Phytotaxa 579 (4): 289–298
https://www.mapress.com/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.579.4.6

Two new species of *Chroogomphus* (Gomphidiaceae, Boletales) with biocultural importance in the Tlahuica-Pjiekakjoo culture from Central Mexico

JESÚS PÉREZ-MORENO[1,3], MAGDALENA MARTÍNEZ-REYES[1,4], CÉSAR RAMIRO MARTÍNEZ-GONZÁLEZ[2,5], ELISETTE RAMÍREZ-CARBAJAL[1,6], ANAITZI CARRERA-MARTÍNEZ[1,7], JAVIER ISAAC DE LA FUENTE[1,8], JOAN WINDHOEK OLVERA-NORIEGA[1,9] & OLIVIA AYALA-VÁSQUEZ[1,10]*

[1]*Colegio de Posgraduados, Campus Montecillo, Edafología, Texcoco, México*
[2]*Tecnológico Nacional de México. Instituto Tecnológico de Ciudad Victoria. Blvd. Emilio Portes Gil #1301Pte. CP87010, Ciudad Victoria, Tamaulipas, México*
[3] *jepemo@yahoo.com.mx; https://orcid.org/0000-0001-5216-8313*
[4] *martinezreyes2012@gmail.com; https://orcid.org/0000-0003-2352-917X*
[5] *cesar.ramiro.mg@gmail.com; https://orcid.org/0000-0002-0256-0840*
[6] *lisrc@hotmail.com; https://orcid.org/0000-0001-5421-589X*
[7] *anaitzicm@gmail.com; https://orcid.org/0000-0002-8184-0217*
[8] *jdelafuenteitcv@gmail.com; https://orcid.org/0000-0003-4667-1574*
[9] *joan8913@hotmail.com; https://orcid.org/0000-0002-0892-684X*
[10] *yootspooj@gmail.com; https://orcid.org/0000-0002-8970-9571*
**Corresponding author: yootspooj@gmail.com*

Abstract

The genus *Chroogomphus* has scarcely been studied in Mexico, with only one species reported in the country despite its great biocultural importance in native cultures. In this study, two new species of *Chroogomphus* are presented based on genetic and morphological characterization. The phylogenetic analysis with DNA sequences from nrITS region confirmed that *C. conacytiensis* sp. nov. and *C. flavovinaceus* sp. nov. are included in independent clades separated from other species within the subgenus *Chroogomphus*. Both species have great biocultural importance in the Tlahuica-Pjiekakjoo native culture from Central Mexico as edible taxa, and are associated with *Pinus pseudostrobus* in *Quercus-Pinus* and in *Pinus hartwegii* forests. Descriptions and photographs of the newly described species are presented.

Keywords: edible wild fungi, ectomycorrhizal fungi, Neotropical forests

Introduction

The order Boletales E.-J. Gilbert, is divided into six suborders Boletineae, Paxillineae, Sclerodermatineae, Suillineae, Tapinellineae, Coniophorineae. The Suillineae suborder includes three families Suillaceae, Rhizopogonaceae and Gomphidiaceae Maire ex Jülich, and they are mainly associated with trees of the Pinaceae family (Miller, 1964; Binder & Hibbett, 2006). The family Gomphidiaceae includes two genera: *Gomphidius* Fr. and *Chroogomphus* proposed by Miller (1964). *Chroogomphus* is divided into three subgenera: *Chroogomphus*, *Floccigomphus* and *Siccigomphus* (Scambler *et al*. 2018). *Chroogomphus* is characterized by pale orange, brown, yellow, ochraceous basidiomata with decurrent, yellow, pale orange to ochraceous lamellae, ochraceous pileal trama, moist to viscid pileipellis and a mycelium at the base of the stipe consisting of amyloid hyphae (Miller 1964; Miller & Aime, 2001); subfusiform to ellipsoid basidiospores, pleuro- and cheilocystidia subfusiform to subclavate with encrustations (Miller, 1964; Li *et al*. 2009; Scambler *et al*. 2018) and some species with secotioid basidiomata, such as *Chroogomphus albipes* (Zeller) Yan C. Li & Zhu L. Yang (Miller, 2003).

Chroogomphus has a forest importance since it forms ectomycorrhizal associations mainly with *Pinus* (Miller, 1964); pharmaceutical importance due to its antioxidant potential, hypoglycemic, hypolipidemic, antidiabetic and antitumor properties (Zhang *et al.* 2017; Zhang *et al*. 2020), and economic importance due to its edibility, currently 11 edible species have been reported mainly in China, Japan and USA (Pérez-Moreno *et al.* 2021). In Mexico, the genus has great biocultural importance due to its edibility in different states such as Oaxaca (Garibay-Orijel & Ruan-Soto,

Accepted by Samantha C. Karunarathna: 23 Dec. 2022; published: 24 Jan. 2023

2014), Tlaxcala (Montoya-Esquivel *et al*. 2001) and State of Mexico (Perez-Moreno *et al*. 2010). However, only *C. jamaicensis* (Murrill) O.K. Mill. has been reported. In this work, we describe two new edible species of *Chroogomphus*, called by the Tlahuica-pjiekakjoo culture from Central Mexico, as "Nchjo t´umendye" or "grandfather mushrooms". These species are characterized by yellow, brown to greyish, small to medium-size basidiomata and are distributed in mixed coniferous forests, *Quercus-Pinus* forests and *Pinus* forests, and form ectomycorrhizal association with *Pinus pseudostrobus* and *P. hartwegii* .

Material and methods

Sampling and morphological characterization

The collection of the studied specimens was made during the rainy season from June to September in the years 2021 and 2022 in different areas the Transverse Neovolcanic Mexican Axis in the states of Mexico and Tlaxcala, in mixed conifer forest (*Abies religiosa* and *Pinus pseudostrobus*), *Pinus-Quercus* mixed forest, and *Quercus-Pinus* forest, in Central Mexico. Additional samplings were conducted in Oaxaca in *Pinus hartwegii* forests in the Mixe culture region. Morphological characters were described according to Miller (1964). Chemical reactions were characterized using KOH. Photographs of basidiomata were taken *in situ*, as well as data on the botanical composition of the sites. The colors for the taxonomic descriptions were based on Kornerup & Wanscher (1978). Microscopic characters were measured with an optical microscope (Carl Zeiss GmbH 37081, Germany); 30–50 basidiospores, basidia, pleurocystidia, pileipellis and stipitipellis were measured. For basidiospores, the Q index (length/width) was calculated. The specimens were deposited in the National Herbarium of Mexico of the Institute of Biology of the National Autonomous University of Mexico (MEXU-HO).

DNA Extraction, PCR amplification, and sequencing

Genomic DNA was obtained with CTAB (Martínez-González *et al*. 2017) from 2–3 mg of dry tissue. DNA quantification was performed with Nanodrop (Thermo, USA). Two molecular markers were used, the Internal Transcribed Spacer (ITS) region was amplified with the primer ITS5-ITS4 (White *et al*. 1990) and the ribosomal large subunit (LSU) region was amplified with the primer LROR and LR5 (Vilgalys & Hester, 1990). The PCR reaction contained the following: enzyme buffer 1 x, Taq DNA polymerase, 0.8 mM deoxynucleoside triphosphates (0.2 mM each), 100 ng DNA, 20 pmol of each primer, and 2 units of GoTaq DNA (Promega, USA), with a final volume of 15 µL. The PCR products were verified by agarose gel electrophoresis run for 1 h at 95 V cm-3 in 1.5% agarose and 1 x TAE buffer (Tris Acetate-EDTA). The products were then dyed with GelRed (Biotium, USA) and viewed in a transilluminator (Infinity 3000 Vilber, Loumat, Germany). Finally, the products were purified using the ExoSap Kit (Affymetrix, USA) according to the manufacturer's instructions and were prepared for the sequencing reaction using the BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied BioSystems). Sequencing was carried out in a genetic analyzer (Sanger sequencing) by Macrogen Inc. (Seoul, Korea). The sequences were analyzed, edited, and assembled using BioEdit v. 1.0.5 (Hall, 1999) to create consensus sequences. The consensus sequences were compared with those in the GenBank database of the National Center for Biotechnology Information (NCBI) using the BLASTN 2.2.19 tool (Zhang *et al*. 2000).

Alignments and phylogenetic analyses

To make the phylogenetic analysis, sequences of previous work were considered including Miller & Aime (2001), Miller *et al*. (2002), Parrent & Vilgalys (2007), Li *et al*. (2009), Kim *et al*. (2015), Scambler *et al*. (2018), Cervini *et al*. (2020), Kiran *et al*. (2020), and GenBank (Table 1). The generated sequences were edited and assembled in the program GENEIOUS PRIME V.2012.2.1(Biomatters Ltd), later they were aligned and the phylogenetic analysis was done in the same program. The matrix was composed of 56 taxa (668 characters) and *Gomphidius glutinosus* was used as outgroup (Schaeff.) Fr. (AF205647, AY077472). The best-fit evolutionary model was estimated with JMODELTEST 2 (Darriba *et al.*, 2012). Sequences were subjected to Maximum Likelihood (ML) analysis with 1000 bootstrap replicates under the GTR+GAMMA+ I model while Bayesian posterior probability phylogeny was performed using MrBayes algorithm (Ronquist *et al*. 2012) using two separate Monte Carlo four chains starting from random trees for 10 million generations each (final standard deviation ± 0.146), trees were sampled every 100 generations. The first 25% of samples were discarded as burn-in, under the GTR+ invgamma model. Trees were visualized and optimized in FigTree v. 1.4.4 (Rambaut 2014), and they were edited in Adobe Illustrator vCS4 (Adobe Systems, Inc., San Jose, CA).

TABLE 1. Taxa used in phylogenetic analysis.

Taxon	Voucher	Locality	ITS	References
Chroogomphus cf. albipes	p038i	USA	EU697239	GenBank
C. asiaticus	AH009862	Eastern Siberia	AH009862	Miller *et al.*, 2002
C. asiaticus	AH009861	Eastern Siberia	AH009861	Miller *et al.*, 2002
C britannicus	H:6001678	Finland	MG457842	Scambler *et al.*, 2018
C. britannicus	H:6025417	Finland	MG457846	Scambler *et al.*, 2018
C. britannicus	H:6045578	Finland	MG457847	Scambler *et al.*, 2018
C. confusus	HKAS49658	China	EF423621	Li *et al.*, 2009
C. confusus	HKAS50537	China	EF423622	Li *et al.*, 2009
C. confusus	HKAS50407	China	EF423623	Li *et al.*, 2009
C. filiformis	KUN-HKAS 52934	China	EU706323	Li *et al.*, 2009
C. filiformis	KUN-HKAS 52935	China	EU706324	Li *et al.*, 2009
C. filiformis	KUN-HKAS 52933	China	EU706326	Li *et al.*, 2009
C. fulmineus	K(M):190394	France	NR_164264	Scambler *et al.*, 2018
C. fulmineus	LIP:0401321	France	MG457864	Scambler *et al.*, 2018
C. fulmineus	TUR-A 208102	Italy	MT328640	GenBamk
C. flavovinaceus	**MEXU-HO- 30429 holotype**	**Mexico**	**OP580493**	**In this study**
C. flavovinaceus	GO-2009-366	Mexico	KC152079	GenBank
C. helveticus	HKAS55293	Czech Republic	FJ652070	Li *et al.*, 2009
C. helveticus	AF205642	USA	AF205642	Miller and Aime 2001
C. jamaicensis	AF205660	USA	AF205660	Miller and Aime 2001
C. jamaicensis	AF205658	USA	AF205658	Miller and Aime 2001
C. leptocystis	PK5361	Canada	FJ157000	Genbank
C. mediterraneus	K:200317	United Kingdom: Scotland	MG457837	Scambler *et al.*, 2018
C. mediterraneus	FR2015401	Greece	MG457868	Scambler *et al.*, 2018
C. mediterraneus	JLS 2887	Spain	LT219432	Scambler *et al.*, 2018
C. conacytiensis	**MEXU-HO- 30426**	**Mexico**	**OP580490**	**In this study**
C. conacytiensis	**MEXU-HO- 30427**	**Mexico**	**OP580491**	**In this study**
C. conacytiensis	**MEXU-HO-30431 holotype**	**Mexico**	**OP580489**	**In this study**
C. conacytiensis	**MEXU-HO- 30452**	**Mexico**	**OP580492**	**In this study**
C. ochraceus	4S2.23.F04	USA	EF619654	Parrent and Vilgalys 2007
C. ochraceus	K(M):46653	Canada	MG457840	Scambler *et al.*, 2018
C. orientirutilus	KUN-HKAS 51180	China	EU706327	Li *et al.*, 2009
C. orientirutilus	KUN-HKAS 52671	China	EU706328	Li *et al.*, 2009
C. pakistanicus	LAH35889	Pakistan	MK509771	Kiran *et al.*, 2020
C. pakistanicus	LAH35890	Pakistan	MK509772	Kiran *et al.*, 2020
C. purpurascens	HKAS 54925	China	FJ481128	Li *et al.*, 2009
C. purpurascens	HMJAU 4634	Russia	EU706333	Li *et al.*, 2009
C. purpurascens	HMJAU 3489	Russia	EU706330	Li *et al.*, 2009
C. pseudovinicolor	iNAT:66848908	USA	MW646782	GenBank
C. roseolus	KUN-HKAS 52901	China	EU706329	Li *et al.*, 2009
C. roseolus	HKAS102283	China	MH827599	Li *et al.*, 2009
C. rutilus	KA13-1203	South Korea	KR673676	Kim *et al.*, 2015
C. rutilus	K(M):82320	United Kingdom	MG457849	Scambler *et al.*, 2018
C. rutilus	K(M):175891	Greece	MG457853	Scambler *et al.*, 2018
C. sibiricus	AH009856	Russia	AH009856	Miller and Aime 2001
Chroogomphus sp.	46653	Canada	MG457840	Scambler *et al.*, 2018
Chroogomphus subfulmineus	LIP:0401318	Cyprus	MG457866	Scambler *et al.*, 2018
Chroogomphus subfulmineus	MCVE-30293	Italy	MN960416	Cervini *et al.*, 2020
Chroogomphus subfulmineus	HBIL-Fungi 2639	Spain	MT712258	GenBank
Chroogomphus tomentosus	AF205648	USA	AF205648	Miller and Aime 2001
Chroogomphus tomentosus	AF205668	USA	AF205668	Miller and Aime 2001
Chroogomphus tomentosus	UBC F19668	Canada	HM240518	GenBank
Chroogomphus vinicolor	AF205645	USA	AF205645	Miller and Aime 2001
Chroogomphus vinicolor	DQ533974	USA	DQ533974	GenBank
Gomphidius glutinosus	AF205647	USA	AF205647	Miller *et al.*, 2002
Gomphidius glutinosus	OKM27567	USA	AY077472	Miller *et al.*, 2002

Results

Phylogenetic analyses

In this work, five nrITS region sequences of *Chroogomphus* were generated and uploaded to GenBank. Our nrITS-based phylogenetic analysis showed that the genus *Chroogomphus* was resolved as monophyletic with full (ML=100, BI=1) support having as outgroup *Gomphidius glutinosus* (Figure 1). The analysis showed that our sequences belonged to 2 new species. The first one, hereinafter named *C. conacytiensis* belonged to the section *Vinicolores* cluster in the subclade American species, having as sister species *C. jamaicensis*, *C. vinicolor* (Peck) O.K. Mill. and *C. pseudovinicolor* O.K. Mill. The second one, hereinafter named *C. flavovinaceus* was located in the cluster of the subgenus *Chroogomphus,* section *Filiformis,* and was found in a terminal clade along with *C. ochraceus* (Kauffman) O.K. Mill. and *C. britannicus* A.Z.M. Khan & Hora.

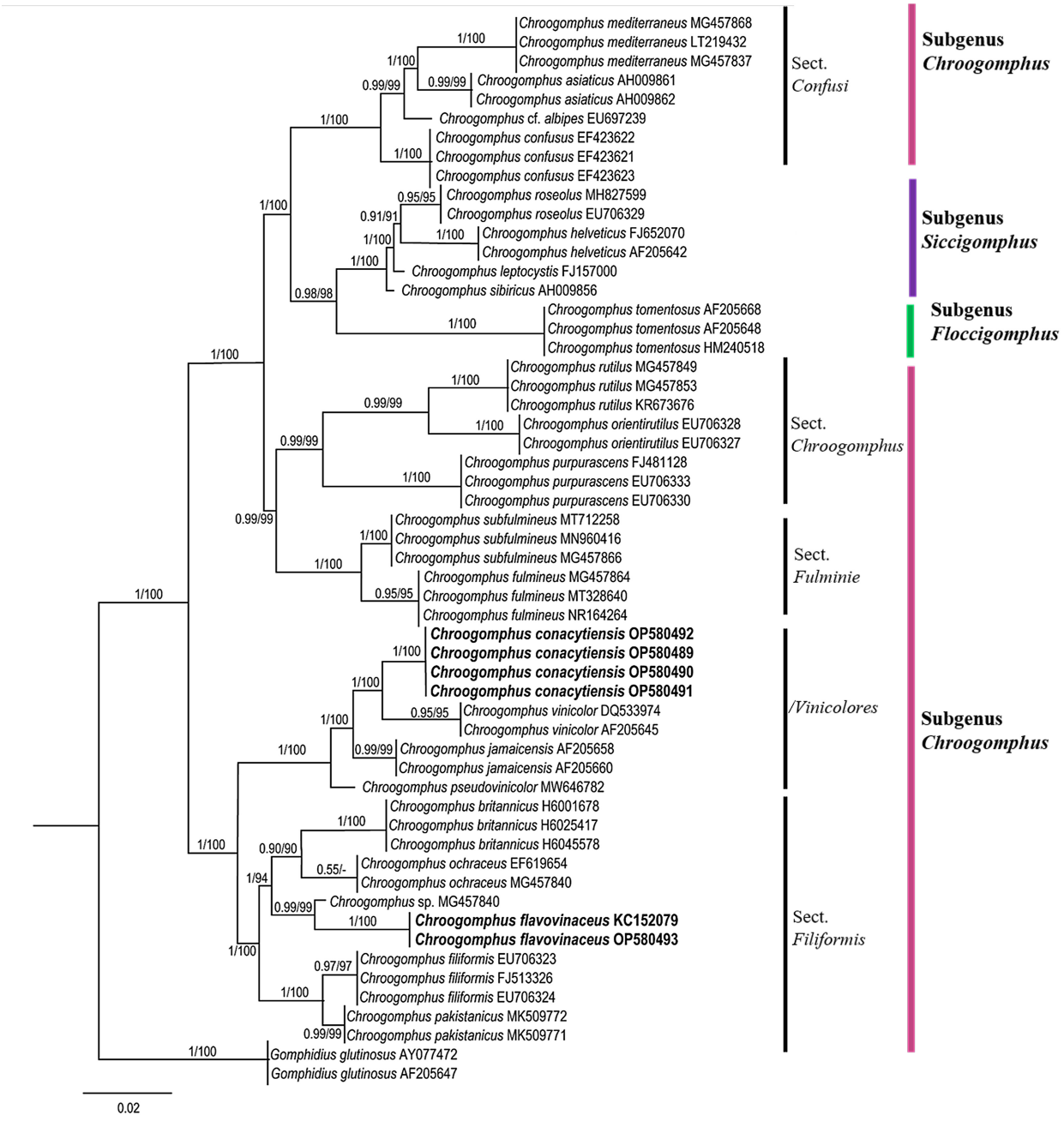

FIGURE 1. The best-scoring BI tree of the genus *Chroogomphus*, reconstructed from the nrITS dataset as outgroup *Gomphidius glutinosus*. Posterior probabilities (> 0.90) and ML bootstraps (> 70%) are indicated above or in front of the branch leading to each node. Newly described species are in boldface.

Taxonomy

Chroogomphus conacytiensis Ayala-Vásquez, Pérez-Moreno, Ramírez-Carbajal & González-Martínez, *sp. nov.* (Fig. 2)

Mycobank no. 845567, GenBank: OP580489 (ITS).

Diagnosis:—Basidiomata small to medium, pileus surface furfuraceous to velvety, brown, brown-yellowish, brown-olivaceus, grey to greyish, sterile margin, when touch reddish-brown to vinaceous. Basidiospores 13–16(–19) × 6–7 (–8) µm, Q=2.2, (n=34), ellipsoid, golden yellow to yellowish-yellow on KOH.

Type:—MEXICO, STATE OF MEXICO: Ocuilan Municipaly, Lomas de Teocaltzingo Town, mixed *Quercus-Pinus* forests, 462878N; 2102619W, 2989 m, 9 September 2021, Martínez-Reyes M., Ayala-Vásquez O, Pérez-Moreno J. (MEXU-HO 30431, holotype).

Description:—*Pileus* 23–35 mm diameter, broadly convex somewhat umbonate when young, hemispherical towards at maturity, pale yellowish-brown (5D8–5E8), brownish-orange (5C3–5D3), grey (30D1–30E1), greyish-brown (5D3), brownish-grey (6D2–6F2) to dark brown (6F8), when touch reddish-brown (8D6–8E6) to vinaceous, humid surface, viscid when wet, smooth, shining when dry, margin enrolled when young, margin with curtain remnants at mature. *Hymenophore* Lamellae, subdecurrent, distant, orange-greyish (5B4–5B5) to light-brown (6D4–6E4), when touch reddish-brown (8D6–8E6) to vinaceous, lamellae 7–10 mm width, slightly serrate, irregular lamellulae common, attenuate. *Context* pileus 8–10mm thick, light orange (5A4), pale orange (5A5) to salmon, basal context of the stipe orange (5A6), immutable to court. *Stipe* 23– 70 × 6– 14 mm, cylindrical, orange-yellow (4A8), orange (5A4), pale-orange (5A3–5A4), when cut it becomes greyish-vinaceous (12E7) to deep vinaceous (12F8), sometimes slightly attenuate downwards, fibrillose surface with ring ephemeral remains very close to the apex whiting to pale pink (12A4). *Chemical reaction* when applying KOH, all basidiomata turn vinaceous.

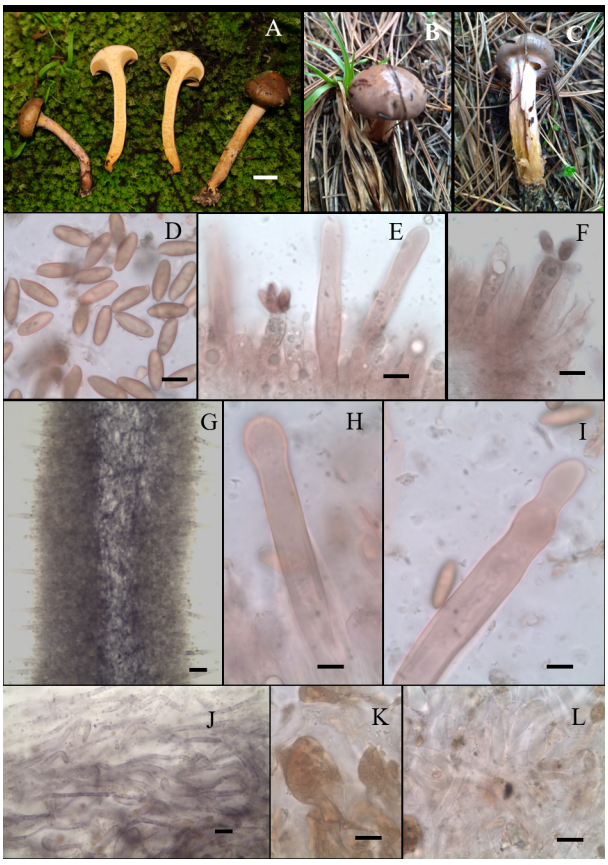

FIGURE 2. *Chroogomphus conacytiensis* (MEXU-HO 30431, holotype, MEXU-HO- 30452 paratype). A. General view of basidiomata. B. Basidioma. C. Stipe surface and partial velum. D. Basidiospores. E. Basidium and cystidium. F. Basidia. G. Lamellar trama. H–I. Two different cystidia forms. J–L Pileipellis. Scale bars: 10 mm (A–C); 10µm (D, E, F, H, I, K, L); 100 µm (G,); 25 µm (J).

Basidiospores (13–)17–18– 20(–23) × 5–6 (–7) µm, (L = 17.89, W = 6.1, Q = 2.5 –2.7 (–2.9), N = 65), cylindrical to elliptical, fusiform, smooth, brown-yellowish, thick-walled. *Basidia* (35–) 37–55–64 (–70) × (7–) 10–14 (–15) µm, clavate, hyaline to pale brown in KOH, brown-chocolate, in Melzer's reagent, with long sterigmata (5–) 7–8× 2–3 µm. *Cystidia* (100–) 120–150 (–150) × (15–) (12–)17–21(–22) µm, fusoid-capitate, subclavate to subfusiform, thick-walled (≤3), hyaline to brown in KOH, brown-yellowish in Melzer's reagent. *Lamellar trama* made up of hyphae 4–6 µm in width, cylindrical, hyaline to brownish in KOH. Pileipellis formed by ixotrichoderm, with terminal cells (14–) 25–45–60 (–70) × (8–) 10–15–20 (–30) µm, clavate, fusiform, to ventricose-fusoid, brown- vinaceous, to chocolate pigment but soon dissolved in KOH, with gelatinous wall that disappears with Melzer's reagent, amyloid. *Stipitipellis* composed of light amyloid, vertical hyphae (4–)5–12 µm in diameter, thick walled (≤2). With clamp connections.

Etymology:—The name is in honor of the Mexican Council of Science and Technology (or CONACYT for its abbreviation in Spanish) which is the public institution responsible for promoting the advancement of scientific research, innovation and technological development, including that of food security and mycocultural heritage, of the country during the last half-century.

Habitat, habit, and distribution:—Solitary to scattered growing under mixed forest to *Quercus-Pinus* forests, forming ectomycorrhizal associations with *Pinus pseudostrobus* and *P. hartwegii*, during July to September at altitudes between 2900 to 3000 m. Only known currently from Central and Southwestern Mexico.

Specimens examined:— MEXICO, STATE OF MEXICO: Ocuilan Municipaly, Lomas de Teocaltzingo Town, mixed forest, 4664720N; 2111033W, 3390 masl, 9 September 2021, Mycoredes (MEXU-HO 30426 paratype); OAXACA: Santa María Tlahuitoltepec Municipaly, Bridge to Zempoaltepetl Town, *Pinus hartwegii* forest, 462878N; 2102619W, 3450 masl, 15 July 2022, Ayala-Vásquez O, Martínez-Gonzalez CR., Martínez-Reyes M., (MEXU-HO-30452); STATE OF TLAXCALA: Nanacamilpa Municipaly, San Felipe Hidalgo Town, El Astillero, 2900 masl, 15 September 2021, Martínez-Reyes M., Ayala-Vásquez O, (MEXU 30451).

Chroogomphus flavovinaceus Ayala-Vásquez, Martínez-Reyes, Pérez-Moreno, *sp. nov.* (Fig. 3)
Mycobank no. 845566, GenBank: OP580493 (ITS).

Diagnosis:—*Small basidiomata, yellow citrus, pale orange, when touching vinaceous, basidiospores (14–)15–16–17(–19) × 6.0–7.5 µm, ellipsoid to fusoid, pleurocystidia with very pronounced granular incrustation.*

Type:—MEXICO, STATE OF MEXICO: Ocuilan Municipality, Lomas de Teocaltzingo Town, *Quercus-Pinus* forests, 462878N; 2102619W, 2989 m,15 July 2021, Ayala-Vásquez O, Martínez-Reyes M., Pérez-Moreno J. (MEXU-HO 30429, holotype).

Description:—*Pileus* 25–45 mm, broadly convex, convex when young, convex-plane at mature, yellow citrus (4A6–4A8), pale orange (5A3–5A4), when touching turn vinaceous (12F8), smooth surface, margin decurvate. *Lamellae* subdecurrent, medium-spaced, yellow (4A4–4A5) to greyish-yellow (4AC), 5mm thick. *Context* 8mm thick, pale yellow (4A3) when cut context stipe base yellowish-olivaceous (2D8). *Stipe* 18–31 × 7–14 mm, cylindrical, slightly tapering toward the base, yellow citrus (4A7), pale orange (5A2), when touching turn vinaceous (12D8). *Basal mycelium* pale yellow, when touching turn olivaceous (2D6). Taste and odor not distinctive.

Basidiospores (14–)15–17(–19) × 6.0–7.5 µm, (L = 13.25, W = 6.0, Q = (2–)2.2 –2.4 (–2.6), N = 48), ellipsoid to fusoid, pale-brown to brown, smooth, with suprahilar depression, thick-walled. *Basidia* (31–) 40–45 (50) × (8–) 9–11 (–13) µm, clavate, hyaline in KOH, 4-sterigmata. Pleurocystidia (86–)94–110–123(–130) × (12–)15–16–18(–20) µm, fusoid, brown-chocolate in KOH, with crystal encrustations embedded in the wall, pale-yellow in Melzer's reagent. *Lamellar trama* composed of inamyloid hyphae 4–6 µm, hyaline to brown-vinaceous in KOH, hyaline to pale-yellow in Melzer's reagent. *Pileipellis* formed by ixotrichoderm with terminal hyphae (17–) 30–58 (68) × (7–) 10–13–15 (–17) µm, cylindrical, subfusoid, with gelatinize wall, hyaline to brown in KOH, pale yellow to brown-vinaceous in Melzer's reagent. *Stipitipellis* formed by vertically intertwined hyphae (6–)8–11 µm, cylindrical, sterile. With clamp connections.

Etymology:—From latin "flavus" by the basidiomata color, "vinaceus" by the color change when touching the basidioma.

Habitat, habit, and distribution:—Solitary, forming ectomycorrhizal association with *Pinus leiophylla* in *Quercus-Pinus* forests, currently only know from the States of Mexico and Michoacán, Mexico.

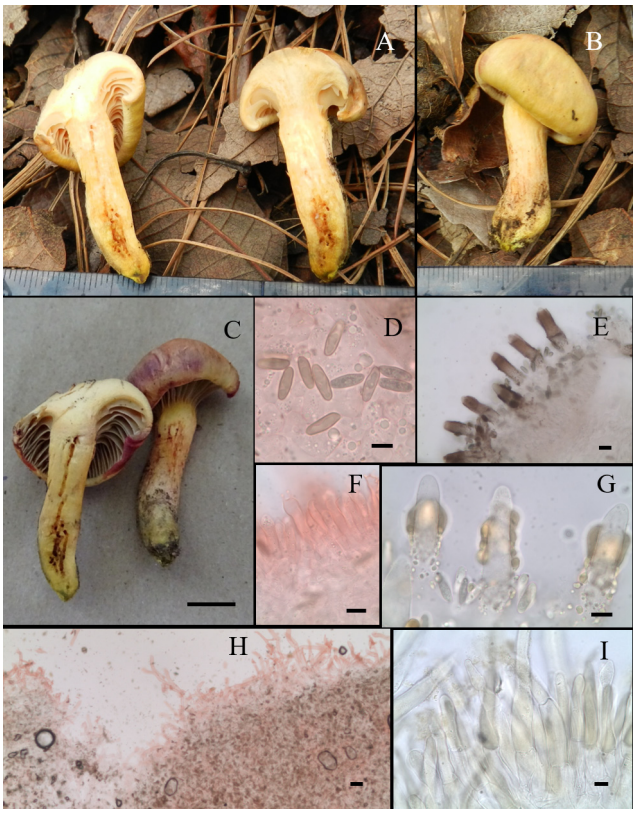

FIGURE 3. *Chroogomphus flavovinaceus* (MEXU-HO 30429, holotype). A. Context of basidiomata. B. Basidioma. C. Change in coloration of the basidiomata when touched. D. Basidiospores. E. Cystidia. F. Basidia. G. Cystidia showing encrustations. H. pileipellis. I. terminal cells of the pileipellis. Scale bars: 10 mm (A–C); 10µm (D, F, G, I); 25 µm (E, H).

Discussion

Chroogomphus species have been described mainly from Asia and Europe. In America, most of the species have been described only from USA. In this work, two species are described for Mexico. Both species belong to subgenus *Chroogomphus* which is characterized by having narrow pileipellis hyphae embedded in a gelatinous layer. *Chroogomphus flavovinaceus* belongs to the section *Filiformis* and is characterized by yellow to orange basidiomata, when touched it turns vinaceous, basidiospores (14–)15–16–17(–19) × 6.0–7.5 µm, ellipsoid to fusoid, pleurocystidia with very pronounced granular crystalline incrustation; it is phylogenetically close to *C. ochraceus* and *C. britannicus* forming a well-supported clade (1BI/94ML); *Chroogomphus flavovinaceus* differs by the characters described above, while *C. ochraceus* has yellowish orange, brown deep chrome to light vinaceous basidiomata and it is distributed in USA and Canada. *Chroogomphus britannicus* differs by the size of its longest basidiospores (17.0–)18.0–23.5(–26.5) × (6.0–)6.5–8 (–9) µm and its distribution in the United Kingdom growing in *Pinus sylvestris* plantations; *Chroogomphus flavovinaceus* is putatively associated with *Pinus leiophylla* which is native to Central-Eastern Mexico. So far, the species has only been found in the *Quercus-Pinus* forests of San Juan Atzingo, Mexico and Tangancicuaro, Patambam, Michoacán based on sequence data KC152079, under the misidentified name "*Chroogomphus jamaicensis*". *Chroogomphus conacytiensis* belongs to the section *Vinicolores*, which is phylogenetically close to *C. vinicolor*, *C. pseudovinicolor* and *C. jamaicensis*, but *C. conacytensis* differs from these species by its colors brown, brown-yellowish, brown-olivaceous, grey to greyish, and its putative association with *Pinus hartwegii* and *P. pseudostrobus*. The diagnostic differences of these related species are shown in table 2. *C. conacytiensis* was previously named based only on morphological characters with the provisional name "*C. jamaicensis"* by Ayala-Vásquez (2021). However, a detailed morphoanatomical, molecular and phylogenetic analysis conducted in the present research indicate that Mexican studied material belongs to a new species.

TABLE 2. Comparative characteristics of the species closely related to *Chroogomphus conacytiensis* and *Chroogomphus flavovinaceus*.

Taxa	Pileus	Stipe	Basidiospores	Cystidia	Host -Country
C. flavovinaceus	Pileus 25–45 mm, yellow citrus, pale orange to salmon, when touching turn vinaceous.	Stipe 18–31 × 7–14 mm, cylindrical	Basidiospores (14–)15–17(–19) × 6.0–7.5 µm, ellipsoid to fusoid, pale-brown to brown.	Pleurocystidia (86–)94–110–123(–130) × (12–)15–18(–20) µm, subfusoid to cilindrycal, with crystal encrustations embedded in the wall.	*Pinus patula* Mexico
C. ochraceus	Pileus 15–35 mm, yellowish orange to ochraceous, deep chrome, zinc orange, light vinaceous.	Stipe 30–60× 4–8(–10) mm wide, tapered downward.	Basidiospores 14–20 X 4.5–7 µm, subfusiform in profile, elliptical in face view	Cystidia 81–160 X 12–20 µm, narrowly clavate to cylindric, thin-walled, rusty brown incrustations.	*P. monticola, P. strobus*. North America
C. conacytensis	Pileus 23–35 mm, pale orange-brown, greyish-yellow brown, pale olivaceous to brown-olivaceous, when touch vinaceous.	Stipe 23– 70 × 6– 14 mm, cylindrical, orange, pale orange, light pink when cut it becomes vinaceous-violet, sometimes slightly attenuate downwards,	Basidiospores (13–)17–18– 20(–23) × 5–6 (–7) µm, cylindrical to elliptical, fusiform, inamyloid.	Cystidia (100–) 120–150 (–150) ×(15–) (12–)17–21(–22) µm fusoid-capitate, subclavate to subfusiform.	*Pinus hartwegii, P. pseudostrobus* Mexico
C. jamaicensis	Pileus 25–50 (–98) mm, dark brown to pale or bright vinaceous.	Stipe 40–100 × 4–15 mm wide, tapering toward the base	Spores 17–20 × 4.5–6 µm, subfusiform in profile, elliptical in face view.	Cystidia 110–165 ×10–25 µm, fusiform to narrowly clavate, thick-walled	Unknown Jamaica
C. vinicolor	Pileus 10–120 mm, orange-red, dingy orange-ochraceous, to dark yellow-brown.	Stipe 50–100× 6–60 mm wide narrowing townward, pale ochraceous, orange-buff to vinaceous red	Spores 17–23 × 4.5–7.5 µm, elliptical in face view, subfusiform in profile.	Cystidia 112–164 × 13–19.5 µm, fusoid-ventricose, clavate to narrowly fusiform, thick-walled.	*Pinus radiata, P. taeda, P. banksiana, P. contorta, P. monticola, P. murrayana, P. resinosa, P. rigida, and P. virginiana.* USA
C. pseudovinicolor	Pileus 60–120 mm, pale orange-ochraceous to red in margin.	Stipe 60–90×20– 40 mm, tapering down to a blunt base.	15– 20×5–7.5 µm, narrowly elliptical in face view, subfusiform in profile.	Cystidia 88–200 × (13–)16–20 µm, fusiform to cylindric.	*Pinus ponderosa* USA

So far, the genus *Chroogomphus* has 11 edible species worldwide (Li *et al*. 2021). However, in this work two more edible species are added giving a total of 13 known edible species of the genus so far. The new species described in the present contribution are accurately distinguished and consumed by the Tlahuica-Pjiekakjoo culture, who inhabits in Central Mexico, and they have biocultural importance as food for this ethnic group. They are called in their native language with the prefix "Nchjo" (meaning mushroom) and the suffix "t'umendye" (which is a diminutive of grandfather). The genus *Chroogomphus* have also medical importance due to its bioactive chemical components. Zhang *et al*. (2017) found that some *Chroogomphus* species have medicinal properties, helping to cleanse the lung, relieve sore throat, detoxification and hemostasis. Therefore, more studies are needed to evaluate the medicinal properties of the two new Mexican species.

Acknowledgments

Olivia Ayala-Vásquez and Javier de la Fuente thank to CONACyT financial support, Id project:3129307; the authors thank the Tlahuica-Pjiekajoo members for all the support provided during the sampling, actually, one the coauthors,

Elissette Ramírez-Carbajal belongs to this ethnic group. This research was funded by the project CONACYT-PRONACES-FOP07-2021-03-316198.

References

Ayala-Vasquez, O. (2021) *Taxonomía, biogeografía y filogenia de los hongos del orden Boletales en la Sierra Norte de Oaxaca, México*. PhD Thesis, Instituto Tecnológico de Ciudad Victoria, Tamaulipas, Mexico.

Binder, M. & Hibbett, D.S. (2006) Molecular systematics and biological diversification of Boletales. *Mycologia* 98: 971–981. https://doi.org/10.1080/15572536.2006.11832626

Garibay-Orijel, R. & Ruan-Soto, F. (2014) *Listado de los hongos silvestres consumidos como alimento tradicional en México*. La etnomicología en México, estado del arte. México.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.

Kim, C.S., Jo, J.W., Kwag, Y.N., Sung, G.H., Lee, S.G., Kim, S.Y., Shin, C.H. & Han, S.K. (2015) Mushroom flora of Ulleung-gun and a newly recorded *Bovista* species in the Republic of Korea. *Mycobiology* 43 (3): 239–257. https://doi.org/10.5941/MYCO.2015.43.3.239

Kiran, M., Sattar, A., Zamir, K., Haelewaters, D. & Khalid, A.N. (2020) Additions to the genus *Chroogomphus* (Boletales, Gomphidiaceae) from Pakistan. *MycoKeys* 66: 23–38. https://doi.org/10.3897/mycokeys.66.38659

Kornerup, A. & Wanscher, J.H. (1978) *Methuen handbook of colour*, 3rd ed. Methuen, London, 252 pp.

Li, Y.C., Yang, Z.L. & Tolgor, B. (2009) Phylogenetic and biogeographic relationships of *Chroogomphus* species as inferred from molecular and morphological data. *Fungal Diversity* 38: 85–104.

Li, H., Tian, Y., Menolli Jr., N., Ye, L., Karunarathna, S.C., Pérez-Moreno, J., Mohammad-Mahmudur-Rahman, M.M., Rashid, M.D.H., Phengsintham, P., Rizal, L., Kasuya, T., Lim, Y.W., Dutta, A.K., Khalid, A.N., Huyen, L.T., Balolong, M.P., Baruah, G., Madawala, S., Thongklang, N., Hyde, K.D., Kirk, P.M., Xu, I., Sheng, J., Boa, E. & Mortimer, P.E. (2021) Reviewing the world's edible mushroom species: A new evidence-based classification system. *Comprehensive Review in Food Science and Food Safety* 20: 1982–2014. https://ift.onlinelibrary.wiley.com/doi/abs/10.1111/1541-4337.12708

Martínez-González, C.R., Ramírez-Mendoza, R., Jiménez-Ramírez, J., Gallegos-Vázquez, C. & Luna-Vega, I. (2017) Improved method for genomic DNA extraction for *Opuntia* Mill. (Cactaceae). *Plant Methods* 13: 1−10. https://doi.org/10.1186/s13007-017-0234-y

Miller, O.K. (1964) Monograph of *Chroogomphus* (Gomphidiaceae). *Mycologia* 56: 526–549. https://doi.org/10.2307/3756358

Miller, O.K. & Aime, M.C. (2001) Systematics, ecology and world distribution in the genus *Chroogomphus* (Gomphidiaceae). *In*: Misra, K. & Horn, B.W. (Eds.) *Trichomycetes and other Fungal Groups*. Science Publishers, Enfield, pp. 314–333.

Miller, O.K., Aime, M.C., Camacho, F.J. & Peintner, U. (2002) Two new species of *Gomphidius* from the Western United States and Eastern Siberia. *Mycologia* 94 (6): 1044–1050. https://doi.org/10.1080/15572536.2003.11833159

Miller, O.K. (2003) The Gomphidiaceae revisited: a worldwide perspective. *Mycologia* 95 (1): 176–183. https://doi.org/10.2307/3761977

Montoya-Esquivel, A., Estrada-Torres, A., Kong, A. & Juárez-Sánchez, L. (2001) Commercialization of wild mushrooms during market days of Tlaxcala, Mexico. *Micología Aplicada International* 13 (1): 31–40.

Montoya, A., Briones-Dumas, E., Núñez-López, R.E., Kong, A., Ortíz-Hernández, V. & Moreno-Fuentes, A. (2019) Los hongos conocidos por la comunidad Yuhmu de Ixtenco, Tlaxcala, México. *Scientia Fungorum* 49: e1230. https://doi.org/10.33885/sf.2019.49.1230.

Parrent, J.L. & Vilgalys, R. (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO2 and nitrogen fertilization. *New Phytologist* 176 (1): 164–174. https://doi.org/10.1111/j.1469-8137.2007.02155.x

Pérez-Moreno, J., Lorenzana-Fernández, A., Carrasco-Hernández, V. & Yescas-Pérez, A. (2010) *Los hongos comestibles silvestres del parque nacional Izta-Popo, Zoquiapan y anexos*. COLPOS-SEMARNAT-CONACyT, Texcoco, 167 pp.

Pérez-Moreno, J., Guerin-Laguette, A., Rinaldi, A.C., Yu, F.G., Verbeken, A., Hernández-Santiago F. & Martínez-Reyes, M. (2021) Edible mycorrhizal fungi of the world: What is their role in forest sustainability, food security, biocultural conservation and climate change?. *Plants, People, Planet* 3: 471–490.

https://doi.org/10.1002/ppp3.10199

Reyes-López, R.C., Montoya, A., Kong, A., Cruz-Campuzano, E.A. & Caballero-Nieto (2020) Folk classification of wild mushrooms from San Isidro Buensuceso, Tlaxcala, Central Mexico. *Journal of Ethnobiology and Ethnomedicine* 16: 53. https://doi.org/10.1186/s13002-020-00408-x

Scambler, R., Niskanen, T., Assyov, B., Ainsworth, A.M., Bellanger, J.M., Loizides, M., Moreau, P.A., Kirk, P.M. & Liimatainen, K. (2018) Diversity of *Chroogomphus* (Gomphidiaceae, Boletales) in Europe, and typification of *C. rutilus*. *IMA Fungus* 9 (2): 271–290. https://doi.org/10.5598/imafungus.2018.09.02.04

Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172 (8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wu, F., Zou, L.W., Yang. Z.L., Bau, T., Li, T.H. & Dai, Y.C. (2019) Resource diversity of Chinese macrofungi: edible, medicinal and poisonous species. *Fungal Diversity* 98: 1–76. https://doi.org/10.1007/s13225-019-00432-7

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000) A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* 7: 203−214. https://doi.org/10.1089/10665270050081478

Zhang, J., Zhao, X., Zhao, L.Q., Zhao, J., Qi, Z. & Wang, L.A. (2017) A primary study of the antioxidant, hypoglycemic, hypolipidemic, and antitumor activities of ethanol extract of brown slimecap mushroom, *Chroogomphus rutilus* (Agaricomycetes). *International Journal of Medicinal Mushrooms* 19 (10): 905–913. https://doi.org/10.1615/IntJMedMushrooms.2017024564

Zhang, Y., Lan, M., Lü, J.P., Li, J.F., Zhang, K.Y., Zhi, H., Zhang, H. & Sun, J.M. (2020) Antioxidant, Anti-inflammatory and Cytotoxic Activities of Polyphenols Extracted from *Chroogomphus rutilus*. *Chemistry & Biodiversity*: 17e1900479. https://doi.org/10.1002/cbdv.201900479