Research Report

BC Journal of Ecosystems and Management

Is the booted tricholoma in British Columbia really Japanese matsutake?

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Abstract

Using DNA sequence information, we compared three collections of the booted tricholoma (*Tricholoma caligatum*) and four collections of the pine mushroom (*Tricholoma magnivelare*) from British Columbia with the Japanese matsutake (*Tricholoma matsutake*) and with other North American collections of booted tricholoma and pine mushroom. We found that, in North America, the booted tricholoma is a distinct species and not the same as Japanese matsutake or pine mushroom. This implies that habitat information describing sites where pine mushroom is commercially harvested in British Columbia may not be relevant to the booted tricholoma. This may be important to forest managers concerned with pine mushroom management because although mushroom buyers purchase the booted tricholoma as pine mushroom, we don't know whether managing forests for pine mushroom would also manage for booted tricholoma.

KEYWORDS: *booted tricholoma, Japanese matsutake, British Columbia,* Tricholoma *sp., DNA sequence, pine mushroom management*.

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Introduction

The pine mushroom or American matsutake (*Tricholoma magnivelare*) is the most valuable mushroom commercially harvested from the forests of British Columbia (deGeus 1995; deGeus and Berch 1997). The fungus is also harvested commercially in Washington, Oregon, and California in the United States (Pilz and Molina 1997), in Mexico (Bandala *et al.* 1997), and to some extent in Quebec (Redhead 1997). Virtually all commercially harvested pine mushroom is shipped fresh to Japan where it is marketed as an acceptable, though less valuable, substitute for the Japanese matsutake (*T. matsutake*). In British Columbia, pine mushroom is commercially harvested from certain well-drained, nutrient-poor sites on coarse-textured soil in the Interior Cedar Hemlock, Sub-Boreal Pine–Spruce, Englemann Spruce–Subalpine fir, Interior Douglas-fir, and Coastal Western Hemlock biogeoclimatic zones (Berch and Wiensczyk 2001; Kranabetter *et al.* 2002).

Another similar species, the booted tricholoma (*T. caligatum*; see Figure 1), can be found mixed with pine mushroom at buying stations in British Columbia (Marty Kranabetter, B.C. Ministry of Forests, and Tyson Ehlers, Tysig Ecological Consultants, pers. comms., 2000) and is sold along with pine mushroom as Hongo Blanco in Mexico (Bandala *et al.* 1997). Both pine mushroom and the booted tricholoma are apparently accepted in the Japanese market as North American versions of matsutake. However, based on similarity in appearance and odour, Arora (1986) suggested that the booted tricholoma on the west coast of North America might be the same species as the Japanese matsutake. If this is correct, the booted tricholoma in British Columbia should be a more valuable commodity than the pine mushroom. For example, in October 1997, a kilogram of Japanese matsutake sold in Japan for the equivalent of CAD$300–741, while Canadian pine mushroom was valued at only CAD$36–83 (Wills and Lipsey 1999).

Bergius and Danell (2000) used DNA sequence information to study the affinities of matsutake in Japan, Korea, and Sweden, and compared them with a Moroccan collection of booted tricholoma (Takao Nakai, retired, Riken Institute of Physical and Chemical Research, pers. comm., 2003). They found that the Swedish, Korean, and Japanese matsutake are all the same species, but the Moroccan booted tricholoma is distinct. Kytövuori (1988) used the name "*Tricholoma matsutake*" as a synonym of the name "*T. nauseosum*" in reference to a species from parts of Europe and North Africa. Bergius and Danell (2000) also confirmed that in Europe, *T. nauseosum* and *T. matsutake* are the same species.

FIGURE 1. *Photograph of pine mushroom* (Tricholoma magnivelare*, bottom) and booted tricholoma* (Tricholoma caligatum*, top) collected in the Robson Valley, British Columbia.

In this study, we used DNA sequence information to determine whether the booted tricholoma in British Columbia is the same species as the Japanese matsutake, as suggested by Arora (1986), or whether it is a distinct species, as determined by Bergius and Danell (2000). DNA sequences from the nuclear ribosomal Internal Transcribed Spacer (ITS) regions are generally useful for sorting mushroom specimens into species or species-groups (Bruns 2001). The ITS regions evolve quickly and accumulate sequence differences that often serve to distinguish different species. As a basis for comparison, ITS sequences from many mushroom species (Bruns 2001), including *Tricholoma* spp. (Bergius and Danell 2002; Bitartondo and Bruns 2002), are already available in GenBank (National Center for Biotechnology Information: *www.ncbi.nlm.nih.gov/)* and other free public databases.

If the British Columbian specimens of booted tricholoma belonged to the same species as Japanese matsutake (i.e., conspecific) the DNA sequence variation from specimens collected under the two names would likely overlap, and sequences from both "species" would intermingle in phylogenies (or family trees) built from ITS sequences. On the other hand, if the two species were distinct, each would be expected to appear as a monophyletic group or "clade" (i.e., a group of biological taxa or species that share features inherited from a common ancestor) in phylogenies, and sequence variation between species would exceed sequence variation within a species. In this paper, seven new ITS sequences from British Columbia collections were compared with 23 sequences from GenBank to help resolve the taxonomic status of commercially important British Columbian pine mushroom species.

Methods

We obtained voucher collections of booted tricholoma (*Tricholoma caligatum*) and pine mushroom (*Tricholoma magnivelare*), which had been collected in British Columbia (Table 1), from the Pacific Forestry Centre in Victoria, British Columbia (Forest Pathology Herbarium [DAVFP]). DNA was extracted from seven different samples, and was sequenced and compared to related species (Table 1). A phylogenetic tree was then built to infer relationships among the samples.

DNA was extracted from dried samples of both species using the methods outlined in the DNeasy plant extraction kit (QIAGEN Inc., Mississauga, Ont.). This DNA was then amplified using puReTaq™ Ready-To-Go™ PCR beads (Amersham Biosciences, Piscataway N.J.) and ITS1F and TW13 as primers (Table 2). Five microlitres of each sample were then electrophoresed on a 1% agarose gel to confirm that the correct DNA had been amplified. These samples were sequenced with primers ITS1F, TW13, cTB6, and ITS4 (Table 2) using an Applied Biosystems AmpliTaq DyeDeoxy™ terminator kit and following the manufacturer's instructions (PE Applied Biosystems, Foster City, Calif.). Sequences were determined by the Nucleic Acid Protein Service at the University of British Columbia using an Applied Biosystems™ automated sequencer.

The resulting sequences were corrected and related sequences were retrieved in a BLAST (Basic Local Alignment Search Tool: *www.ncbi.nlm.nih.gov/blast/)* search, which rapidly scans nucleotide databases for similar sequences. Three sequences of booted tricholoma and five of pine mushroom (Table 1) from specimens not collected in British Columbia were selected from GenBank to add to the phylogenetic tree. Additional *Tricholoma* species were also included in the phylogenetic tree (Table 1).

These sequences were aligned using the computer program SeqApp (available through: *ftp://iubio.bio.indiana.edu/molbio/seqapp/)* and then analyzed in PAUP version 4.0b8 (Phylogenetic Analysis Using Parsimony, Swofford 1999) to create a phylogenetic tree. The ITS phylogenetic analysis was based on a parsimony analysis (Swofford 1999) using the bootstrap method without branch swapping ("fast" stepwise-addition, 500 replicates). *Tricholoma huronense, T. mutabile, T. pardinum*, and *T. venenatum* were used as the outgroups. According to Bidartondo and Bruns (2002), these four species are closely related, but they form a separate group from *T. magnivelare, T. matsutake*, and *T. caligatum*. We confirmed the outcome of this phylogenetic analysis by running another using parsimony analysis with branch-and-bound search (score of best tree found = 1237; number of trees retained = 18) with the same outgroups. This analysis (phylogram not shown) confirmed the other.

Results and Discussion

When sequences from 30 collections, reported from eight *Tricholoma* species, were analyzed phylogenetically (see Figure 2, page 6), collections of booted tricholoma *(T. caligatum*) from British Columbia differed from a South Korean matsutake (*T. matsutake* [synonym = *Tricholoma nauseosum*]) and from North American pine mushroom (*T. magnivelare*). The three British Columbia booted tricholoma collections grouped with collections from California, and their high sequence similarity suggested that they belong to the same species. The four pine mushroom collections from British Columbia grouped with *T. magnivelare,* and unidentified (*Tricholoma* sp.) collections from Oregon, California, and Mexico, were all *Tricholoma magnivelare*. The high bootstrap numbers in Figure 2 (over 98% for each species) indicate that the species clusters would not have been likely to result by chance. Average percent differences between pairs of sequences from different species were 3.5–10 times greater than the average percent differences within a species group. The average distance between pairs of sequences from within the *T. caligatum* clade was 0.2% (range: 0–0.7%); the average distance between pairs within the *T. magnivelare* clade was 0.4% (range: 0–1.3%); and the average distance in the *T. matsutake* clade was 0.8% (range: 0.5–1.2%). In contrast, the average distance

TABLE 1. Collections used for phylogenetic analysis of nuclear internal transcribed spacer (nrITS) sequences of fungal species related to pine mushroom (*Tricholoma magnivelare*)

Species	GenBank accession number	Collection location	Date of collection	Herbarium and voucher collection
Tricholoma caligatum (Viv.) Ricken	AF527373	McBride, B.C., Canada	20-Sep-01	DAVFP26219
	AF527374	McBride, B.C., Canada	18-Sep-01	DAVFP26217
	AF527372	Tete Jaune, B.C., Canada	20-Sep-01	DAVFP26218
	AF309523	Yuba County, Calif., U.S.A.	—	SFSU HDT48319
	AF309533	Tuolomne County, Calif., U.S.A.	—	SFSU MGW48
	AF377225	Yuba County, Calif., U.S.A.	—	SFSU HDT48319
Tricholoma fulvocastaneum Hongo	AB036901	Wakayama Prefecture, Japan	1988	? MR28
Tricholoma huronense A.H. Smith	AF377229	Olympic National Forest, Wash., U.S.A.	—	SFSU KMS248
Tricholoma magnivelare (Peck) Redhead	AB036893	Exported to Japan from Canada	1994	—
	AF309531	Oaxaca, Mexico		I. Chapela personal collection
	AF309540	Oregon, U.S.A.	—	I. Chapela personal collection
	AF377223	Olympic National Forest, Wash., U.S.A.	—	SFSU DED5372
	AF377224	Yuba County, Calif., U.S.A.	—	SFSU KMS232
	AF527370	Valemount, B.C., Canada	04-Oct-00	DAVFP25966
	AF527368	Valemount, B.C., Canada	05-Oct-00	DAVFP25945
	AF527369	Valemount, B.C., Canada	20-Sep-01	DAVFP26221
	AF527371	McBride, B.C., Canada	18-Sep-01	DAVFP26220
Tricholoma matsutake (S. Ito & Imai) Singer	TMU62964	Uljin, Gyungsang-buk- do, South Korea	Sep-93	—
Tricholoma mutabile Shanks	AF349703	Yuba County, Calif., U.S.A.	—	SFSU KMS428
Tricholoma nauseosum (Blytt) Kytovuori	AB036891	Exported to Japan from Mexico	1992	—
Tricholoma pardinum Quél.	AF377228	Sonoma, Calif., U.S.A.	—	SFSU KMS197
Tricholoma ponderosum (Sacc.) Singer	AF204811	Canada	05-Oct-97	—
Tricholoma sp.	AF349706	Lane County, Oreg., U.S.A.	2000	—
Tricholoma sp.	AF377217	Lane County, Oreg., U.S.A.	2000	—
Tricholoma sp.	AF377218	Napa County, Calif., U.S.A.	1999	—
Tricholoma sp.	AF377219	Siuslaw National Forest, Oreg., U.S.A.	1999	—
Tricholoma sp.	AF377220	Klamath County, Oreg., U.S.A.	2000	—
Tricholoma sp.	AF377221	Umpqua National Forest, Douglas County, Oreg., U.S.A.	1999	—
Tricholoma sp.	AF377222	Siskyou National Forest, Curry County, Oreg., U.S.A.	1999	—
Tricholoma venenatum G.F. Atk.	AF377230	Sierra, Calif., U.S.A.	—	SFSU KMS396

TABLE 2. Primers used for sequencing[a] (Bruns Lab Web site: *http://plantbio.berkeley.edu/~bruns/*)

Primer	Sequence
ITS1-F	CTTGGTCATTTAGAGGAAGTAA
TW13	GGTCCGTGTTTCAAGACG
cTB6	GCATATCAATAAGCGGAGG
ITS4	TCCTCCGCTTATTGATATGC

[a] Sequences are written 5'–3'.

between sequences of *T. caligatum* and *T. magnivelare* was 8.1% (range: 4.7–9.5%); the average distance between sequences of *T. caligatum* and *T. matsutake* was 8.7% (range: 5.6–10.5%); and the average distance between sequences of *T. magnivelare* and *T. matsutake* was 3% (range: 2.2–3.8%). This indicates (with 93% bootstrap support) that *T. matsutake* is not conspecific with *T. caligatum* from the West Coast and that *T. magnivelare*, not *T. caligatum*, is most closely related to *T. matsutake*.

One remaining taxonomic question concerns the identity of two collections from Mexico. The collections—identified in GenBank as *T. magnivelare* (AF309531) and *T. nauseosum* (AB036891)—grouped together, even though (if they had been correctly identified) the first (AF309531) should have grouped with other *T. magnivelare* collections and the second (AB036891) with *T. matsutake* (see Bergius and Danell 2000) (Figure 2). Further study of Mexican collections may reveal whether those populations represent an undescribed species closely related to *T. matsutake* (note the 98% bootstrap support in Figure 2), or merely a high level of diversity within *T. matsutake*.

A second remaining question concerns the Moroccan versus the North American booted tricholoma. Our results support Ito and Yanagi's (1999) conclusion, based on random amplified polymorphic DNA (RAPD) analysis, that matsutake and booted tricholoma are distinct species. Also, like Bergius and Danell (2000), we found that ITS sequences in *T. matsutake* and *T. caligatum* were different. However, Bergius and Danell (2000) reported that their Moroccan *T. caligatum* sequences (GenBank Accession No. D86572) differed from other *T. matsutake* sequences at 4% of the ITS sequence sites. We found considerably more divergence (8.7%) between North American *T. caligatum* and *T. matsutake*. Because Bergius and Danell's (2000) *T. matsutake* sequences are in GenBank and are 99% identical to the matsutake sequence used in this study, the *T. matsutake* sequences cannot account for the difference in divergence levels. The difference in divergence may indicate that "*Tricholoma caligatum*" is applied to different species in North America and in North Africa.

Based on their phylogenetic clustering (Figure 2) and sequence divergence, British Columbian pine mushroom (*T. magnivelare*) and booted tricholoma (*T. caligatum*) appear to be distinct species. Because the booted tricholoma and the pine mushroom are different species, they may also have distinct habitat preferences.

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In British Columbia, research on pine mushroom has revealed that the fungus occurs in commercial amounts in specific forest types (Berch and Wiensczyk 2001; Kranabetter *et al.* 2002). This is important in forest planning that addresses both timber and pine mushroom harvesting. Little is currently known about the habitat preferences of booted tricholoma in British Columbia, so managing for pine mushroom habitat might not preserve the booted tricholoma. At present, the Forest Pathology Herbarium at the Pacific Forestry Centre lists only four collections of *Tricholoma caligatum* for British Columbia—three from the Robson Valley and one from Jordan River on Vancouver Island. The Fungal Collection at the University of British Columbia has two collections, one from Lac La Jeune near Kamloops and one from the Sechelt Peninsula. Further research is needed to determine the true distribution and habitat preferences of booted tricholoma in the forests of British Columbia. Additional research is also required to help clarify how much of what is collected as pine mushroom is actually booted tricholoma.

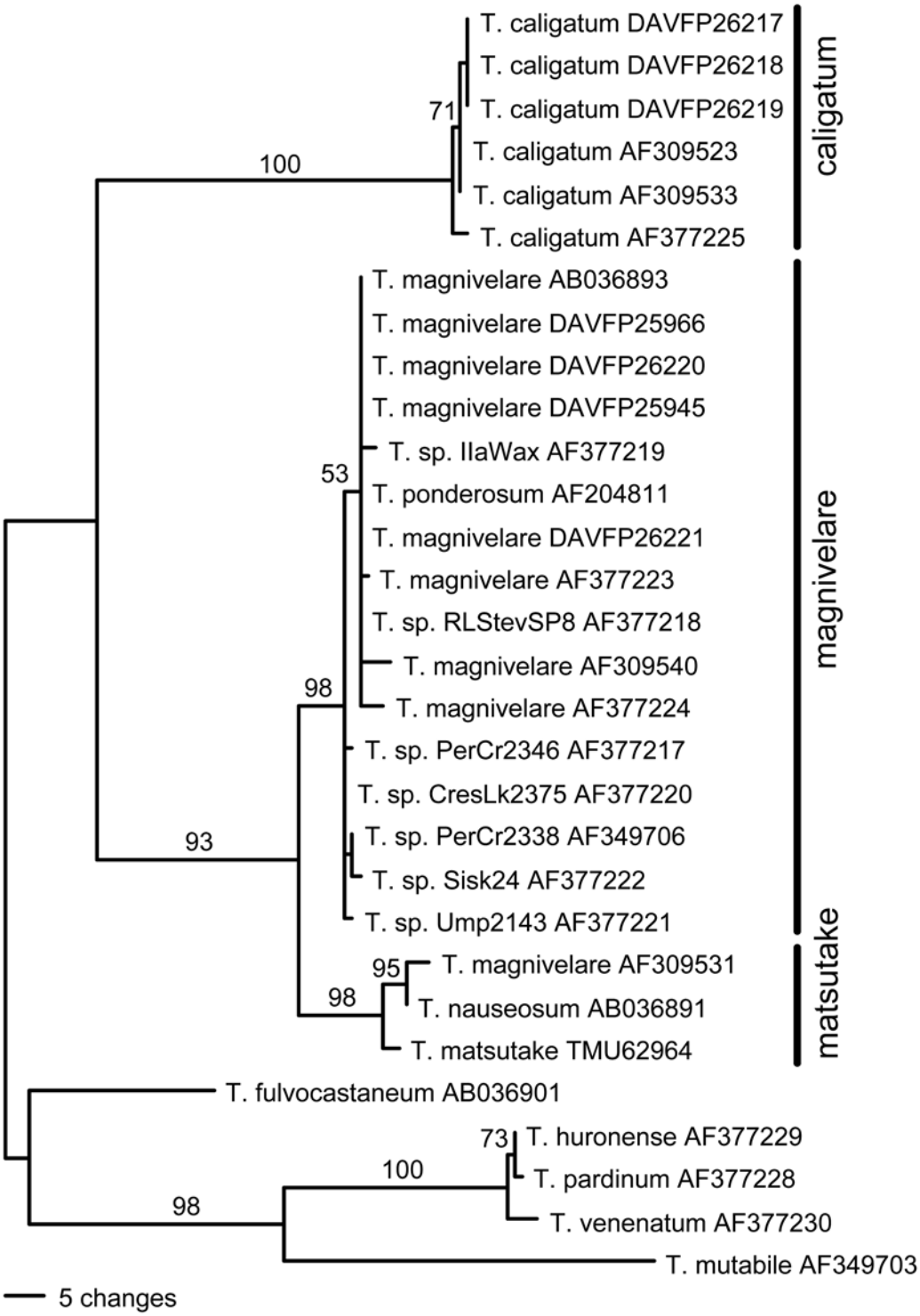

FIGURE 2. *Phylogenetic tree of 30 tricholoma collections from North America and Asia. This fungal nuclear internal transcribed spacer (nrITS) phylogram is based on parsimony analysis, a bootstrap method with fast-heuristic search ("fast" stepwise-addition, 500 replicates).* Tricholoma huronense, T. mutabile, T. pardinum, *and* T. venenatum *were used as the outgroups. Note: Each sequence has different length of nucleotides. Not all sequences have 5' partial 18S, ITS1, 5.8S, ITS2, and 3' partial 28S genes.*

Acknowledgements

This study was initiated by Sea Ra Lim and Alison Fischer as an undergraduate project in the Botany Department at the University of British Columbia. Thanks to Paul Kroeger for pointing out that our collections in the Robson Valley consisted of both pine mushroom and booted tricholoma and thereby inspiring this research. Thanks to Sharmin Gamiet for providing authorities for some of the species. Thanks also to Dr. Brenda Callan, Curator, Forest Pathology Herbarium for the loan of specimens.

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