nanopore sequencing at Mycota (Plymouth, Michigan, USA; mycota.com), following the MinION barcoding workflow designed by S.D. Russell. A small fragment of dried basidiome (<1mm) was extracted with 20 µL of DNA extraction solution in strip microtubes, heated for 10 minutes at 80°C in a thermocycler, and then 50 µL Low TE Solution (Russell 2025) was added. Each specimen was dual-indexed and amplified with one of 10 uniquely tagged forward primers (ITS1F) and one of 96 uniquely tagged reverse primers (ITS4) (Eurofins Genomics), designed for 96-well plates. Sequencing was performed on a MinION Mk1B device (Oxford Nanopore Technologies, Oxford, UK) using Flongle R10.4.1 flowcells (FLO-FLG114) and the Ligation Chemistry Kit V14 (SQK-LSK114) (Russell 2025). Resulting reads were base-called with Dorado (v0.9.1), demultiplexed with Specimux (v0.63, <https://github.com/joshuaowalker/specimux>), and consensus sequences were formed with NGSpeciesID (v0.3.1, Shalin *et al.* 2021).

Phylogenetic analyses

A total of 54 ITS and 37 *TEF1-α* sequences were included in the phylogenetic analyses, based on the preliminary results from the phylogenetic analysis of the “*P. nanus* group”. *Pluteus phlebophorus* and *P. pallidus* were used as outgroup taxa. All sequences are listed in Table 1. Sequences were aligned using MAFFT version 7 (Katoh *et al.* 2019) and the FFT-NS-i strategy. The alignments were inspected and manually corrected in AliView (Larsson 2014). Phylogenetic analyses (detailed below) were conducted on the individual ITS and *TEF1-α* datasets, and no topological conflicts were detected among the resulting phylogenies. A combined ITS+*TEF1-α* dataset was analyzed and served as the basis for the phylogenetic and taxonomic discussion presented here.

TABLE 1. GenBank accession numbers for the sequences used in this study (- refers to the data unavailability).

Taxon	Collection	ITS	<i>TEF1-α</i>
<i>P. californicus</i>	iNat 166510945	PP791272	PX578817
<i>P. californicus</i>	iNat 205954425	PV020856	PX578816
<i>P. californicus</i>	Kelkar01022020	PX646976	PX578812
<i>P. californicus</i>	MO352725	PX646977	PX578813
<i>P. californicus</i>	MO92764	MH613716	-
<i>P. californicus</i>	NY775456	PX634675	-
<i>P. californicus</i>	NY775457 (Lectotype)	PX634676	-
<i>P. californicus</i>	SBBG000095F	PX646978	PX578814
<i>P. californicus</i>	SBBG000229F	PX646979	PX578815
<i>P. ochroviridans</i>	FSI202002	PX646987	PX578819
<i>P. ochroviridans</i>	FSI202004	PX646988	PX578821
<i>P. ochroviridans</i>	GM2746	PX646990	PX578823
<i>P. ochroviridans</i>	GM3932	PX646986	-
<i>P. ochroviridans</i>	GM4236	PX646980	PX578818
<i>P. ochroviridans</i>	GM4254	PX646989	PX578822
<i>P. ochroviridans</i>	GM4363-b	PX646984	-
<i>P. ochroviridans</i>	GM4366	PX646985	-
<i>P. ochroviridans</i>	GM4390	PX646982	-
<i>P. ochroviridans</i>	GM4408	PX646983	-
<i>P. ochroviridans</i>	GM4425 (Holotype)	PX646981	-
<i>P. ochroviridans</i>	HAY-F-009538	PP971289	PX578820
<i>P. olivaceus</i>	AJ229	HM562108	PX578799
<i>P. olivaceus</i>	AJ34	HM562248	OQ332371
<i>P. olivaceus</i>	GM1847	PX646991	PX578800
<i>P. olivaceus</i>	GM2659	PX646992	PX578801
<i>P. olivaceus</i>	GM2695	PX646994	PX578803

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TABLE 1. (Continued)

Taxon	Collection	ITS	TEF1- α
<i>P. olivaceus</i>	GM2696	PX646995	PX578804
<i>P. olivaceus</i>	GM2739	PX646993	PX578802
<i>P. olivaceus</i>	GM2764	PX646998	PX578807
<i>P. olivaceus</i>	GM2794	PX646997	PX578806
<i>P. olivaceus</i>	GM2795	PX646996	PX578805
<i>P. olivaceus</i>	GM4255	PX646999	PX578808
<i>P. olivaceus</i>	K-M000093481 (I)	PX634651	-
<i>P. olivaceus</i>	K-M000093481 (II)	PX634652	-
<i>P. olivaceus</i>	LB23011407	PX647000	PX578809
<i>P. olivaceus</i>	LB23011408	PX647001	PX578810
<i>P. pallidus</i>	HRL427	PX647002	PX578832
<i>P. phaeocyanopus</i>	iNat 191770594	PX634687	PX578824
<i>P. phaeocyanopus</i>	iNat 194340051	PX650095	-
<i>P. phaeocyanopus</i>	iNat 201445247	PX647003	PX578825
<i>P. phaeocyanopus</i>	iNat 203542692	PX634678	-
<i>P. phaeocyanopus</i>	iNat 257438357	PX634677	-
<i>P. phaeocyanopus</i>	MICH69511 (Paratype)	PP938223	-
<i>P. phlebophorus</i>	AJ81	HM562039	ON133554
<i>P. sternbergii</i>	PRM154258 (Epitype)	ON864116	-
<i>Pluteus</i> sp. CN4	GM1764	PX647005	PX578827
<i>Pluteus</i> sp. CN4	GM2163	PX647006	PX578828
<i>Pluteus</i> sp. CN4	GM3587	PX647004	-
<i>Pluteus</i> sp. CN5	AJ216	HM562046	OQ332375
<i>Pluteus</i> sp. CN5	GM1808	PX647007	PX578829
<i>Pluteus</i> sp. CN5	GM3936	PX647009	PX578831
<i>Pluteus</i> sp. CN5	GM537	PX647008	PX578830
<i>Pluteus</i> sp. CN77	iNat 197161170	PX647010	PX578811
<i>Pluteus</i> sp. CN78	SGS20230930-12	PX647011	PX578826

Two different phylogenetic analyses were conducted for all datasets: (i) Maximum Likelihood (ML) analyses using RAxML 8.2.10 (Stamatakis *et al.* 2008, Stamatakis 2014) under a GTRGAMMAI model with 100 rapid bootstrap (BS) replicates; (ii) Bayesian Inference (BI) analyses using MrBayes 3.2.2 (Ronquist *et al.* 2012) for 10 million generations under a GTRGAMMAI model with four chains, and trees sampled every 1000 generations. The initial burn-in phase was set to 2.5 million generations. We examined the graphical representation of the likelihood scores of the sampled trees, and checked that the standard deviation of split frequencies was < 0.05 , and that PRSF values were close to 1, as detailed in Ronquist *et al.* (2011). Both methods confirmed that the burn-in values were appropriate for the dataset. All analyses were performed using resources at the CIPRES Science Gateway (Miller *et al.* 2010).

We compared the ITS and TEF1- α sequences of *P. ochroviridans* with all other species represented in our datasets. This was done by visually inspecting the alignments in AliView. The number of evolutionary changes is defined as the minimum number of events, including indels (multiple-base indels treated as a single change), transitions, and transversions. Only differences shared by all sequences of the same species were counted (missing data for sequences of different lengths were not considered). The term “evolutionary events” refers to all possible evolutionary changes between two sets of sequences.

Results

Sequencing of type collections

We were able to generate full or partial ITS sequences for the lectotype collection of *P. californicus* (NY775457, designated in Minnis & Sundberg 2010), one additional original collection of *P. californicus* (NY775456), the holotype collection of *P. olivaceus* (K-M000093481), and the paratype of *P. phaeocyanopus* (MICH69511).

The *P. californicus* sequences were generated using nanopore sequencing. Both sequences are 673 bp long and span the full ITS1, 5.8S, and ITS2 regions.

The sequences for *P. olivaceus* were both generated from the holotype using Illumina sequencing. Both reads are 293 bp long (ITS2 region only) and differ in one single base pair, in position 209 of the sequence: "G" in the sequence marked as (I) in Fig. 1 and "A" in the sequence marked as (II). This particular position has also been shown to be polymorphic in the other *P. olivaceus* sequences included here, all generated by Sanger sequencing. Collections AJ34, AJ229, GM2794, LB23011407, and LB23011408 have an "A" in this polymorphic position, while the rest of the collections have a "G".

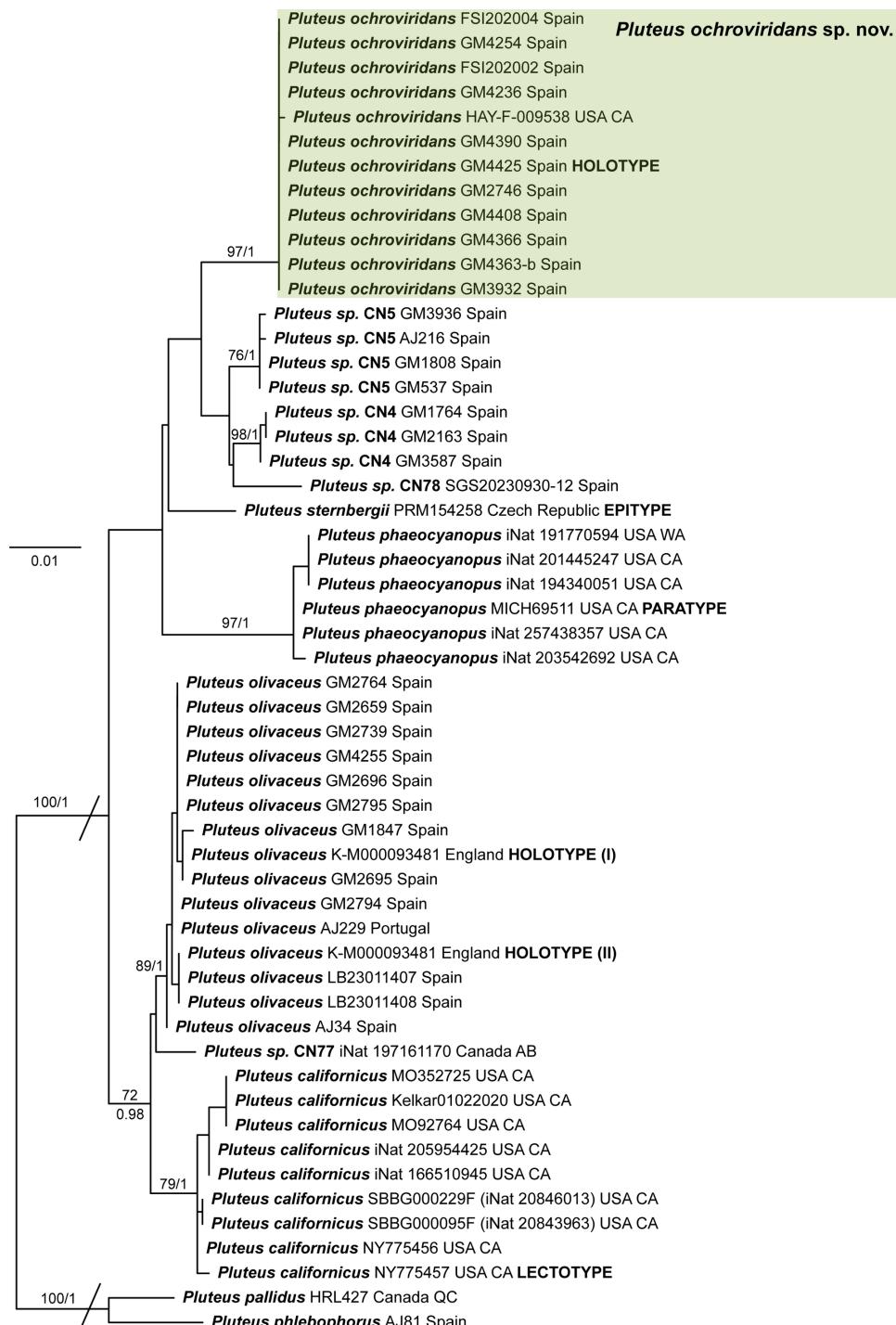


FIGURE 1. Best tree from the ML analysis of the ITS+TEF1-α dataset. Bootstraps (≥ 70) and Posterior Probabilities (≥ 0.90) from the BI analysis are shown on/below the branches. The root length has been shortened to facilitate graphical representation.

The paratype sequence of *P. phaeocyanopus* was generated by Sanger sequencing. The sequence is 656 bp long and spans the full ITS1, 5.8S, and ITS2 regions.

Phylogenetic analyses

The ITS+ *TEF1- α* dataset consisted of 54 concatenated sequences totaling 1,278 nucleotide positions. The best tree from the ML analyses of this dataset is presented in Figure 1, with support values from the ML and BI analyses.

The 12 collections of *P. ochroviridans* appear as a well-supported clade, separate from all other species included in the analysis. The grouping of *P. californicus*, *P. olivaceus*, and *Pluteus* sp. CN77 is moderately supported (72/0.98). The rest of the tree's internal topology received little support, and only the terminal species-level nodes were moderately or well-supported in the analyses. *Pluteus californicus*, *P. olivaceus*, and *P. phaeocyanopus* are recovered as monophyletic and distinct from one another. Two currently unnamed species from Spain, represented by multiple collections (*Pluteus* sp. CN4 and *Pluteus* sp. CN5), are also recovered as monophyletic. Two additional taxa (*P. sternbergii* and *Pluteus* sp. CN78) are currently represented by a single collection each.

Taxonomy

Pluteus ochroviridans Justo, G. Muñoz, F. Sánchez, Arrillaga, Plá & Ševčíková sp. nov.

Figures 2–4

Mycobank: 861534

Etymology: A combination of the Latin terms *ochros* (brown, yellow-brown) and *viridans* (turning green), for the overall colors and color changes of the pileus.

Diagnosis:—Closely related and morphologically similar to *Pluteus californicus* and *Pluteus olivaceus*, differing in the more markedly hygrophanous pileus, with mixed gray-brown, yellow-brown, and olivaceous tinges, and different ITS (7 and 9 evolutionary events respectively) and *TEF1- α* (36 and 32 evolutionary events respectively) sequences.

Typification:—SPAIN: La Rioja, Calahorra, 42.269348°N, 2.009818°W, in an olive plantation, among herbaceous remains and compost, on clay soil or small half-buried twigs, in a Mediterranean environment, 1 December 2024, G. Muñoz GM4425 (AH61716, Holotype).

Description:—*Pileus* 10–43 mm in diameter, hemispherical or campanulate when young, expanding to convex, plano-convex, often with a low, broad central umbo; surface often rugose to markedly rugose, especially around center; when young with very dark brown or gray-brown colors (5YR 3/2–3/4; 10YR 3/2–3/6, 4/3–4/6), in mature specimens brown, yellow-brown or gray-brown, often developing distinct olive tinges (7.5YR 4/2–4/6; 10YR 5/3–5/8, 2.5Y 4/3–4/6), generally with a rather matte aspect, strongly hygrophanous, on drying with much paler colors (7.5YR 7/4–4/6, 10YR 6/3–6/6, 7/3–7/4; 10YR 5/3–5/8, 2.5Y 7/4–4/6, 8/2–8/4), sometimes almost white; margin translucently striate. *Lamellae* crowded, free, ventricose, up to 6 mm broad, white when young, often with olive, or olive-gray tinges before turning pink, with white, flocculose edges. *Stipe* 20–50 × 1–6 mm, cylindrical, with slightly broadened base; surface gray, brown-gray or olive-gray (2.5Y 7/1–7/2, 5Y 7/1–7/4), often with a shiny or translucent aspect, with longitudinal white fibrils. *Context* in stipe and pileus white or pale grey, olive-grey under the surface. *Smell* and *taste* indistinct.

Basidiospores [232, 14, 12] (5.4–)6.2–8.1(–8.6) × (5.1–)5.4–7.2 μ m, avl × avw = 6.5–7.6 × 5.8–6.7, Q = 1.00–1.34 (–1.40), avQ = 1.10–1.19, globose to ellipsoid, some ovoid. **Basidia** 12–28 × 6.5–8.5 μ m, 4-spored, clavate. **Pleurocystidia** 39–94 × 14–32 μ m, most often (narrowly) utriform, clavate or ovoid, some obovoid; most hyaline, some with light brown intracellular pigment, thin-walled; common all over lamellar faces. **Lamellar edge** sterile. **Cheilocystidia** 26–88 × 10–25 μ m, polymorphic, narrowly utriform or lageniform, some ovoid or (narrowly) clavate, rarely mucronate or subcapitate; most hyaline, some with light brown intracellular pigment, thin-walled, crowded, covering the lamella edge completely. **Pileipellis** an epithelioid hymeniderm or a transition between hymeniderm and epithelium, with individual elements 34–76 × 26–50 μ m, spheropedunculate, globose or broadly clavate, rarely with a shortly mucronate apex, very rarely fusiform; most elements with evenly dissolved brown intracellular pigment, slightly thick-walled. **Stipitipellis** a cutis of cylindrical, slightly thick-walled hyphae, 7–15 μ m wide, tightly packed together; hyaline, or some with pale brown intracellular pigment. **Caulocystidia** (not always present) (50–)60–90(–105) × (8–)10–18 (–23) μ m, cylindrical, narrowly fusiform; hyaline; scattered. **Clamp-connections** absent in all parts examined.



FIGURE 2. *Pluteus ochroviridans*. A–B. Basidiomes. A (GM4425, holotype); B. (FSI202002). Photos by G. Muñoz (A), F. Sánchez (B). Bar = 1cm.

Habit, habitat, and phenology:—Gregarious, growing terrestrially, often directly on soil, or among accumulating leaves, more rarely directly on decaying plant stems (e.g. *Caroxylon vermiculatum*). In Mediterranean forests,

xerophytic Mediterranean scrubland, and gardens. Mostly October–December, two collections were made in February and March.

Distribution:—Spain and the USA (California).



FIGURE 3. *Pluteus ochroviridans*. A–E. Basidiomes. A (GM4390); B. (GM4326); C (GM4254); D (GM4366); E (FSI202004). Photos by G. Muñoz (A, B, D); F. Sánchez (E); Felipe Plá (C). Bar 1cm.

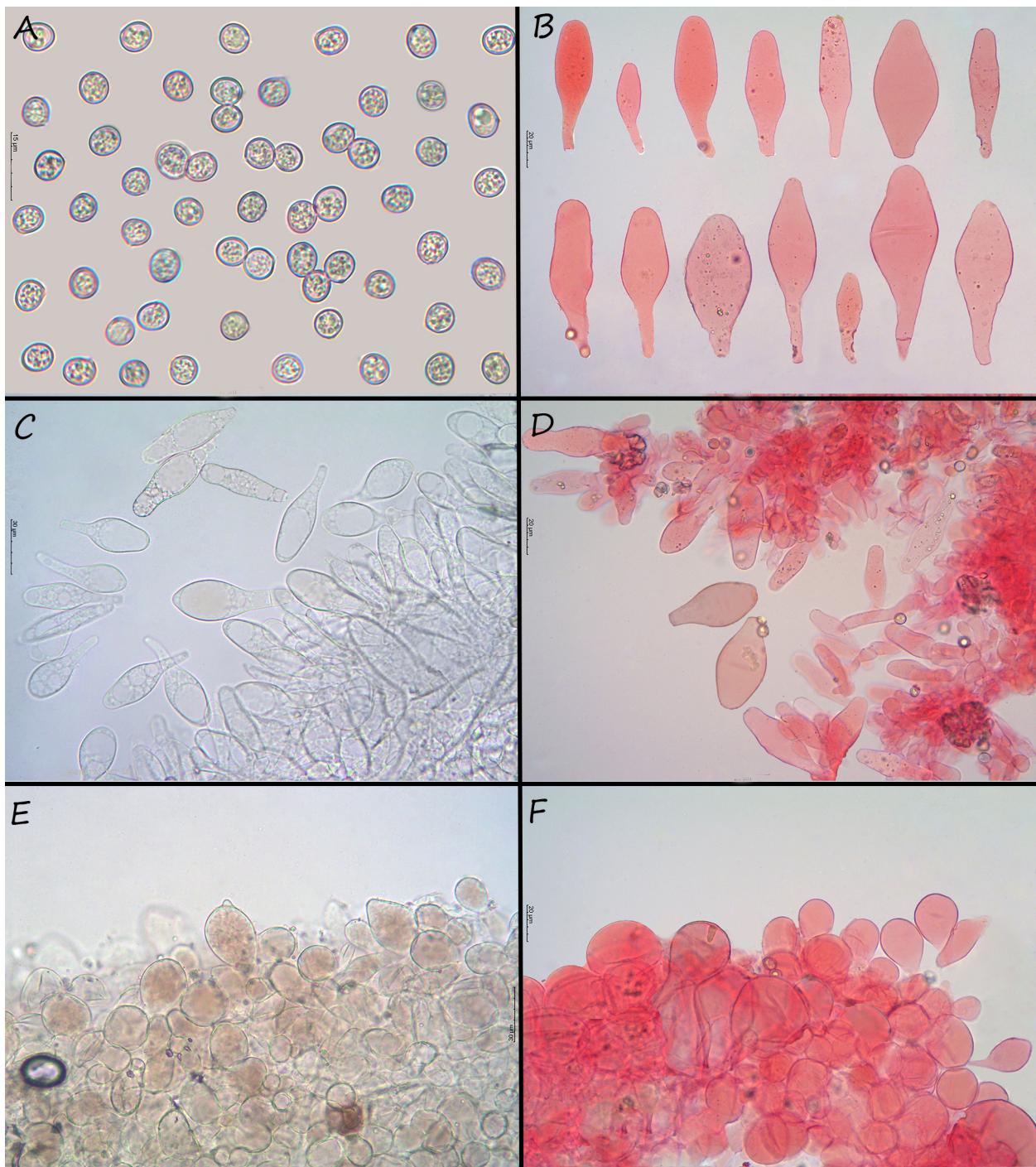


FIGURE 4. *Pluteus ochroviridans*. A. Basidiospores in water; B. Pleurocystidia in Congo Red; C. Cheilocystidia in water; D. Cheilocystidia in Congo Red; E. Pileipellis in water; F. Pileipellis in Congo Red. Bar: (A) = 15 μ m; (B, C, D, F) = 20 μ m; (E) = 30 μ m. Photos by G. Muñoz.

Additional collections examined. SPAIN. Badajoz: Badajoz (city), 38.8685787°N, 6.9355012°W, in an urban garden under *Melia azedarach* and *Arbutus unedo*, growing directly on the ground, 17 November 2023, F. Plá, GM4254 (AH61717). La Rioja: Calahorra, 42.269348°N, 2.009818°W, in an olive plantation, among herbaceous and compost remains, on clay soil or small half-buried twigs, in a Mediterranean environment, 17 November 2012, G. Muñoz, GM2746 (AH61718); *ibid.*, 27 March 2022, G. Muñoz, GM3932 (AH61719); *ibid.*, 12 November 2023, G. Muñoz, GM4236 (AH61720); *ibid.*, 1 November 2024, G. Muñoz, GM4408 (AH61721). Sevilla: Sevilla (city), Parque Jardines del Guadaira, 37.363496°N, 5.946951°W, under *Tamarix* sp., on soil, with abundant plant material, 14 November 2020, F. Sánchez, FS1202002 (ARAN-Fungi 22820); *ibid.*, 37.361954°N, 5.949888°W, 25 December 2020, FS202004. Zaragoza: Gallur, 41.8830858°N, 1.3476015°W, in a clearing of a *Pinus halepensis* grove, on clay

soil in a xerophytic Mediterranean environment, with *Caroxylon vermiculatum*, *Atriplex halimus*, *Thymus vulgaris* and other xerophytic plants, 16 October 2024, G. Muñoz, GM4363-b (AH61722); *ibid.*, 16 October 2024, G. Muñoz, GM4366 (AH61723); Mallén, 41.8911634°N, 1.3633259°W, in a clearing of a *Pinus halepensis* grove, on clay soil in a xerophytic Mediterranean environment, growing on soil or on dry woody stems of *Caroxylon vermiculatum*, 21 October 2024, G. Muñoz, GM4390 (AH61724). UNITED STATES OF AMERICA. California: Los Angeles Co., Long Beach, El Dorado Nature Center, 33.94743537°N, 118.084201°W, growing beneath *Salix* near a creek, 26 February 2024, B. Vardeh, iNaturalist 201268494, (HAY-F-009538).

Observations:—*Pluteus ochroviridans* is characterized by the combination of an overall brown to yellow-brown pileus in mature basidiomes, which develop distinct olivaceous tinges; the strongly hygrophanous pileus; the terrestrial habit in Mediterranean environments; and basidiospores with $avL \times avW = 6.5-7.6 \times 5.8-6.7$ and $avQ = 1.10-1.19$.

Pluteus olivaceus is another European species with distinct green colors in the pileus (Orton 1960). Some have considered this species to be synonymous with *P. cinereofuscus* (Vellinga & Schreurs 1985, Vellinga 1990), but our current research indicates that both taxa are best kept separate. We obtained two almost identical ITS2 sequences from the holotype of *Pluteus olivaceus*, which match several modern collections from Spain. *Pluteus cinereofuscus* lacks a type collection suitable for sequencing, but the clade we have identified as the best candidate to retain this name is not closely related to the species discussed here. Some of the collections included in the initial phylogenies of *Pluteus* (Justo *et al.* 2011a, b) originally identified as *P. cinereofuscus* (AJ34, AJ229), as well as many additional collections from Spain, are considered here to represent *P. olivaceus*. Morphologically, *P. olivaceus* differs from *P. ochroviridans* in the more uniformly colored pileus, usually some shade of olive, olive-gray, or bluish-gray, which is also less markedly hygrophanous, and the larger basidiospores with higher avQ values ($avL \times avW = 7.9-8.3 \times 5.8-6.3$, $avQ = 1.29-1.37$). Molecularly, the ITS sequences of both species differ in 9 evolutionary events, and their *TEF1- α* sequences in 32 evolutionary events.

Pluteus californicus was originally described from Pasadena (California), and one of the characters highlighted in its original description was the green color of the pileus (McClatchie 1897). The species was subsequently mentioned by Murrill in his catalogue of North American *Pluteus* (1917), and by Minnis & Sundberg (2010) in their monograph of *Pluteus* sect. *Celluloderma*. By 2025 (more than 125 years after its description), the two original collections made by McClatchie, deposited at NY, were still the only known collections representing *P. californicus*. We have generated ITS sequences from the two collections at NY, including the lectotype. Sequences from the lectotype match several modern collections that have been sequenced by us, or as part of broader community-science efforts, such as the CA FunDIS project (<https://www.funidis.org/ca-fundis>). Morphologically, the mature basidiomes of *P. californicus* differ from *P. ochroviridans* in the more uniformly colored, less-markedly hygrophanous, and olive to brown-olive pileus. Molecularly, the ITS sequences of both species differ in 7 evolutionary events, and their *TEF1- α* sequences in 36 evolutionary events.

Pluteus phaeocyanopus was described from California by Minnis & Sundberg (2010) based on two herbarium collections by Alexander Smith. We sequenced the paratype of this species deposited at MICH, and it matches several modern collections from California and Washington (Fig. 1). *Pluteus phaeocyanopus* differs from *P. ochroviridans* in the rather uniformly brown-colored pileus, which lacks olivaceous discoloration, and it is not as markedly hygrophanous, and the stipe that often has distinct blue or blue-gray colors. *Pluteus phaeocyanopus* often fruits on wood of *Quercus* and *Notholithocarpus densiflorus* (Minnis & Sundberg 2010, Siegel & Schwarz 2016). This apparent preference for Fagaceae wood also separates this species from *P. ochroviridans*, which fruits more frequently terrestrially or among decaying plant material, not associated with wood remnants. Molecularly, the ITS sequences of both species differ in 14 evolutionary events, and their *TEF1- α* sequences in 32 evolutionary events.

Being able to sequence >125-year-old collections using nanopore technology represents a crucial step in bringing older, validly published names back into modern use. The nanopore-generated sequences from the original collections of *P. californicus* (NY775457 and NY775456) match modern collections of the same species generated either with nanopore (iNat 166510945 and iNat 205954425) or Sanger sequencing (Kelkar01022020, MO352725, MO92764, SBBG000229F, and SBBG000095F). Similarly, the Sanger-generated sequence of the paratype of *P. phaeocyanopus* (MICH69511) matches modern sequences of the same species generated with nanopore (iNat 191770594, iNat 194340051, iNat 257438357, and iNat 203542692), and Sanger sequencing (iNat 201445247).

Full modern descriptions of *P. californicus*, *P. olivaceus*, and *P. phaeocyanopus* will be included in the upcoming monographs of the Holarctic species of the “*P. nanus* group”. All the older available names mentioned in the Introduction were carefully considered, but none of these species are a good morphological and/or molecular match for the species here described as *P. ochroviridans*.

Acknowledgements

We gratefully acknowledge the curators and collections managers at California State University East Bay (HAY-F), University of Michigan (MICH), The New York Botanical Garden (NY), Royal Botanic Gardens, Kew (K), and Santa Barbara Botanic Garden (SBBG) for managing the loans of their respective collections, including invaluable type and original material, and authorizing their use for DNA sequencing. Relevant collections included in the phylogenetic analysis were made by Luis Ballester, Michael Beug, Evan Casey, Chris Dietel, Kris Fleming, Santi Gibert, Jacob Kalichman, Saumitra Kelkar, Renée Lebeuf, Dora Panayotova, Stu Pickell, Alan Rockefeller, Taylor Routledge, Adam Searcy, Douglas Smith, Christian Schwarz, and Bat Vardeh. We thank Ida Broman Nielsen and Tobias Guldberg Frøslev (Globe Institute, University of Copenhagen) for sequencing the type collection of *Pluteus olivaceus*.

The research of H. Ševčíková received institutional support for the long-term conceptual development of research institutions, provided by the Ministry of Culture (Moravian Museum, ref. MK000094862).

References

Alvarado, P., Moreno, G. & Manjón, J.L. (2012) Comparison between *Tuber gennadii* and *T. oligospermum* lineages reveals the existence of the new species *T. cistophilum* (Tuberaceae, Pezizales). *Mycologia* 104 (4): 894–910.
<https://doi.org/10.3852/11-254>

Atkinson, G.F. (1909) Preliminary notes on some new species of Agaricaceae and *Clavaria*. *Annales Mycologici* 7 (4): 365–376.

Boudier, J.L.É. (1905) Note sur quatre nouvelles espèces de Champignons de France. *Bulletin de la Société Mycologique de France* 21 (2): 69–73.

Fayod, V. (1889) Prodrome d'une histoire naturelle des Agaricinés. *Annales des Sciences Naturelles Botanique Séries* 7, 9: 181–411.

Fries, E. (1836) *Corpus Florarum provincialium suecicae I. Floram Scanicam*. Upsaliae, Typis Palmblad, Sebell & Co, 394 pp.
<https://doi.org/10.5962/bhl.title.47083>

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
<https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>

Homola, R.L. (1972) Section *Celluloderma* of the genus *Pluteus* in North America. *Mycologia* 66 (6): 1211–1247.
<https://doi.org/10.1080/00275514.1972.12019374>

Justo, A. & Hibbett, D.S. (2011) Phylogenetic classification of *Trametes* (Basidiomycota, Polyporales) based on a five-marker dataset. *Taxon* 60: 1567–1583.
<https://doi.org/10.1002/tax.606003>

Justo, A., Minnis, A.M., Ghignone, S., Menolli, N. Jr., Capelari, M., Rodríguez, O., Malyshева, E., Contu, M. & Vizzini, A. (2011b) Species recognition in *Pluteus* and *Volvoplateus* (Pluteaceae, Agaricales): morphology, geography and phylogeny. *Mycological Progress* 10: 453–479.
<https://doi.org/10.1007/s11557-010-0716-z>

Justo, A., Vizzini, A., Minnis, A.M., Menolli, N. Jr., Capelari, M., Rodríguez, O., Malysheva, E., Contu, M. & Hibbett, D.S. (2011a) Phylogeny of Pluteaceae (Agaricales, Basidiomycota): taxonomy and character evolution. *Fungal Biology* 115: 1–20.
<https://doi.org/10.1016/j.funbio.2010.09.012>

Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20: 1160–1166.
<https://doi.org/10.1093/bib/bbx108>

Keerthi, V., Justo, A. & Pradeep, C.K. (2024) Two new species of *Pluteus* (Pluteaceae) from India based on morphology and ITS-based phylogeny. *Phytotaxa* 670 (3): 161–74.
<https://doi.org/10.11646/phytotaxa.670.3.2>

Kühner, R. & Romagnesi, H. (1956) Espèces nouvelles, critiques ou rares de Volvariacées. *Bulletin trimestriel de la Société mycologique de France* 72: 181–249.

Kummer, P. (1871) *Der Führer in die Pilzkunde*. C. Luppe, Zerbst, 146 pp.

Lange, J.E. (1917) Studies in the Agarics of Denmark. Part III. *Pluteus, Collybia, Inocybe*. *Dansk botanisk Arkiv* 2 (7): 1–50.

Largent, D.L., Johnson, D. & Watling, R. (1977) *How to identify mushrooms to genus III: microscopic features*. 3rd ed. Mad River Press Inc., Eureka, California, USA, 148 pp.

Larsson, A. (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30: 3276–3278.

https://doi.org/10.1093/bioinformatics/btu531

McClatchie, A.J. (1897) Seedless plants of southern California. *Proceedings of the Southern California Academy of Sciences* 1: 338–395.

Menolli, N., Justo, A. & Capelari, M. (2015) Phylogeny of *Pluteus* section *Celluloderma* including eight new species from Brazil. *Mycologia* 107 (6): 1205–1220.
<https://doi.org/10.3852/14-312>

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. In: *2010 Gateway Computing Environments Workshop (GCE)*. IEEE, pp. 1–8.
<https://doi.org/10.1109/GCE.2010.5676129>

Minnis, A.M., Sundberg, W.J. (2010) *Pluteus* section *Celluloderma* in the USA. *North American Fungi* 5 (1): 1–107.
<https://doi.org/10.2509/naf2010.005.001>

Munsell, C. (Ed.) (2009) *Munsell Soil Color Charts*. Grand Rapids, Michigan (USA).

Murrill, W.A. (1945) [1944] New Florida Fungi. *Proceedings of the Florida Academy of Sciences* 7 (2–3): 107–127. [<http://www.jstor.org/stable/24313148>]

Murrill, W.A. (1911) The Agaricaceae of tropical North America IV. *Mycologia* 3 (6): 271–282.
<https://doi.org/10.1080/00275514.1911.12017682>

Murrill, W.A. (1917) Agaricaceae subtribe Pluteanae. *North American Flora* 10 (2): 77–144.
<https://doi.org/10.1080/00275514.1918.12016739>

Murrill, W.A. (1946) New and interesting Florida fungi. *Lloydia* 9: 315–330.

Orton, P.D. (1960) New checklist of British Agarics and Boleti, part III (keys to *Crepidotus*, *Deconica*, *Flocculina*, *Hygrophorus*, *Naucoria*, *Pluteus* and *Volvaria*). *Transactions of the British Mycological Society* 43 (2): 159–439.
[https://doi.org/10.1016/S0007-1536\(60\)80065-4](https://doi.org/10.1016/S0007-1536(60)80065-4)

Orton, P.D. (1984) Notes on British Agarics VIII. *Notes from the Royal Botanic Garden Edinburgh* 41 (3): 565–624.
<https://doi.org/10.24823/nrbge.1984.3358>

Polhorský, A., Justo, A., Szabóová, D., Konyarikova, Z., Dima, B. & Ševčíková, H. (2023) *Pluteus flammans*, a new brightly coloured species in *Pluteus* sect. *Celluloderma* (Pluteaceae) from Central Europe. *Phytotaxa* 598 (1): 69–79.
<https://doi.org/10.11646/phytotaxa.598.1.5>

Quélet, L. (1883) [1882] Quelques espèces critiques ou nouvelles de la Flore Mycologique France. *Compte Rendu de l'Association Française Pour l'Avancement des Sciences* 11: 387–412.

Rathnayaka, A.R., Tennakoon, D.S., Jones, G.E., Wanasinghe, D.N., Bhat, D.J., Priyashantha, A.H., Stephenson, S.L., Tibpromma, S. & Karunaratna, S.C. (2025) Significance of precise documentation of hosts and geospatial data of fungal collections, with an emphasis on plant-associated fungi. *New Zealand Journal of Botany* 63 (2–3): 462–489.
<https://doi.org/10.1080/0028825X.2024.2381734>

Rehner, S.A. & Buckley, E.P. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98.
<https://doi.org/10.1080/15572536.2006.11832842>

Romagnesi, H. (1979) [1978] Quelques espèces rares ou nouvelles de macromycètes. 7. Agarics rhodosporés (Volvariacées). *Bulletin de la Société Mycologique de France* 94 (4): 371–377.

Ronquist, F., Huelsenbeck, J.P. & Teslenko, M. (2011) MrBayes Version 3.2 Manual: Tutorials and Model Summaries. Available from: http://mrbayes.sourceforge.net/mb3.2_manual.pdf (accessed 15 November 2025)

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology* 61 (3): 539–542.
<https://doi.org/10.1093/sysbio/sys029>

Russell, S.D. (2023) ONT DNA Barcoding Fungal Amplicons 1221 w/ MinION & Flongle V.3 (Version1222 3) [Protocol]. protocols.io [<https://dx.doi.org/10.17504/protocols.io.36wgq7qykvk5/v3>]

Russell, S.D. (2025) Extract-N-Amp Equivalent DNA Extraction Protocol V.3 [Protocol]. protocols.io.
<https://doi.org/10.17504/protocols.io.5jyl8p458g2w/v3>

Ševčíková, H., Ferisin, G., Malysheva, E., Justo, A., Heilmann-Clausen, J., Horak, E., Kalinina, L., Kaygusuz, O., Knudsen, H., Menolli, N. Jr., Moreau, P.-A., Muñoz González, G., Saar, I., Türkekul, İ. & Dovana, F. (2022a) *Pluteus insidiosus* complex, four new species described and *Pluteus reisneri* resurrected. *Journal of Fungi* 8 (6): 623.
<https://doi.org/10.3390/jof8060623>

Ševčíková, H., Malysheva, E., Ferisin, G., Dovana, F., Horak, E., Kalichman, J., Kaygusuz, O., Lebeuf, R., Muñoz González, G., Minnis, A.M., Russell, S.D., Sochor, M., Dima, B., Antonín, V. & Justo, A. (2022b) Holarctic species in the *Pluteus romellii* clade. Five new

species described and old names reassessed. *Journal of Fungi* 8 (8): 773.
<https://doi.org/10.3390/jof8080773>

Ševčíková, H., Malysheva, E.F., Antonín, V., Borovička, J., Dovana, F., Ferisin, G., Eyssartier, G., Grootmyers, D., Heilmann-Clausen, J., Kalichman, J., Kaygusuz, O., Lebeuf, R., Muñoz González, G., Minnis, A.M., Russell, S.D., Saar, I., Nielsen, I.B. & Frøslev, T.G. (2023) Holarctic species in the *Pluteus podospileus* clade: description of six new species and reassessment of old names. *Journal of Fungi* 9 (9): 898.
<https://doi.org/10.3390/jof9090898>

Sahlin, K., Lim, M.C. & Prost, S. (2021) NGSpeciesID: DNA barcode and amplicon consensus generation from long-read sequencing data. *Ecology and Evolution* 11 (3): 1392–1398.
<https://doi.org/10.1002/ece3.7146>

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312–1313.
<https://doi.org/10.1093/bioinformatics/btu033>

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* 75: 758–771.
<https://doi.org/10.1080/10635150802429642>

Thiers, B. (2025) (continuously updated) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. [<http://sweetgum.nybg.org/ih/>]

Velenovský, J. (1947) *Novitates mycologicae novissimae*. Sumptibus Societatis Botanicae Čechoslovacae, 197 pp.

Velenovský, J. (1929) Novinky Z Oddel. bedlovytch cernenovytrusnych. (Agaricineae rhodosporeae aliquot novae). *Mykologia* 6 (2): 25–29.

Velenovský, J. (1940) [1939] *Novitates mycologicae*. L. Souček, Pragae, 211 pp.

Vellinga, E.C. & Schreurs, J. (1985) Notulae ad Floram Agaricinam Neerlandicam – VIII. *Pluteus* Fr. in West-Europe. *Persoonia* 12 (4): 337–373.

Vellinga, E.C. (1988) Glossary. In: Bas, C., Kuyper, Th.W., Noordeloos, M.E. & Vellinga, E.C. (Eds.) *Flora Agaricina Neerlandica*, vol. 1. A.A. Balkema, Rotterdam, pp. 54–64.

Vellinga, E.C. (1990) *Pluteus* Fr. In: Bas, C., Kuyper, Th.W., Noordeloos, M.E. & Vellinga, E.C. (Eds.) *Flora agaricina Neerlandica*, Vol. 2. Balkema, Rotterdam, pp. 31–55.

White, T.J., Bruns, T., Lee, S.S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: A Guide to Methods and Applications*. Academic Press, New York, pp. 315–322.
<https://doi.org/10.1016/B978-0-12-372180-8.50042-1>

Wichanský, E. (1966) Štitovka—*Pluteus brunneoater* Wich. n. sp. *Časopis Československých houbařů* 43: 30.