

North American matsutake: Names clarified and a new species described

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SHORT TITLE: North American matsutake

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described**

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ABSTRACT

Tricholoma matsutake, known widely as “matsutake,” has great commercial and cultural significance in Japan. Because Japanese production is insufficient to meet the high domestic demand, morphologically similar mushrooms, thought by many to belong to *T. magnivelare*, are imported from western North America. However, molecular data produced since the early 2000s have indicated that more than one species of matsutake occur in North America and this raises the question of correct naming for the different species. To address this question, we assessed the phylogenetic diversity within North American matsutake based on nuc rDNA ITS1-5.8S-ITS2 (ITS barcode) sequences, including newly obtained sequences from the type collections for *Agaricus ponderosus* and *Armillaria arenicola*, and morphological characters. Our results agree with earlier indications that three matsutake species occur in North America and allow us to clarify the correct application of names—*T. magnivelare* from the eastern USA and Canada, *T. murrillianum* from the western USA and Canada, and *T. mesoamericanum* from Mexico, newly described here. The existence of the three North American species is further supported by the results of evolutionary divergence analysis, geographical distributions, and morphological characters.

KEY WORDS: *Agaricus magnivelaris*, *Armillaria ponderosa*, commercial mushroom harvesting, molecular phylogenetics, North American mycoflora, pine mushroom, *Tricholoma ponderosum*

INTRODUCTION

Tricholoma matsutake (S. Ito & S. Imai) Singer is an ectomycorrhizal fungus with great commercial and cultural significance in Japan. Known widely as “matsutake” (“pine mushroom” in Japanese), it is a large firm mushroom with a characteristic spicy odor that contributes greatly to its desirability. As a result of significantly reduced production from Japan’s declining native pine forests, a substantial import market has developed to meet the demand for the mushroom (Hosford et al. 1997; Redhead 1997; Wang et al. 1997). The imported mushrooms include *T. matsutake* from Korea and China, and similar species from western North America (Redhead 1997; Wang et al. 1997). The latter were thought to belong to *T. magnivelare* (Peck) Redhead (Hosford et al. 1997; Redhead 1997; Zamora-Martínez and Nieto de Pascual-Pola 2004) and are usually referred to in the USA/Canadian commercial trade as “matsees/matsis,” “pine mushrooms,” or “pines,” and “hongo blanco de ocote” in Mexico. *Tricholoma magnivelare* is included in the “matsutake group” (Ota et al. 2012), along with *T. matsutake*, *T. anatolicum* H.H. Doğan & Intini, and an until-now-undescribed species from Mexico. Together, these form a monophyletic group in phylogenetic analyses and share similar macro- and micromorphology. All also share the characteristic “matsutake” odor, with the possible exception of *T. anatolicum*, whose odor has been described as being that of extract of *Cedrus libani* (Intini et al. 2003). The matsutake group is classified in *Tricholoma* Section *Caligata* Bon, along with species such as *T. bakamatsutake* Hongo, *T. fulvocastaneum* Hongo, *T. dulciolens* Kytövuori, *T. ilkkæ* Mort.Chr., Heilm.-Claus., Ryman & Niclas Bergius, and *T. caligatum* (Viv.) Ricken.

The taxonomic history of the matsutake group begins with the description of *Agaricus* (*Armillaria*) *ponderosus* by Peck (1873) based on a specimen collected in New York state. After finding that this name was illegitimate, he corrected it to *Agaricus* (*Armillaria*) *magnivelaris* (Peck 1878). Saccardo (1887) transferred it to *Armillaria* as *A. ponderosa* and Singer (1949) transferred it to *Tricholoma* as *T. ponderosum* and it is by these names that American matsutake were known for most of the 20th century. However, both of those names are illegitimate and Redhead (1984) concluded that the correct basionym for the species is *Agaricus magnivelaris* Peck and transferred it to *Tricholoma* as *T. magnivelare*.

Based on a collection made along the immediate coast near Newport, Oregon, Murrill (1912) described a new species, *Armillaria arenicola*, which Singer (1942) transferred to *Tricholoma* as *T. murrillianum*. In his original description, Murrill stated that “In general appearance, it resembles *Armillaria magnivelaris* Peck.” In his notes that accompany the holotype, he stated “General style of *Armillaria magnivelaris* Peck but cannot be same.” Thus, Murrill considered his western material to constitute a similar, but separate, species from Peck’s eastern species. Kauffman (1922) also noted the similarity of the western and eastern material, but he too treated them as separate species.

Zeller and Togashi (1934) made a comparison of matsutake collections from the Pacific Coast of North America (Oregon and Washington) with collections from Japan to determine whether the material from the two regions represented one species, as suggested by some western American collectors. They concluded that the Japanese and American matsutake should be considered separate, although similar, species. Furthermore, they concluded that American matsutake from western and eastern North America should be considered one

species and synonymized *A. arenicola* with *A. ponderosa* (*A. magnivelaris*). However, there is no indication in their paper that either author examined any eastern material in fresh condition, making it likely that their concept of Peck's species was based only on the brief published descriptions, which are similar for the two taxa (cf Peck 1873; Murrill 1912). A small piece of a pileus from Peck's holotype exists in OSC and probably represents the extent of Peck's New York type specimen, and perhaps eastern matsutake material in general, examined by Zeller and Togashi (1934). From that time forward, North American authors (i.a., Hotson 1940; Smith 1949; Miller 1972; Redhead 1984; Hosford et al. 1997) accepted the synonymy and considered that "American matsutake" consisted of a single species—*Armillaria ponderosa* (*A. magnivelaris*, *T. magnivelare*).

Early molecular analyses of species in the matsutake group focused on assessing whether local species were the same as *T. matsutake* (Bergius and Danell 2000; Lim et al. 2003). Chapela and Garbelotto (2004) took a broader view, using ITS sequencing of a worldwide sample to attempt to define the presence and geographic distribution of phylogenetic groupings within matsutake and species traditionally regarded as its close relatives, such as *T. caligatum*. They stated that, "Our evidence points consistently to the existence of three main groups of matsutake: western North America (WNA), Mesoamerica (MX) and Asia-Europe-North Africa-eastern North America (CB), with further substructuring within each group. These groups do not coincide with currently used nomenclature." These conclusions were generally supported and refined by subsequent studies (Matsushita et al. 2005; Ota et al. 2012; Murata et al. 2013). This led to publication of *T. anatolicum*, from Turkey and North Africa, as a separate species (Intini et al. 2003, 2015) and discussion of potential impacts on the application

of species concepts in North America (e.g., Kuo 2006; Bravi and Voitek 2011; Bunyard 2013; Voitek 2014; Siegel 2016). Results of more recent molecular analyses (Ovrebo et al. 2009; Gulden et al. 2014) provided further support for the existence of eastern, western, and Mexican species in North America but, because the original descriptions are very brief and molecular information from type collections was lacking, knowing which names to apply to the different species had not been possible.

Our main objectives were to assess the phylogenetic diversity and clarify the names within North American matsutake based on ITS sequences and morphological characters, including newly obtained sequences from the type collections for *Agaricus ponderosus* and *Armillaria arenicola*. Specifically, we sought to determine whether *T. magnivelare* and *A. arenicola* should be considered the same, or different, species and, if different, what the correct name for each species should be. Additionally, we formally describe the new species that previous studies had indicated exists in Mexico to complete the current picture for North America.

MATERIALS AND METHODS

The type specimens of *Agaricus ponderosus* (*Armillaria magnivelaris*, *T. magnivelare*) and *Armillaria arenicola* were obtained from NYS and NY, respectively, including permission to sample the collections for sequencing. Nine more recent exsiccates also were analyzed, including six from eastern Canada (three each from Quebec and Newfoundland) and one each from Massachusetts, Oregon, and Mexico. Information about these samples is presented in

SUPPLEMENTARY TABLE 1.

We targeted the two internal transcribed spacers nuc rRNA region (nuc rDNA ITS1-5.8S-ITS2 = ITS), commonly used for fungal species identification and representing the universal fungal barcode (Schoch et al. 2012). Our use of ITS also was dictated by the very limited availability in public databases, for the matsutake group and related species, of sequences from other loci.

DNA extraction and ITS sequencing.—The genomic DNA from the nine recent exsiccates was extracted using the CTAB protocol described by Xu et al. (1994). To obtain ITS sequences from these samples, we first amplified this locus through PCR using the universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990), following the PCR protocol described by Xu et al. (2000). Clean PCR products were then sequenced using both forward and reverse primers by the Sanger method with ABI3700.

Because of degradation of their DNA associated with age and long-term storage, the *Agaricus ponderosus* and *Armillaria arenicola* type specimens had to be treated differently from more recent samples. Genomic DNA was extracted with a High Pure PCR Template Preparation Kit from Roche Applied Science (Germany) following the manufacturer's protocol. PCR amplification and sequencing for these two specimens used two separate sets of primer pairs that amplified smaller sections within the ITS region: (i) ITSOF (5′-ACTTGGTCATTTAGAGGAAGT-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) (Tedersoo et al. 2008; White et al. 1990); (ii) 58SF (5′-ATGCATCGATGAAGAACGC-3′) and ITS4B (5′-CAGGAGACTTGTACACGGTCCAG-3′) (Tedersoo unpublished; Gardes and Bruns 1993). Each PCR reaction sample included 5x HOT FIREPol® Blend Master Mix Ready to Load (with 10 mM MgCl₂; Solis BioDyne, Estonia), 0.4 μmol of each primer, and 5 μL of DNA solution, in a total volume of 25 μL. The PCR amplification and

purification followed the protocols described by Saar and Voitk (2015). Sequences from these two samples were obtained by MacroGen Europe (the Netherlands) using primers ITS5 (5′–GGAAGTAAAAGTCGTAACAAGG–3′), ITS2, ITS3 (5′–GCATCGATGAAGAACGCAGC–3′), and ITS4 (White et al. 1990).

For each ITS fragment, the forward and reverse sequences were inspected and assembled using SEQUENCHER 5 (Gene Codes, Michigan). All sequences were deposited in GenBank, and those from the two type collections also were deposited in UNITE.

Phylogenetic analysis.—Additional sequences identified during review of previous studies and others similar to our sequenced specimens were retrieved from GenBank, using the BLASTn search option, to assemble a dataset that represents the broad geographic distribution of the matsutake group species as well as representative species from outside the group. We set the E-value cutoff parameter at values $<10^{-10}$ and only included sequences with query coverage $\geq 98\%$. *Tricholoma bakamatsutake* was chosen as outgroup for the analyses because previous studies showed it to be a member of Section *Caligata*, but not of the matsutake group (Matsushita et al. 2005; Ota et al. 2012; Murata et al. 2013).

The sequences were aligned using MAFFT 7 (Kato and Toh 2008; <http://mafft.cbrc.jp/alignment/server/>), using the Q-INS-i strategy. The alignments were edited and manually corrected using ALIVIEW (Larsson 2014). The alignment file is deposited in TreeBASE under accession number S19397. The aligned sequence set was analyzed using two methods: (i) maximum likelihood analyses (ML) were run using RAXML 8.2.8 (Stamatakis 2014), under a GTR model with 100 rapid bootstrap replicates; (ii) Bayesian inference (BI) analyses were run using MRBAYES 3.2.6 (Ronquist et al. 2012) for 10 million generations, under a GTR

model, with four chains, and trees sampled every 100 generations. The initial burn-in phase was set to 2.5 million generations and, after examining the graphic representation of the likelihood scores of the sampled trees, that was confirmed to be an adequate value for all datasets. A 50% majority-rule consensus tree was computed using the remaining trees. Both ML and BI analyses were run at the CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/>).

Estimation of evolutionary divergence.—The number of base substitutions per site from averaging over all sequence pairs between groups was determined using the Kimura 2-parameter model (Kimura 1980) within MEGA6 (Tamura et al. 2013). The analysis included 83 nucleotide sequences. Ambiguous positions for each sequence pair were removed and there were 694 positions in the final dataset.

Measurement of spores from the Tricholoma magnivelare and T. murrillianum type collections.—To eliminate inter-observer variation, all spore measurements were carried out by the first author. Fifty spores each from the NYS holotype and OSC isotype of *T. magnivelare*, and 75 (a larger sample could be sacrificed because of the greater volume of material in this collection compared to the *T. magnivelare* type collections) from the NY holotype of *A. arenicola* were measured. Bits of gill tissue (the collections do not include spore prints) were mounted in 5% KOH and examined using an American Optical One-Hundred Microstar compound microscope. Q values were calculated as length/width for each spore. Mean values for length, width, and Q are reported ± 1 standard deviation.

Abbreviations and color codes.—Herbarium abbreviations follow those in *Index Herbariorum* (Thiers, continuously updated). Alphanumeric color codes follow Kornerup and Wanscher (1978). Spore measurements for the type collection of *T. mesoamericanum* are given as [total

number of spores measured / number of sporocarps from which spores were measured /
number of collections from which sporocarps were obtained].

RESULTS

ITS sequences.—We successfully obtained full ITS sequences from 10 specimens and an incomplete sequence, containing only ITS-1, from one specimen (*A. arenicola*). The ML and BI analyses returned five clades in the matsutake group (FIG. 1), with mean pairwise Kimura-2-parameter distances between the clades being three to more than 10 times greater than those from within individual clades (TABLE 1). One clade is represented by a large number of specimens of *T. matsutake* from Europe and Asia, and one collection from the southeastern USA. A second clade is represented by specimens of *T. anaticum* from the Mediterranean region. The three remaining clades are represented exclusively by samples from North America—one group from eastern USA/Canada, one from western USA/Canada, and the third from Mexico. Based on the inclusion of Peck's type collection in the eastern clade, those samples represent *Tricholoma magnivelare* (Peck) Redhead. Based on the inclusion of Murrill's type collection in the western clade, those samples represent *Armillaria arenicola*, now correctly called *Tricholoma murrillianum* Singer. The third clade includes samples from Mexico and represents the new species, *Tricholoma mesoamericanum*, described below.

Spore measurements.—Spores from the *Agaricus ponderosus* / *T. magnivelare* and *Armillaria arenicola* / *T. murrillianum* type collections were nearly the same in size and shape (TABLE 2).

TAXONOMY

Descriptions of the three North American species are provided below.

Tricholoma magnivelare (Peck) Redhead, *Trans Mycol Soc Jpn* 25:6. 1984. FIG. 2F, G.

Typification: USA. NEW YORK: Copake, Columbia Co., Oct (1872?), *CH Peck* (holotype NYS f2421!; isotype OSC 5598!). GenBank / UNITE accession: ITS = LT220177 / UDB024110.

≡ *Agaricus magnivelaris* Peck, *Ann. Rep. NY St. Mus. Nat. Hist.* 29:66. 1878 [1875]

≡ *Agaricus ponderosus* Peck, *Bull Buffalo Soc. Nat. Sci.* 1: 42. 1873 [“1873–1874”]. nom. illegit. (ICBN Art. 53.1)

≡ *Armillaria magnivelaris* (Peck) Murrill, *N. Amer. Fl. (New York)* 10:37. 1914.

≡ *Armillaria ponderosa* Sacc., *Syll. fung. (Abellini)* 5:78. 1887. nom. illeg. (ICBN Art. 52.1, 52.2, see Ex. 4)

≡ *Tricholoma ponderosum* (Sacc.) Singer, *Lilloa* 22:227. 1951 [“1949”]. nom. illeg. (ICBN Art. 52.1, 52.2, see Ex. 4)

Misapplied name: *Tricholoma matsutake*

Description: Pileus 50–200+ mm diam, convex when young, expanding to plano-convex; surface smooth to scaly and dry, beige to tan with light brown radial fibrils, disc usually slightly darker than margin, browning more with age, dry to slightly viscid; margin inrolled under the partial veil when young, expanding and mostly losing the veil fibrils with age. Lamellae close, sinuate, whitish when young, darkening to yellowish tan and spotting or bruising reddish brown with age, margin concolorous, entire. Stipe 40–250 × 20–50 mm, cylindrical, tapered toward base, surface dry or slightly viscid, white above annulus, concolorous with cap below. Partial veil forming a membranous annulus attached at midpoint of stipe or higher, persistent or

wholly or in part fugacious, often flaring upward. Context white, firm; odor fragrant with distinctive spicy character, taste mild.

Basidiospores white in mass, smooth, non-amyloid, $5\text{--}7.5 \times 3.5\text{--}5.5 \mu\text{m}$, mean $6.1 \times 4.5 \mu\text{m}$; $Q = 1.1\text{--}1.8$, mean 1.4, subglobose to mostly ellipsoidal. Basidia $32\text{--}40 \times 6.5\text{--}7.5 \mu\text{m}$, 4-spored, clavate. Lamella margin fertile with basidia intermixed with basidioles, the latter sometimes narrow and possibly representing cheilocystidia, pleurocystidia absent. Lamella trama parallel, composed of hyaline cylindrical hyphae with thin, smooth walls, $3\text{--}19 \mu\text{m}$ wide. Pileipellis a cutis, composed of hyphae $3\text{--}10 \mu\text{m}$ wide, hyaline. Stipitipellis a cutis; hyphae up to $11 \mu\text{m}$ wide; hyaline to pale yellow; with thin, smooth walls. Partial veil composed of cylindrical hyphae up to $12 \mu\text{m}$ wide; hyaline; with thin to slightly thickened, smooth walls. Clamp-connections absent.

Ecology and distribution: Solitary, scattered, or gregarious; locally common in often sandy soil under conifers in Pinaceae, usually including pine (e.g., *Pinus banksiana*, *P. resinosa*, *P. rigida*) and/or *Tsuga canadensis*; reported most commonly from northeastern USA and southeastern Canada, extending at least as far west as Michigan, where it occurs with *P. banksiana*, and as far south as North Carolina, where it occurs with *T. canadensis*; quite likely occurs in Ontario and farther west (perhaps following the distribution of *P. banksiana* to Northwest Territories), but its westward extent in the Canadian boreal forest and geographic relationship to *T. murrillianum* are uncertain. Peak fruiting typically in late Sep.

Representative illustrations: Gulden et al. 2014 (title banner; FIGS. 1, 4C; all as *T. matsutake*); Millman 2011; Spahr 2009; Voitk 2007.

Notes: *Tricholoma magnivelare* is best distinguished from *T. matsutake* by its North American distribution, the paler coloration of its sporocarps, an overall light tan as opposed to the darker reddish or blackish brown tones in the Eurasian species, and its smaller spores. Compared to the usually white *T. murrillianum*, *T. magnivelare* is generally darker and has a more consistently scaly pileus surface. Their ITS sequences and relative eastern versus western distributions also distinguish the latter two species.

Tricholoma murrillianum Singer, *Lloydia* 5:113. 1942.

FIG. 2H, I.

Typification: USA. OREGON: Newport, Lincoln Co., 13 Nov 1911, WA Murrill 1044 (holotype NY 586560!). GenBank / UNITE accession: ITS = LT220179 / UDB024112.

≡ *Armillaria arenicola* Murrill, *Mycologia* 4:212. 1912.

non *Tricholoma arenicola* (Murrill) Murrill, *Mycologia* 5: 223. 1913.

Misapplied name: *Tricholoma magnivelare* auct.

Description: Pileus 50–210 mm diam, convex or broadly umbonate when young, expanding to plano-convex, margin sometimes uplifted in age; surface dry to subviscid, shining to dull when dry, smooth or becoming slightly fibrillose, at first whitish, disc and fibrils becoming ochraceous to brownish with age; margin inrolled under the partial veil, white to pale cream, sometimes with attached veil remnants, expanding and mostly losing the veil remnants with age. Lamellae adnexed to sinuate, close to crowded, white to pale cream, spotted orange- to red-brown in age or when bruised; margin concolorous, entire. Stipe 60–150 × 20–40 mm, cylindrical, tapered towards the base; surface white and furfureous above the annulus, concolorous with pileus below the annulus, becoming orange- to red-brown with age or where bruised. Partial veil forming a white, cottony, membranous annulus in the upper part, flaring

upwards at first and then becoming appressed, with brown spots in age. Context white, firm; odor fragrant, with somewhat spicy (cinnamon) character, taste mild.

Basidiospores white in mass, smooth, non-amyloid, $5\text{--}7.5 \times 3.5\text{--}5.5 \mu\text{m}$, mean $5.9 \times 4.6 \mu\text{m}$; $Q = 1.1\text{--}1.6$, mean 1.3, ellipsoidal. Basidia $30\text{--}43 \times 6.0\text{--}7.0 \mu\text{m}$, 4-spored, clavate. Cheilocystidia and pleurocystidia absent; lamella edge fertile with basidia intermixed with basidioles. Lamella trama parallel, composed of hyaline cylindrical hyphae with thin, smooth walls, mostly $6\text{--}10 \mu\text{m}$ wide. Pileipellis a cutis, composed of hyphae $3.5\text{--}10 \mu\text{m}$ wide, hyaline to pale yellow. Stipitipellis a cutis; composed of hyphae up to $10 \mu\text{m}$ wide; hyaline; with thin, smooth walls. Partial veil composed of cylindrical hyphae up to $10 \mu\text{m}$ wide; hyaline; with thin, smooth walls. Clamp-connections absent.

Ecology and distribution: Solitary to gregarious, sometimes cespitose, often in sandy soils with a cover of mosses, fruticose lichens, or leaf litter and with only a few cm of the pileus exposed while the stipe is buried; can be quite abundant locally; along the coast especially common in open pine (*Pinus contorta* var. *bolanderi* or other pine species, typically with serotinous cones) woodlands, elsewhere with a wide variety of conifers in Pinaceae (i.e., *Abies magnifica*, *Pseudotsuga menziesii*, and *Tsuga heterophylla*) as well as a variety of angiosperm trees and shrubs such as *Arbutus menziesii*, *Arctostaphylos* spp, and *Notholithocarpus densiflorus*; mycelium can be parasitized by the mycoheterotrophic plant, *Allotropia virgata*; most abundant in British Columbia, Washington, Oregon, and northern California, extending south at least to Santa Barbara Co., also occurring farther east in Canada (at least to Alberta) and southward in the Rocky Mountains. Generally fruiting after the onset of cold weather in late fall in the north, later farther south.

Representative illustrations: Arora 1986 (as *A. ponderosa*); Arora 1991 (as *T. magnivelare*); Bessette et al. 2013 (as *T. magnivelare*); Davis et al. 2012 (as *T. magnivelare*); Desjardin et al. 2015 (as *T. magnivelare*); Siegel and Schwarz 2016 (as *T. magnivelare*; but see their discussion of the name, *T. murrillianum*); Smith 1949, 1975 (as *A. ponderosa*); Trudell and Ammirati 2009 (as *T. magnivelare*).

Notes: The very white overall color of *T. murrillianum*, especially in young specimens, tendency for the pileus surface to be less scaly, ITS sequence, and western North American distribution distinguish it from *T. matsutake* and *T. magnivelare*. The tendency to develop reddish brown stains on the gills and elsewhere when handled and in age can make older specimens difficult to distinguish from the other two species, especially the latter one. Although older specimens can become brownish, this is less consistent than it is in *T. mesoamericanum*.

Tricholoma mesoamericanum Justo & Cifuentes sp. nov.

FIG. 2D, E

MycoBank MB816649

Typification: MEXICO. STATE OF OAXACA: Ixtlán de Juárez, 0.5 km north of “Rancho de Los Torres.” 1 Aug 2005, Pérez Ramírez 2922 and Cifuentes 2005-130 (**holotype** FCME 21585).
GenBank accession: ITS = KX037037.

Etymology: *Mesoamericanum* refers to the general area where the species is known to occur.

Misapplied name: *Tricholoma magnivelare* auct.

Diagnosis: A member of the *Tricholoma matsutake* group by virtue of its size, stature, well developed annulus, distinctive odor, and ecological occurrence, but distinct in its

combination of overall brown color in age, reduced scaliness of the pileus surface, ITS sequence, host-tree associates, and geographic distribution.

Description: Pileus 40–165 mm diam, convex or occasionally broadly subumbonate when young, expanding to plano-convex, becoming depressed at center in older specimens; surface white and smooth in young specimens, fibrils more evident in age, pale yellow (4A1–2) to brown (5–6D5), distinctly brown all over in older specimens, dry to viscid; margin inrolled when young, with attached fibrillose veil remnants, expanding and losing the veil fibrils with age. Lamellae crowded to very crowded, adnate to sinuate, white, spotted brown in age, with concolorous, entire edge. Stipe 40–175 × 15–40 mm, cylindrical, tapered toward the base; surface dry or slightly viscid, white to pale yellow (4A1). Partial veil sheathing from stipe base, entire or breaking up in patches, forming a white, cottony annulus in the upper part, with yellow or brown spots all over in age. Context white or very pale yellow (4A1), firm; odor fragrant, sweet with somewhat spicy character, taste mild to somewhat sweet.

Basidiospores white in mass, smooth, non-amyloid, 4.5–6.5 × 3.5–4.5 μm, mean 5.4–5.5 × 4.0–4.1 μm, Q = 1.27–1.48, mean 1.37–1.39, broadly ellipsoidal [60/2/1]. Basidia 22–38 × 4.5–6.5 μm, mostly 4-spored, but 2-spored also present, narrowly clavate. Cystidia absent; lamella edge fertile with basidia intermixed with basidioles. Lamella trama parallel, composed of hyaline cylindrical hyphae with thin, smooth walls, up to 6.5 μm wide, with some inflated elements up to 15 μm wide. Pileipellis a cutis, in areas transitional to an ixocutis, composed of hyphae 5–11 μm wide, mostly hyaline, some with pale brown intracellular pigment. Stipitipellis a cutis; hyphae up to 10 μm wide; mostly hyaline; with thin, smooth walls. Partial veil

composed of cylindrical hyphae up to 9 μm wide; hyaline; with thin, smooth walls. Clamp-connections absent.

Ecology and distribution: Solitary to gregarious, often in complete or incomplete rings, in montane (ca. 2000–3000 m elevation) mixed *Pinus-Quercus* forests subject to summer rainfall, with species such as *Pinus teocote*, *P. douglasiana*, *P. patula*, *Quercus scytophylla*, *Q. crassifolia*, *Q. laurina*, *Q. rugosa*, and *Q. conzattii*; most abundant in the states of Hidalgo, Oaxaca, Veracruz, and México but also occurs at least in the states of Chihuahua, Guerrero, Durango, Michoacán, Puebla, Colima, Morelos, and Tlaxcala; fruiting mainly Jul through Oct, with peak abundance in Aug and Sep.

Notes: *Tricholoma mesoamericanum* is morphologically very similar to the other matsutake, the principal differences being its geographic distribution, frequent association with many endemic Mexican tree species, and ITS sequence. Its tendency to become brown overall with age helps distinguish it from *T. murrillianum* and *T. magnivelare*, and the less scaly aspect of the pileus helps distinguish it from *T. magnivelare* and *T. matsutake*. The existence of a possible new Mexican species was suggested by Hosford et al. (1997) and later supported by the molecular studies of Lim et al. (2003), Chapela and Garbelotto (2004), and Ota et al. (2012), but the species was not formally described by those authors.

Other specimens examined: MEXICO. CHIHUAHUA STATE: Bocoyna Co., road Nerócnacai-Goracai, 16 Aug 1998, *Moreno-Fuentes* 503. GUERRERO STATE: Taxco Co.: El Huizteco Hill Park, 14 Jul 1987, *Villegas* 838 and *Cifuentes* 37-33. HIDALGO STATE: Zacualtipán Co., 15 Jul 1978, *Villegas* and *Cifuentes* 27. (All FCME).

DISCUSSION

Tricholoma matsutake, *T. magnivelare*, and *T. murrillianum* are distinct species. Our results support the existence of the five clades within the matsutake group suggested by previous work (Chapela and Garbelotto 2004; Gulden et al. 2014; Ota et al. 2012) and, with the inclusion of North American type collections, confirmed that there are at least three species in the matsutake group in North America. Thus, contrary to some suggestions following Chapela and Garbelotto's (2004) study, the northeastern matsutake is *T. magnivelare*, not *T. matsutake*. Further, as believed by Murrill (1912, 1914), the matsutake of western and eastern USA/Canada represent two similar, but distinct, species. Although closely related, the genetic distance between the two within the matsutake group is great—phylogenetically, *T. magnivelare* is much closer to *T. matsutake* than it is to *T. murrillianum*, its most distant relative in the group. Their main morphologic difference is one of color—typical young sporocarps of *T. murrillianum* are white or almost so, whereas those of *T. magnivelare* are light tan. Both are considerably lighter than the dark brown sporocarps of *T. matsutake* (FIG. 2A-C). Although this might seem to be a rather subtle feature, for someone familiar with the species, the difference in most cases is clear. For example, Norwegian mycologist Gro Gulden, who has studied *Tricholoma* for many years, found it difficult to accept that the *T. magnivelare* found during a visit to Newfoundland could be the same species as *T. matsutake* (at a time when the eastern North American matsutake was being considered by some to be *T. matsutake*). Her scepticism is borne out by the results of our analysis. In his original observations of *T. murrillianum*, Murrill was convinced that it differed from the eastern species. Assistant Director of the New York Botanical Garden at the time, he was familiar with Peck's *A. magnivelaris* and, although noting

its similarity to his new species, recognized the latter as different. Therefore, it is fitting that this mushroom should now bear Murrill's name.

The third North American clade reported here, represented by samples from Mexico, confirms the suggestions of previous studies that an unrecognized species could exist there. Thus, here we describe the new species, *Tricholoma mesoamericanum*.

All of the species in the matsutake group grow in similar ecological settings. They are ectomycorrhizal and typically occur in coarse-textured, well drained soils, such as dune sands, that are low in both moisture and nutrients. As a consequence, the soils usually are low in organic matter, although often overlain by a ground cover of moss or fruticose lichens (Murrill 1912; Zeller and Togashi 1934; Hosford et al. 1997; Kranabetter et al. 2002). Although referred to as "pine mushrooms," *T. matsutake*, *T. magnivelare*, *T. mesoamericanum*, and *T. murrillianum* all are reported to occur in association with a variety of other conifers in Pinaceae (Farlow 1929; Zeller and Togashi 1934; Hosford et al. 1997; Kranabetter et al. 2002; Zamora-Martínez and Nieto de Pascual-Pola 2004; Siegel and Schwarz 2016). In addition, both *T. mesoamericanum* and *T. murrillianum* also are reported with woody angiosperm species (Hosford et al. 1997; Zamora-Martínez and Nieto de Pascual-Pola 2004; Siegel and Schwarz 2016). The mycelium of *T. murrillianum* is frequently parasitized by the mycoheterotrophic plant, *Allotropia virgata* (Bidartondo and Bruns 2001, 2002; Trudell et al. 2003; Leake 2005), however, to our knowledge, no similar relationship has been reported for other species of matsutake.

As is often the case when what has been thought to be a single widespread species is found to comprise more than one species, determining the precise distributions of the three

North American matsutake species on the basis of the existing information is not possible. Thus, we described distributions as accurately as existing data and our collective experience allow. However, it is clear that matsutake (usually referred to as "*Tricholoma magnivelare*" in the following cited accounts) occurs in areas not covered by our evaluation, particularly in the boreal forest zone of Canada, possibly extending into Alaska. Redhead (1984) cited specimens from Northwest Territories, then presented a map that shows it occurring in Manitoba (Redhead 1989), and he later (Redhead 1997) described a seemingly bimodal distribution including "from Alaska, the Yukon and the Northwest Territories to California and Colorado, along the west coast, and in eastern North America, from northern Québec and the Canadian maritime region to Tennessee in the Great Smoky Mountains." That description is very similar to the ones we present for *T. murrillianum* and *T. magnivelare*, respectively. Matsutake is not included in a recent brochure illustrating common mushrooms of the Yukon Territory (Government of Yukon Territory 2014). Although this does not exclude its occurrence in the Territory, it could suggest that the species (singular or possibly plural) is not abundant there. Visser (1995) reported *T. magnivelare* from a mature jack pine (*P. banksiana*) stand in northern Alberta. Bossenmaier (1997) provided a description and photograph from the vicinity of Prince Albert National Park in Saskatchewan. To us, the photograph appears more like *T. murrillianum* than *T. magnivelare* but it does not allow a confident species determination. Pomerleau (1951) did not mention matsutake in eastern Canada but later (Pomerleau 1980) stated that it is "Found rarely in southern Québec in conifer and mixed woods." Miron (1994) described it as occurring with jack pine in the boreal forest of Quebec. This observation, along with the documented occurrence of *T. magnivelare* with jack pine in Michigan, suggests the high

likelihood of it occurring in similar habitat in Ontario and perhaps throughout the range of jack pine, which extends northwesterly beyond Ontario through much of Manitoba, the northern two-thirds of Saskatchewan, east-central Alberta, and the south-central and southeastern portions of Northwest Territories. This would suggest that the Rocky Mountain chain, including Canadian ranges such as the Selwyn and MacKenzie, could represent the main dividing feature between *T. murrillianum* and *T. magnivelare*. However, future collecting in these areas and close examination of morphological features along with DNA sequence analyses will be necessary before details of the species ranges can be ascertained.

Our spore measurements from the types of *T. magnivelare* and *T. murrillianum* show they are nearly the same in size and shape. These measurements were slightly larger than those reported by Zeller and Togashi (1934) for *T. murrillianum* (as *Armillaria ponderosa*; $4\text{--}7 \times 3\text{--}5 \mu\text{m}$, mean $5.16 \times 4.17 \mu\text{m}$). Intraspecific variability and differences in equipment and observer procedure likely contribute to the difference. In the same paper, those authors also reported that spore length and width differed by as much as 0.50 and $0.34 \mu\text{m}$, respectively, in separate spore prints obtained from a single maturing sporocarp over a 5-day period. Thus, the maturity of the sporocarps from which the measured spores were derived also could have been a factor. The spores from the *T. magnivelare* and *T. murrillianum* types are only slightly larger than those from the type collection of *T. mesoamericanum* and so spore size appears to be of little, if any, practical taxonomic value for differentiating the three North American species. While our measurements for *T. magnivelare* are similar to the mean values reported by Zeller and Togashi (1934) for *T. matsutake* ($3\text{--}8 \times 3\text{--}6 \mu\text{m}$, mean $5.57 \times 4.40 \mu\text{m}$), they are distinctly smaller than those reported for the latter species by Kytövuori (1988; $6.6\text{--}8.4\text{--}(9.1) \times 5.0\text{--}6.3 \mu\text{m}$),

Christensen and Heilmann-Clausen (2013; $5.8\text{--}9.5 \times 4.4\text{--}7.6 \mu\text{m}$, mean $6.9\text{--}7.9 \times 5.3\text{--}6.3 \mu\text{m}$), and some Japanese authors (Imazeki et al. 1957, 1970, 1988; $6.5\text{--}7.5 \times 4.5\text{--}6.5 \mu\text{m}$) for that species. Thus, spore size appears to be valuable for distinguishing *T. magnivelare* from *T. matsutake*.

We are aware of a suggestion that the name "*Tricholoma magnivelare*" be conserved for application to what is now correctly called "*T. murrillianum*" because of the economic value of western North American matsutake. We do not support this suggestion. First, our sequencing of the type collections allows us to apply the correct names to the three currently known North American matsutake. Second, the name "*Tricholoma magnivelare*" is not typically used in the commercial trade. Rather, the mushrooms usually are referred to in the USA/Canadian trade as "matsees/matsis," "pine mushrooms," or "pines," and as "hongo blanco de ocote" in Mexico. When offered for sale in Japan, they are simply referred to as "matsutake," with the country of origin indicated. Thus, the correction of the scientific name reported here should have little, if any, impact on the commercial trade. Third, the suggestion ignores the fact that Mexico exports substantial quantities of *T. mesoamericanum* and it is not clear how this species would be treated under the suggested conservation. We believe that the group most impacted by the correction of names will not be the commercial industry, but rather the western North American professional and amateur mycological community. However, the latter are used to name changes and should not be affected to any great degree. After all, they have adjusted to "*Cantharellus cibarius*" becoming *C. formosus* and *C. californicus*, and the spring/summer "*Boletus edulis*" becoming *B. rex-veris*, as well as many other name changes.

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FOOTNOTES

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LEGENDS

FIG. 1. Best tree from the ML analysis of the nrITS dataset. Bootstrap values $\geq 80\%$ (above) and posterior probabilities ≥ 0.95 (below; from the BI analysis, which returned essentially the same tree topology as ML), are shown on the branches. The root length has been reduced to facilitate graphical representation.

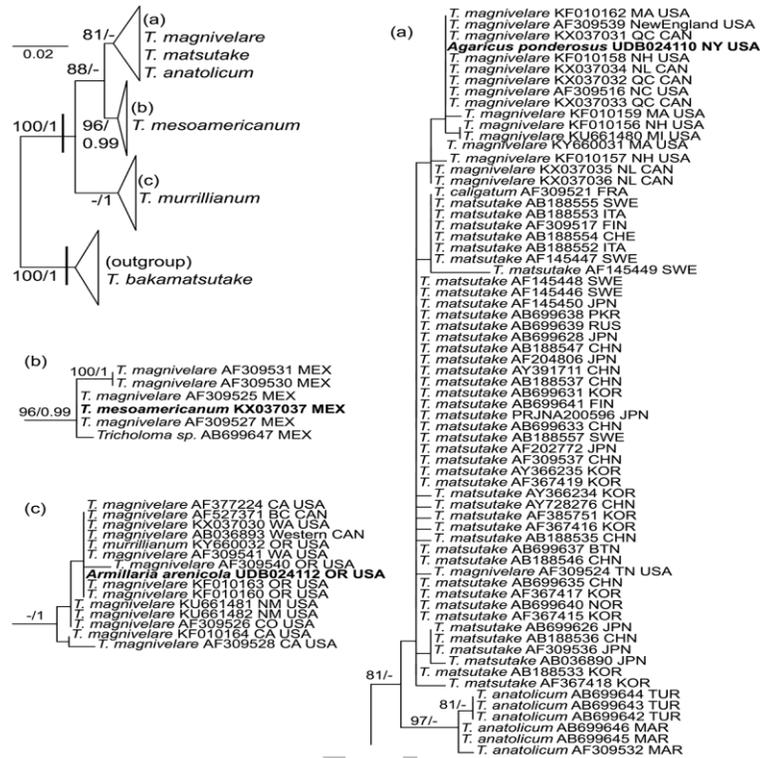


FIG. 2. Images of *Tricholoma matsutake* and the three North American matsutake species. A, B. *T. matsutake*, Japan (A. Yamada 2101021-001, 2071012-001). C. *T. matsutake*, Finland (J. Vauras 23243F; TUR-A170938). D, E. *T. mesoamericanum*, Oaxaca (holotype). F. *T. magnivelare*, New Brunswick (from Farlow 1929, courtesy of Archives of the Farlow Herbarium of Harvard University). G. *T. magnivelare*, Massachusetts (NS-16-307-01; WTU-F-068734). H. *T. murrillianum*, Washington (SAT-00-283-55; WTU-F-049207). I. *T. murrillianum*, Oregon (SAT-16-319-01; WTU-F-068823).



TABLE 1. Estimates of evolutionary divergence among sequence pairs within and between species analyzed here.

	<i>T.bakamatsu</i> <i>take</i>	<i>T.murrillia</i> <i>num</i>	<i>T.matsut</i> <i>ake</i>	<i>T.magnive</i> <i>lare</i>	<i>T.anatolic</i> <i>um</i>	<i>T.mesoameric</i> <i>anum</i>
<i>T.bakamatsu</i> <i>take</i>	0.01174					
<i>T.</i> <i>murrillianum</i>	0.12974	0.00229				
<i>T. matsutake</i>	0.11773	0.03656	0.00148			
<i>T.</i> <i>magnivelare</i>	0.11708	0.03665	0.00364	0.00080		
<i>T.</i> <i>anatolicum</i>	0.12487	0.03609	0.00951	0.01155	0.00095	
<i>T.</i> <i>mesoamerica</i> <i>num</i>	0.12670	0.03699	0.01167	0.01365	0.01651	0.00186

TABLE 2. Summary of spore measurements from the type collections of *Agaricus ponderosus* (*T. magnivelare*) and *Armillaria arenicola* (*T. murrillianum*).

	Length	Width	Q
<i>Agaricus ponderosus</i> (NYS f2421, n=50)			
Minimum	5.0	4.0	1.1
Maximum	7.5	5.5	1.8
Mean	5.89	4.59	1.29
Standard deviation	0.67	0.49	0.13
<i>Agaricus ponderosus</i> isotype (OSC 5598, n=50)			
Minimum	5.0	3.5	1.11
Maximum	7.5	5.5	1.75
Mean	6.23	4.42	1.42
Standard deviation	0.76	0.51	0.17
<i>Agaricus ponderosus</i> type and isotype combined (n=100)			
Mean	6.06	4.51	1.35
Standard deviation	0.73	0.51	0.16
<i>Armillaria arenicola</i> (NY 586560, n=75)			
Minimum	5.0	3.5	1.00
Maximum	7.5	5.5	1.57
Mean	5.93	4.63	1.29
Standard deviation	0.56	0.51	0.13