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Armillaria mexicana, a newly described species from Mexico

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ABSTRACT

Armillaria mexicana (Agaricales, Physalacriaceae) is described as a new species based on morphology, DNA sequence data, and phylogenetic analyses. It clearly differs from previously reported *Armillaria* species in North, Central, and South America. It is characterized by the absence of fibulae in the basidioma, abundant cheilocystidia, and ellipsoidal, hyaline basidiospores that are apparently smooth under light microscope, but slightly to moderately rugulose under scanning electron microscope. It is differentiated from other *Armillaria* species by macromorphological characters, including annulus structure, pileus and stipe coloration, and other structures. DNA sequence data (nuc rDNA internal transcribed spacers [ITS1-5.8S-ITS2 = ITS], 28S D-domain, 3′ end of 28S intergenic spacer 1, and translation elongation factor 1-α [*TEF1*]) show that *A. mexicana* sequences are quite distinct from sequences of analogous *Armillaria* species in GenBank. In addition, sequences of ITS of the *A. mexicana* ex-type culture reveal an ITS1 of 1299 bp and an ITS2 of 582 bp, the longest ITS regions reported thus far in fungi. Phylogenetic analysis based on *TEF1* sequences place *A. mexicana* in a well-separated, monophyletic clade basal to the polyphyletic *A. mellea* complex.

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INTRODUCTION

Armillaria (Fr.) Staude includes approximately 35 species (Volk and Burdsall 1995; Kirk et al. 2008), many of which are root pathogens that can cause major damage to diverse woody hosts worldwide. *Armillaria* species also perform other ecological roles, such as saprophytic decomposers of diverse organic substrates and mycorrhizal associates of orchids (Baumgartner et al. 2011). In North America, nine *Armillaria* species are currently recognized by the application of morphological, biological, and phylogenetic species concepts (Anderson and Ullrich 1979; Anderson and Stasovsky 1992; Burdsall and Volk 1993, 2008; Kim et al. 2006; Brazee et al. 2012; Ross-Davis et al. 2012). Recently, *A. tabescens* (Scop.) Emel, which also occurs in North America, was reassigned to the genus *Desarmillaria* (Herink) R.A. Koch & Aime, based on multigene phylogenetic analyses (Koch et al. 2017).

Most previous phylogenetic analyses of *Armillaria* have focused on nuc rDNA, such as internal transcribed spacers (ITS1-5.8S-ITS2 = ITS), the 28S gene (large subunit), intergenic spacer 1 (IGS1), and 5S (e.g., Anderson and Stasovski 1992; Chillali et al. 1998; Coetzee et al. 2001, 2003, 2005, 2015; Mueller et al. 2001; Dunne et al. 2002; Keča et al. 2006; Kim et al. 2006; Lima et al. 2008; Hasegawa et al. 2010; Keča and Solheim 2010). Although 28S, ITS, and/or IGS sequences provided useful information for phylogenetic studies among widely divergent taxa, these sequences do not reliably resolve closely related species (Kim et al. 2006). Some eastern North American *Armillaria* species were resolved by phylogenetic analysis based on RNA polymerase II (*RPB2*) sequences, but the closely related *A. gallica* Marxm. & Romagn. and *A. calvescens* Bérubé & Dessur. were not resolved (Brazee et al. 2011). Sequences of ITS, translation elongation factor 1-α (*TEF1*), and β-tubulin (*TUB2*) were used to distinguish phylogenetic lineages of *Armillaria* associated with *Gastrodia elata* in China; *TEF1* was the most parsimony-informative (Guo et al. 2016). Recently, 28S, *TEF1*, *RPB2*, actin-1 (*ACT*), glyceraldehyde-3-phosphate dehydrogenase (*GPD*), and *TUB2* were used to effectively assess phylogenetic relationships among

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species of *Guyanagaster, Armillaria*, and *Desarmillaria* (Koch et al. 2017). Among the six loci examined in that study, no single locus was sufficient for a well-supported phylogeny; however, *TEF1* was a major determinant of phylogenetic relationships. In this regard, Brazee et al. (2011) indicated that the phylogenetic analysis of concatenated sequences (*TEF1, RPB2*, and 28S) of North American *Armillaria* species supported the results obtained from partial *TEF1* sequences, which is evidence that the majority of phylogenetically informative characters were in this gene.

Sequences of *TEF1* have displayed great utility for examining differences among even closely related *Armillaria* species from different geographical regions, including Europe (Antonín et al. 2009; Mulholland et al. 2012; Tsykun et al. 2013), Asia (Hasegawa et al. 2010; Ota et al. 2011; Coetzee et al. 2015), and North America (Brazee et al. 2011; Ross-Davis et al. 2012; Elías-Román et al. 2013). In these studies, *TEF1* has consistently demonstrated utility for species delimitation among closely related *Armillaria* species in Europe, Asia, and North America (Klopfenstein et al. 2017). Gene trees based on *TEF1* show that *Armillaria* and *Desarmillaria* species from the Northern Hemisphere generally comprise the following four superclades, which were named according to the specific epithet of the most frequently cited species within: (i) Socialis/Tabescens (exannulate) superclade including Eurasian *D. ectypa* (Scop.) R.A. Koch & Aime, North American *D. tabescens*, and Eurasian *D. tabescens* clades; (ii) Mellea superclade including an undescribed annulate North American *Armillaria* sp. (Mexico), the species described here, and four separate clades of *Armillaria mellea* (Vahl) P. Kumm. (Europe and Iran, eastern Asia, and two groups from North America); (iii) Gallica superclade including *Armillaria* Nag E (Japan), multiple clades of *A. gallica* (Asia and Europe), *A. calvescens* (eastern North America), *A. cepistipes* Velen. (North America), *A. altimontana* Brazee, B. Ortiz, Banik & D.L. Lindner (western USA), *A. nabsnona* T.J. Volk & Burds. (North America and Japan), and at least two *A. gallica* clades (North America); and (iv) Solidipes/Ostoyae superclade including two *A. solidipes/ostoyae* clades (North America), *A. gemina* Bérubé & Dessur. (eastern USA), *A. solidipes/ostoyae* (Eurasia), *A. cepistipes* (Europe and Japan), *A. sinapina* Bérubé & Dessur. (North America and Japan), and an *A. borealis* (Eurasia) clade (Klopfenstein et al. 2017).

In Mexico, where *Armillaria* studies are largely devoted to local marketing, ethnobotany, and mushroom consumption, identification of *Armillaria* species has been primarily based on macro- and micromorphological characters. The following *Armillaria* spp. have been identified in Mexico based on morphology: *A. borealis* Marxm. & Korhonen (Pérez-Silva et al. 2006), *A. mellea* (Villareal and Pérez-Moreno 1989; Montoya et al. 2003), *A. polymyces* (Pers.) Singer & Clémençon (Termorshuizen and Arnolds 1987; Villareal and Pérez-Moreno 1989), *Desarmillaria tabescens* (Scop.) R.A. Koch & Aime (as *A. tabescens*; Farr and Rossman 2014), and *Armillaria* spp. (Montoya-Ezquivel et al. 2001). The taxonomic standing of *A. polymyces* is uncertain because this species was previously considered synonymous with *A. solidipes* Peck. (as *A. ostoyae* (Romagn.) Herink; Volk and Burdsall 1995). The report of *A. borealis* also requires confirmation, because this is the only report of this species in the Western Hemisphere. However, identification of *Armillaria* species based on morphological characters is difficult (Watling et al. 1991), especially for closely related species (Brazee et al. 2011). In Mexico, a few studies have identified species of *Armillaria* by considering other criteria, such as compatibility reactions, which revealed the presence of *A. solidipes* (as *A. ostoyae*; Shaw 1989), *A. mellea, A. gallica*, and an undetermined species (Alvarado-Rosales and Blanchette 1994; Alvarado 2007). Only limited DNA sequence–based identification has been conducted for *Armillaria* in Mexico. The presence of *D. tabescens* (as *A. tabescens*) and *A. gallica* were validated by partial 28S-IGS1 and/or *TEF1* sequences (Kim et al. 2010; Klopfenstein et al. 2014). Recently, 5.8S rDNA-ITS2-28S D-domain, partial 28S-IGS1, and *TEF1* sequences were used to identify *Armillaria* species associated with root disease of peach trees in orchards of the State of Mexico (Elías-Román 2013; Elías-Román et al. 2013). In those studies, a *TEF1*-based phylogeny of North American *Armillaria* species indicated that an undescribed species, widely distributed across commercial peach orchards, was positioned within the Mellea superclade and was quite distinct from, but phylogenetically adjacent to, other clades comprising characterized isolates of *A. mellea*. On the basis of partial *TEF1* sequences, Klopfenstein et al. (2017) confirmed the phylogenetic position of the undescribed *Armillaria* species from Mexico in relation to other *Armillaria* species from the Northern Hemisphere using neighbor-net and Bayesian analyses. This previously undescribed *Armillaria* species from Mexico is described in this paper.

MATERIALS AND METHODS

Fungal isolates.—Five basidiome specimens collected in the State of Mexico were used for the species description (TABLE 1), and three cultured isolates (MEX85, MEX87, MEX88) derived from context tissue of the specimens were

Table 1. List of *Armillaria mexicana* specimens (with associated cultures) used for species description and DNA analysis.

Herbarium basidiomata collection numbers[a]	Basidiome-derived culture isolate number[b]	Host	Location Coordinates	Altitude (m a.s.l.)	Source
IZTA4599	MEX85	Dead peach (*Prunus persica*) tree with *Armillaria* symptoms	Coatepec Harinas, State of México N18°56′41.1″, W99°48′52.3″	2345	Basidiome
IZTA4595 (holotype)	MEX87 (CM-CNRG 399)	Dead peach (*P. persica*) tree	Coatepec Harinas, State of Mexico N18°55′34.9″, W99°48′ 43″	2227	Basidiome
IZTA4597	MEX88 (CM-CNRG 365)	Dead peach (*P. persica*) tree	Coatepec Harinas, State of Mexico N18°55′35″, W99°48′ 43.1″	2225	Basidiome
IZTA4596	MEX115 (CM-CNRG 372)	Symptomatic olive (*Olea europaea*) tree	Coatepec Harinas, State of Mexico N18°55′16.8″, W99°45′ 41.1″	2228	Basidiome
IZTA4598	MEX114	Fallen trunk of *Quercus* sp.	Coatepec Harinas, State of Mexico N18°55′17.7″, W99°45′ 33.5″	2213	Basidiome

[a]In previous reports, basidiome collection numbers of R. D. Elías-Román were the same as the associated culture isolate numbers, from which DNA was extracted and sequences obtained.
[b]All isolates were deposited at Forestry Sciences Laboratory, Forest Service, Rocky Mountain Research Station, USDA, in Moscow, Idaho, USA.

used for DNA sequence analysis. Representative isolates were deposited in the microorganism collection (CM) at Centro Nacional de Recursos Genéticos-Instituto Nacional Investigaciones Forestales, Agrícolas y Pecuarias (CNRG-INIFAP), Tepetitlán de Morelos, Jalisco, México. Basidiome-derived cultures also were deposited in the fungal culture collection of US Department of Agriculture (USDA) Forest Service Rocky Mountain Research Station, Forestry Sciences Laboratory, Moscow, Idaho, USA.

DNA extraction and sequencing.—For DNA extraction, basidiome-derived culture isolates (TABLE 1) were subcultured on sterile nylon filters overlaid on 3% malt agar (3% Bacto malt extract [Becton Dickinson & Co., Sparks, Maryland, USA], 3% dextrose, 1% Bacto peptone, 1.5% Bacto agar) in Petri dishes maintained at 22 C in darkness for 3 wk. DNA extraction and purification was conducted using a FastPrep FP120 tissue homogenizer (Bio 101; Thermo-Savant, Carlsbad, California, USA) and ZR Fungal/Bacterial DNA MiniPre Kit (Zymo Research, Irvine, California, USA). The quantity and quality of DNA and polymerase chain reaction (PCR) products were assessed by a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA), Qubit 2.0 fluorometer (ThermoFisher Scientific, Waltham, Massachusetts, USA), and TapeStation 2200 DNA Analysis System (Agilent Technologies, Santa Clara, California, USA). PCR was used to amplify three products for sequencing: (i) partial 18S (nuclear small subunit), ITS, and partial 5′ 28S D-domains (18S-ITS-28S); (ii) partial 3′ 28S, IGS1, and partial 5S rDNA (28S-IGS1-5S); and (iii) partial *TEF1* using methods modified from those of Kim et al. (2006) and Ross-Davis et al. (2012). PCR protocols for 18S-ITS-28S and 28S-IGS1-5S followed Kim et al. (2006) except 100 ng template DNA was used instead of mycelial scrapings, the annealing temperature was set to 42 C, and only 21 cycles were used. Thermocycler settings for *TEF1* followed those of Ross-Davis et al. (2012) with number of cycles reduced to 21. PCR and sequencing primer pairs used in the reactions are shown in SUPPLEMENTARY TABLE 1.

DNA sequence comparison and phylogenetic analysis.—Sequences were edited and aligned with BioEdit 7.1 (Hall 1999). Polymorphic sites were coded using the International Union of Pure and Applied Chemistry (IUPAC) codes for ambiguous nucleotides. Resulting sequences were compared with sequences available in GenBank using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis was based on *TEF1* sequences of undescribed *Armillaria* sp. isolates (MEX85, MEX87, MEX88) and other *Armillaria* spp. for which sequences were previously reported (Maphosa et al. 2006; Antonín et al. 2009; Hasegawa et al. 2010; Ota et al. 2011; Mulholland et al. 2012; Ross-Davis et al. 2012; Elías-Román et al. 2013; Tsykun et al. 2013; Coetzee et al. 2015; Hood and Ramsfield 2016; Klopfenstein et al. 2017). For these studies, we use *A. solidipes* as the species name provisionally assigned by Guo et al. (2016) to the North

American species previously called *A. ostoyae* (Redhead et al. 2011). In addition, *TEF1* sequences of *A. puiggarii* Speg. (FJ618636, KU289104, KU289113), *A. mellea* (AY881023), and *A. heimii* Pegler (FJ618651, FJ618644) were included in the analysis. *Desarmillaria tabescens* (JF313113, JF313112, JF313111) and *D. ectypa* (EU251403, FJ618643, FJ875698) were used as outgroups. Gaps in sequence alignments were treated as missing data. Sequences of *TEF1* were analyzed using maximum parsimony (MP) in PAUP* 4.0 b10 (Swofford 2002). The MP analysis was performed using heuristic search; starting trees were obtained via stepwise using random addition sequences with 100 replicates, followed by tree-bisection-reconnection branch swapping. Gaps were excluded from the analysis, and multistate taxa were interpreted as uncertain. The Bootstrap method with 1000 replicates was performed to evaluate the clade support and obtain bootstrap 50% majority-rule consensus trees (FIG. 1). Bootstrap support values greater than 70% were considered significant in this study.

The edited 18S-ITS-28S, 28S-IGS1-5S, and *TEF1* sequences were deposited in GenBank (TABLE 2),

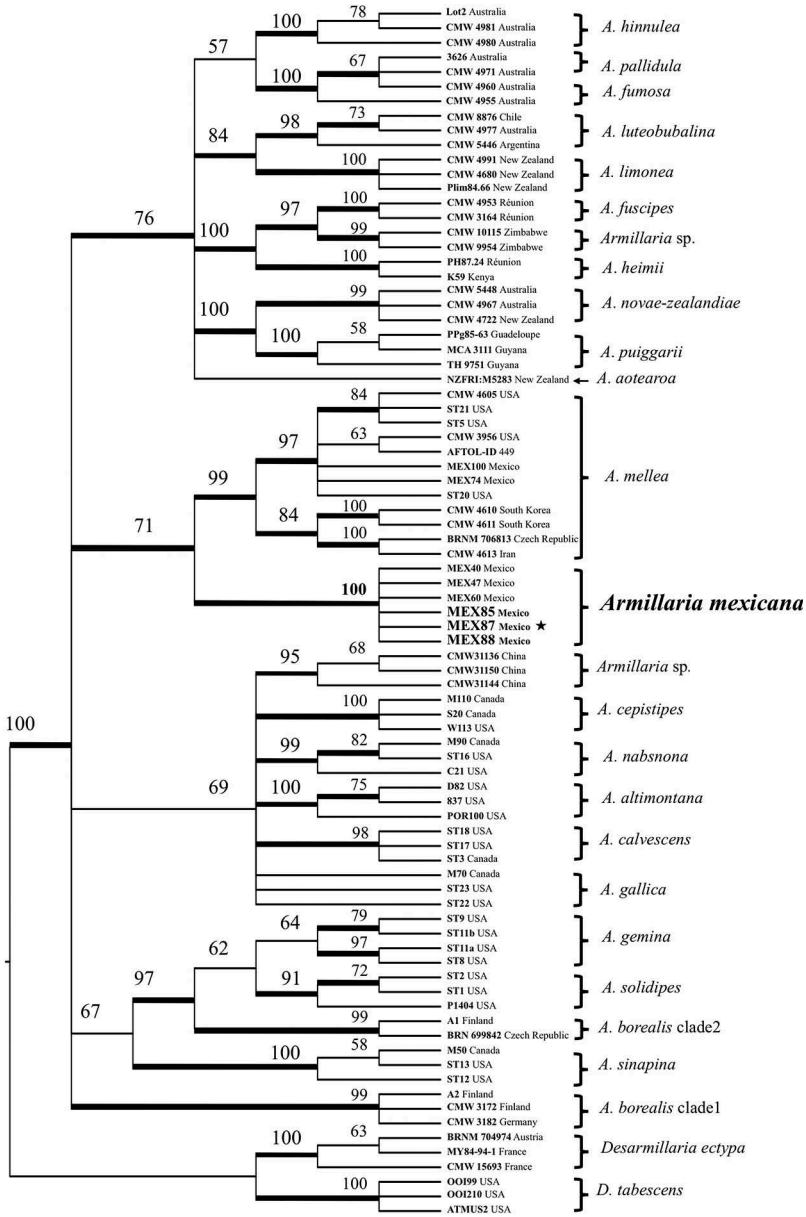


Figure 1. Bootstrap 50% majority-rule consensus tree of *Armillaria* spp. based on partial translation elongation factor 1-α (*TEF1*) sequences using maximum parsimony, with gaps excluded from the analysis. Bootstrap supports are indicated above branch nodes based on 1000 bootstrap replicates. Bold branches indicate ≥70% bootstrap support. Ex-type (MEX87) is indicated with a star.

Table 2. GenBank accession numbers of *Armillara mexicana* isolates, with product length and percent similarity to the holotype.

	GenBank accession numbers (product length[a], percent similarity to holotype)		
Isolate	18S-ITS-28S[b]	28S-IGS1-5S[c]	*TEF1*[d]
Holotype-derived isolate			
MEX87	KR061310 (3054 bp)	KR061306 (729 bp)	KR061314 (1178 bp)
Paratype-derived isolates			
MEX85	KR061308 (2059 bp, 100%), KR061309 (1142 bp, 99%)	KR061305 (787 bp, 99%) KR061307 (860 bp, 100%)	KR061313 (1178 bp, 100%) KR061315 (1125 bp, 100%)
MEX88	KR061311 (2946 bp, 99%), KR061312 (2282 bp, 100%)		
Other A. mexicana *isolates*			
MEX40[e]	KC111007 (1641 bp, 100%)	KC111002 (660 bp, 99%)	KC111016 (887 bp, 100%)
MEX43[f]	JX281813 (1662 bp, 100%)	JX281802 (660 bp, 99%)	—
MEX46[f]	JX281812 (1660 bp, 99%)	JX281803 (660 bp, 99%), JX281804 (658 bp, 99%)	—
MEX47[e]	KC111008 (1599 bp, 99%)	KC111003 (660 bp, 99%), KC111004 (658 bp, 99%)	KC111017 (887 bp, 100%)
MEX57[f]	JX281814 (1673 bp, 99%)	JX281805 (660 bp, 99%)	—
MEX60[e]	KC111009 (1661 bp, 99%)	KC111005 (660 bp, 99%)	KC111018 (887 bp, 100%)

[a]The products of some isolates are only partial to those of the holotype and do not contain all regions and/or have sequences with partial regions compared with that of the holotype.
[b]Partial 18S, internal transcribed spacer 1 (ITS1), 5.8S, internal transcribed spacer 2 (ITS2), and partial 5′ 28S D-domains of nuc rDNA.
[c]Partial 3′ 28S, intergenic spacer 1 (IGS1), and partial 5S of nuc rDNA.
[d]Partial translation elongation factor 1-α (*TEF1*).
[e]These isolates are included in phylogenetic analyses reported by Elías-Román et al. (2013).
[f]These isolates are mentioned by Elías-Román et al. (2013).

and *TEF1* sequence data used in phylogenetic analysis were deposited into TreeBASE (study no. 20029).

Basidiome morphology.—The description of basidiomes was based on freshly collected material. Dimensions (length and diam) of the pileus and stipe were measured with a digital vernier caliper (Truper, Mexico City, Mexico). Color determinations for the macromorphological structures were based on the Royal Horticultural Society color chart (Royal Horticultural Society 1986). The microscopic study was performed on a Primo Star iLED compound light microscope (Carl Zeiss, Jena, Germany). Sections of gills, pileus, annulus, and stipe were made by hand with razor blades and mounted in 3% KOH and 1% Congo red. For each sample, measurements (N = 40) were determined for (i) length and width of basidiospores, basidia, cheilocystidia, annulus hyphae; and (ii) length of pileipellis, context cells, and stipe hyphae.

Spore ornamentation was observed using scanning electron microscopy (SEM), as follows: fresh pieces (<4 mm^3) of stipe and lamellae were fixed in 2.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer, pH 7.2. After samples were subjected to three 5-min washes in Sorensen's phosphate buffer, they were dehydrated through a graded ethanol series (30, 40, 50, 60, 70, 80, and 90%) for 45 min at each concentration, before completing the dehydration with three 15-min treatments in 100% ethanol. Samples were dried in a critical-point dryer (Tousimis Sandri-780A; Rockville, Maryland, USA), mounted on a slide with carbon conductive adhesive tape, coated with gold for 4 min using an ionizer (fine coat ion sputter; JFC-1100; JEOL, Tokyo, Japan), and examined with SEM (JEOL JSM-6390; Tokyo, Japan) at the Electron Microscope Unit of Colegio de Postgraduados (Montecillo, Texcoco, State of Mexico, Mexico).

Specimens were deposited at the mycological collections of the Herbarium IZTA Facultad de Estudios Superiores Iztacala UNAM, Tlalnepantla, State of Mexico, Mexico, and mycological collections of XAL Herbarium, Instituto de Ecología A.C. Xalapa, Veracruz, México.

RESULTS

DNA sequences analysis.—DNA sequences of the partial 28S-IGS1-5S from the basidiome-derived culture isolates of the undescribed *Armillaria* sp. isolates showed 99% similarity with the undescribed *Armillaria* sp. (GenBank accession numbers KC111002, KC111003, KC111004, KC111005; based on 76–90% coverage) from the previous study of peach orchards in the State of Mexico (Elías-Román et al. 2013) and 99–100% similarity with *Armillaria* sp. collected from avocado trees in Michoacán State, México (GenBank accession numbers KU378660, KU378657, KU378654, KU378658, KU378655, KU378659; based on 56–87% coverage). However, partial 28S-IGS1-5S sequence comparisons with other *Armillaria* spp. sequences were limited because IGS1

sequences from the undescribed *Armillaria* sp. showed <5% similarity with any sequences in GenBank. Thus, any sequence similarities with other *Armillaria* spp. were based only on the 3′ partial 28S region. For example, sequences of the undescribed *Armillaria* sp. showed 90–94% similarity with *A. singula* J.Y. Cha & Igarashi (D89926), *A. mellea* (AY509188), and *A. altimontana* (AY509181), based on 12–30% coverage, which represented the 3′ end of 28S. Thus, phylogenetic analyses could not be conducted for 28S-IGS1-5S because sequences from the undescribed *Armillaria* sp. could not be aligned with sequences from other *Armillaria* spp.

18S-ITS-28S sequences of the undescribed *Armillaria* sp. (MEX87) showed 99–100% similarity, based on 52–54% coverage (which represented ITS2-28S), with isolates of the undescribed *Armillaria* sp. (KC111007, JX281813, JX281812, KC111008, JX281814, KC111009) reported by Elías-Román et al. (2013) from peach orchards in the State of Mexico (TABLE 2). However, 18S-ITS-28S sequences of the undescribed *Armillaria* sp. showed no close similarity with sequences from any of the 10 currently recognized *Armillaria* and *Desarmillaria* species in North America or species from Central and South America that were represented in GenBank. The closest sequences were from *A. mellea* of USA (AY213584, AY213586, AY213587) and Mexico (JX281807, JX281806, JX281808), with 93% similarity based on 45–52% coverage. Similarities of 90–93% with 26–31% coverage were observed with sequences of *A. affinis* (Singer) T. J. Volk & Burds. (JMER.126), *A. luteobubalina* Watling & Kile (DQ338562, DQ338558), *A. montagnei* (Singer) Herink (FJ711623), *A. puiggarii* (FJ618722), and *A. sparrei* (Singer) Herink (FJ618750). As with 28S-IGS1-5S, phylogenetic analyses of 18S-ITS-28S were also prevented because sequences could not be aligned with confidence.

Sequences of *TEF1* from the undescribed *Armillaria* sp. (MEX85, MEX87, MEX88) showed high similarity (100%) with the sequences of the undescribed *Armillaria* sp. (KC111016, KC111017, KC111018; based on 75% coverage) previously reported by Elías-Román et al. (2013). Similarities of 88–92% with 88–89% coverage were observed with *TEF1* sequences of nine annulate *Armillaria* species reported by Ross-Davis et al. (2012).

Phylogenetic analysis.—The *TEF1* data comprised 83 sequences with 510 characters, of which 16 sites were variable and 188 were informative for the parsimony analysis. The heuristic search resulted in 10 000 trees with a length of 504 steps, a consistency index (CI) of 0.573 and a retention index (RI) of 0.886. The analysis of *TEF1* sequences showed that *Desarmillaria* and *Armillaria* spp. were grouped into two major clades (FIG. 1). The first primary clade contained *Desarmillaria* (exannulate) species (*D. tabescens* and *D. ectypa*), and the second primary clade contained four major subclades that comprised 24 annulate *Armillaria* species from worldwide locations. One major subclade is composed of the undescribed *Armillaria* sp. (MEX40, MEX47, MEX60, MEX85, MEX87, MEX88) from Mexico and *A. mellea* isolates, which were divided into at least four subclades (FIG. 1). The undescribed *Armillaria* sp. clade appears distinct from, but phylogenetically adjacent and basal to, the *A. mellea* complex clades.

TAXONOMY

Armillaria mexicana R. Elías, Medel, Alvarado, Hanna, Ross-Davis, Kim, & Klopfenstein sp. nov.

(FIGS. 2–19)

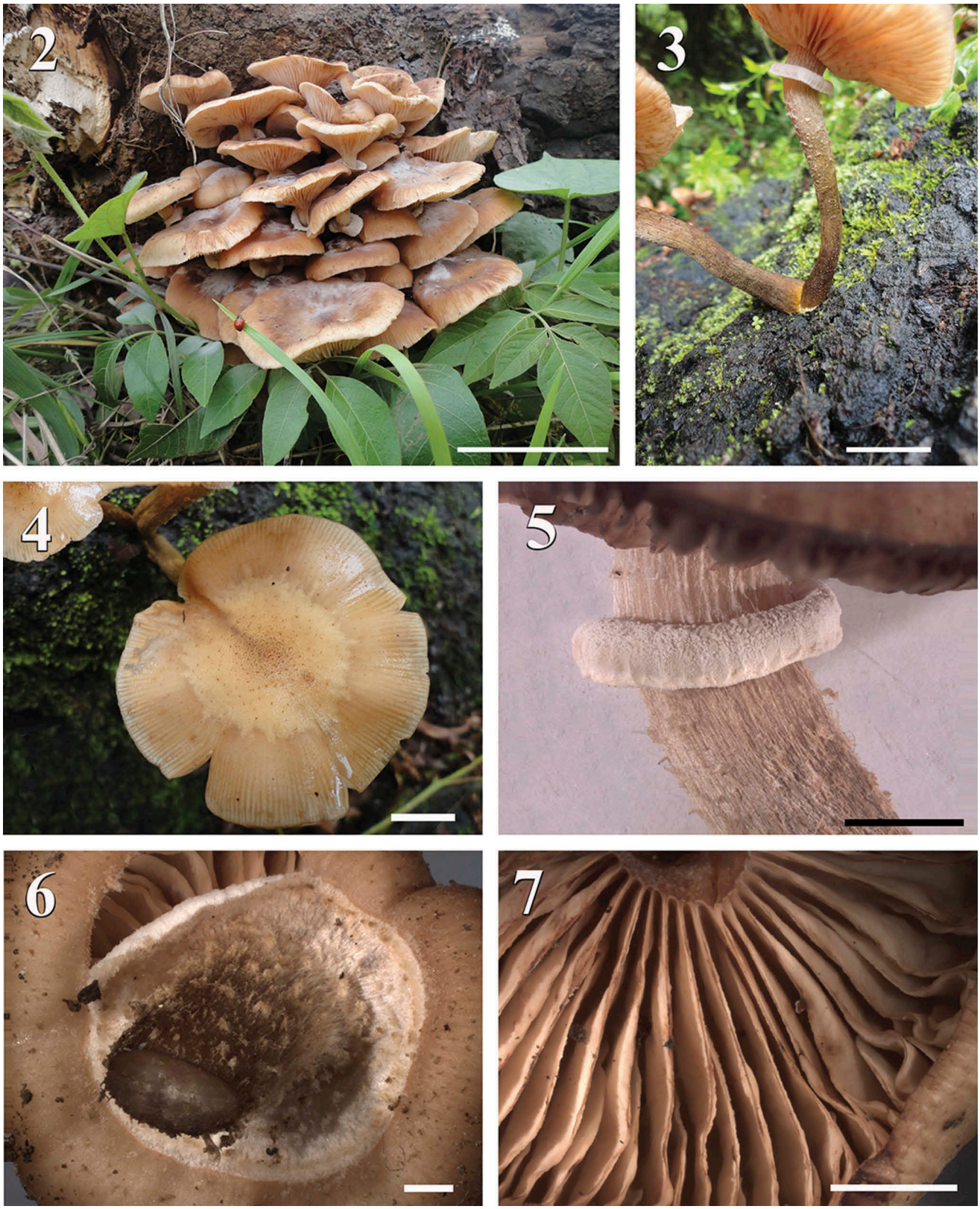
MycoBank MB814489

Typification: MEXICO. STATE OF MEXICO: Municipality Coatepec Harinas, Cruz de Piedra, road to San Pedro, found on a *Prunus persica* tree in a commercial orchard at 2200 m above sea level, 13 Jul 2011, *R.D. Elías-Román*, MEX87 (**holotype** *IZTA4595;* **isotype** XAL Mycological collection). GenBank: 18S-ITS-28S = KR061310; 28S-IGS1-5S = KR061306; *TEF1* = KR061314. Ex-type culture: CM-CNRG 399 = MEX87, derived from basidiome tissue.

Etymology: "*mexicana*," referring to its known distribution in the State of Mexico, Mexico. Common names of this fungus in Coatepec Harinas, State of Mexico, Mexico are "pata seca" and "escuape."

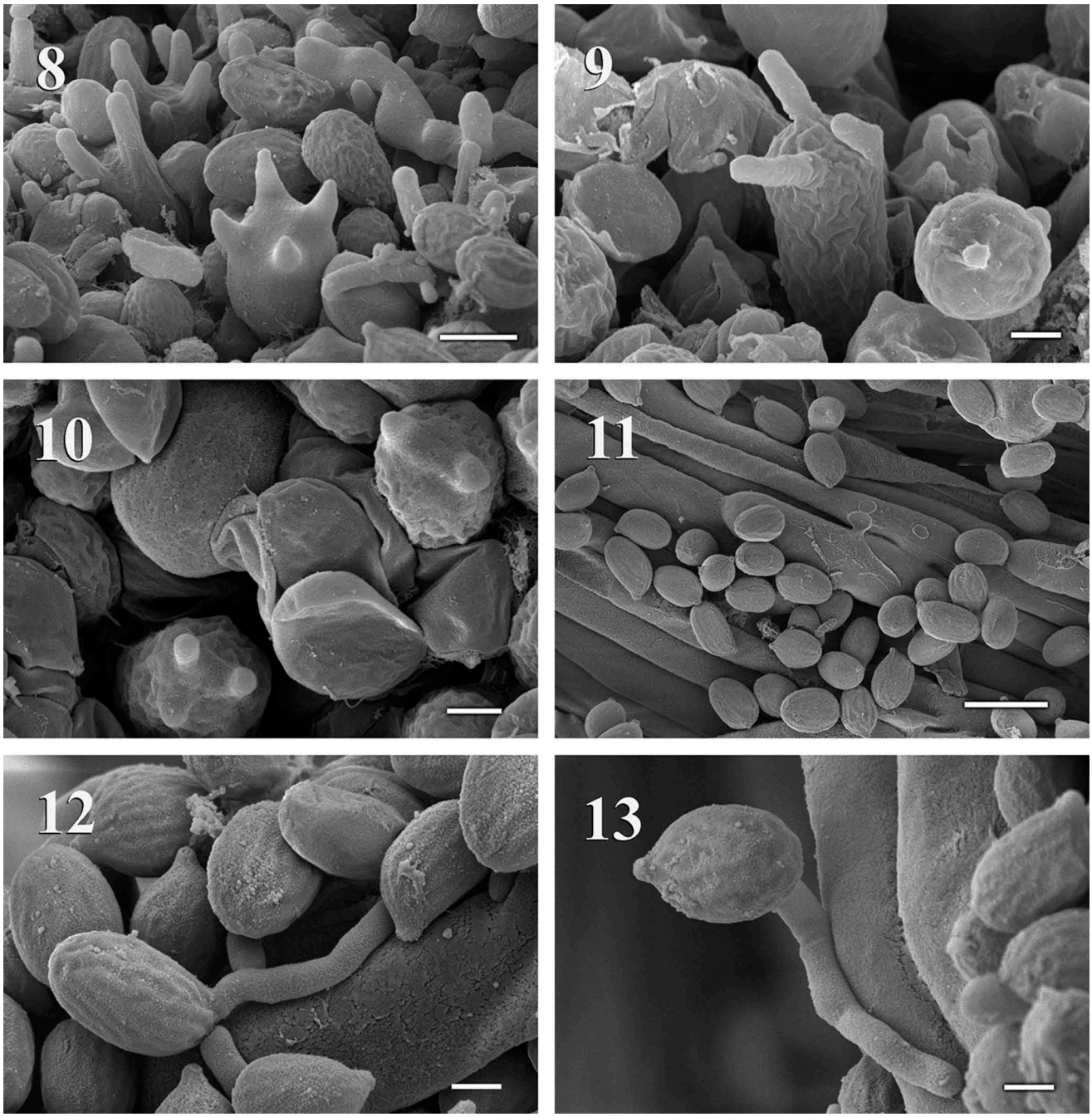
Diagnosis: Macroscopic characters that distinguish this species include color and shape of the pileus and stipe, presence of squamules on the pileus and a peronate annulus slightly striate on upper surface; absence of fibulae in the basidia and anywhere in the basidiomata; spores with a pattern of longitudinal ridges under SEM. This species can be identified by comparison of the 18S-ITS-28S D-domain, partial 28S-IGS1, and *TEF1* sequences. Additionally, *A. mexicana* has the largest ITS region reported thus far in fungi. Phylogenetic analysis based on *TEF1* sequences place *A. mexicana* in a well-separated, monophyletic clade that appears basal to the polyphyletic *A. mellea* complex.

Macromorphology: Pileus umbonate, convex or plane to plane-convex when mature, center slightly umbonate to depressed, orbicular in apical view, margin deflexed to lobed, edge entire to dentate, surface of the margin



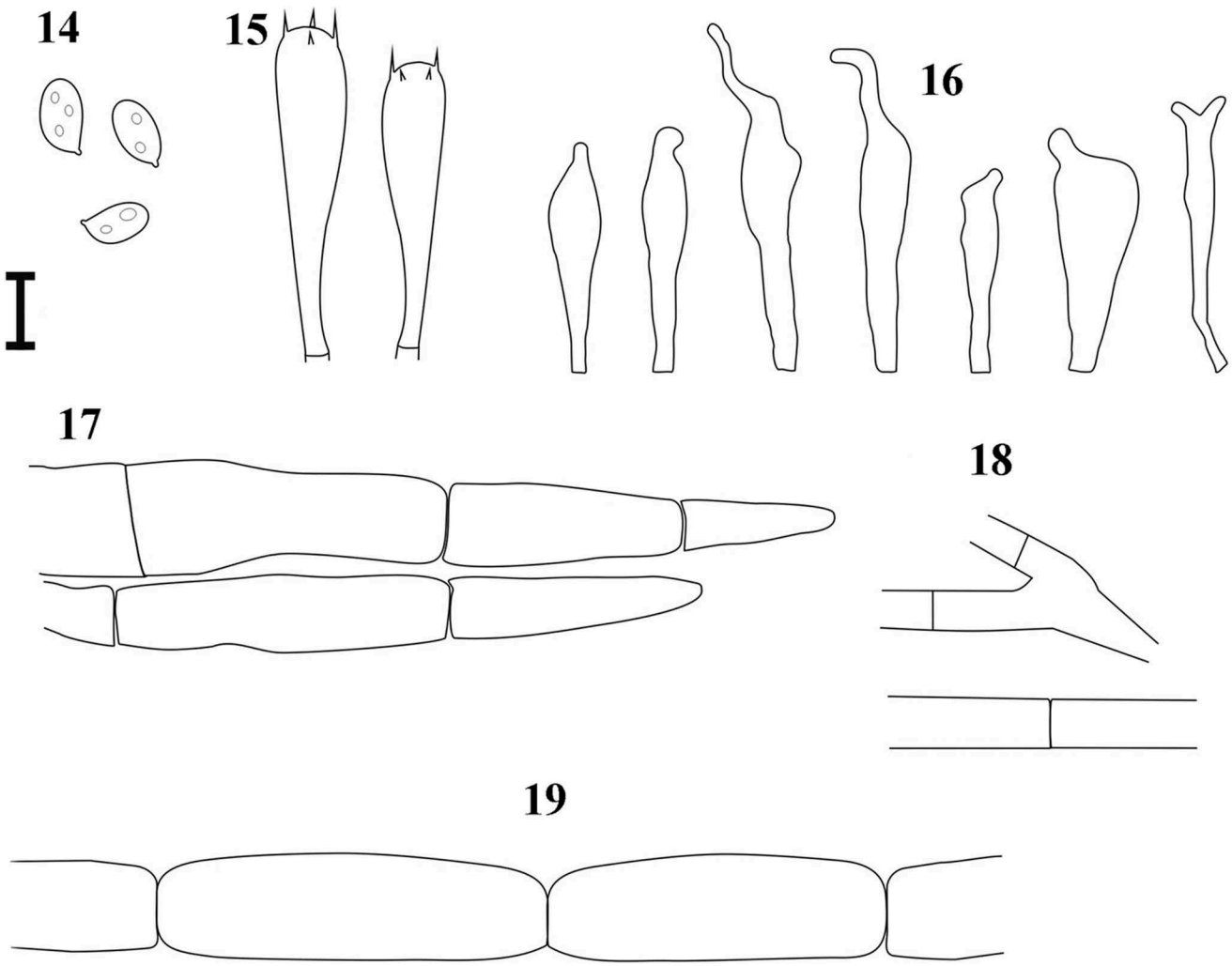
Figures 2–7. *Armillaria mexicana*. 2. Mature basidiomata (IZTA4597). 3. IZTA4598, lamellae, stipe, and annulus. 4. IZTA4598, pileus surface. 5–6. IZTA4598, annulus. 7. IZTA4598, lamellae. Bars: 2 = 5 cm; 3, 4 = 1 cm; 5 = 5 mm; 6 = 1 mm; 7 = 5 mm.

striate, pileus surface moderate orange yellow (164C in the center), and pale yellow (164D) at margin, 28–107 (–128) mm diam when fresh. Pileus with fibrillose squamules moderate brown (165A) dispersed toward the margin, hygrophanous. Lamellae close, decurrent, adnate, thick, smooth edges, 9 mm deep, light yellowish



Figures 8–13. *Armillaria mexicana* (IZTA4598). 8. 4-Spored basidium. 9. 3-Spored basidium. 10. Basidia, 2- and 3-spored. 11–12. Basidiospores on stipe. 13. Basidiospore germination on stipe. Bars: 8 = µm; 9, 10, 12, 13 = 2 µm; 11 = 10 µm.

pink (27B, 27C, and 27D) and pale yellowish pink (159D); lamellulae, attenuate, concolorous with lamellae, with smooth edges. Partial veil with rounded irregular scales on its under surface, light pink (49C), falling apart from the stipe forming an annulus. Annulus apical, persistent, simple, membranous, slightly striate on upper surface, peronate, light orange (27C), and pale yellowish pink (159C, 159D), 5–7 mm width. Stipe central, cylindrical, solid, slightly squarrose, pale greenish yellow (160C) and pale yellow (162D) scales, stipe color ranging from light orange (27C) under the annulus and light olive gray (197A), moderate olive brown (199A), and dark grayish reddish brown (200A) toward the base in fresh specimens, (39–)74–109 mm long × 5–8 mm wide in fresh specimens, base without rhizomorphs (although these were produced abundantly *in vitro*). Spore print white. Taste and odor in fresh samples not tested. Basidiome caespitose, gregarious, rarely solitary when growing in association with *Prunus persica* roots.



Figures 14–19. Microscopic features of *Armillaria mexicana* (IZTA4597). 14. Basidiospores. 15. Basidia. 16. Cheilocystidia. 17. Pileipellis hyphae. 18. Hyphae of inferior surface of the annulus (edge). 19. Hyphae of the superior surface (near stipe). Bars: 14–19 = 10 µm.

Micromorphology: Pileipellis of parallel hyphae 40–60 µm wide, hyphae 8–13 µm diam, hyaline to slightly yellowish, thin-walled, without clamp connections. Subpellis (hypodermium) 20–30 µm wide hyaline to slightly yellowish. Context pale pink (49D), 100–130 µm wide, hyaline hyphae, 6–10 µm diam, clamp connections absent. Hymenophoral trama regular, of hyaline hyphae, 5–10 µm diam, clamp connections absent. Stipe hyphae hyaline to slightly yellowish, constricted at the septum, cylindrical, 10–30 µm thick with scarce amber pigment, 31–47 × 20–27 µm. Hyphae of the superior surface (near stipe) of the annulus hyaline to slightly yellowish, thin-walled, septate, 28–55(–58) × (15–)17–25 µm; hyphae from inferior surface (edge) of the annulus of 7–10 µm long, without fibulae. Caulocystidia scarce, clavate, ventricose rostrate, hyaline, ca. 30 × 10 µm, thin-walled. Basidia clavate, with 2–4 sterigmata, hyaline, (32–)36–45 × 8–10 µm, mean 39.6 ± 3.9 × 8.9 ± 0.8 µm, thin-walled, without clamp connection at the base. Sterigmata 3.5–4.5 µm long, rarely up to 10 µm (under SEM). Basidiospores ellipsoidal, smooth under light microscope but ornamented with a slightly to moderately rugulose surface in a pattern of longitudinal ridges under SEM, hyaline, guttulate, 7–10(–12) × 5–7 µm, mean 8.7 ± 1.2 × 5.9 ± 0.6 µm, thin-walled, inamyloid. Cheilocystidia cylindrical, clavate to mucronate, some with a long neck hyaline, 20–40(–46) × (3–)4–7(–11) µm, thin-walled, abundant, lacking clamp connections at the base.

Habitat and distribution: On stem bases of dead peach trees in commercial orchards established on deforested land previously occupied by native forest *Pinus-Quercus* species, on dead *Quercus* sp., and on living olive (*Olea europaea*) in the State of Mexico.

Additional specimens studied: MEXICO. STATE OF MEXICO: Municipality of Coatepec Harinas, Cruz de

Piedra, in a commercial orchard at 2300 m above sea level (a.s.l.), on a dead *Prunus persica* tree, Jul 2009, *R. D. Elías-Román*, *IZTA4599*, XAL, (MEX85); Cruz de Piedra, road to San Pedro, in a commercial orchard at 2200 m a.s.l., on a dead *Prunus persica* tree, 13 Jul 2011, *R.D. Elías-Román*, *IZTA4597*, XAL, (CM-CNRG 365 = MEX88); on a dead oak (*Quercus* sp.) tree at 2200 m a.s.l., *R.D. Elías-Román*, *IZTA4598*, XAL, (MEX114); on a living olive (*Olea europaea*) tree at 2200 m a.s.l., *R.D. Elías-Román*, *IZTA4596*, XAL, (CM-CNRG 372 = MEX115).

DISCUSSION

Morphologically, the absence of fibulae or clamp connections in the basidia of *A. mexicana* clearly distinguish it from seven of nine currently recognized North American species of *Armillaria* (i.e., *A. calvescens, A. cepistipes, A. gallica, A. gemina, A. nabsnona, A. sinapina*, and *A. solidipes*) that possess fibulae in some or all basidia (Bérubé and Dessureault 1988, 1989; Volk et al. 1996; Burdsall and Volk 2008). The absence of fibulae in the basidia and anywhere in the basidiomata are typical of *A. mexicana*, but this character is also shared with *A. mellea* s.s. (Motta and Korhonen 1986; Termorshuizen and Arnolds 1987; Pegler 2000); however, the shape and color of the stipe, pileus coloration, and annulus structure that are found in *A. mellea* (Motta and Korhonen 1986; Termorshuizen and Arnolds 1987; Wingfield et al. 2010) differ in *A. mexicana* (TABLE 3; FIGS. 2–6). Our results show that the morphological differences between *A. mexicana* and *A. mellea* are mainly macromorphological and support a conclusion of Watling et al. (1982) that the taxonomic significance of the absence/presence of fibulae in basidia, as a character to separate *Armillaria* species, should be considered carefully.

Table 3. Comparative morphological characteristics of *Armillara mexicana* and other *Armillaria* species.

Element	*A. mexicana*	*A. mellea*[a]	*A. puiggarii*[b]	*A. affinis*[c]	*A. melleorubens*[d]
Pileus					
Shape	Umbonate when young, convex to planate, rarely infundibuliform at maturity	Convex to plane or broadly subumbonate	Convex, sometimes flattened to slightly subumbonate to umbonate center, eventually subapplanate	Convex, obtuse, then flattened; pileus center subdepressed and minutely squarrulose, the rest almost denuded; subviscid, transparently striate to slightly sulcate at pileus margin	—
Color	Moderate orange yellow and pale yellow	Dark honey color when young, nearly "amber yellow" fading to a weak yellow in age	Pale melleous beige or paler	Brown	Pileus pale brown, shaded to pink on edge
Size[f] (mm)	28.4–106.8(–128)	40–90	11–33	29–31	26–51
Stipe					
Shape	Cylindrical	Cylindrical, broad at apex	Often bulbous base	Cylindrical, stipe base not bulbous, rarely attenuate to the apex, generally 3 mm broad at the apex	Solid, slightly thickened at the base
Color	Light orange under the annulus and light olive gray and dark grayish reddish brown toward the base	Nearly white when young, yellowish to brown to olive when old	Whitish to almost concolorous with the pileus, fuscous in lower part	Brown below the annulus, fuliginous	—
Size[g] (mm)	(39–)73.9–109 × 4.6–7.74	45–80 long	30–50 × 2–3.5	42–43 × 4	38 × 6
Annulus	Membranous, slightly striate on upper surface	Thick, citron yellow and white	Subcortinoid to thin-membranous	Annuliform, white, thinly membranaceous	—
Basidia (µm)	(32–)36–45 × 8–10(–11)	—	14.5–29 × 6.2–9.3	24–26.8 × 5.5–7.2	28–39 × 7–10, 4-sterigmata
Clamp connection on basidia	Absent	Absent	Absent and present	Absent	Absent
Cheilocystidia (µm)	20–40(–46) × (3–)4–7(–11)	Not observed	14.5–32.5 × 9–12, scattered	20–32 × 6–9.5 abundant, polymorphic	Not mentioned
Spores (µm)	Cylindrical 7–10(–12) × 5–7 Slightly to moderately rugulose surface	6–7 × 8.5–12 Surface with irregular longitudinal ridges[h,i]	6.5–10.5 × 3.5–6	(6.5–)7–8(–9) × (4.5–)4.5–5.5(–6)	8–10 × 5.5–7.5 Minutely ornamented and appearing somewhat wrinkled in KOH
Distribution	State of Mexico, Mexico	Wide, Northern Hemisphere[e]	South America[e]	Montane zone of Central America (Costa Rica)	Cuba

[a]Motta and Korhonen (1986). [b]Singer (1970). [c]Singer (1989). [d]Baroni (1981). [e]Volk and Burdsall (1995). [f]Diameter. [g]Diameter × length. [h]Pegler and Young (1971). [i]Bennell et al. (1985).

The stipe form of *A. mexicana* distinguishes it from *A. altimontana*, which has a swollen stipe base (Brazee et al. 2012).

Another *Armillaria* species, *A. affinis*, reported by Singer (1989) in North America (Costa Rica and Caribbean region), has basidia without fibulae and that are smaller (24–26.8 × 5.5–7.2 µm) than *A. mexicana* basidia [(32–)36–45 × 8–10(–11) µm] (TABLE 3). Additionally, 28S sequences of *A. mexicana* are only 93% similar (31% coverage) to *A. affinis* (Moncalvo et al. 2002).

Regarding South American *Armillaria* species, *A. paulensis* Capelari has basidia without fibulae, cheilocystidia are present, characteristic pinkish lamellae, and stipe with variations ranging from pinkish toward the apex, grayish brown under the annulus, and olive at the base (Lima et al. 2008). *Armillaria paulensis* is phylogenetically close to *A. luteobubalina* (Pildain et al. 2009) and morphologically similar to *A. melleorubens* (Berk. & M. A. Curtis) Sacc. from Cuba (Lima et al. 2008), which has basidiospores with a pattern of ornamentation (Baroni 1981) similar to that of *A. mexicana* (TABLE 3; FIGs. 11–13). Pegler and Young (1971) documented irregular longitudinal ridges on the surface of *A. mellea* basidiospores, and Bennell et al. (1985) mentioned that several *Armillaria* species (e.g., *A. mellea, A. hinnulea* Kile & Watling, and *A. novae-zelandiae* (G. Stev.) Boesew.) have ornamented (irregularly scattered shallow hollows and longitudinal ridges) basidiospores under SEM. They also indicated that the variability of spore morphology depends on *Armillaria* species and the developmental maturity of spores, among other factors.

Other *Armillaria* species reported by Singer (1970) differ from *A. mexicana* by (i) bulbous stipes and basidia with or without fibulae (*A. tigrensis* (Singer) T. J. Volk & Burds.); (ii) 2–4-spored basidia with fibulae and gray cortinoid annulus (*A. yungensis* (Singer) Herink); and (iii) stipe usually subbulbous, membranous with ornate annulus (*A. procera* Speg.). Unfortunately, these three South American species were not part of our phylogenetic analysis (FIG. 1) because no DNA sequences were available in GenBank.

Armillaria mexicana also differs in macromorphological features from other species reported by Pildain et al. (2010) and Kile and Watling (1983) in the following respects: (i) stipe cylindrical and robust (*A. montagnei* (Singer) Herink); (ii) pileus with abundant, relatively large and dense squamules (*A. umbrinobrunnea* (Singer) Pildain & Rajchenb.); and (iii) pileus ornamented and scrobiculae (*A. griseomellea* (Singer) Kile & Watling).

The permanence of the annulus (after drying) is distinctive of some *Armillaria* taxa (e.g., *A. montagnei* and *A. mexicana*). In contrast, the annuli of *A. novae-zelandiae, A. sparrei*, and *A. umbrinobrunnea* break down during drying or only traces remain (Pildain et al. 2010). *Armillaria viridiflava* (Singer) T.J. Volk & Burds. is also reported to occur in South America (Volk and Burdsall 1995) and Spain (Singer 1989) and has abundant basidia with fibulae, which clearly distinguishes it from *A. mexicana*.

Phylogenetic analysis of *TEF1* sequences showed that *A. mexicana* clustered in a well-separated clade that appears basal to *A. mellea* groups. Similar phylogenetic structure was documented for *A. mexicana* isolates MEX40, MEX47, and MEX60 using Bayesian phylogenetic analyses of *TEF1* and partial 28S (3′ and 5′ ends) (Elías-Román et al. 2013), and for *A. mexicana* isolates MEX49, MEX85, MEX87, and MEX88 using neighbor-net and Bayesian phylogenetic analyses of *TEF1* (Klopfenstein et al. 2017). Thus, multiple phylogenetic analyses provide strong support that *A. mexicana* represents a distinct species within the Mellea superclade (Klopfenstein et al. 2017).

Additionally, successful sequencing of the ITS regions of the ex-type culture CM-CNRG 399 (MEX87; TABLE 2) reveals an ITS1 of 1299 bp and an ITS2 of 582 bp, which represent the largest fungal ITS regions reported thus far in fungi. Most fungal ITS regions have a total length of between 600 and 800 bp (Gardes and Bruns 1993; Platas et al. 2004). Previously reported extremes in fungi include an ITS1 of 833 bp in Xylariales (Platas et al. 2004) and size variation in the ITS of between 694 and 1480 bp in *Leccinum* (den Bakker et al. 2004). ITS lengths for other *Armillaria* species average <800 bp (Kim et al. 2006) compared with the 2036 bp we report for *A. mexicana* isolate MEX87 (TABLE 2). This newly discovered extreme ITS length of *A. mexicana*, and consequent extreme variation within the *Armillaria* genus, is akin to other organisms, such as ladybird beetles (Coleoptera: Coccinellidae) or downy mildew fungi (Peronosporaceae), considered to have extreme ITS sequences (von der Schulenburg et al. 2001; Thines 2007); however, the evolutionary implications of extreme ITS length are largely unexplored.

In conclusion, our morphological and DNA sequence–based phylogenetic analyses clearly separate *A. mexicana* from the nine currently recognized *Armillaria* species from North America and the other eight *Armillaria* species for which sequences are publicly available and that have been reported in Central and South America (Volk and Burdsall 1995; Lima et al. 2008; Pildain et al. 2010) but have not been found in North America. DNA sequence–based phylogenetic analyses also demonstrate that isolates of *A. mexicana* are quite distinct from *Armillaria* spp. reported in Europe, Asia, Africa, and Australia. DNA sequences

of the 18S-ITS-28S D-domain, partial 28S-IGS1, and *TEF1* can be used to confirm the identity of *A. mexicana*. This information should prove useful for continued studies to determine the full geographic distribution, host range, and ecological role of *A. mexicana*.

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LITERATURE CITED

Alvarado RD. 2007. Pudrición de raíz por *Armillaria*/*Armillaria* root rot. *Armillaria* (Fr.) Staude. (Agaricales, Marasmiaceae). In: Cibrián TD, Alvarado, García S, eds. Enfermedades forestales en México [Forest diseases in Mexico]. Universidad Autónoma Chapingo, Chapingo, México: Mundi-Prensa. p. 211–215.

Alvarado-Rosales D, Blanchette RA. 1994. *Armillaria* species from forests of central Mexico. Phytopathology 84:1106.

Anderson JB, Stasovsky E. 1992. Molecular phylogeny of Northern Hemisphere species of *Armillaria*. Mycologia 84:505–516, doi:10.2307/3760315

Anderson JB, Ullrich RC. 1979. Biological species of *Armillaria mellea* in North America. Mycologia 71:402–414, doi:10.2307/3759160

Antonín V, Tomšovský M, Sedlák P, Májek T, Jankovský L. 2009. Morphological and molecular characterization of the *Armillaria cepistipes–A. gallica* complex in the Czech Republic and Slovakia. Mycological Progress 8:259–271, doi:10.1007/s11557-009-0597-1

Baroni TJ. 1981. Collections in the Farlow Herbarium: new species of *Melanophyllum* and *Gastrocybe*, type studies on *Armillaria* and *Stropharia*. Mycologia 73:181–197, doi:10.2307/3759634

Baumgartner K, Coetzee MPA, Hoffmeister D. 2011. Secrets of the subterranean pathosystem of *Armillaria*. Molecular Plant Pathology 12:515–534, doi:10.1111/j.1364-3703.2010.00693.x

Bennell A, Watling R, Kile G. 1985. Ornamentation in *Armillaria* (Agaricales). Transactions of the British Mycological Society 84:447–455.

Bérubé JA, Dessureault M. 1988. Morphological characterization of *Armillaria ostoyae* and *Armillaria sinapina* sp. nov. Canadian Journal of Botany 66:2027–2034.

Bérubé JA, Dessureault M. 1989. Morphological studies of the *Armillaria mellea* complex: two new species: *A. gemina* and *A. calvescens*. Mycologia 81:216–225, doi:10.2307/3759703

Brazee NJ, Hulvey JP, Wick RL. 2011. Evaluation of partial tef1, rpb2, and nLSU sequences for identification of isolates representing *Armillaria calvescens* and *Armillaria gallica* from northeastern North America. Fungal Biology 115:741–749, doi:10.1016/j.funbio.2011.05.008

Brazee NJ, Ortiz-Santana B, Banik MT, Lindner DL. 2012. *Armillaria altimontana*, a new species from the western interior of North America. Mycologia 104:1200–1205, doi:10.3852/11-409

Burdsall HH Jr, Volk TJ. 1993. The state of taxonomy of the genus *Armillaria*. McIlvainea 11:4–11.

Burdsall HH Jr, Volk TJ. 2008. *Armillaria solidipes*, an older name for the fungus called *Armillaria ostoyae*. North American Fungi 3:261–267, doi:10.2509/naf2008.003.00717

Chillali M, Wipf D, Guillaumin J-J, Mohammed C, Botton B. 1998. Delineation of the European *Armillaria* species based on the sequences of the internal transcribed spacer (ITS) of ribosomal DNA. New Phytologist 138:553–561.

Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS. Kile GA, Wingfield MJ. 2001. Phylogenetic relationships of Australian and New Zealand *Armillaria* species. Mycologia 93: 887–896.

Coetzee MPA, Wingfield BD, Bloomer P, Ridley GS, Wingfield MJ. 2003. Molecular identification and phylogeny of *Armillaria* isolates from South America and Indo-Malaysia. Mycologia 95:285–293.

Coetzee MPA, Wingfield BD, Bloomer P, Wingfield MJ. 2005. Phylogenetic analyses of DNA sequences reveal species partitions amongst isolates of *Armillaria* from Africa. Mycological Research 109:1223–1234.

Coetzee MPA, Wingfield BD, Zhao J, van Coller SJ, Wingfield MJ. 2015. Phylogenetic relationships among biological species of *Armillaria* from China. Mycoscience 56:530–541, doi:10.1016/j.myc.2015.05.001

den Bakker HC, Gravendeel B, Kuyper TW. 2004. An ITS phylogeny of *Leccinum* and an analysis of the evolution of minisatellite-like sequences within ITS1. Mycologia 96:102–118, doi:10.2307/3761992

Dunne CP, Glen M, Tommerup IC, Shearer BL, Hardy GE St J. 2002. Sequence variation in the rDNA ITS of Australian *Armillaria* species and intra-specific variation in *A. luteobubalina*. Australasian Plant Pathology 31:241–251.

Elías-Román RD. 2013. Etiología, patrón espacio-temporal y análisis filogenético de *Armillaria* spp., en árboles de durazno [*Prunus persica* (L.) Batsch.] en el sur del Estado de México. Tesis de Doctor en Ciencias, Colegio de Posgraduados, Montecillo, Texcoco, Estado de México, México.

Elías-Román RD, Guzmán-Plazola RA, Klopfenstein NB, Alvarado-Rosales D, Calderón-Zavala G, Mora-Aguilera JA, Kim M-S, García-Espinosa R. 2013. Incidence and phylogenetic analyses of *Armillaria* spp. associated with root disease in peach orchards in the State of Mexico, Mexico. Forest Pathology 43:390–401, doi:10.1111/efp.12043

Farr DF, Rossman AY. 2014. Fungal Databases. Systematic Mycology and Microbiology Laboratory, Agricultural Research Service, US Department of Agriculture. [cited 2014 Apr 21]. Available from: http://nt.ars-grin.gov/fungaldatabases/

Gardes D, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118, doi:10.1111/j.1365-294X.1993.tb00005.x

Guo T, Wang HC, Xue WQ, Zhao J, Yang ZL. 2016. Phylogenetic analyses of *Armillaria* reveal at least 15 phylogenetic lineages in China, seven of which are associated with cultivated *Gastrodia elata*. PLoS ONE 11: e0154794, doi:10.1371/journal.pone.0154794

Hall TA. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Hasegawa E, Ota Y, Hattori T, Kikuchi, T. 2010. Sequence-based identification of Japanese *Armillaria* species using elongation factor-1 alpha gene. Mycologia 102:898–910, doi:10.3852/09-238

Hood IA, Ramsfield TD. 2016. *Armillaria aotearoa* species nova. New Zealand Journal of Forest Science 46:2, doi:10.1186/s40490-016-0058-y

Keča N, Bodles WJA, Woodward S, Karadžić D, Bojović S. 2006. Molecular-based identification and phylogeny of *Armillaria* species from Serbia and Montenegro. Forest Pathology 36:41–57.

Keča N, Solheim H. 2010. Ecology and distribution of *Armillaria* species in Norway. Forest Pathology 41:120–132.

Kile GA, Watling R. 1983. *Armillaria* species from south-eastern Australia. Transactions of the British Mycological Society 81:129–140, doi:10.1016/S0007-1536(83)80212-5

Kim M-S, Klopfenstein NB, Hanna JW, Cannon P, Medel R, López A. 2010. First report of Armillaria root disease caused by *Armillaria tabescens* on *Araucaria araucana* in Veracruz, México. Plant Disease 94:784, doi:10.1094/PDIS-94-6-0784B

Kim M-S, Klopfenstein NB, Hanna JW, McDonald GI. 2006. Characterization of North American *Armillaria* species: genetic relationships determined by ribosomal DNA sequences and AFLP markers. Forest Pathology 36:145–164.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth & Bisby's dictionary of the fungi, 10th ed. Wallingford, UK: CABI. 771 p.

Klopfenstein NB, Hanna JW, Cannon PG, Medel-Ortiz R, Alvarado-Rosales, D, Lorea-Hernández F, Elías-Román RD, Kim M-S. 2014. First report of the Armillaria root-disease pathogen, *Armillaria gallica*, associated with several woody hosts in three states of Mexico. Plant Disease 98:1280, doi:10.1094/PDIS-04-14-0349-PDN

Klopfenstein NB, Stewart JE, Ota Y, Hanna JW, Richardson, BA, Ross-Davis AL, Elías-Román RD, Korhonen K, Keča N, Iturritxa E, Alvarado-Rosales D, Solheim H, Brazee NJ, Łakomy P, Cleary MR, Hasegawa E, Kikuchi T, Garza-Ocañas F, Tsopelas P, Rigling, D, Prospero S, Tsykun T, Bérubé JA, Stefani FOP, Jafarpour S, Antonín V, Tomšovský M, McDonald GI, Woodward S, Kim M-S. 2017. Insights into the phylogeny of Northern Hemisphere *Armillaria*: neighbor-net and Bayesian analyses of translation elongation factor 1-α gene sequences. Mycologia 109:75–91, doi:10.1080/00275514.2017.1286572

Koch RA, Wilson AW, Séné O, Henkel TW, Aime MC. 2017. Resolved phylogeny and biogeography of the root pathogen *Armillaria* and its gasteroid relative, *Guyanagaster*. BMC Evolutionary Biology 17:33, doi:10.1186/s12862-017-0877-3

Lima MLA, Asai T, Capelari M. 2008. *Armillaria paulensis*: a new South American species. Mycological Research 112:1122–1128, doi:10.1016/j.mycres.2008.03.006

Maphosa L, Wingfield BD, Coetzee MPA, Mwenje E, Wingfield MJ. 2006. Phylogenetic relationships among *Armillaria* species inferred from partial elongation factor 1-alpha DNA sequence data. Australasian Plant Pathology 35:513–520, doi:10.1071/AP06056

Moncalvo J-M, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clemencon H, Miller OK Jr. 2002. One hundred and seventeen of CLADES of Euagarics. Molecular Phylogenetics and Evolution 23:357–400, doi:10.1016/S1055-7903(02)00027-1

Montoya A, Hernández-Totomoch O, Estrada-Torres A, Kong A, Caballero J. 2003. Traditional knowledge about mushrooms in a Nahua community in the State of Tlaxcala, México. Mycologia 95: 793–806, doi:10.2307/3762007

Montoya-Esquivel A, Estrada-Torres A, Kong A, Juárez-Sánchez L. 2001. Commercialization of wild mushrooms during market days of Tlaxcala, México. Micologia Aplicada International 13:31–40.

Motta JJ, Korhonen K. 1986. A note on *Armillaria mellea* and *Armillaria bulbosa* from the Middle Atlantic states. Mycologia 78:471–474, doi:0.2307/3793052

Mueller GM, Wu Q-X, Huang Y-Q, Guo S-Y, Aldana-Gomez R, Vilgalys R. 2001. Assessing biogeographic relationships between North American and Chinese macrofungi. Journal of Biogeography 28:271–281.

Mulholland V, MacAskill GA, Laue BE, Steele H, Kenyon D, Green S. 2012. Development and verification of a diagnostic assay based on EF-1α for the identification of *Armillaria* species in Northern Europe. Forest Pathology 42:229–238.

Ota Y, Kim M-S, Neda H, Klopfenstein NB, Hasegawa E. 2011. The phylogenetic position of an *Armillaria* species from Amami-Oshima, a subtropical island of Japan, based on elongation factor and ITS sequence. Mycoscience 52:53–58, doi:10.1007/S10267-010-0066-3

Pegler DN. 2000. Taxonomy, nomenclature and description of *Armillaria*. In: Fox RTV, ed. *Armillaria* root rot: biology

and control of honey fungus. Andover, UK: Intercept Press. p 81–93.
Pegler DN, Young TWK. 1971. Basidiospore morphology in the Agaricales. Nova Hedwigia 35:1–210.
Pérez-Silva E, Esqueda M, Herrera T, Coronado M. 2006. Nuevos registros de Agaricales de Sonora, México. Revista Mexicana de Biodiversidad 77:23–33.
Pildain MB, Coetzee MPA, Rajchenberg M, Petersen RH, Wingfield MJ, Wingfield BD. 2009. Molecular phylogeny of *Armillaria* from the Patagonian Andes. Mycological Progress 8:181–194, doi:10.1007/s11557-009-0590-8
Pildain MB, Coetzee, MPA, Wingfield BD, Wingfield MJ, Rajchenberg M. 2010. Taxonomy of *Armillaria* in the Patagonian forests of Argentina. Mycologia 102:392–403, doi:0.3852/09-105
Platas G, Ruibal C, Collado J. 2004. Size and sequence heterogeneity in the ITS1 of *Xylaria hypoxylon* isolates. Mycological Research 108:71–75, doi:10.1017/S0953756203008815
Redhead SA, Bérubé J, Cleary MR, Holdenrieder O, Hunt RS, Korhonen K, Marxmüller H, Morrison DJ. 2011. Proposal to conserve *Armillariella ostoyae* (*Armillaria ostoyae*) against *Agaricus obscurus Agaricus occultans* and *Armillaria solidipes* (*Basidiomycota*). Taxon 60:770–771.
Ross-Davis AL, Hanna JW, Kim M-S, Klopfenstein NB. 2012. Advances toward DNA-based identification and phylogeny of North American *Armillaria* species using elongation factor-1alpha gene. Mycoscience 53:161–165, doi:10.1007/S10267-011-0148-X
Royal Horticultural Society. 1986. Royal Horticultural Society colour chart. London: Royal Horticultural Society, and Leiden: Flower Council of Holland.
Shaw CG III. 1989. *Armillaria ostoyae* associated with mortality of new hosts in Chihuahua, Mexico. Plant Disease 73:775, doi:10.1094/PD-73-0775B
Singer R. 1970. A monograph of the subtribe Omphalinae. Flora Neotropica Monograph No. 3 Omphalinae (Clitocybeae-Tricholomataceae-Basidiomycetes), 84 p.
Singer R. 1989. New taxa and new combinations of Agaricales (Diagnoses fungorum novorum agaricalium IV). Fieldiana (Bot.) New series 21. 133 p.
Swofford D. L. 2002 PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4]. Sinauer Associates, Sunderland, Massachusetts.
Termorshuizen AAD, Arnolds EEF. 1987. On the nomenclature of the European species of the *Armillaria mellea* group. Mycotaxon 30:101–116.
Thines M. 2007. Characterisation and phylogeny of repeated elements giving rise to exceptional length of ITS2 in several downy mildew genera (Peronosporaceae). Fungal Genetics and Biology 44:199–207, doi:10.1016/j.fgb.2006.08.002
Tsykun T, Rigling D, Prospero S. 2013. A new multilocus approach for reliable identification of *Armillaria* species. Mycologia 105:1059–1076.
Villareal L, Pérez-Moreno J. 1989. Los hongos comestibles silvestres de México, un enfoque integral. Micologia Neotropical Aplicada 2:77–114.
Volk TJ, Burdsall HH Jr. 1995. A nomenclatural study of *Armillaria* and *Armillariella* species (Basidiomycotina, Tricholomataceae). Synopsis Fungorum 8. Oslo, Norway: Fungiflora. 121 p.
Volk TJ, Burdsall HH Jr, Banik MT. 1996. *Armillaria nabsnona*, a new species from western North America. Mycologia 88:484–491, doi:10.2307/3760888
von der Schulenburg JHG, Hancock JM, Pagnamenta A, Sloggett JJ, Majerus MEN, Hurst GDD. 2001. Extreme length and length variation in the first ribosomal internal transcribed spacer of ladybird beetles (*Colleoptera: Coccinellidae*). Molecular Biology and Evolution 18:648–660, doi:10.1093/oxfordjournals.molbev.a003845
Watling R, Kile GA, Burdsall HH Jr. 1991. Nomenclature, taxonomy, and Identification. In: Shaw CG III, Kile GA, eds. *Armillaria* root disease. Agricultural Handbook No. 691. Washington, DC: USDA Forest Service. p. 1–9.
Watling R, Kile GA, Gregory NM. 1982. The genus *Armillaria*—nomenclature, typification, the identity of *Armillaria mellea* and species differentiation. Transactions of the British Mycological Society 78:271–285, doi:10.1016/S0007-1536(82)80011-9
Wingfield MJ, Coetzee MPA, Crous PW, Six D, Wingfield BD. 2010. Fungal phoenix rising from the ashes? IMA Fungus 1:149–153, doi:10.5598/imafungus.2010.01.02.06