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Article

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Chroogomphus gracilis (Gomphidiaceae, Boletales), a new species with mycocultural relevance in a Nahua-origin community in Central Mexico, and validation of four names of Boletes

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Abstract

Chroogomphus gracilis sp. nov. is described from Mexico, occurring in pine-oak mixed forests on the slopes of the Popocatépetl volcano. The species is characterized and documented based on both morphological features and molecular data. Phylogenetic analyses were performed using DNA sequences from the nuclear ribosomal internal transcribed spacer (nrITS) region. Comprehensive descriptions and photographic documentation of the new species are provided. *C. gracilis* is consumed by members of the local Nahua community of San Pedro Nexapa, state of Mexico, and commercialized in local markets located in the eastern part of the State of Mexico. Additionally, four bolete species described from Mexico in 2023 are here validated, as their protologues did not initially satisfy the stipulations of Articles F.5.1 and 40.7 of the International Code of Nomenclature for Algae, Fungi, and Plants.

Key words: Boletaceae, ectomycorrhizal fungi, edible species, mycocultural heritage, temperate forests

Introduction

Chroogomphus (Gomphidiaceae, Boletales), as classified by Miller (1964:56), is divided into three subgenera. The subgenus *Chroogomphus*, comprising five sections, is distinguished by a pileipellis composed of compressed, gelatinized hyphae. Its type species is *Chroogomphus rutilus* (Schaeff.) O.K. Mill., and it is primarily distributed across Eurasia and North America. The subgenus *Floccigomphus* (Imai) Niskanen, Scambler, & Liimat. is characterized by a non-gelatinized pileipellis with tomentose to fibrillose innate hyphae. Its type species, *Chroogomphus tomentosus* (Murrill) O.K. Mill., occurs in North America and Asia (Miller 1964, Kiran *et al.* 2020). The third subgenus, *Siccigomphus* (Imai) Niskanen, Scambler, & Liimat., is defined by its broad, non-gelatinized hyphae in the pileipellis. The type species, *Chroogomphus roseolus* Y.C. Li & Zhu L. Yang, has been reported to date from Eurasia and North America (Scambler *et al.*, 2018). Species of *Chroogomphus* are typically found in coniferous and mixed forests, particularly in association with species of *Pinus* L., and other members of the Pinaceae family, including *Picea* A. Dietr. and *Abies* Mill. (Miller 1964; Scambler *et al.* 2018). In Mexico, two species have been described. *Chroogomphus conacytiensis* Ayala-Vásquez, Pérez-Moreno, Ram.-Carb., & González-Martínez, which is distributed in the Trans-Mexican Volcanic Belt and the North mountain range of Oaxaca, according to the biogeographic classification of Morrone (2019). It is mainly associated with *Pinus pseudostrobus* Lindl. and *P. hartwegii* Lindl., at elevations ranging from 2,900 to 3,000

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meters. Meanwhile, *Chroogomphus flavovinaceus* Ayala-Vásquez, Martínez-Reyes, & Pérez-Moreno is currently known only from the boundary of the Mexican Trans-volcanic Belt and the Southern Mother Mountain range. It grows in *Quercus-Pinus* forests at altitudes between 2,900 and 2,989 meters, in association with *Pinus leiophylla* Schltdl. & Cham. (Pérez-Moreno *et al.*, 2023). In this study, a detailed description of a new Mexican species, *Chroogomphus gracilis*, is provided, based on both morphological and molecular data. Additionally, we formally validate four bolete species described from Mexico in 2023, whose initial protologues failed to satisfy the stipulations of Articles F.5.1 and 40.7 of the International Code of Nomenclature for Algae, Fungi, and Plants.

Materials & Methods

Sampling and Morphological Characterization

Specimens for this study were collected during the rainy season, from August to September 2024, in forests located on the slopes of the Popocatépetl Volcano, specifically in the San Pedro Nexapa region of the State of Mexico. The collection site was in a mixed forest comprising *Pinus ayacahuite*, *Cupressus lindleyi*, and *Quercus* spp. Morphological characteristics were described following the guidelines of Rathnayaka *et al.* (2024). Chemical reactions were assessed using KOH. Photographs of the basidiomata were taken *in situ*, and data on the botanical composition of the site were also recorded. The taxonomic descriptions were based on the color standards provided by Kornerup and Wanscher (1978). Microscopic characters were measured using an optical microscope (Leica DM3000, Germany). Measurements were taken for 75 basidiospores, basidia, pleurocystidia, pileipellis, and stipitipellis. The total number of basidiospores measured is denoted as *n/m*, where *n* refers to the total number of spores measured, and *m* refers to the number of basidiomata sampled. *Lm/Wm* refers to the standard deviation of length to width, and *Q* represents the length/width ratio of the basidiospores. The specimens were deposited in the National Herbarium of Mexico (MEXU), located at the Institute of Biology, National Autonomous University of Mexico.

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted using the CTAB method (Martínez-González *et al.* 2017) from 2-3 mg of dry tissue. DNA quantification was performed using a Nanodrop spectrophotometer (Thermo, USA). Two molecular markers were utilized for amplification. The Internal Transcribed Spacer (ITS) region was amplified using the primers ITS5-ITS4 (White *et al.* 1990). The PCR reaction mixture included the following components: 1× enzyme buffer, Taq DNA polymerase, 0.8 mM deoxynucleoside triphosphates (0.2 mM of each), 100 ng DNA, 20 pmol of each primer, and 2 units of GoTaq DNA polymerase (Promega, USA), with a final reaction volume of 15 µL. PCR products were verified by agarose gel electrophoresis, which was run for 1 hour at 95 $V \cdot cm^{-3}$ in 1.5% agarose and 1× TAE buffer (Tris-Acetate-EDTA). The products were stained with GelRed (Biotium, USA) and visualized using a transilluminator (Infinity 3000 Vilber, Loumat, Germany). The PCR products were purified with the ExoSap Kit (Affymetrix, USA) following the manufacturer's instructions and then prepared for sequencing with the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems). Sequencing was performed using a genetic analyzer (Sanger sequencing) by Macrogen Inc. (Texas, USA).

Alignments and Phylogenetic Analyses

The sequences obtained from both ITS primers (ITS5 and ITS4) were assembled using Sequencher 4.1 (Gene Codes Corporation, www.genecodes.com). Consensus sequences were subjected to BLAST analysis using MegaBLAST (Morgulis *et al.* 2008) through the NCBI platform. A molecular matrix was constructed using our sequences and several other ITS sequences, primarily representing the section *Filiformis* and the subgenus *Chroogomphus*, including those with high BLAST identities and those used by Pérez-Moreno *et al.* (2023). The alignment was performed using MAFFT v. 7 (Katoh & Kuma 2002), visually revised, and trimmed using BioEdit (Hall 1999). The nucleotide substitution model was estimated using jModelTest v. 2.1.7 (Darriba *et al.* 2012). Bayesian Inference Analysis was conducted using MrBayes v. 3.2.5 (Ronquist & Huelsenbeck 2003), with 10,000,000 generations, using the GTR+I+GAMMA nucleotide substitution model, with the remaining parameters set to default values. Additionally, a Maximum Likelihood analysis was performed using RAxML v. 8.2.10 (Stamatakis 2014) with 1,000 rapid bootstrap inferences.

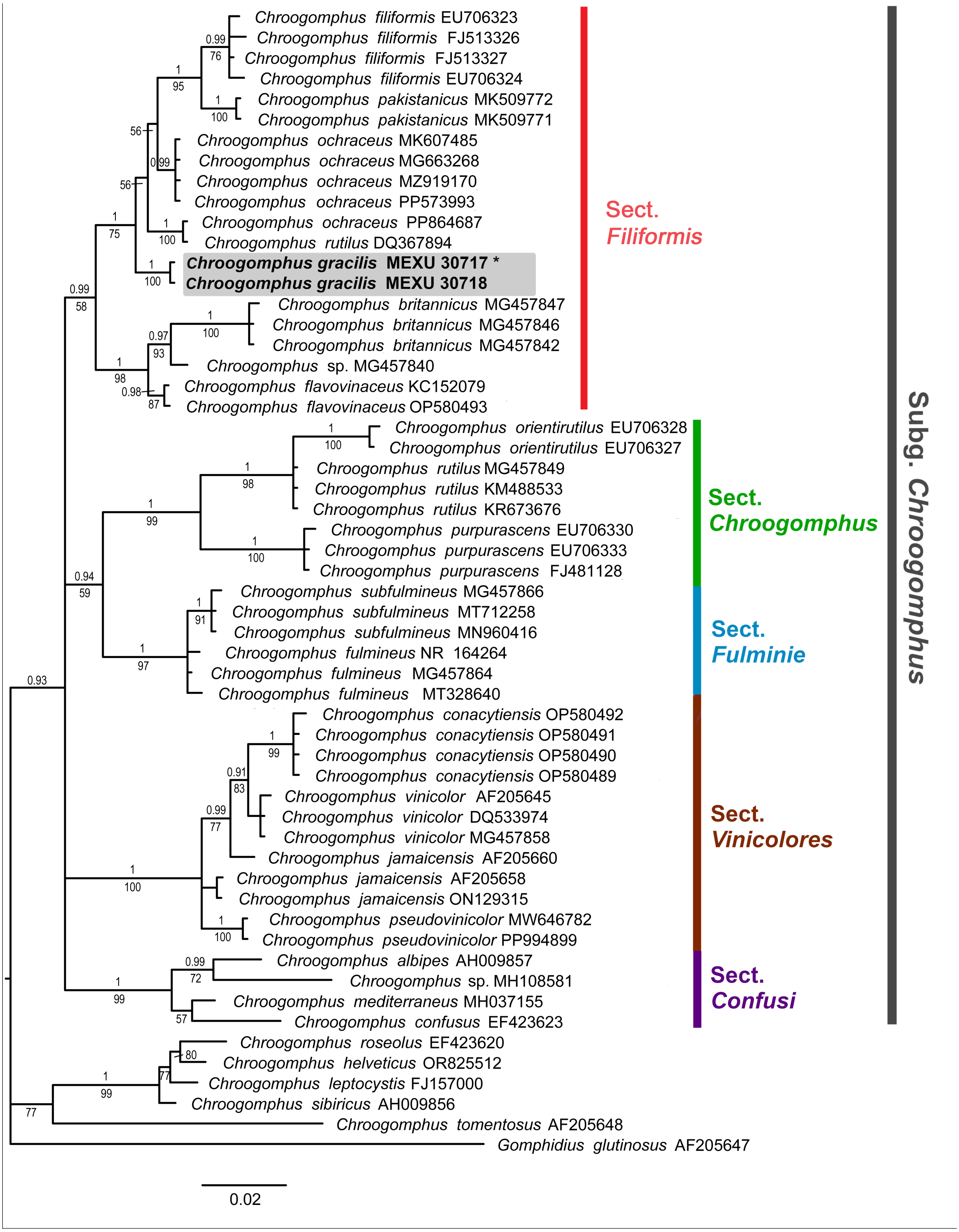

FIGURE 1. Phylogram from the Bayesian inference analysis of a molecular matrix of nrITS of selected species of the genus *Chroogomphus*. The specimens of the newly described species, *C. gracilis*, are highlighted in bold and a gray background. The holotype specimen is marked with an asterisk.

Results

The Bayesian inference (BI) phylogram and the maximum likelihood (ML) tree exhibited nearly identical topologies. The BI reconstruction, however, provided only a marginally higher topological resolution, unambiguously recovering the clade *Filiformis* as sister to the clade comprising sections *Chroogomphus* and *Fulminei* (Figure 1). In both analyses, the subgenus *Chroogomphus* resulted monophyletic (PP = 0.93), separated from the species of the subgenus *Siccigomphus* (*C. roseolus*, *C. helveticus*, *C. leptocystis*, and *C. sibiricus*) and the subgenus *Foccigomphus* (*C. tomentosus*). The two specimens of the newly described species, *C. gracilis*, form a highly supported clade (PP = 1, BS = 100) that is nested within the monophyletic section *Filiformes* (PP = 1, BS = 58). *Chroogomphus gracilis* turned out to be sister to a clade composed of *C. flavovinaceus*, *C. rutilus*, *C. ochraceus*, *C. pakistanicus,* and *C. filiformis*.

FIGURE 2. A–B Forests in the foothills of the Popocatepetl volcano, located in Central Mexico, showing the vegetation type and sampled sites in the State of Mexico. C–D Basidiomata of *Chroogomphus gracilis* showing different stages.

Taxonomy

Chroogomphus gracilis Ayala-Vásquez & Balbuena-Carrasco *sp. nov.* Figure 3
MycoBank No: MB858840

Diagnosis:—Basidiomata small to medium, basidiomata orange-greyish, apricot with shades of grey in the middle to orange-brownish, staining vinaceous when handled, only in mature specimens. Basidiospores (14–)15–17(–20) × 5–7(–8) µm, cylindrical with suprahilar depression, hyaline, olivaceous to brown.

Holotype:—MEXICO, STATE OF MEXICO: San Pedro Nexapa locality, 19°04´18´´N, 98°41´30´´W, altitude 3012 m, mixed coniferous forest; 13 August 2024, Soledad Balbuena Carrasco BCS_1 (MEXU-30717, GenBank: ITS: PV563460).

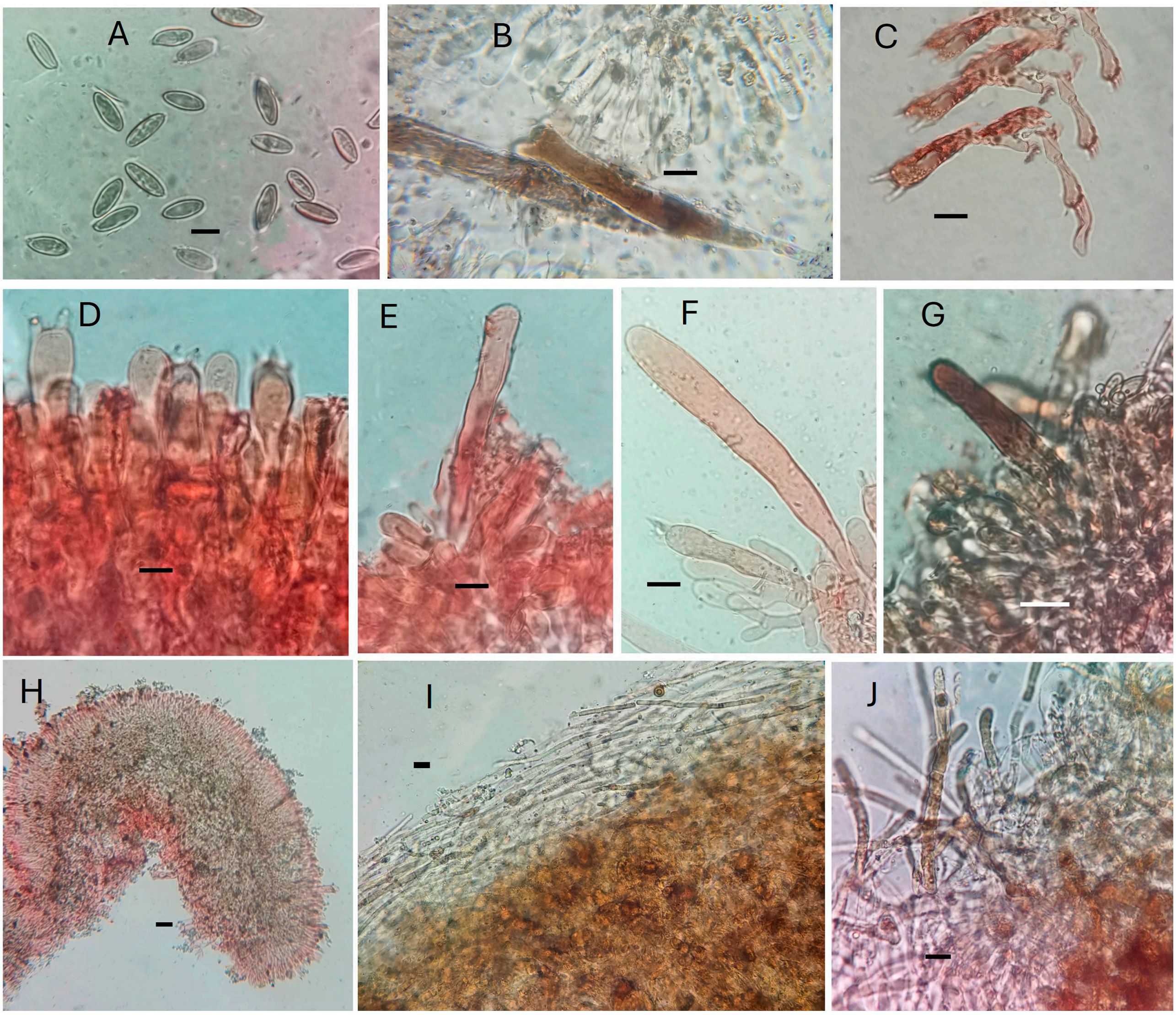

FIGURE 3. *Chroogomphus gracilis sp. nov.* A. Basidiospores (100×). B. Bifurcate cystidia (100×). C–D Basidia (100×). E–G. Cystidia (100×). H. Hymenophoral trama (10×). I Pileipellis (40×). J. Pileipellis terminal cells (100×). All scale bars = 10 µm.

Etymology:—The name is taken from the Latin word "*gracilis*", slender, fragile, subtle, due to the shape of the basidiomata.

Description:—Pileus of 15–42 mm, umbonate, convex when young to later becoming low convex to plane at maturity, surface pileus smooth, orange-greyish (5E2), apricot (5A6–5A8) with shades of grey in the middle (5E2) to orange-brownish (5C7) at maturity, staining vinaceous (12F8) when handled, only in mature specimens. Hymenophore lamellar, subdecurrent, with regular lamellulae, entirely covered by a fibrillose ring when young, lamellae 3–6mm wide, margin regular, pale orange (5A3), orange-greyish (5B2) to yellowish-brown (5E5–5E7) at maturity. Context 2–5mm thick, pale orange (5A3), immutable to cut. Stipe of 45–121 × 6–10 mm, fusoid to cylindrical, surface fibrillose, with apricot (5A6–5A7) veil remnants and pale orange fibrils at the apex, whitish to pale apricot (10 A3) at the base. Basidiospores [75/3] (14–)15–17(–20) × 5–7(–8) µm, (Lm = 15.87, Wm = 5.58), Q = 2.84, cylindrical with suprahilar depression, hyaline to olivaceous, some brown in KOH, thick wall. Basidia (47–) 50–60 × 10–12 (–13) µm, clavate, with contents granular, hyaline to golden content in KOH, tetrasporic, with long sterigmata 7–8 µm. Cystidia 90–110 (–146) × (11–)12–15(–20) µm, fusoid, subclavate thick-walled (≤2-2.5), rarely fusoid-bifurcated, hyaline, yellow-brown to brown in KOH. Lamellar trama made up of hyphae 8–12µm in width, cylindrical, hyaline in KOH, invested in a gelatinous substance of golden color. The pileipellis was structured as an ixotrichoderm, composed of terminal elements measuring (46–)50–75(–85) µm in length by 4–6 µm in width. These terminal cells were predominantly cylindrical to clavate in morphology and had a distinctly gelatinized wall. Under microscopic examination with KOH as the mounting medium, these hyphal elements exhibit a hyaline to pale golden coloration. Stipitipellis composed vertical hyphae (4–)5–12 µm in diameter, thick-walled (≤2). With clamp connections.

TABLE 2. Comparative morphological characteristics of species within the section *Filiformis*, with emphasis on taxa closely related to *Chroogomphus gracilis sp. nov.*, according to descriptions of the holotype species [a].

Taxa	Pileus	Stipe	Basidiospores	Cystidia	Vegetation; Country
C. filiformis	10–60 mm wide; olive gray to orange-gray when young, grayish orange to orange at maturity, to pink and purplish pink when dried.	Subcylindric, sometimes slightly enlarged at base; orange-yellow with pink to golden yellow apex and pink to pinkish base at maturity.	Basidiospores of (15–)16– 19(–21.5) × (5.5–)6–7(–7.5) µm, subfusiform, dextrinoid.	90–170× 14–21 µm, subclavate with attenuate or obtuse apex, often with 1-3 constrictions; hyaline or with pink to brownish pink vacuolar pigment in KOH,	Mixed forest (*P. armandii* and *P. yunnanensis*); China
C. gracilis sp. nov.	15–42 mm wide, orange-greyish to apricot, with grayish tones centrally, to orange-brownish at maturity, staining vinaceous when handled, only in mature specimens.	Fusoid to cylindrical; apricot to pale orange with veil remnants; whitish to pale apricot at base	Basidiospores of (14–)15–17(–20) × 5–7(–8) µm; cylindrical; hyaline to olivaceous, some turning brown in KOH.	90–110 (–146) × (11–)12–15(–20) µm, fusoid to subclavate, rarely bifurcated; thick-walled.	Mixed conifer forest (*Pinus ayacahuite*): Mexico
C. ochraceus	15–50 mm wide, deep chrome, yellowish orange to bright ochraceous.	Concolorous with pileus, becoming reddish to magenta near the base.	Basidiospores of 14–20 × 4.5–7 µm, subfusiform in profile, elliptical in face view, gray-brown in KOH.	81–160× 12–20 µm; clavate to cylindric; thin-walled; hyaline, yellow-brown to brown, often with incrusting material in KOH.	Conifer forest (*P. monticola, P. strobus*); Canada and USA.
C. pakistanicus	20–50 mm wide; greyish-yellow brown, dark bluish-grey to orange.	Cylindrical; orange to reddish-brown, surface fibrillose, pruinose to squamulose.	(15–)16–19.5(–20.5) × (5.5–)6–7.5(–8) µm, elongate to oblong.	75–107 × 17.5–25.5 µm, clavate to slightly utriform; pale brown to brown in KOH; encrusted.	Mixed conifer forest; Pakistan.
C. rutilus	25–120 mm wide, grayish, ochraceous, vinaceous to dingy vinaceous brown.	Tapering toward base; pale ochraceous, orange-buff to vinaceous red.	14–22×6–7.5µm; subfusiform in profile, elliptical in face view, light gray-brown in KOH.	82–178× 13– 22 µm, narrowly fusiform, fusiform, or narrowly clavate to cylindric; thin-walled.	*Pinus* forest; Europe.

[a] The data are based on the descriptions provided by Scambler *et al.* (2018) and Kiran *et al.* (2020).

Habitat, habit, distribution: scattered growing under mixed forest, establishing ectomycorrhizal associations with *Pinus ayacahuite*, at altitudes between 2900 and 3000 m.

Material additional: **Mexico**. Estado de México, San Pedro Nexapa Locality, Clatepec Place, 19°03’39´N, 98°41.0´ 45´´O, altitude 2967 m, mixed coniferous forest, 13 August 2024, Balbuena Carrasco S., BCS_2 (MEXU-30718, GenBank: ITS: PV563461).

Taxonomic Note—*Chroogomphus gracilis* belongs to the subgenus *Chroogomphus,* sect. *Filiformes* is characterized by orange-greyish, with shades of grey in the middle to orange-brownish, staining vinaceous when handled, only in mature specimens. With basidiospores of (14–)15–17(–20) × 5–7(–8) µm, very similar to that of *C. flavovinaceus*, although slightly (1µm) wider and longer than the latter species; *Chroogomphus flavovinaceus* differs from *C. gracilis* by the median basidiomata, fully cylindrical stipe, basidiomata yellow citrus, pale orange, staining vinaceous when touched (Perez-Moreno *et al*. 2023), while *C. gracilis* has a smaller pileus and a very long stipe, fusoid to cylindrical, apricot, range-greyish, apricot with shades of grey in the middle to orange-brownish, vinaceous-coloured stain only on mature specimens. Meanwhile, *C. conacytiensis* differs by the colours of its basidiomata: brown, brown-yellowish, grey, brown-olivaceous, to greyish, sterile margin, when touched reddish-brown to vinaceous; and their basidiospores measuring (13–)17–20(–23) × 5–6 (–7) µm, elliptical, cylindrical to fusoid. This species is part of Mexico’s mycocultural heritage, as it is traditionally consumed as a food source. It is commonly known as "ombligo rosa" (pink navel) by residents of various communities located along the slopes of the Popocatépetl volcano. Additionally, the species is also present in the Ozumba marketplace, supplied by mushroom gatherers mainly based in San Pedro Nexapa, State of México. This trade was documented as *Chroogomphus* sp. by Pérez-Moreno *et al.* (2010).

Validation of the species names

Additionally, in this contribution, four bolete species described from Mexico in 2023 are validated, as their protologues did not initially meet the requirements of Articles F.5.1 and 40.7 of the International Code of Nomenclature for Algae, Fungi, and Plants. Specifically, *Aureoboletus elvirae* and *Chalciporus perezsilvae* are validated as new species, while *Garcileccinum violaceotinctum* and *Garcileccinum viscosum* are formally recombined within the genus *Garcileccinum.*

Aureoboletus elvirae Ayala-Vásquez, García-Jim. & J.I. Fuente. ex. Ayala-Vásquez & Pérez-Moreno
Mycobank No: MB858842

Description and illustration in Ayala-Vásquez *et al. J. Fungi* **9**(10, no. 1041): 9 (2023).

Diagnosis:—Small basidioma, honey to brown-orange color. Pileus broadly convex, mamelonate to umbonate, surface with sharp, small triangular scales, crenulate margin, somewhat decurved, hymenophore with angular to circular pores; basidiospores (7–) 8–10 (–12) × 4–5 (–6) µm, elliptical; pileipellis formed by trichoderm, with thick-wall terminal cells.

Holotype:—Mexico, Oaxaca state, Mixistlán de la Reforma Municipality, Santa María Mixistlan Town, Paaxoom place, 2211 m.a.s.l., 17◦08018.28" N 96◦05017.64" W, in *Quercus scytophylla* forest, 4 November 2016, Ayala-Vásquez O., AVO-794-ITCV, (MEXU-29006, LSU GenBank Num: OQ975746, RPB2 GenBank Num: OQ938893).

Etymology:—Named in honor of Elvira Aguirre-Acosta, eminent Mexican mycologist and a great human being, curator of the collection of mushrooms of MEXU from the National Autonomous University of Mexico for more than 49 years, expert in Agaricales and Gasteroid fungi.

Chalciporus perezsilvae Pérez-Moreno J., Ayala-Vásquez O., Martínez-Reyes M., Martínez-González. ex. Ayala-Vásquez & Pérez-Moreno
Mycobank No: MB858843

Description and illustration in Ayala-Vásquez *et al. J. Fungi* 9 (10, no. 1041): 9 (2023).

Diagnosis:—Small basidiomata pileate-stipitate. Hymenial with circular to hexagonal pores, pale orange, red-brown to cinnamon.

Holotype:—MEXICO, Oaxaca, Santa María Tlahuitoltepec Municipality, Santa Ma-ría Yacochi Town, Zempoaltepetl Hill, cloud forest, 27 June 2022, Ayala-Vásquez O, Martínez-Reyes M., Martínez-González CR., 1571, (MEXU-30459, ITS: OR421044, LSU: OR421572, RPB2: OR43553, TEF1: OR44012).

Etymology:—Named in honor of Dra. Evangelina Pérez-Silva, a pioneering figure in Mexican mycology. A specialist in Ascomycetes and the genus *Inocybe,* she had a distinguished career spanning four decades, during which she inspired successive generations of scientists.

Garcileccinum violaceotinctum (B. Ortiz & T.J. Baroni) Ayala-Vásquez & Pérez-Moreno, *comb. nov.*
Mycobank: MB858857

Basionym: *Leccinum violaceotinctum* B. Ortiz & T.J. Baroni, Fungal Diversity 27: 352 (2007).

Holotype:—Belize, Belize District, Belize Zoo area near Democracia, at the Tropical Education Center, N 17_ 210270 0 W88_32'30", 30 m.a.s.l., 6 October 2002, (CMFR).

Description and recombination details in Ayala-Vásquez *et al.*, Journal of Fungi 2023,9,1126:21

Garcileccinum viscosum (Halling & B. Ortiz) Ayala-Vásquez & Pérez-Moreno, *comb. nov.*
Mycobank: MB858858

Basionym*:* *Leccinum viscosum* Halling & B. Ortiz, Brittonia 61 (2): 172 (2009).

Holotype:—Belize. Cayo: Mountain Pine Ridge, entrance road to Five Sisters Lodge, 335 m.a.s.l., 6 Oct 2003, Halling 8528 (holotype: NY; isotypes: BRH, CFMR).

Description and recombination details in Ayala-Vásquez *et al.*, Journal of Fungi 2023,9,1126:21. https://doi.org/10.3390/jof9121126

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