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Article

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Agaricus cervinoculus sp. nov. (Agaricaceae), a new wild edible mushroom from Mexico

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Abstract

In Mexico, there are 55 wild species of *Agaricus* representing approximately 10 % of the world's diversity of the genus. 22 species of these wild mushrooms are traditionally consumed. The lack of keys for the identification of Mexican species, the difficulties in recognizing distinctive morphological characters and the need for comprehensive studies in some sections of the genus, makes it difficult to distinguish species complexes. The edible species known locally as "*ojo de venado*" was previously identified as *A. sylvaticus* in Mexico. Our phylogenetic analyses revealed that this ethnotaxon, which is sold in the markets of Xalapa (Veracruz, Mexico), includes at least two species of *A.* sect. *Subrutilescentes*. One of them, *Agaricus cervinoculus,* is described as a new species. A full description, photographs and comparisons to other similar species are provided. This new species constitutes a well-supported monophyletic clade, sister to the "*A. subrutilescens*" group.

Keywords: *Agaricus* sect. *Subrutilescentes*, edible mushroom, *Pinus-Quercus* forest

Introduction

The genus *Agaricus* L. (1753:1171) includes more than 600 species, according to the Catalogue of Life (Bánki *et al.* 2023). Species of *Agaricus* are distributed throughout the world, except in Antarctica (Jaichaliaw *et al.* 2021). Zhao *et al.* (2016) proposed the segregation of this genus into five subgenera and 20 sections, considering the evolutionary divergence time as a standard taxonomic criterion. Other studies (Kerrigan 2016, Chen *et al.* 2017, He *et al.* 2018, Parra *et al.* 2018, Ortiz-Santana *et al.* 2021) have contributed to a better knowledge of the systematics of the genus and it currently consists of seven subgenera and 32 sections, although *A.* subg. *Conioagaricus* Heinem. (Heinemann, 1956), which contains two sections, has not been studied molecularly because sequencing of type specimens has not been successful.

In Mexico, 55 species of *Agaricus* belonging to six subgenera and 10 sections are known (Chen *et al.* 2020, Palestina-Villa *et al.* 2020, Medel-Ortiz *et al.* 2022), however, only 10 taxa have been studied both morphologically and molecularly. From material collected in Mexico, the novel species *A. guzmanii* Linda J. Chen & G. Mata (2019:95), *A. macrochlamys* Medel, Garibay-Orijel, Argüelles-Moyao, G. Mata, Kerrigan, Bessette, Geml, Angelini, L.A. Parra & Linda J. Chen (2022:16), *A. tlaxcalensis* Callac & G. Mata (2008:881) and *A. tollocanensis* Callac & G. Mata (2004:31) have been described to date (Callac & Mata 2004, Kerrigan *et al.* 2008, Chen *et al.* 2019, Medel-Ortiz *et al.* 2022).

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However, none of these previously described species belong to *A*. sect. *Subrutilescentes* Kerrigan (Kerrigan 2016), which is the section to which the taxa known as "*ojo de venado*" belong. *Agaricus* sect. *Subrutilescentes* is one of the six sections (Ortiz-Santana *et al.* 2021) that make up *A*. subg. *Spissicaules* (Heinem.) R.L. Zhao & Moncalvo (Zhao *et al.* 2016). It is a 19.5 Ma old monophyletic clade (Zhao *et al.* 2016) that is distributed throughout the world, currently encompassing 14 species. Two species of *A*. sect. *Subrutilescentes* have been recorded in Mexico: *A. impudicus* (Rea) Pilát (1951:403) and *A. subrutilescens* (Kauffman) Hotson & D.E. Stuntz (1938:219). These species are distributed in nine states of the country, in various types of vegetation such as *Pinus*, *Quercus* or mixed *Pinus*-*Quercus* forests and tropical deciduous forests (Medel-Ortiz *et al*. 2015, Palestina-Villa *et al.* 2020).

In Mexico the consumption of edible mushrooms dates back to pre-Hispanic times and persists to this day. There are more than 370 species that are consumed in the country (Moreno-Fuentes & Garibay-Orijel 2014), of which 22 correspond to wild *Agaricus* species (Palestina-Villa *et al.* 2020). These species are known by many vernacular names such as "*codorniz*", "*gavilán*", "*gavilancillo*", "*hongo de San Juan*", "*llanero*", "*mazayel*", "*ojo de venado*", "*pechuga de gavilán*" and "*pípila*" (Guzmán 1997). The ethnotaxon "*ojo de venado*" ("*gavilancillo*" or "*pechuga de gavilán*") sold in the markets of the city of Xalapa (central area of Veracruz, Mexico) has previously been identified as *Agaricus sylvaticus* Schaeff. (1774:62) by Villarreal & Pérez-Moreno (1989), Medel-Ortiz *et al.* (2015) and López & García (2018). It was reported to develop in *Pinus*-*Quercus* and mountain cloud forests (Chacón *et al.* 1995). When the macroscopic characters of the specimens from the market were examined in detail, they did not correspond to *A. sylvaticus* (Parra 2008), because in this species the context turns intensely red in the pileus and the upper half of the stipe when cut. Therefore, to resolve the identity of the wild edible mushroom called "*ojo de venado*" we conducted morphological and phylogenetic analyses of specimens collected from markets and the wild, and determined that they did not belong to *A. sylvaticus*, a member of *A*. sect. *Sanguinolenti* L.A. Parra (Parra 2008), but to a new species belonging to *A*. sect. *Subrutilescentes*, which is described here. Additionally, we tested the presence of *A. sylvaticus* in Mexico using environmental DNA sequences.

Material & Methods

Sampling and morphological study

During the 2019–2021 rainy seasons, specimens were obtained from the markets of the city of Xalapa, and wild specimens were collected in mixed pine-oak forests in Cofre de Perote National Park. Morphological descriptions followed taxonomic treatments published by Parra (2008, 2013) and Kerrigan (2016). Microscopic characters were observed using a Carl Zeiss Primo Star compound microscope, with at least 20 measurements taken for each structure. For spores, the Q coefficient, and its mean (Q, Qm, respectively) were calculated, underlined values are the average of the length and the width. Specimens were deposited in the mycological collection of the "*Herbario Nacional de México*" (MEXU-HO) and in Elvia Naara Palestina-Villa's private herbarium (PV).

DNA extraction, PCR, and sequencing

DNA was extracted from the context of the dried material using XNAP-REDExtract-N-Amp™ (Sigma-Aldrich) kit following Mata *et al.* (2016). The internal transcribed spacer region (ITS) of the nuclear ribosomal DNA, including ITS1, 5.8S and ITS2 was amplified using the primers ITS1F and ITS4 (White *et al.* 1990). A segment of the translation elongation factor 1-α (*tef1-α*) was amplified using the primers EF1-983 and EF1-1567R (Rehner & Buckley 2005). PCR products were verified on a 1 % agarose gel and were purified with ExoSAP-IT (USB-Affimetrix) kit following Mata *et al.* (2016). Sequencing was performed with BigDye Terminator 3.1 (Applied Biosystems) kit using the same primers for ITS and *tef1-α* at the "*Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud*, *Instituto de Biología*, UNAM, Mexico" with an ABI 3100 (Applied Biosystems) sequencer.

Phylogenetic analysis

Phylogenetic analyses were performed using a multigene dataset comprised of 56 ITS and 31 *tef1-α* sequences representing 35 validly named species, as well as undescribed taxa from *A*. subg. *Spissicaules*; *A. campestris* the type of the genus *Agaricus* was chosen as the outgroup (Parra *et al.* 2018, Ortiz-Santana *et al.* 2021). Among these sequences, 11 were newly generated in the present study (Table 1). Newly generated sequences were edited and assembled in Geneious R10 (Biomatters). Both regions were aligned individually in MAFFT (Katoh & Standley 2013) and manually adjusted in BioEdit v. 7.2.5 (Hall 1999). The rest of the sequences were retrieved from GenBank and previous studies (Ling *et al*. 2021, Ortiz-Santana *et al.* 2021). The sample´s origin and GenBank accession numbers are listed in Table 1.

TABLE 1. Sample origin and GenBank accession numbers used in phylogenetic analysis. New species and sequences produced in this work are in bold. Vouchers from types are indicated with "T". Sections within the table are ordered as in the phylogenetic tree. Unpublished = Indicates sequences retrieved from GenBank not included in previous phylogenetic analyses.

Subgenus Section Species	**Collection**	**GenBank Access Number**		**Country**	**Reference**
		ITS	***tef1-α***		
Spissicaules					
Subrutilescentes					
A. variegans	LAPAG1063	**PP85518**		Spain	This work
A. variegans	LAPAG1076	**PP85519**		Italy	This work
A. variegans	LAPAG408	**PP85517**		Spain	This work
A. koelerionensis	LAPAG177	**PP85520**		Portugal	This work
A. impudicus	CA583	JF797192		France	Zhao *et al.* 2011
A. impudicus	LAPAG1289	**PP85520**		Spain	This work
A. linzhiensis	Isolate 119	JN182898		China	Ling *et al.* 2021
A. linzhiensis T	ZRL2012618	KT951378	KT951582	China	Ling *et al.* 2021
A. linzhiensis	ZRL2015448	MT102738	MT114180	China	Ling *et al.* 2021
A. thiersii	RWK1940	AY943973		USA	Kerrigan *et al.* 2005
A. linzhiensis	ZRL2014289	KT951418		China	Ling *et al.* 2021
A. linzhiensis	SHY2012070604	KT951341	KT951581	China	Ling *et al.* 2021
A. linzhiensis	ZRL20170662	MN872966	MN866374	China	Ling *et al.* 2021
A. parasubrutilescens	ZRL2012025	KT951362	KT951584	China	Ling *et al.* 2021
A. parasubrutilescens T	ZRL2014076	KT951410	MN866387	China	Ling *et al.* 2021
Agaricus cervinoculus	MEXU-HO30388	**OR142432**		Mexico	This work
Agaricus cervinoculus	MEXU-HO30387	**OR142431**		Mexico	This work
***Agaricus cervinoculus* T**	MEXU-HO30375	**OR142433**	**PP084621**	Mexico	This work
A. subrutilescens	ZRLWXH3276	KT951392	KT951585	USA	Ling *et al.* 2021
A. subrutilescens	RWK2122	KJ877775	MT424830	USA	Kerrigan 2016
A. ciferrianus	PR6585	MT436328	MT424829	Puerto Rico	Ortiz-Santana *et al.* 2021
A. ciferrianus T	PR6822	MT436327	MT424828	Puerto Rico	Ortiz-Santana *et al.* 2021
A. aff. *subrutilescens*	HC-PNNT-222	KT874932		Mexico	Unpublished
A. aff. *subrutilescens*	HC-PNNT-049	KT874934		Mexico	Ling *et al.* 2021
A. aff. *subrutilescens*	CB08378	KT874933		Mexico	Ling *et al.* 2021
A. aff. *subrutilescens*	CB08115	KT874931		Mexico	Unpublished
A. aff. *subrutilescens*	HC-PNNT-303	KT874935		Mexico	Unpublished
A. vinosobrunneofumidus	MGW1363	KY777365		USA	Ortiz-Santana *et al.* 2021
A. vinosobrunneofumidus	NC-1-8341	AY456333		USA	Ortiz-Santana *et al.* 2021
A. vinosobrunneofumidus	RWK2012	KJ877781		USA	Ortiz-Santana *et al.* 2021
A. sp.	CP1829	**PP083184**		Mexico	This work
A. inthanonensis T	ZRLLD014	KT951422		Thailand	Zhao *et al.* 2016
A. inthanonensis	ZRL160	KT951345	KT951586	Thailand	Zhao *et al.* 2016
A. inthanonensis	LDMS22	KT951423		China	Zhao *et al.* 2016
A. brunneopileatus	ZRL2014144	KT951414	MN866388	China	Ling *et al.* 2021

......continued on the next page

TABLE 1 (Continued)

Subgenus Section Species	Collection	GenBank Access Number ITS	 *tef1-α*	Country	Reference
A. brunneopileatus T	ZRL2012115	KT951404	KT951587	China	Ling *et al.* 2021
A. planiceps T	ZRL2015166	KX186722	MN866389	China	Ling *et al.* 2021
A. planiceps	ZRL20151566	KX186720	MN866390	China	Ling *et al.* 2021
A. sp.	TL2321	JF495040		New Zealand	Lebel & Syme 2012
A. sp.	PV70B	**OR142430**		Mexico	This work
A. catenariocystidiosus	ZRL20151199	KX186712	MN866391	China	Ling *et al.* 2021
A. catenariocystidiosus T	ZRL2015038	KX186706	MN866393	China	Ling *et al.* 2021
A. omphalodiscus T	F2255	JF797198	MT424834	France, Martinique	Ortiz-Santana *et al.* 2021
"*A*"					
A. sp.	JBSD130759	MT436332	MT424835	Dominican Republic	Ortiz-Santana *et al.* 2021
Rarolentes					
A. baronii T	DR2523	MT436352	MT424854	Dominican Republic	Ortiz-Santana *et al.* 2021
A. parvisporus T	PR6173	MT436351	MT424853	Puerto Rico	Ortiz-Santana *et al.* 2021
Amoeni					
A. brunneovariabilis T	ZRL2014277	MN872985	MN866394	China	Ling *et al.* 2021
A. amoenomyces T	ZRL2010072	KT951348	KT951638	China	Ling *et al.* 2021
Globoterminales					
A. basicingulatus	JBSD130760	MT436339	MT424841	Dominican Republic	Ortiz-Santana *et al.* 2021
A. basicingulatus T	JBSD130754	MT436338	MT424840	Dominican Republic	Ortiz-Santana *et al.* 2021
Fulventes					
A. bellanniae	CA486	JF797189	MT424837	France	Ortiz-Santana *et al.* 2021
A. bellanniae T	RWK2008	KJ877782		USA	Kerrigan 2016
A. nanofulvens T	AH47621	MT436334	MT424838	Argentina	Ortiz-Santana *et al.* 2021
Spissicaules					
A. lanipedisimilis T	ZRL2012193	KT951399	MN866413	China	Ling *et al.* 2021
A. planipileus T	ZRL2011250	KT951398	MN866409	China	Ling *et al.* 2021
Agaricus					
Agaricus					
A. campestris T	LAPAG370	JQ903618	KR006636	Spain	Kerrigan 2016; Zhou *et al.* 2016

The Maximum Likelihood (ML) analysis was performed with RAxMLGUI v. 2.0 (Edler *et al.* 2020) under a GTRGAMMA+I model with one thousand rapid bootstrap (BS) replicates for each gene. There was no significant incongruence between the datasets, so a concatenated alignment was made in Mesquite v. 3.70 (Maddison & Maddison 2021). The dataset was partitioned into ITS and *tef1-α* regions, and the best substitution model was determined with jModelTest v. 2.1.10 (Darriba *et al.* 2012): HKY+I+G for ITS, and SYM+I for *tef1-α*. Bayesian Inference (BI) analysis was performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Six Markov chains were run for one million generations sampling every 100th generation. Burn-in was determined by checking the likelihood trace plots in Tracer v. 1.6 and subsequently the first 2000 trees were discarded; the remaining trees were used to calculate posterior probabilities. The outputs are displayed in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Distribution of Agaricus sylvaticus

As *A. sylvaticus* is the most cited species of the genus in Mexico (Palestina-Villa *et al.* 2020) and it has been identified as the "*ojo de venado*" several times, we tested its real distribution using reference DNA sequences and environmental DNA sequences. For this, a search with the linux function AWK was conducted in the Global Soil Mycobiome (GSM) database (Tedersoo *et al*. 2021), looking for OTUs annotated as *A. sylvaticus* in the field "taxonomy". Additionally, to identify other potential *Agaricus sylvaticu*s OTUs, four reference sequences of this species (GenBank ZRL2012568, LAPAG382, ZRL2012013, ALG07_213) (Zhao *et al*. 2016) were used as queries in a BLAST search (Altschus *et al*. 1990) against the GSM sequence database. The correct taxonomic assignation of the OTUs was revised by the percentage of nucleotide similarity between its sequences and those of reference sequences in an ITS alignment run in MAFFT removing ambiguous positions. To retrieve the read abundance and geographic location of each *A. sylvaticus* OTU, a search and extraction with AWK function in Linux was made with the OTU-Id in the OTU-table and the Metadata-table from the GSM database.

Results

Phylogenetic analyses

The final alignment contained 1222 characters including gaps. The resulting trees from the Maximum Likelihood and Bayesian Inference analysis had very similar topologies. The Maximum Likelihood tree is shown in Figure 1 and branches with Bayesian posterior probabilities higher than 90 % are thicker. *Agaricus* subg. *Spissicaules* is monophyletic, and the seven sections revealed in a previous study (Ortiz-Santana *et al*. 2021) are supported with strong values in our analysis, except for the undescribed *A.* sect. "A".

The four Mexican samples are all placed in *A.* sect. *Subrutilescentes*. Sequences of *A. cervinoculus* (MEXU-HO30388, MEXU-HO30387 y MEXU-HO30375) formed a well-supported monophyletic clade (BS=99, PP=1), which is sister to *A. subrutilescens* (BS=73, PP=98). Indeed, they differ at 10 and eight positions, in their ITS and *tef1-α* sequences, respectively. Therefore, combined with morphological characteristics, *A. cervinoculus* is described as a new species.

The phylogenetic position of *Agaricus sp.* (PV70B) remains unresolved within *A.* sect. *Subrutilescentes*. In comparison to *A. cervinoculus*, it differs at 11 positions of the ITS sequence. Given that only a single voucher specimen was obtained from the market, and the macroscopic and organoleptic characteristics were incomplete, this sample remains undescribed in the present study.

Taxonomy

Agaricus subg. ***Spissicaules*** (Heinem.) R.L. Zhao & Moncalvo, Fungal Diversity 78: 44. 2016
Agaricus sect. ***Subrutilescentes*** Kerrigan, *Agaricus* of North America: 333. 2016

Agaricus cervinoculus Palestina-Villa, Medel, R. Garibay-Orijel, Linda J. Chen & L.A. Parra, *sp. nov.* (Figs. 2–3)
MycoBank: MB 852840

Type:—MEXICO. Veracruz: Perote municipality, Tembladeras, 2998 m elev., 19°33'11.4'' N, 97°06'35.9'' W, in zacatonal (*Muhlenbergia spp.*) near *Pinus-Quercus* forest, 23 September 2022, *coll. E. N. Palestina-Villa* (Holotype: MEXU-HO 30375; Isotype: *PV129*)

Etymology:—From the Latin *cervinus* (belonging to a deer) and *oculus* (eye), a Latin translation of the Mexican traditional name of this species "*ojo de venado*".

Diagnosis:—*A. cervinoculus* can be distinguished from any other species of *A.* sect. *Subrutilescentes* in having ochre to blackish discoloration on the stipe surface when rubbed or over time, the yellowish context at the junction of the stipe with the pileus when cut, the complex, variable and peculiar smell of wood, chocolate or fruit, the more positive bluish KOH reaction on the pileus surface.

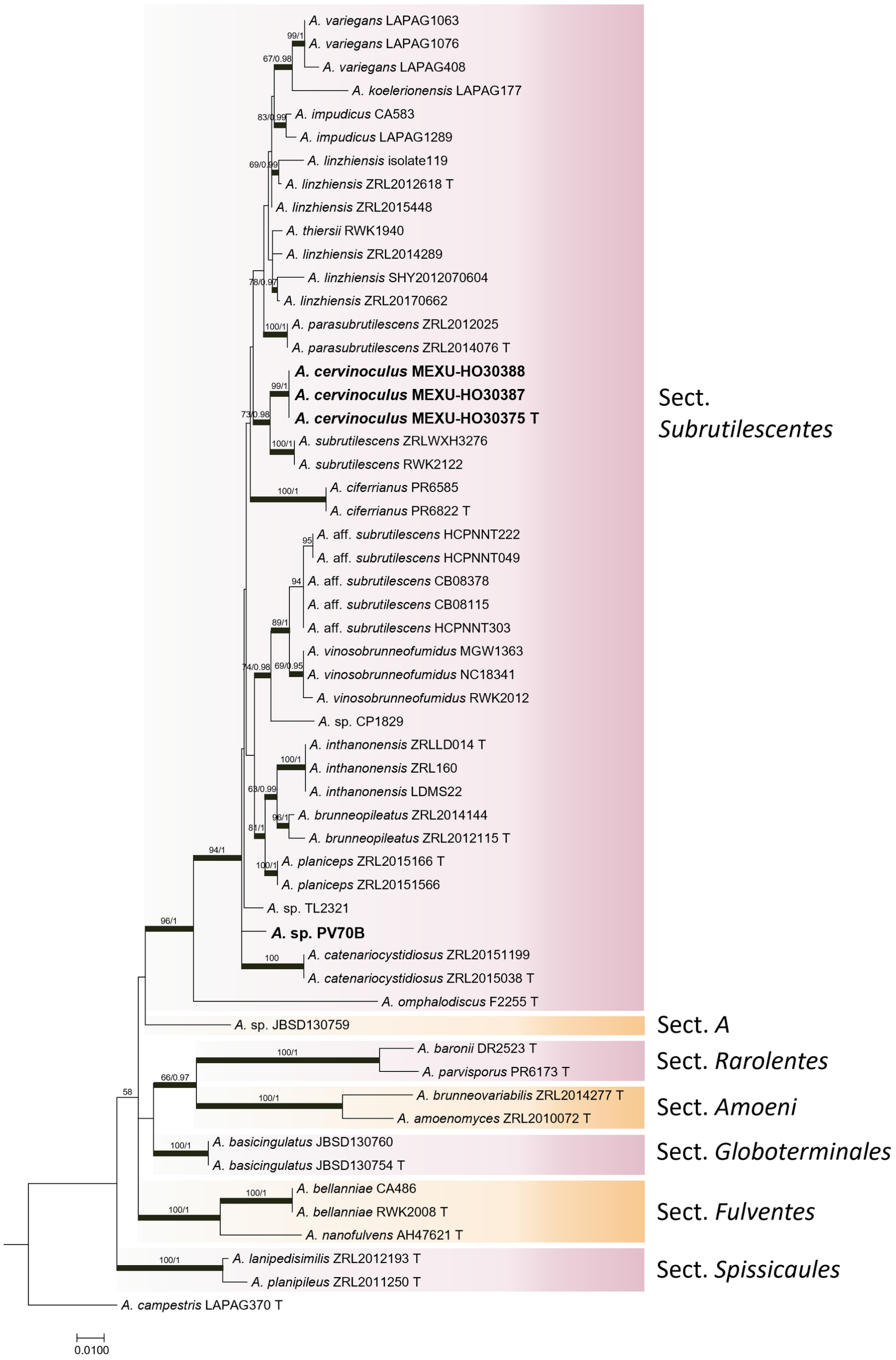

FIGURE 1. Maximum likelihood phylogram of *Agaricus* subg. *Spissicaules* resulting from analysis of ITS and *tef1-α* concatenated alignment. The bootstrap support values greater than 50 % are indicated, branches with Bayesian posterior probabilities greater than 0.9 are in bold. Species sampled from Mexico are in bold. T = Type specimen.

Description:—*Pileus* (7.5)9.5–13.5(16.5) cm in diam., at first convex, then plano-convex, slightly umbonate at the center, covered by small reddish brown to dark brown appressed triangular scales, less dense towards the margin on a whitish background, smooth and dry. *Margin* initially decurved, smooth, sometimes undulating or crenulate, not exceeding the lamellae. *Lamellae* free, up to 0.6 cm broad, crowded, straight, intercalated with numerous lamellulae, at first white, then grayish pink, finally dark brown, with concolorous even edge. *Stipe* (13.4)13.6–15.6 × 1.5–2.6 cm, cylindrical, bulbous at the base (up to 3.5 cm), fistulose, with an annulus on the upper third, above annulus smooth to slightly striate, orange to grayish brown, below annulus covered with abundant semi-erect white fibrils arranged in horizontal zig-zag bands; surface whitish, becoming ochre on handling or over time, then blackish. Base covered with felted mycelium. *Annulus* superous, up to 1.7 cm broad, white, membranous, double, upper surface smooth, lower surface floccose-fibrillose with thin, narrow, dark brown bands of universal veil around the margin. *Context* white on cutting, becoming pinkish on the cortex of the stipe and yellowish in the central area at the junction with the pileus. *Odor* strong and variable among collections, of molasses, chocolate, wood, or slightly citric.

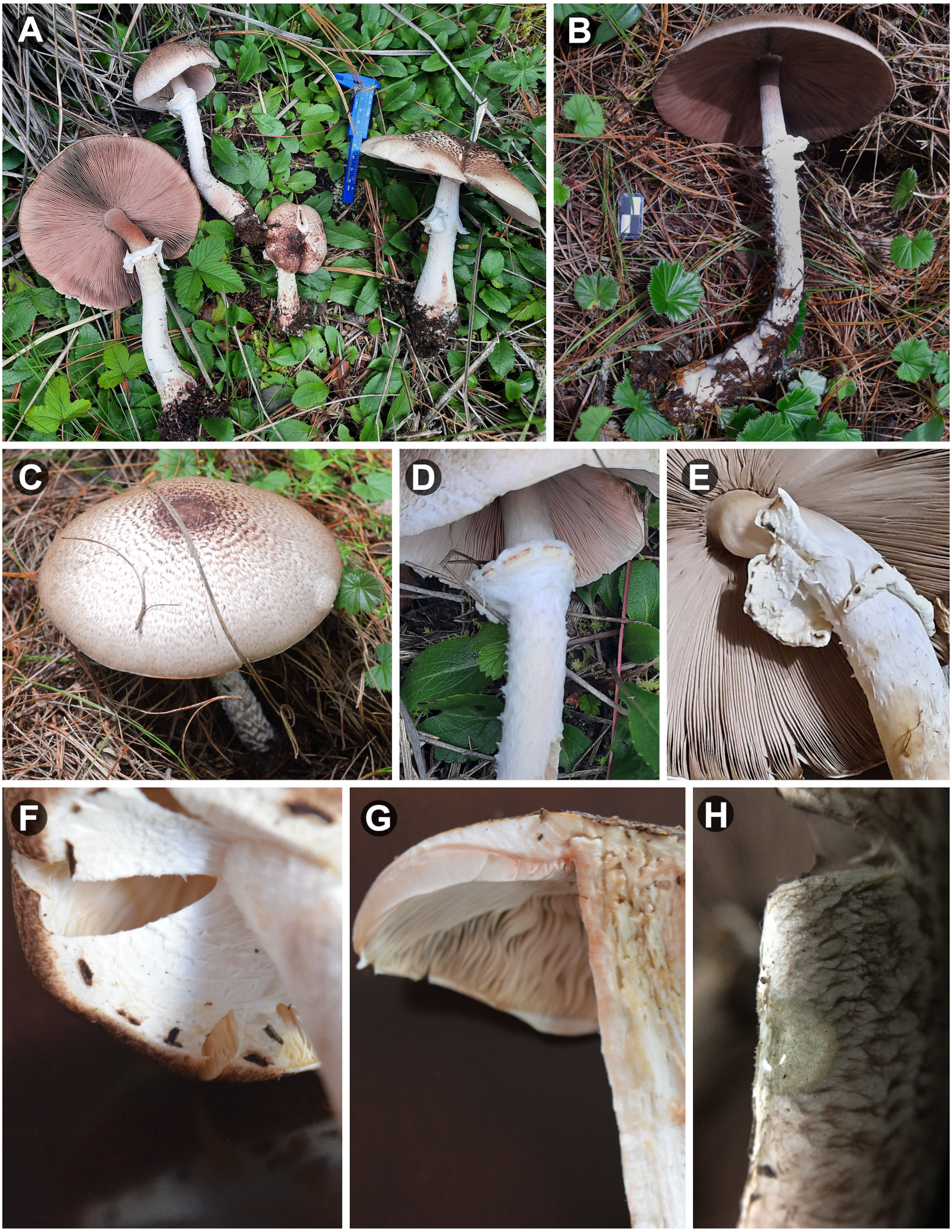

FIGURE 2. Macroscopic characteristics of *Agaricus cervinoculus*. **A–C.** Basidiomata (A: MEXU-HO30375 holotype, B–C: MEXU-HO30387). **D–F.** Annulus detail (D: MEXU-HO30375 holotype, E: PV105, F: PV70A). **G.** Color change in context (PV70A). **H.** KOH reaction (PV62). Photos by E.N. Palestina-Villa.

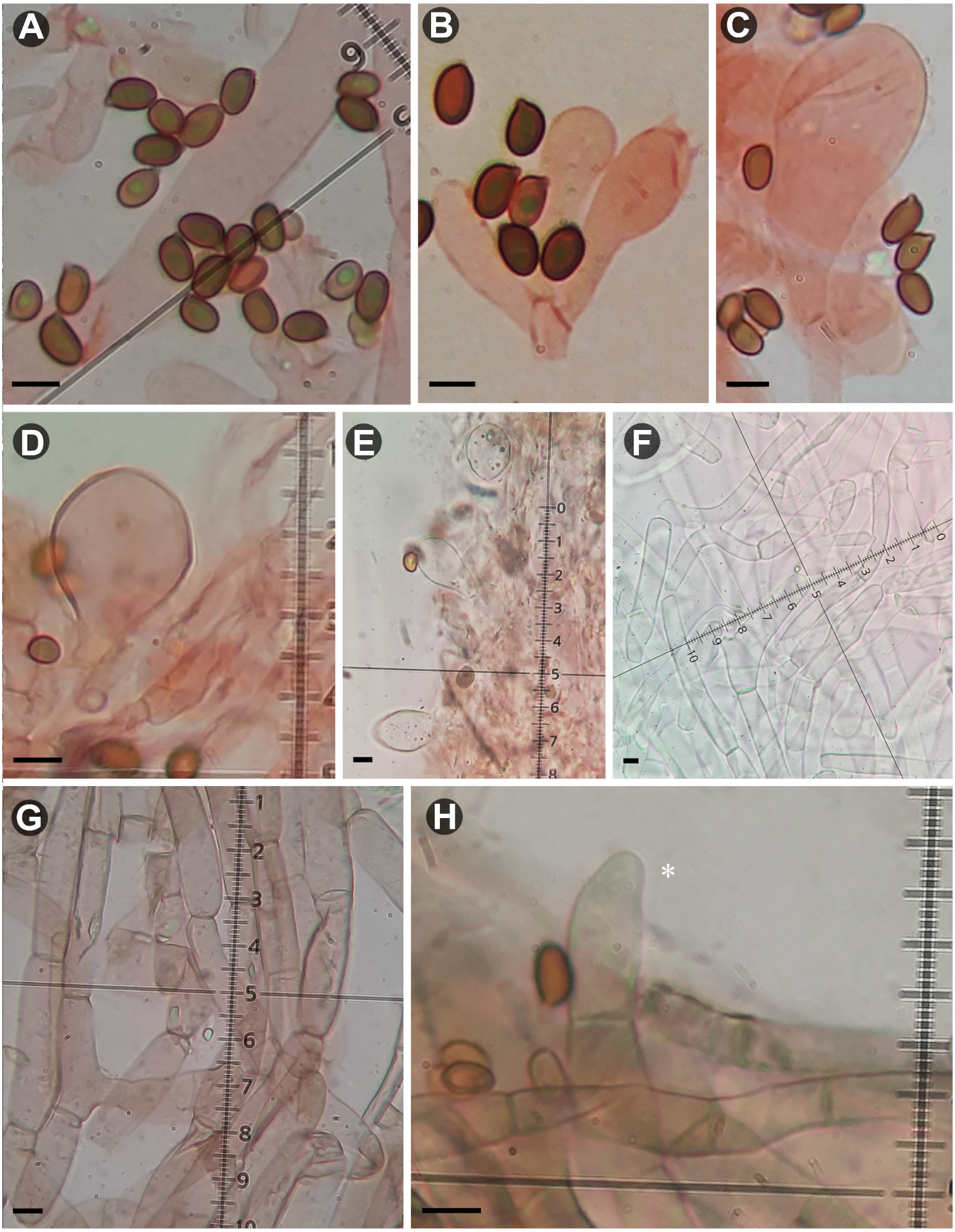

FIGURE 3. Microscopic characteristics of *Agaricus cervinoculus*. **A.** Spores (PV83). **B.** Basidia (PV83). **C–E.** Cheilocystidia (C-D: MEXUHO30387, E: PV98). **F.** Hyphae of the lower surface of the annulus (PV72). **G–H.** Pileipellis hyphae and a terminal element with an asterisk (G: MEXU-HO30387, H: PV105). Scale bars = 5 µm. Photos by E. N. Palestina-Villa.

Basidiospores (5)5.1–5.7–6.2(6.5) × (3)3.2–3.6–3.9(4.2) µm, Q = (1.3)1.4–1.6–1.7(1.8), ellipsoid to ellipsoid-elongate, smooth, brown, thick-walled, without apical pore, unigutulate, bigutulate or with granular content. *Basidia* (15.3)16–20.8(23.2) × (5.9)6.4–7.9(8) µm, tetrasporic, clavate, hyaline, sterigmata up to 2.8 µm long. *Cheilocystidia* (16.9)17.7–24.4(38.7) × (11.3)11.7–16.7(18.3) µm, hyaline, usually pyriform or sphaero-pedunculate, some globose or broadly clavate, abundant. *Pleurocystidia* absent. *Lower surface of the annulus* composed of hyaline hyphae, 6.2–

8.7 µm wide, cylindrical, not or slightly constricted at the septa. *Pileipellis* composed of cylindrical hyphae, 8.5–12 µm, constricted at the septa with some terminal elements progressively attenuated with rounded apex. Intracellular diffuse brown pigment. *Clamp connections* not observed.

Macrochemical reactions:—KOH on pileus surface of fresh specimens olive green to slightly bluish.

Habitat:—Solitary to gregarious, on ground with decomposed leaves, in *Pinus-Quercus* forests. Occurring from September to October.

Edibility:—Edible, a combination of mature and immature sporocarps is generally sold in local markets.

Additional specimens examined:—MEXICO. Veracruz: Xalapa municipality, San José Market, specimens bought from markets, 3 October 2019, *coll. E. N. Palestina-Villa PV62*. 8 October 2020, *coll. E. N. Palestina-Villa PV70A*, 3 September 2021, MEXU-HO 30386 (duplicate *PV83*). 16 September 2021, *coll. E. N. Palestina-Villa PV92*. 30 September 2021, *coll. E. N. Palestina-Villa PV98*, *PV100*. 03 October 2021, *coll. E. N. Palestina-Villa, PV103*. 10 October 2021, *coll. E. N. Palestina-Villa* MEXU-HO 30388 (duplicate *PV106*). Veracruz: Perote municipality, Tembladeras, 2952 m elev., 19°32'01'' N, 97°06'04'' W, in *Pinus-Quercus* forest, under *Pinus*, 04 September 2021, *coll. E. N. Palestina-Villa* MEXU-HO 30387 (duplicate *PV86*).

Comments:—Morphologically, *A. cervinoculus* can be distinguished from other species of *A.* sect. *Subrutilescentes* in having ochre to blackish discoloration on the stipe surface when rubbed or over time, the yellowish context at the junction of the stipe with the pileus when cut, the complex, variable and peculiar smell of wood, chocolate or fruit, the more bluish positive KOH reaction on the pileus surface. The complex odor of this species is due to the combination of volatile compounds (an alcohol, an aldehyde, and an acid) that will be described in a later publication.

In Mexico, two species from this section have been cited: *A. impudicus* and *A. subrutilescens*, which are morphologically similar to *A. cervinoculus*. However, they differ in having a context not becoming yellow when cut (Kerrigan 1986, 2016). Furthermore, *A. impudicus* has no bluish olive-green positive KOH reaction, and *A. subrutilescens* has smaller spores (5.2 × 3.3 µm on average). The remaining species in the section do not present a yellow context when cut at the junction of the stipe with the pileus or a bluish KOH reaction on the pileus surface. Molecularly, *A. cervinoculus* lacks any species-specific ITS marker within *A.* sect. *Subrutilescentes*. However, *A. cervinoculus* and *A. omphalodiscus* L.A. Parra, Fiard, Callac & B. Ortiz (2021:406) share an exclusive single nucleotide agcag[A]–tgc@159 as a relevant distinguishing character from all other species of the section. *Agaricus omphalodiscus*, recently described from the Caribbean, can be easily distinguished from other species because it possesses many species–specific ITS markers (Ortiz-Santana *et al*. 2021).

Distribution of Agaricus sylvaticus

Only one OTU (f869374f7f2e96de7da0254c98eb58b97cf655e7) identified as *A. sylvaticus* was found in the GSM database. The ITS sequence of this OTU has a 99.5 % nucleotide similarity with the reference sequence of *A. sylvaticus* var. *sylvaticus* KM657929 and 99.1 % with the ITS sequence of the type specimen of *A. sylvaticus* var. *occidentalis* (Suppl. Table 1). From more than 3000 sites distributed globally in the GSM database, this OTU is distributed in 49 sites; 47 of these sites are located in Europe (95.8 %), one in Kyrgyzstan (2.1 %), and another in Hawaii (2.1 %) (Suppl. Table 2).

The BLAST search with *A. sylvaticus* reference sequences of both varieties against the GSM database, retrieved only one other OTU (583f31b3c8e70da58c845159b339583bb3dd1cd2) close to *A. sylvaticus*. However, its nucleotide similarity ranged from 98.9 %–98.4 % to this species and it belongs to *A. cordillerensis* Kerrigan (2016:208) (Suppl. Table 1). In the GSM database this species is present in 17 sites, 16 in Europe and 1 in Canada (Suppl. Table 3).

Discussion

The "*ojo de venado*" ethnotaxon is requested and valued by chefs in local restaurants and now includes at least two species, *A. cervinoculus* and a similar undescribed species (PV70B) differing from *A. cervinoculus* at 11 positions in the ITS sequence. The name *A. sylvaticus* continues to be applied in Mexico to the "*ojo de venado*" (López & García 2022), even though *A. sylvaticus* has a strong reddening of the context at the pileus and the upper half of the stipe. It is necessary to continue collecting "*ojo de venado*" in surrounding areas and cities, since the consumption of *A. sylvaticus* and other species known as "*gavilán*" or "*totolita*" has been reported, which could refer to the pattern of the pileus scales that are characteristic of *A. cervinoculus* or some similar species (Contreras-Cortés *et al*. 2018).

Our analyses demonstrate that *A. sylvaticus* is a predominantly European species and likely is not present in Mexico, even though it is the most cited species of the genus in Mexico (Palestina-Villa *et al.* 2020). In consequence, any collection identified as *A. sylvaticus* in Mexico requires further verification.

The gastronomic potential of *A.* sect. *Subrutilescentes* remains to be explored. Kerrigan (2016) mentions that in some parts of the United States the primordia of *A. subrutilescens* are consumed. It is evident that, in Mexico, there are other edible and yet undescribed species of the genus, many of which are traditionally consumed, such as *A. cervinoculus* and *Agaricus sp*. (PV70B) in the center of the state of Veracruz, as well as *A.* aff. *subrutilescens* (HCPNNT222, HCPNNT049, HCPNNT222, CB08115, CB08378), a clade whose sequences belong to edible specimens from the State of Mexico, with *A. vinosobrunneofumidus* being its sister clade. These comprise one of the few species of *A.* subg. *Spissicaules* that are consumed.

There is a high diversity of species in the genus *Agaricus* in Mexico (Mata *et al*. 2011) and more research is needed to characterize the morphological and phylogenetic diversity of the genus. The need for a comprehensive approach becomes evident when considering that the precise identification of species is difficult in many cases, as demonstrated by the phylogenetic analysis of *A. cervinoculus*.

Conclusion

The edible ethnotaxon "*ojo de venado*" includes at least two species previously identified as *A. sylvaticus*. Phylogenetic analyses showed that these are two undescribed species belong to *A.* sect. *Subrutilescentes*. One of them, *Agaricus cervinoculus*, described here, is a unique and well-differentiated species, with distinctive morphological and genetic characteristics, which separate it from the related species. The other taxon, *Agaricus sp*. (PV70B), requires more collections representing the phenotypic variability of the species to be formally described. *Agaricus sylvaticus* is a European species not distributed in Mexico and Mexican collections labeled as *A. sylvaticus* should be re-examined in detail. The diversity of *A.* sect. *Subrutilescentes* in Mexico requires more taxonomic and molecular studies to clarify and fully understand the variability and distribution of its species. The identification and description of new species within *A.* sect. *Subrutilescentes*, such as the "*ojo de venado*", demonstrates the importance of a more precise taxonomy of the genus. Research on the ecology, distribution and phylogenetic relationships of these species can provide valuable information for potential use in biotechnological or medicinal applications.

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