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NOTE

DNA-based geographic typing of the gourmet mushroom *Tricholoma matsutake* traded in China

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Abstract *Tricholoma matsutake* is among the most valuable mushrooms in the world, and natural populations of this species from different geographic regions are priced very differently. To examine the geographic origins of 'matsutake' mushrooms traded in China, we analyzed the mushrooms from two major production and trading regions in China, the Southwest and the Northeast, using a recently published retroelement-based DNA marker. Our analyses showed that 67% of commercial matsutake claimed to be from the northeast were in fact genetically identical to those from the southwest but were different from authentic northeast samples. The finding suggests that caution should be applied to the authentication of commercial matsutake from northeast China.

Keywords Traceability · DNA fingerprinting · Gourmet mushroom · Matsutake

Mushrooms have been widely used since ancient times, not only as foods or food flavoring materials but also for spiritual and medicinal purposes (Stamets 1993). The ectomycorrhizal matsutake (or pine-mushroom, *Tricholoma matsutake* Singer, Phylum Basidiomycota) is among the most revered and valuable mushrooms in the world. This species is distributed in Europe, North America, and Asia (Xu et al. 2001, 2008; Chapela and Garbelotto 2004; Murata et al. 2008). Matsutake collected from different parts of the world are priced very differently in Japan—the world's preeminent consumer market—from less than US$100 to more than US$4000/kg (Murata et al. 2008). In Asia, matsutake is produced in two major regions: Eastern Himalaya, which includes Southwest (SW) China and Bhutan, and the Far East, which includes Japan, the Korean Peninsula, and Northeast (NE) China. The NE Chinese matsutake is distributed in the Changbai Mountain range bordering North Korea and covers parts of two provinces, Jilin and Heilongjiang (Xu et al. 2001). In addition, matsutake from North Korea is often traded in this region of NE China. This region produces about 500–600 tons per year with the majority exported to Japan (Xu et al. 2001; Ge 2004). The SW Chinese matsutake is distributed in eastern Tibet and western parts of Yunnan and Sichuan provinces (Xu et al. 2008). This region produces about 1500 tons per year, with 1100–1300 tons exported to Japan (Zhang 2004). In Japan, the NE Chinese matsutake is sold for an average of about US$200/kg, similar to those from North Korea, slightly lower than South Korean matsutake, and about half the price of those from within Japan. However, the matsutake from SW China is traded at about US$100/kg, significantly lower than their NE counterpart and other Far East matsutake (Murata et al. 2008). Such a price variation is also seen within the Chinese domestic consumer market. Despite its high price tag, matsutake's unique aroma (Takama et al. 1984) and its demonstrated health benefits to humans (Ebina et al. 2002; Hoshi et al. 2005) are attracting a growing number of people within China.

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Table 1 Chinese *Tricholoma matsutake* samples analyzed in this study

Source/company location	True or claimed geographic origin	Sample size	Geotype (geographic affiliation, % isolates)
#1, Erdaobaihe, Changbai, Jilin, NEC	#1-1: Wangqing, Jilin	13	B (SWC, 100)
	#1-2: Tumen, Jilin	15	B (SWC, 100)
#2, Yanji, Yanbian, Jilin, NEC	#2-1: Dongning, Heilongjiang, NEC	15	B (SWC, 100)
	#2-2: Ning'an, Heilongjiang, NEC	15	B (SWC, 100)
#3, Yanji, Yanbian, Jilin, NEC	#3: Antu, Jilin, NEC	14	B (SWC, 100)
#4, Yanji, Yanbian, Jilin, NEC	#4-1: Hunchun, Jilin	15	A (NEC, 100)
	#4-2: Tumen, Jilin	20	A (NEC, 100)
#5, Shangri-La, Yunnan, SWC	Shangri-La, Yunnan	15	B (SWC, 100)
#6, Shangri-La, Yunnan, SWC	Deqin, Yunnan, SWC	15	B (SWC, 100)
#7, Shangri-La, Yunnan, SWC	Weixi, Yunnan, SWC	15	B (SWC, 100)
Authentic matsutake collected by authors and colleagues[a]	Dongning, Heilongjiang, NEC	7	A
	Antu, Jilin, NEC	10	A
	Hunchun, Jilin, NEC	8	A
	Longjing, Jilin, NEC	13	A
	Baiyu, Sichuan, SWC	12	B
	Linzhi, Tibet, SWC	2	B
	Ailaoshan, Yunnan	19	B
	Deqin, Yunnan	8	B
	Weixi, Yunnan	10	B
	Shangri-La, Yunnan	30	B
	Lijiang, Yunnan	15	B
	Lanping, Yunnan	11	B
	Longling, Yunnan	2	B
	Yongping, Yunnan	7	B
	Jianchuan, Yunnan	9	B
	Lincang, Yunnan	9	B
	Nanhua, Yunnan	9	B
	Lufeng, Yunnan	8	B
	Chuxiong, Yunnan	9	B
	Luquan, Yunnan	7	B
	Yimen, Yunnan	13	B
	Luliang, Yunnan	3	B

NEC, Northeastern China; SWC, Southwestern China

[a] Of the 183 authentic matsutake samples analyzed here from southwestern China, 154 were from Xu et al. (2008)

To examine the geographic origins of matsutake traded in China, we analyzed DNA profiles of *T. matsutake* fruiting bodies obtained from Chinese trading companies and compared them with known specimens from NE China and SW China. The commercial matsutake included 107 fruiting bodies from four companies in NE China and 45 from three companies in SW China (Table 1). There are 24 officially licensed and about 30 unlicensed matsutake trading companies in NE China, and all our 107 commercial samples from this region were obtained from licensed traders. The commercial NE China samples were claimed to be from six geographic areas from Jilin and Heilongjiang provinces (both in NE China) whereas those from SW China were claimed to be from three counties in Yunnan province. The two cities, Yanji in Jilin and Shangri-La in Yunnan, where our commercial matsutake were obtained, are the main sites for matsutake trading in NE and SW China, respectively. The geographically authentic matsutake samples included 38 mushrooms from four local populations in Jilin and Heilongjiang in NE China (longitude 127°48′ E–131°17′ E; latitude 42°24′ N–44°52′ N), and 183 samples from 18 local populations in Yunnan, Tibet, and Sichuan provinces in SW China (longitude 94°23′ E–104°65′ E; latitude 23°15′ N–32°23′ N) (see Table 1). The authentic samples were collected by us and our collaborators directly from their natural habitats. The sampled

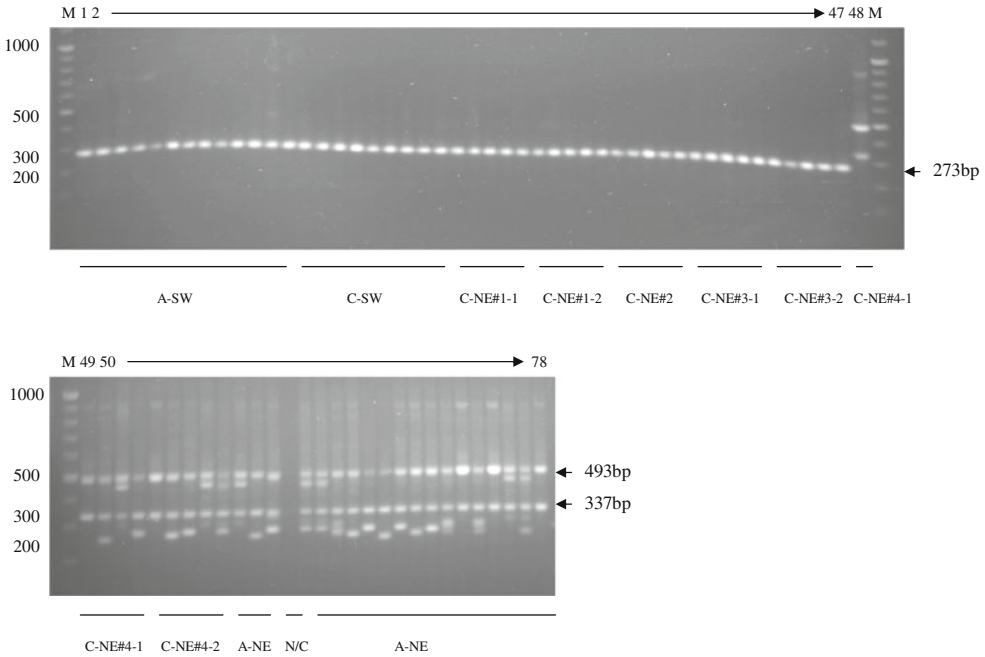

Fig. 1 Polymerase chain reaction (PCR) profiles of representative Chinese isolates of *Tricholoma matsutake* based on the pDGSL313-1/pS48 primer typing system. The lane numbers are indicated at the *top* and the origins of specimens are given at the *bottom* of gels: *A-SW*, authentic southwestern matsutake (*lanes 1–13*); *C-SW*, commercial southwestern matsutake (*lanes 14–22*); *C-NE#1-1*, commercial northeast matsutake claimed to be from Wangqing, Jilin (*lanes 23–27*); *C-NE#1-2*, commercial northeast matsutake claimed to be from Tumen, Jilin (*lanes 28–32*), *C-NE#2-1*, commercial northeast samples claimed to be from Dongning, Heilongjiang (*lanes 33–37*); *C-NE#2-2*, commercial northeast samples claimed to be from Ning'an, Heilongjiang (*lanes 38–42*); *C-NE#3*, commercial northeast samples claimed to be from Antu, Jilin (*lanes 43–47*), *C-NE#4-1* commercial northeast samples claimed to be from Hunchun, Jilin (*lanes 48–52*); *C-NE#4-2*, commercial northeast samples claimed to be from Tumen, Jilin (*lanes 53–57*); *A-NE*, authentic northeast matsutake (*lanes 58–60*, *62–78*), *N/C*, negative control (water, no sample DNA); *lanes M*, molecular markers (200–1000 bp). *Right-hand labels* indicate signature DNA fragments corresponding to SW (273 bp) and NE (493 and 337 bp) Chinese matsutake samples

areas spanned almost the entire matsutake distribution range in both NE and SW China (Xu et al. 2001, 2008).

The genotypes of all 373 isolates were determined using a recently described polymerase chain reaction (PCR) genotyping system based on specific primers targeting the *gypsy*-type retroelements (Murata et al. 2008). Briefly, genomic DNA from the silica-dried internal cap tissue of each of the 373 mushroom isolates was extracted using the CTAB protocol described previously (Xu et al. 1994) and stored at –20°C until use. PCR was carried out in 15 µl reaction mixtures containing 200 µM deoxynucleoside triphosphates, 0.5 µM primers (the pDGSL313-1 and pS48 primer pair described in Murata et al. 2008), 10 ng template genomic DNA, and 0.5 U *Taq* polymerase (Fermentas, Burlington, ON, Canada) and a universal buffer provided with the enzyme. Cycle reactions were performed as follows: 1 cycle of 94°C for 4 min; 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min; and 1 cycle of 72°C for 10 min in an Eppendorf Mastercycler (VWR CanLab, Missisauga, ON, Canada). The amplified PCR products were electrophoresed in a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide in the TAE buffer and run for 3 h at 200 V.

Murata et al. (2008) showed that the primer pair pDGSL313-1/pS48 unambiguously separated the Asian matsutake into two types: geotype A from the Far East and geotype B from Eastern Himalaya. Our analyses of the 221 authentic matsutake samples showed results consistent with the earlier findings: all authentic NE samples had the geotype A-specific 493 and 337-bp bands while all the authentic SW matsutake had only the geotype B-specific 273-bp band (Murata et al. 2008) (Fig. 1; Table1). The 45 commercial matsutake from all three trading companies in SW China were indistinguishable from the authentic SW isolates, consistent with no evidence of counterfeiting in this region. However, 72 (of 107) matsutake from three of the four trading companies in NE China had a genotype profile completely identical to those from SW China and distinctly different from those authentic NE Chinese matsutake (Fig. 1, Table1). Our assay would predict that only one (#4) of the four companies in NE China was selling authentic NE Chinese matsutake (Table 1).

The apparent counterfeiting [three of four surveyed trading companies (75%) in NE China including 72 of the 107 analyzed isolates (67%)] will likely increase in the near future because of the large price differences among

regions, a large and unsaturated market in Japan, expanding popularity within China, and the shrinking supply of matsutake in Japan, China, and Europe (Arnolds 1991; Wang and Hall 2004; Xu et al. 2001, 2008). Whether this traceability system is broadly applicable to other matsutake production areas both within and outside Asia remains to be determined. Our results confirmed the feasibility of a matsutake traceability system in China and indicated that caution should be applied to authenticate *T. matsutake* from NE China. Furthermore, our finding raises the need for a scientific traceability system in food crops in China.

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Conflict of interest statement We declare no conflicts of interest.

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